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LONG-TERM POTENTIATION AND LEARNING

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ABSTRACT

Long-term potentiation (LTP), a relatively long-lived increase in synaptic strength, remains the most popular model for the cellular process that may underlie information storage within neural systems. The strongest arguments for a role of LTP in memory are theoretical and involve Hebb's Postulate, Marr's theory of hippocampal function, and neural network theory. Considering LTP research as a whole, few studies have addressed the essential question: Is LTP a process involved in learning and memory? The present manuscript reviews research that attempts to link LTP with learning and memory, focusing on studies utilizing electrophysiological, pharmacological, and molecular biological methodologies. Most evidence firmly supports a role for LTP in learning memory. However, an unequivocal experimental demonstration of a contribution of LTP to memory is hampered by our lack of knowledge of the biological basis of memory and of the ways in which memories are represented in ensembles of neurons, the existence of a variety of cellular forms of LTP, and the likely resistance of distributed memory stores to degradation by treatments that incompletely disrupt LTP.

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INTRODUCTION

All neurobiologists would agree that information is acquired, stored, and retrieved by the brain; memory is a thing in a place in a brain. Unfortunately, we do not understand completely how any brain encodes memory as a biological entity. However, the brain's cellular architecture provides clues. All brains consist of individual cellular units or neurons. Most neurons have the same parts: a dendritic tree, cell-body, axon, and synaptic buttons. The majority of neurons communicate with each other across a synaptic space via neurotransmitters and neuromodulators. In mammalian brains, billions of neurons interconnect in vast networks via even more billions of synapses. This fact leads to our first assertion about memory.

Assertion 1: Memory Is Stored in Networks of Neurons

The brain accomplishes all of its remarkable activity through networks of neurons. A single neuron is unlikely to encode a specific memory; rather, ensembles of neurons participate in maintaining a representation that serves as a memory. Such ensembles require dynamic interactions among neurons and an ability to modify these interactions. This implies a need for use-dependent changes in synaptic function and leads to the second assertion about memory.

Assertion 2: Memory Is Stored through Changes in Synaptic Function

Hebb (1949) increased our understanding of how networks of neurons might store information with the provocative theory that memories are represented by reverberating assemblies of neurons. Hebb recognized that a memory so represented cannot reverberate forever and that some alteration in the network must occur to provide integrity both to make the assembly a permanent trace and to make it more likely that the trace could be reconstructed as a remembrance. Thus, our second assertion is that, because neurons communicate with

each other only at synapses, the activity of the assembly or network is most easily (perhaps only) altered by changes in synaptic function. Hebb (1949) formalized this idea in what is known as Hebb's Postulate: "When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased." Hebb's Postulate is very close to a modern-day definition of long-term potentiation (LTP) and leads to two more assertions about why LTP could be a mechanism of memory storage.

Assertion 3: LTP Could Operate in Networks of Neurons to Store Memory in a Manner Similar to That in Hebb's Postulate

Bliss & Lomo (1973) first reported that tetanic stimulation of the perforant path in anesthetized rabbits increased the slope of the population excitatory post-synaptic potential (EPSP) recorded extracellularly in the dentate gyrus and reduced the threshold for eliciting a population action potential (population spike). They defined LTP as potentiation that lasted longer than 30 min, although they observed LTP for several hours. Later studies showed that LTP recorded in animals with permanent indwelling electrodes lasted from weeks to months (Barnes 1979). Moreover, LTP is found in many areas of neocortex (Bear & Kirkwood 1993).

A line of reasoning that led to the conclusion that LTP is a mechanism of memory is derived from theoretical studies on neural networks. Marr (1971) described an associative network in area CA3 of the hippocampus in which distributed patterns of activity were imposed on principal cells; the trace became established as a result of strengthening synaptic connections. Since the work of Hebb (1949) and the discovery of LTP (Bliss & Lomo 1973), these theoretical connections among neurons that strengthen as a result of activity are referred to as Hebb Synapses.

Synaptic strengthening as described by the Hebb Rule could increase without bound. Because such a Hebbian mechanism would lead to saturation, anti-Hebb processes were suggested (Stent 1973, Sejnowski 1977). Recently there has been a surge of interest in long-term depression (LTD) both as a memory mechanism (homosynaptic or associative LTD) and as a process that normalizes synaptic weights in networks (homosynaptic and heterosynaptic LTD; cf Morris 1989b, Linden & Conner 1995, Rolls 1989, Derrick & Martinez 1995).

The use of the Hebb Rule in a distributed memory system can lead to efficient storage of a number of representations within the same network (also called correlation matrix memories; see McNaughton & Morris 1987), which can be regenerated with partial input (pattern completion). The notion of correlation matrix memories resolves the seeming paradox of how specific

memories or representations are stored in nonspecific (distributed) stores. Further, any particular part of the network is not essential for pattern completion; the performance of the entire network deteriorates gradually as more and more units are damaged or eliminated. This feature, referred to as graceful degradation, is a natural by-product of distributed memory stores (Rolls 1989, Rumelhart & McClelland 1986) and is characteristic of neural systems (see Rumelhart & McClelland 1986). Moreover, storage of memory within distributed systems rests on the ability of neurons to form synapse-specific alterations in synaptic strength. Thus we come to our third assertion about memory. If memory is stored in networks of neurons and if network efficiency is mediated by persistent activity (Hebb's Postulate), then LTP induced by persistent stimulation of an afferent pathway is at least one likely mechanism by which the brain stores information.

Together these three assertions provide a powerful rationale for the claim that LTP is a substrate of memory. However, because no one has isolated a memory trace, LTP cannot be studied in a known memory network. Thus the evidence reviewed in this paper is correlational and inferential. Before we consider the evidence, we discuss three other similarities between LTP and learning that some consider support the notion that LTP is a memory mechanism: LTP is specific to tetanized inputs, it is associative, and it lasts a long time. In our view, these arguments unfortunately focused discussion on similarities between classical conditioning and LTP that, to date, remain merely similarities.

LTP Is Specific to Tetanized Inputs

Since the time of Pavlov (1927), conditioned reflexes have been thought to involve specific neural pathways. In fact, simple neural reflexes may be incorporated into conditioned reflexes. LTP is specific in this way in that only tetanized afferents show potentiation, so-called homosynaptic LTP. Unfortunately, the idea of specificity of tetanized afferents has become clouded with reports that LTP induction might involve gases, such as nitrous oxide (NO), that readily diffuse into adjacent neurons (O'Dell et al 1991, Schuman & Madison 1991). Also, evidence suggests that maintenance of LTP involves retrograde messengers that also may affect neighboring neurons (Bonhoeffer et al 1989). This lack of specificity has advantages over a strict Hebb Rule in that diffuse alterations in presynaptic elements (referred to as volume learning) may permit the storage of the temporal order of inputs (Montague & Sejnowski 1994).

LTP Is Associative

Another interesting property of LTP, which led some researchers to suggest that it is a memory mechanism, is associativity. If weak non-LTP-inducing

stimulation in one afferent is paired with strong LTP-inducing stimulation in another afferent to the same cell population, then the weakly stimulated afferent exhibits LTP (Levy & Steward 1979, McNaughton et al 1978). The property of associativity is reminiscent of classical conditioning, in which a neutral CS is associated with a strong UCS to induce conditioning (Makintosh 1974). As the argument goes, because neural afferents in associative LTP act in a way similar to neural activity in classical conditioning, and because the mechanism of associative LTP is the same as in LTP, at least in *N*-methyl-D-aspartate (NMDA) receptor–dependent systems LTP is a memory mechanism. This proposition has been roundly criticized. The critics' view (Gallistel 1995) is that the temporal constraints of associative LTP are dissimilar to those of classical conditioning. In addition, the necessary ordering of CS and UCS are absent in associative LTP, and a mechanism as simple as associative LTP cannot account for the behavioral complexity observed in classical conditioning.

Today most researchers would agree that associative LTP is not classical conditioning (Diamond & Rose 1994). LTP does, however, bear comparison to a psychological example of learning. Associative LTP, described by Hebb (1949) as the simultaneous activity of sensory afferents, is more similar to sensory preconditioning than classical conditioning (Mackintosh 1974). Sensory preconditioning is the association of two sensory stimuli—for example, a tone and a light—by repeated pairing. The comparison of associative LTP and sensory preconditioning is straightforward: The stimuli need not be presented in a particular order, nor does a UCS need be present, as in classical conditioning. However, temporal contiguity for the presentation of the two stimuli is required (Kelso & Brown 1986, Mackintosh 1974). In our view, it is more proper to compare associative LTP to sensory preconditioning than to classical conditioning. An interesting observation in this regard is that hippocampal lesions appear to abolish sensory preconditioning (Port et al 1987).

From a behavioral point of view, LTP is more analogous to sensitization, and LTD is more analogous to habituation—both forms of nonassociative learning—than either is to classical conditioning. Habituation may be defined authoritatively as a "response decrement as a result of repeated stimulation" (Harris cited in Thompson & Spencer 1966). Sensitization may be defined as a response increment as a result of repeated (usually strong) stimulation (Thompson & Spencer 1966). LTP and LTD are response increments and decrements that result from repeated stimulation (Bliss & Lomo 1973, Dudek & Bear 1993). Most researchers would not agree that LTP is analogous to sensitization because induction of LTP requires that a threshold number of fibers have to be simultaneously active (McNaughton et al 1978). Cooperativity could involve associative interactions within the postsynaptic target or

among presynaptic fibers (whereas Hebbian associativity implies a postsynaptic associative effect of multiple fibers).

The comparison of LTD and habituation has not been made, but a parametric analysis of habituation is available (Thompson & Spencer 1966). Habituation and sensitization were recognized quite early to be separate processes, and dishabituation was viewed as sensitization induced simultaneously with habituation (Thompson & Spencer 1966). An analogous contemporary conundrum is whether depotentiation represents the addition of separate and oppositely signed processes, or the cellular reversal of LTP (Bear & Malenka 1994). While the comparisons of LTP and LTD to psychological phenomena will undoubtedly continue, it seems that simple isomorphisms do not exist.

LTP Lasts a Long Time as Does Long-Term Memory

The lasting nature of LTP has been used as an argument both for (Barnes 1979) and against (Gallistel 1995) LTP as a memory mechanism; the latter is supported by the fact that LTP does not last a lifetime, as do some memories (Squire 1987). However, any number of properties of networks—for example, reactivation (Hebb 1949)—may extend the biological integrity of a memory. Further, most studies characterizing LTP longevity observed LTP at hippocampal sites. Because the hippocampus is viewed as having a temporally restricted role in memory in both animals and humans (Barnes 1988, Zola-Morgan & Squire 1993), there is no a priori reason to expect permanent changes within the hippocampus. Thus, longevity comparisons between hippocampal LTP and long-term memories are not meaningful. Memory is not a unitary phenomenon, and memory systems likely include anatomically distinct structures and even perhaps distinct neural mechanisms (Schacter & Tulving 1994). Perhaps synaptic plasticity within other parts of the brain—in neocortical regions, for example—lasts longer than hippocampal LTP.

In our view the findings discussed to this point offer compelling reasons to consider LTP (and LTD) likely biological mechanisms of memory. This extensive prologue was required because the evidence supporting such an interpretation is not convincing to some (Keith & Rudy 1990, Gallistel 1995) and because each set of studies supporting this view carries interpretational difficulties. We now turn to a discussion of the evidence. First, we briefly list the known cellular mechanisms for LTP; for more extensive reviews of cellular mechanisms, see Bliss & Collingridge (1993), Bramham (1992), and Johnston et al (1992). Then we discuss electrophysiological correlations between LTP and learning, induction of LTP and its effect on learning, the pharmacological properties of learning and LTP, and new studies that attempt to determine simultaneously the genetic basis of LTP and learning.

CELLULAR MECHANISMS OF LTP INDUCTION

Several different forms of LTP have been described (Bliss & Collingridge 1993). In the hippocampus, two major forms of LTP are NMDA receptor-dependent (Collingridge et al 1983) or opioid receptor-dependent (Bramham 1992). Each is discussed.

NMDA-Receptor-Dependent LTP and Associative LTP

NMDA is a voltage-dependent glutamate receptor subtype. For LTP induction, the NMDA receptor must be activated by the neurotransmitter glutamate and simultaneously there must be sufficient depolarization of the postsynaptic membrane to relieve a Mg^{2+} block in the NMDA-associated ion channel, which allows the entry of Ca^{2+} into the postsynaptic terminal. Ca^{2+} activates any number of Ca^{2+}-sensitive second messenger processes. Because NMDA receptors are sensitive to both presynaptic transmitter release and postsynaptic depolarization, they act as Hebbian coincidence detectors. This property can explain cooperativity and associativity through temporal and spatial summation. Thus, activated NMDA receptors at synapses that are proximal to active sites of depolarization may be depolarized sufficiently to relieve the Mg^{2+} block and initiate the cascade of events that leads to LTP induction. This cascade may occur even though the activity of that particular synapse alone was not sufficient to induce LTP. Thus, NMDA receptors can account for the association of two separate afferent projections to the same cell, one strongly and the other weakly active (Kelso & Brown 1986, Levy & Steward 1979), and for the cooperative requirement that a threshold number of fibers be active. Recently Bashir et al (1993) suggested that other glutamate receptors, particularly the metabotropic subtype, may contribute to the induction of LTP.

The maintenance of NMDA-receptor-dependent LTP is less well understood. In a contemporary review a distinction was suggested between short-term potentiation (STP), which decays in about one hour, followed by three stages of LTP ($LTP_{1–3}$) requiring, respectively (*a*) protein kinase activation and protein phosphorylation, (*b*) protein synthesis from existing mRNAs, and (*c*) gene expression (Bliss & Collingridge 1993). Behavioral approaches to learning suggested that these same cellular processes are involved in the establishment of long-term memory (Brinton 1991).

Opioid-Receptor-Dependent LTP and Associative LTP

Although less well known and less completely studied (Bramham 1991a,b; Breindl et al 1994; Derrick et al 1991; Ishihara 1990; Martin 1983), this form of LTP is the predominant form of plasticity within extrinsic afferents to the hippocampal formation (mossy-fiber CA3, lateral-perforant-path dentate gyrus, lateral-perforant-path CA3) and is present in more afferent projections

to the hippocampal formation than is NMDA-receptor-dependent LTP (medial-perforant-path dentate gyrus, medial-perforant-path CA3). Thus if the hippocampus is important in memory formation, as much data suggests, then opioid-receptor-dependent LTP and its relationship to NMDA-receptor-dependent LTP need to be understood.

LTP induction in the mossy-fiber CA3 and lateral-perforant-path CA3 pathways depends on the activation of μ-opioid receptors (Derrick et al 1992, but see Weisskopf et al 1993) and induction in the perforant-path dentate pathway depends on δ-opioid receptors (Bramham et al 1991a, 1992). Therefore, more than one form of opioid-receptor-dependent LTP exists in the hippocampus. We refer to the different forms as LTP_{μ} (mossy-fiber CA3 and lateral-perforant-path CA3) and LTP_{δ} (lateral-perforant-path dentate).

The time courses of NMDA-receptor-dependent and LTP_{μ} differ in that the former reaches its maximum almost immediately and can begin to decay, whereas the latter takes approximately an hour to reach its maximum and shows no decay (Derrick & Martinez 1989). These different time courses of augmentation and decay are relevant to our understanding of the operation of these forms of LTP in neural networks.

Associative opioid-receptor-dependent LTP in the mossy-fiber CA3 system appears to have constraints regulating induction that are different from those regulating associative NMDA-receptor-dependent LTP. The mossy fibers also show cooperativity in that a sufficient number of fibers have to be activated in order to observe LTP (Derrick & Martinez 1994b, McNaughton et al 1978, but see Chattarji et al 1989). Induction of LTP in the mossy fibers also is dependent on a sufficient number of tetanizing pulses, presumably to insure the release of opioid peptides (Derrick & Martinez 1994a); peptides in general are only released after trains of impulses (Peng & Horn 1991). Associative LTP of mossy-fiber responses can be observed with stimulation of the convergent commissural pathway only when trains of mossy-fiber pulses are used (Derrick & Martinez 1994b). The commissural-CA3 system expresses NMDA-receptor-dependent LTP (Derrick & Martinez 1994b), and the induction of associative mossy-fiber LTP is blocked by both opioid- and NMDA-receptor antagonists (Derrick & Martinez 1994b).

Research findings in the area of mossy-fiber LTP are controversial. Although it is generally agreed that LTP in this pathway depends on trains of pulses and the presence of extracellular Ca^{2+}, the site of Ca^{2+} entry, either pre- or postsynaptically, is in dispute (Williams & Johnston 1989, Zalutsky & Nicoll 1990), as is the necessity of postsynaptic depolarization (Jaffe & Johnston 1990). One group of researchers even refuses to ascribe the lofty title of LTP to the phenomenon of synaptic enhancement in mossy fibers and refers to LTP in this pathway as mossy-fiber potentiation because it is nonassociative and, according to them, rapidly decremental (Staubli 1992, Staubli et al 1990).

The controversy may arise because the preparation of the hippocampal in vitro slice may compromise the integrity of the mossy-fiber system (Dailey et al 1994), and different species, particularly rat and guinea pig, which are favorite subjects, have different distributions of opioids and opioid receptors (McLean et al 1987). Future research, particularly in vivo, should resolve some of the controversy.

ELECTROPHYSIOLOGICAL APPROACHES TO RELATING LTP TO LEARNING

Studies addressing the contribution of LTP to learning have been approached at an electrophysiological level to answer two major questions: Does learning induce changes in synaptic responses that are similar to LTP? Does the induction of LTP alter learning?

Does Learning Produce LTP-like Changes?

We limit our review to those studies that measured changes in the population EPSP rather than the population spike, owing to general agreement that excitatory postsynaptic potentials (EPSPs) changes reflect changes in synaptic function, whereas changes in the population spike amplitude may reflect other mechanisms (Bliss & Lynch 1988).

Changes in population EPSPs can be observed in perforant-path dentate gyrus responses during exploratory behaviors. The phenomenon was initially named short-term exploratory modulation, or STEM (Sharp et al 1985). This initial study demonstrated that exploration produced increases in perforant-path synaptic responses over the course of exploration and that the increases persisted for short periods of time after exploration. The initial and subsequent studies (Green et al 1990) revealed that STEM was not dependent on handling, novelty, repeated stimulation, or increased locomotion. Like LTP, STEM results in an apparent increase in the field EPSP and can be blocked by the NMDA-receptor antagonist MK 801 (Erickson et al 1990). However, unlike LTP, STEM is relatively short lived: It lasts only 20–40 min (Sharp et al 1985).

Evidence suggesting that STEM was not an LTP-like process emerged in 1993 with the report of additive effects of STEM and LTP (Erickson et al 1993) and changes in STEM that are distinct from those observed with LTP (Erickson et al 1993). A strong correlation between the magnitude of STEM and simultaneously recorded 2–3°C fluctuations in brain temperature (Moser et al 1993a), presumably resulting from physical activity that occurred during exploratory behavior, also was reported. STEM-like changes could also be induced with intense activity or with passive heating. More recent studies (Moser et al 1993b) suggest that, when temperature-induced alterations in

conduction velocity are controlled, small changes in perforant-path dentate field potentials may actually reflect changes due to exploration. However, this effect is short lived. STEM may represent endogenously occurring short-term potentiation (STP), the rapidly decaying process that precedes the generation of stimulation-induced LTP.

Ex vivo study is a different approach to the problem of detecting electrophysiological changes in evoked responsiveness following learning. The responsiveness of in vitro hippocampal slices removed from animals exposed to an enriched environment were compared with responsiveness of slices from animals exposed to a standard laboratory environment (Green & Greenough 1986). Rearing animals in complex environments produces anatomical changes in cortex that are thought to be a result of learning (Bennett et al 1964, Greenough et al 1973, Rosenzweig et al 1962). In this study, the slope of perforant-path dentate responses was assessed. The magnitude of field EPSP slopes was larger in rats raised in a complex environment than in rats housed in standard laboratory conditions, effects that are similar to those observed after LTP induction in this pathway (Bliss & Lomo 1973). Electrophysiological measures of antidromic (nonsynaptic) volleys and of the presynaptic-fiber volley (number of fibers activated) revealed no differences between the rearing conditions. Thus the field EPSP slopes elicited by equivalent volleys were significantly larger, which suggests that the differences arise from an enhancement of perforant-path synaptic transmission. The increased dentate responsiveness was not observed in animals that were removed from complex housing three to four weeks prior to testing, which suggests the effects were transient, as is LTP (Barnes 1979).

More recently, one group of researchers recorded responses in another hippocampal system, the mossy-fiber projections, as animals learned a radial arm maze (Mitsuno et al 1994). Incremental increases were observed in mossy-fiber field EPSPs over the course of learning. Changes in evoked responsiveness were evident three days after learning. Taken together, these studies show that learning induces changes in hippocampal responsiveness that resemble those observed following LTP induction.

Why should changes in evoked-response amplitude following a single learning episode be detectable? According to the view of distributed memory systems, changes underlying learning should occur in a very small fraction of the available synapses, and there is no reason to expect that such sparse changes would be evident in synaptic activation evoked by the stimulation of thousands of afferent fibers activated by a stimulating electrode. However, the hippocampal memory system could have a small capacity and utilize most synapses when storing information. In such a system an evoked response might reveal the existence of a stored memory. However, in order for new information to be stored, the information in this low-capacity system would

either have to be erased or have to decay rapidly. Some researchers suggest that the mossy-fiber projections to CA3 represent a low-capacity store (Lynch & Granger 1986) because LTP in mossy fibers can decay quite rapidly (within hours) in vitro (Mitsuno et al 1994). However, learning-induced LTP-like changes in evoked mossy-fiber responses are observed three days after the cessation of training, arguing against the neural changes representing a transient, low-capacity store.

One clever strategy eliminates this problem of "looking for a needle in a haystack." Synapse-specific changes in responses mediated by a large number of afferents need not be observed. Rather, the evoked response is employed as an integral part of the learning task. Detection of salient learning-induced change in a large number of randomly stimulated fibers is not necessary; instead, the activity of the fibers is incorporated into the learning task. This strategy was employed by several laboratories and provides consistent and convincing electrophysiological evidence for a role of LTP in learning.

In one set of studies, a shuttle avoidance task with a footshock as an unconditioned stimulus was employed (Matthies et al 1986, Ott et al 1982, Reymann et al 1982). High-frequency perforant-path stimulation was the conditioned stimulus. Low-frequency evoked responses were recorded in the dentate gyrus before, during, and after 10 daily training sessions. Overall daily changes of the field EPSP slope roughly corresponded to changes in learned behavior. However, the relationships among the measures each day were more complex; improved performance was not correlated with response magnitude within the daily trials. The LTP-like increase in responses was apparent only at the start of the second day of training, which suggests that a consolidation process occurs after the training and prior to the session the following day. Nevertheless, the increases in the field EPSP paralleled learning across days, with asymptotic performance occurring on the days of asymptotic LTP. An important observation was that animals that were poor learners and did not acquire the task also failed to show an increase in dentate responses. The stimulation may have induced LTP that was independent of any learning-induced changes in neural function. However, the stimulation trains used as a CS did not produce any changes in the EPSP during the initial 40 trials on the first day of training. Thus, it is not likely that the CS stimulation induced LTP.

An interpretational difficulty of the above study is that the hippocampus is not necessary for learning of the active-avoidance task; in fact, hippocampal lesions or NMDA-receptor antagonists can facilitate active- or passive-avoidance learning, respectively (Mondadori et al 1989, Nadel 1968, Ohki 1982, Shimai & Ohki 1980). Thus increases observed in perforant-path responses that parallel learning may reflect ancillary learning of other aspects of the CS, such as context (Kim & Fanselow 1992). However, in a subsequent study, colchicine lesions of the dentate gyrus eliminated both the evoked response

and the ability of perforant-path stimulation to serve as a CS (Ruthrich et al 1987). These lesions did not alter conditioning to other CSs nor did they alter conditioned emotional response to the footshock. Together, these data suggest that the increases in responses of activated perforant-path dentate synapses contributed to the learning of the CS aspects of an active-avoidance response.

In a similar study (Laroche et al 1989), high-frequency stimulation served as a CS for a footshock that elicited behavioral suppression. Learning of the perforant-path stimulation-shock association occurred only when the trains were of an intensity sufficient to elicit LTP. Further, inhibition of LTP induction by prior tetanization of commissural afferents, which inhibits LTP induction by engaging inhibitory mechanisms, produced substantial deficits in learning. Furthermore, chronic infusion of AP5, a selective NMDA antagonist, blocked both LTP induction and the ability of the stimulation to serve as a CS. A significant correlation existed between the magnitude of LTP produced by these various treatments and the acquisition of the conditioned response. The decay of LTP induced in this behavioral paradigm was observed in the following 31-day period and correlated with retention of the conditioned response (Laroche et al 1991).

In the experiments mentioned above, it was assumed that stimulation of the perforant path can serve as a sensory-like conditioning stimulus. However, the degree to which the perforant path is normally involved in representing a sensory CS is unknown. Further, because the stimulation produced a potentiated synaptic response, the correlation between LTP and learning may reflect merely an increase in the salience of the perforant-path stimulation. For this reason such an approach may be of limited utility. An alternative strategy is to stimulate structures or pathways that actually mediate sensory input. Studies by Roman and colleagues (Roman et al 1987, 1993) used such an approach by recording monosynaptic responses in the olfactory (piriform) cortex elicited by stimulation of sensory projections from the olfactory bulb (the lateral olfactory tract, or LOT). These studies are notable in that they depart from the study of LTP restricted to the hippocampus and address the contribution of LTP to learning at other cortical sites. In these studies, patterned LOT stimulation was used as a discriminative cue for the presence of water. Stimulation of this olfactory pathway apparently produced something like a sensory event, because rats responded to burst stimulation with sniffing and exploring, as though they detected an odor, and such stimulation served as a CS in an olfactory discrimination learning task. Performance in this task using stimulation as a CS was remarkably similar to that observed with actual odors as CSs. Comparison of monosynaptic responses during the acquisition of discrimination learning revealed increases in the monosynaptic LOT piriform cortex responses, an effect that persisted at least 24 hours after training. Thus patterned stimulation did not produce synaptic potentiation unless the association

of the cue and the water reward was learned. A significant correlation was found between the increase in the field EPSP slope and the number of correct responses. Although the magnitude of LTP and behavioral responses among animals was quite variable, better responding was associated with larger changes in the field EPSP slopes within individual animals (Roman et al 1993). Of particular interest is the observation that the burst stimulation, which is thought to be optimal for LTP induction at other sites (Staubli & Lynch 1987), was ineffective by itself in inducing LTP. Rather, a long-term depression of responses was observed following stimulation of naive rats in a non-learning situation. Because the LOT pathway is known to be resistant to LTP induction in vivo (Racine et al 1983, Stripling et al 1991) but not in vitro (Jung et al 1990, Kanter & Haberly 1993) or during learning (Roman et al 1987, 1993), these data suggest that LTP induction is actively inhibited in vivo. It is tempting to speculate that attentional or other mechanisms are engaged during conditioning that enable LTP induction in this cortical structure.

Together these studies provide positive support for the idea that LTP may be involved in conditioning because LTP-like increases in evoked potentials exist following learning in CS pathways that are chosen for experimental convenience. A more direct experimental approach to the question of whether LTP is a mechanism of learning is to induce LTP and then determine whether it influences later learning.

Does the Induction of LTP Influence Learning?

LTP induced prior to learning might impair learning by saturating LTP processes that normally participate in the learning; LTP induced after learning might obscure prior learning by occluding any distributed pattern of synaptic changes that were formed as a result of learning. Alternatively, LTP may enhance or impair learning by activating modulatory mechanisms (Martinez et al 1991).

In one study the effects of LTP induction on the acquisition of classically conditioned nictitating membrane response (NMR) were assessed (Berger 1984). The rationale for this study arose from the observation that changes in hippocampal pyramidal-cell activity parallel changes in the acquisition of the conditioned behavioral response (Berger et al 1983, Berger 1984) as well as from the possibility that the increase in hippocampal unit firing resulted from plastic events within the hippocampus. LTP induced unilaterally in the perforant path facilitated the subsequent acquisition of a classically conditioned NMR in rabbits (Berger 1984). Given that the hippocampus is not essential for learning of simultaneous classical conditioning of the NMR (although it appears important in the acquisition of more complex aspects of classical conditioning; see Berger & Orr 1983), this effect may be of a modulatory nature, rather than a direct effect on an essential learning mechanism.

An opposite effect was observed using spatial learning in a circular maze (McNaughton et al 1986). Bilateral, supposedly saturating LTP stimulation of the angular bundle, which carries both the lateral and medial aspects of the perforant-path projections, disrupted performance either prior to or immediately after learning. In an important control procedure, LTP that was induced after the task was well learned did not disrupt performance. Subsequent studies (Castro et al 1989) expanded this initial observation. The strategy was to saturate LTP by stimulating rats every day for a 19-day period. On the final day, the ability of the rats to find a hidden platform in the Morris water maze was assessed. A single probe trial was used to measure performance of rats when the hidden platform was removed, and the time a rat spent in each quadrant was determined. Rats that received LTP-inducing stimulation displayed deficits in learning, whereas rats that received only low-frequency non-LTP-inducing stimulation acquired the task and spent more time in the quadrant where the hidden platform was during acquisition. As a control, the ability to locate a visible platform was assessed, and in this case no difference was observed between the stimulation groups, which indicates that the stimulation did not affect any sensory capacity. Rats in which LTP was induced and then allowed to decay did not show any learning deficits. Taken together, these data suggest that LTP itself, rather than nonspecific effects of stimulation, is essential for learning because saturation-impaired acquisition of the spatial learning task and the ability to learn returned with the decay of the LTP.

Several laboratories, including the laboratory of origin, reported difficulties in replicating the LTP saturation effect (Jeffery & Morris 1993, Robinson 1992, Sutherland et al 1993). A number of reasons may explain the failure to replicate. First, although the stimulation parameters used may have resulted in the saturation of LTP in those afferents stimulated, stimulation of the angular bundle with a single stimulation electrode may not sufficiently tetanize all fibers that course through this structure. Second, LTP saturation does not prevent the induction of LTD (Linden & Conner 1995), which also is a potential memory mechanism (Sejnowski 1977, Stent 1973). Other reasons for lack of replication of the LTP saturation effect were delineated in a recent study (Barnes et al 1994) in which LTP saturation induced deficits in reversal training to a circular maze, but not in a water maze, which suggests different task susceptibility to LTP saturation. The extent of saturation was addressed by measuring the induction of the immediate early gene *zif*, whose induction was correlated with the quantity of LTP induction in the dentate. LTP saturation procedures induced *zif* mostly in the dorsal hippocampus. Thus, if *zif* marks those cells that potentiated, then perhaps LTP was neither saturated nor induced in the more ventral regions of the hippocampus in those experiments that did not replicate the saturation effect. Barnes et al (1994) believe this interpretation is supported by findings from the same study in which maximal

electroconvulsive shock (ECS) treatments, which produce a synaptic potentiation (Stewart et al 1994) that is NMDA-receptor-dependent (Stewart & Reid 1994), also led to significant deficits in acquisition and reversal of the water maze task. The potentiation produced by either ECS or LTP-inducing stimulation was not additive, and ECS induced *zif* throughout the hippocampus. Seizures were observed in some animals, which apparently did not influence learning: When ECS treatment induced seizures without inducing LTP, no deficits were observed. The deficits were highly correlated with the amount of LTP induced. Although an interpretational problem is that multiple ECS treatments may produce effects that alter learning as a result of actions that are unrelated to the induction of LTP, the results of Barnes et al (1994) are consistent with the view that a large degree of hippocampal inactivation is needed to reliably induce learning deficits (Jarrard 1986, McNaughton et al 1989). In this view, information stored in a distributed memory system is quite resistant to degradation, and the partial saturation of LTP or preservation of a process such as LTD may be sufficient to permit substantial learning.

Although the enhancement of classical conditioning (Berger 1984) and the impairment of spatial maze learning (Barnes et al 1994, Castro et al 1989, McNaughton et al 1986) apparently are contradictory effects, the differences in the findings of these studies reflect, in our view, a differential contribution of the hippocampus, and therefore hippocampal LTP, to classical conditioning of the NMR and spatial learning, which are distinctly different memory tasks that appear to require distinct memory systems (Thompson 1992). Because the hippocampus is not required for acquisition of the NMR response but is required for acquisition of spatial mazes, the roles of LTP in these two kinds of learning are likely different, and thus the studies cannot be compared directly.

PHARMACOLOGICAL APPROACHES RELATING LTP TO LEARNING

Subsequent to the demonstration of the important role for the NMDA-type glutamate receptors in LTP induction, a number of behavioral researchers rushed to characterize the effects of NMDA-receptor antagonists on learning. As in all pharmacological studies attempting to study learning, the inference of causality from a specific action of a drug is problematic (Martinez et al 1991). Drug-related side effects and determination of the drug's specific site of action are always issues. Further, in the studies reviewed below, the drug has to be administered before the initiation of conditioning if it is to block the induction of any LTP that might contribute to the learning. Being thus present early, the drug might induce an effect on learning through a sensory, motor, motiva-

tional, attentional, or other variable (Martinez et al 1991). As noted below, these concerns complicate the interpretation of studies using this strategy.

Many studies examined the effect of selective NMDA-receptor antagonists on a variety of learning tasks (Kim et al 1991, Walker & Gold 1991), including tasks thought to depend on hippocampal function (Robinson et al 1990, Staubli et al 1986). Here we limit our discussion to pharmacological studies that address both hippocampus-based learning and LTP induction and that use relatively localized, or at least intra-CNS, administration of drugs, so that as far as possible the effects described are the result of an action of the drug in a circumscribed area of the brain. The most comprehensive and elegant studies (Morris et al 1986) examined intracerebroventricular (ICV) administration of AP5, the selective NMDA antagonist, on learning in a Morris water maze task. Prior research indicated that the hippocampus is important in the acquisition of this task, that is, when the rats are required to learn the location of the platform with respect to distal cues in the environment (Morris et al 1982). In the initial studies (Morris et al 1986), the nature of the memory impairment induced by the NMDA antagonist was assessed with (*a*) measures of latency on acquisition trials, (*b*) measures of performance on a probe trial with the platform removed, and (*c*) a reversal procedure, by which animals were additionally trained with the platform in a different location. For each of these measures a significant impairment was observed in the animals infused with AP5. Potential sensorimotor impairments induced by the drug were assessed with a visual discrimination task using the same water maze apparatus. In this circumstance, the NMDA antagonist had no apparent effect. The effect of AP5 on LTP induction also was assessed in these studies to compare the behavior-impairing and LTP-induction-impairing action of AP5. LTP was induced by stimulation of the perforant-path dentate synapse. The drug had no effect on the low-frequency evoked responses; however, AP5 impaired acquisition of the maze and AP5 completely blocked LTP induction.

A striking impairment of task acquisition was not observed; although the animals receiving AP5 showed longer latencies to escape than control animals, learning in the drug-treated group paralleled that in the control animals. Thus a learning curve was observed. However, because animals with hippocampal lesions show a similar early acquisition deficit (Morris et al 1982), the authors suggested that learning in the Morris water maze can involve nonspatial elements and that other, hippocampus-independent strategies are employed in the initial stages of learning. In this view, spatial deficits should be most apparent at the point of asymptotic learning, and performance in the probe trials should be sensitive to spatial-learning deficits. Thus, for many researchers, the most convincing indication of memory deficits is observed in the probe trials. As noted earlier, in this test the platform is removed, and the amount of time an animal spends in the quadrant where the platform was located is measured.

Animals treated with the NMDA antagonist showed no preference for the original location of the platform. By contrast, animals that received either saline or the inactive stereoisomer of AP5 showed a significant preference for the quadrant where the platform had been located, which indicates that the animals treated with AP5 had no spatial memory of the platform. The acquisition curve, as measured by decreased latencies, therefore indicates that the animals had learned to escape from the maze using a nonspatial strategy.

The results of reversal tests are more ambiguous (Morris et al 1986). In a reversal test the platform is moved to a location different from that of the original training. The degree of animals' learning is reflected by the persistence of the animals in returning to the place of original learning and by the acquisition of the new platform location. The animals that received AP5 showed no acquisition of the new location of the escape platform, whereas the control groups showed substantial preference for the quadrant of original training and readily learned the new location of the platform. The interpretational problem with this study is that the AP5-treated animals' performance at the beginning of reversal training was as poor as the control animals', which suggests a negative transfer effect of some original learning.

Other critics noted that some rats fell off the platform during training and suggested that the impairment produced by AP5 was because of motor deficits (Keith & Rudy 1990). Further control experiments suggest that falling off the platform did not have an aversive effect on performance in water maze learning (Morris 1990). As an added measure, pretraining within the water maze using the visual discrimination task prior to ICV infusion demonstrated that the apparent sensorimotor deficit revealed by platform instability could be overcome by pretraining. Spatial learning impairments resulting from AP5 administration were still observed in these pretrained rats. It has been noted (Keith & Rudy 1990) that the rats receiving AP5 showed performance deficits on the first trials before learning had occurred, and that this deficit may reflect a side effect of the drug on sensorimotor function. However, later studies that more closely examined learning in the early trials showed no effect of moderate doses of AP5 on performance in the first trial (Davis et al 1992). Goddard (1986) objected that the discrimination learning experiment is not a good test of sensorimotor impairment because ICV administration of AP5 probably results in lower concentrations of AP5 at sites important for visual discrimination. However, actual measurement of the dispersion of AP5 following ICV administration showed that it was evenly distributed within the brain (Butcher et al 1990). Subsequent studies indicated that localized infusion of AP5 within the visual cortex did not produce impairments in the visual discrimination task (Butcher et al 1991). Together these results suggest that the impairment of performance in the water maze produced by AP5 is the result of the effects mediated by the actions of this drug at hippocampal sites.

As noted by the embattled originators of these NMDA-antagonist studies, it would be erroneous to conclude that AP5 causes the learning deficit because AP5 blocked LTP (Morris 1989a). AP5 may affect learning, for example, because AP5 has an effect on hippocampal theta rhythm, and treatments that disrupt theta rhythm can block acquisition of learning tasks (Winson 1978). Thus, as discussed above, many factors impede the interpretation of a drug effect, including the selectivity of the drug's actions, side effects, drug dispersion, and the site of drug action.

Another way to demonstrate that two separate drug effects, such as impaired spatial learning and impaired induction of LTP, are related is to compare the dose response curves of the drug's separate effects. Different dose response functions may show that the drug was acting on different processes, and identical dose response functions may show that the drug was acting on a common process. In subsequent studies (Davis et al 1992) identical dose response curves were observed for both impairment of spatial learning and blocking of LTP induction. Furthermore, concentrations of AP5, measured in the brain using high-performance liquid chromatography (HPLC) microdialysis, that impaired learning and that blocked LTP were the same; no concentration of AP5 was observed to block LTP without affecting learning (Butcher et al 1991). Lastly, the extracellular concentrations that were measured during the block of LTP induction in vivo matched the concentrations that were effective in blocking LTP induction in vitro.

Further studies (Morris 1989a) addressed the question of the effect of AP5 on both the acquisition and retrieval of a spatial-learning task. The reasoning in these studies was as follows: NMDA-receptor activation, although essential for LTP induction in many hippocampal pathways, is not essential for either the expression or the maintenance of LTP. If AP5 alters memory by blocking LTP induction, then any deleterious effects of AP5 should be limited to the acquisition period, and AP5 should not impair performance on a spatial-learning task when administered following training. This strategy also addresses, to some degree, the possible sensorimotor and LTP-independent effects of NMDA-receptor antagonists, because any performance deficit seen in these conditions could not be because of any effect on acquisition. AP5, when infused into rats by ICV administration following asymptotic acquisition of the water maze task, has no effect on the retrieval of learned spatial information, as assessed using probe trials. Moreover, in these same rats, the doses of AP5 that had no effect on performance following training effectively blocked new learning in a subsequent reversal test. The lack of effects on performance of an already learned task suggests that the AP5 is not producing sensorimotor impairment that interferes with performance of the task. Taken together, these studies provide striking evidence that AP5 may impair learning through blocking the induction of LTP.

The recent data implicating metabotropic glutamate receptors in the induction of LTP prompted assessment of the role of these glutamate receptors in spatial learning. Richter-Levin et al (1994) reported that perfusion of the metabotropic antagonist [RS]-α-methyl-4-carboxyphenylglycine (MCPG) did not produce deficits in animals during acquisition of a Morris water maze, although a significant deficit was observed in probe trials given 24 h after the last training trial. In these same animals, equivalent quantities of MCPG attenuated the magnitude but did not block the induction of perforant-path dentate LTP. Thus antagonism of metabotropic glutamate receptors produces some deficits in LTP and spatial learning.

The studies employing NMDA-receptor antagonists to assess the contribution of hippocampal LTP to learning have been the subject of particularly intense scrutiny (see Keith & Rudy 1990). However, in our view, the fact that spatial learning is not blocked completely by NMDA-receptor antagonists is not surprising. Several pathways in the hippocampus, including the mossy-fiber pathway (Derrick et al 1992), the lateral perforant path to area CA3 (Breindl et al 1994), and the lateral perforant path to dentate (Bramham et al 1991a,b; but see Zhang & Levy 1992) display LTP_{μ} and LTP_{δ}, which are both opioid receptor-dependent and NMDA receptor-independent. In addition, both NMDA-receptor-dependent and NMDA-receptor-independent mechanisms of LTP induction are observed within the CA1 region (Teyler & Grover 1993). As mentioned above with respect to the saturation experiments of McNaughton and colleagues, when viewed from the perspective of distributed memories, partial sparing of function may be sufficient to permit learning. Such reasoning leads to the conclusion that the alteration of any one of the LTP systems within the hippocampus may not be sufficient to produce a total or even a profound deficit in spatial learning. That localized NMDA-receptor blockade does produce observable deficits, and that these deficits are similar to, although less severe than, those observed with extensive hippocampal lesions, suggest not only that NMDA-receptor-dependent mechanisms, and perhaps LTP, contribute to spatial learning, but also that they may be a fundamental mechanism of information storage.

Does Learning of a Spatial Task Involve Hippocampal Opioid Systems?

Given that opioid receptor antagonists impair the induction of LTP in opioidergic afferents, opioid receptor antagonists would be expected to impair spatial learning. However, systemic administration of naloxone is reported to facilitate acquisition of a spatial water maze as measured by latency to find the platform (Decker et al 1989). These studies employed intraperitoneal administration of naloxone 5 min prior to training, which may be insufficient time for

intraperitoneally administered naloxone to block sufficiently opioid receptors at central sites. For example, intraperitoneal naloxone effects on evolved hippocampal responses are observed only 10–15 min following intraperitoneal naloxone administration (Martinez & Derrick 1994). Thus training may not have been given at an optimum time following drug administration. In addition, opioid antagonists exert effects on opioid systems that influence learning that may be independent of hippocampal opioid systems (Martinez et al 1991), and alterations in these opioid systems by systemic administration of opioid receptor antagonists may also alter learning. In support of this interpretation, other studies employing local application of opioids into the hippocampus produce an impairment of spatial learning. For example, local administration of dynorphins impairs spatial learning (McDaniel et al 1990), and dynorphins impair LTP induction in both the mossy-fiber CA3 and perforant-path dentate synapses via actions on kappa receptors (Wagner et al 1993, Weisskopf et al 1993). To date, no studies have addressed the effect of selective blockade of hippocampal μ or δ receptors in spatial learning, but local blockade of opioid receptors is likely to produce spatial learning deficits because, like opioid receptor blockade (Derrick et al 1992), elimination of specific metabotropic glutamate receptors selectively impairs mossy fiber LTP, and elimination of these metabotropic receptors also impairs spatial learning (Conquet et al 1994).

KNOCKOUT MUTANTS, LTP, AND HIPPOCAMPALLY DEPENDENT LEARNING

The molecular biological revolution has arrived in force in the area of LTP and learning. A paradox of learning is that it is expressed as activity among neurons, though the biological changes that underlie memories are stored within neurons. The molecular biological revolution taught us that enduring alterations of cell function, as must occur in long-term memory storage, are controlled by gene expression and resultant protein production. Thus, for every sustained memory there is likely a chain of events leading from the initiation of activity at a synaptic receptor, to the activity of second messenger systems, to intermediate early gene induction, and to secondary gene induction in every cell that participates in the memory network. The same is likely true for LTP (but see Lisman 1989).

A number of research groups are endeavoring to trace the chain of cellular events that underlie induction and maintenance of LTP (Grant et al 1992, Silva et al 1992a,b). In these studies single genes, controlling what are hoped to be specific events within cells, can be eliminated and the resultant effect can be studied simultaneously in whole animals minus one gene, so-called knockouts, for LTP and learning. In this method the gene of interest, usually a well-char-

acterized gene, is cloned and in most cases altered so that important regulatory regions of the gene are nonfunctional. This altered DNA is introduced into embryonic stem cells derived from blastocysts. The gene combines with the DNA of the stem cells, and those cells in which the gene is inserted at appropriate regions of the DNA (via homologous recombination) can be isolated and inserted into developing blastocysts. Subsequent cells arising from these altered cells all lack the knockout gene. The resulting animal is a heterozygous chimera (combination of normal and mutant cells) that, with cross breeding, can generate progeny that are homozygous for the knocked-out targeted gene.

One reason to target genes is that these genetic procedures have the potential to overcome the current limitations of pharmacology. In studies of genes related to LTP, an area of focus in the study of transgenes has been kinases. Although data strongly suggest LTP induction involves a variety of kinases, including protein kinase C (Malinow et al 1989), calmodulin kinase (Malenka et al 1989), and tyrosine kinases (O'Dell et al 1991), these studies are limited by the fact that currently available kinase inhibitors lack a high degree of selectivity. Further, for a given kinase, the kinase family to which it belongs is composed of a number of subtypes, which appear to have varied functions. It would be of great utility to selectively impair the function of specific kinase isoforms, a feat that is achieved by the use of knockout mutants.

The first study that attempted to trace the events underlying induction and maintenance of LTP (Grant et al 1992) compared various knockouts of genes coding for particular tyrosine kinases. Deletion of one specific tyrosine kinase found in the *fyn* gene altered the amount of current necessary to induce LTP in area CA1. Traditional measures of synaptic function appeared normal, such as the maximal EPSP amplitudes and measures of paired-pulse facilitation, a short-term augmentation of synaptic response that appears to depend on residual presynaptic Ca^{2+}. The *fyn*-knockout rats appeared incapable of learning the location of a hidden platform in a Morris water maze.

Unfortunately, this study is difficult to interpret. First, the hippocampus displayed obvious anatomical abnormalities, including an increase in granule and pyramidal cells. The dendrites of pyramidal cells in stratum radiatum showed disorganization and were less tightly packed, as were the cell bodies. Given the altered neural architecture, the synaptic volume might have been reduced, which may explain the reduced ability of high-intensity stimulation to produce LTP, although this is perhaps unlikely because low-frequency evoked EPSP amplitudes in the *fyn* knockouts are not different from those of wild-type controls. There were impairments in visual function because *fyn* knockouts were initially poor at performing a visual discrimination where the platform was visible, although they eventually reached latencies comparable to wild-type controls. The authors also noted that "overtraining in spatial tasks masked

the *fyn* learning deficit." Apparently then, the animals could learn, and the deletion of the *fyn* gene only altered the sensitivity of the knockout animals to such parametric aspects of training as the number of training trials needed to evidence learning. Because the *fyn* knockouts could express LTP, these data do not support a conclusion that LTP is a substrate of memory, because LTP and learning clearly do not depend on the presence of the *fyn* gene (Deutsch 1993).

In a second wave of studies other researchers (Silva et al 1992b) engineered knockout mice that were deficient in α-calcium-calmodulin-dependent kinase II (α-CaMKII). The kinase α-CaMKII, in contrast with tyrosine kinase FYN, is localized to the brain and is neuron specific. The α-CaMKII mutants showed no overt physical or neuroanatomical abnormalities. Measures of post-synaptic function, such as the maximal EPSP amplitudes, in Schaffer-CA1 responses appeared normal, but paired-pulse potentiation was reduced in mutant mice. Activation of NMDA receptors appeared to elicit normal responses. Although the probability of induction of LTP was greatly reduced in the mutants, LTP in some animals was virtually indistinguishable from LTP observed in wild-type controls.

A subsequent study (Silva 1992a) assessed the ability of α-CaMKII mutants to learn the Morris water maze. These mutants apparently had a defect in their visual function, because they showed an initial deficit in the visual discrimination task. However, these mutant mice eventually matched the wild-type animals in performance. The α-CaMKII mutants were also impaired in their ability to find the hidden platform on the first session of training in the Morris water maze and were always slower than the wild-type control mice; that the mutants did learn is shown by the fact that their latencies to find the platform decreased over sessions. For the probe trial, the mutant mice took roughly twice as long to find the platform. An additional test employed a randomly located platform. Some trials were conducted with the hidden platform randomly located at other sites. Mutant mice took as long to find refuge at the random sites as to find refuge at the original location, whereas wild-type mice took less time to find the original location and longer times to find the random platforms, which indicates negative transfer. The results of the random probe test therefore suggest that the mutant mice did not know the spatial location of the hidden platform, although they apparently were able to use some strategy to escape the maze. Mutant animals were the equal of their wild-type cousins in learning a + maze, which does not exact any spatial ability from its students. The α-CaMKII mutants showed greater activity in open field and did not evidence habituation of activity. Thus the evidence suggests that the α-CaMKII mutants did have a deficit in the ability to learn the spatial maze. What is not so clear is whether this spatial deficit is related to LTP. In the mutant mice only the probability of LTP induction was altered; LTP induction was not abolished. If a mutant did show LTP, then the LTP was

indistinguishable from that observed in wild-type controls. The deficit in paired-pulse potentiation in the mutant mice is also problematic. Such an alteration could be important for hippocampal function that is unrelated to LTP but that is manifested as a spatial deficit.

Another group targeted protein kinase C (Abeliovich et al 1993) and selected the PKC_γ isoform, both because inhibitors of PKC prevent induction of NMDA-receptor-dependent LTP in CA1 (Malinow et al 1989) and because PKC_γ is specific to neurons in the CNS and is expressed postnatally. The probability of LTP induction was reduced in the mutants much as it had been in previous studies employing knockouts; but if the mutant mice were first treated with low-frequency stimulation, then the LTP was indistinguishable from that observed in wild-type controls. An interesting finding, however, was that expression of LTD was not impaired. In spite of coordination deficits, the mutant mice learned the Morris water maze at the same rate as did the wild-type controls and performed similarly in the probe and random probe tests. The authors believe the mutant mice did exhibit a mild spatial deficit because during the probe test the mutants crossed the hidden platform site less often than the controls, even though they were searching the correct quadrant. In contrast with their behavior in the spatial maze, the PKC_γ mutants did show deficits in contextual-fear conditioning in that they froze significantly less after return to a chamber where they experienced footshock. There is evidence that acquisition of a contextual-fear task depends on both the hippocampus and NMDA receptors (Kim et al 1991, 1992; Kim & Fanselow 1992). Conditioned fear (measured by observing freezing in response to a tone in a novel environment), which is thought to be independent of hippocampal function, was not impaired. The results do not support a role for PKC_γ in either LTP or spatial learning because the mutant mice could learn the Morris water maze and, if stimulated appropriately, displayed LTP.

Departing from the study of the kinases, other groups targeted genes specific for subtypes of the glutamate receptor. One group (Sakimura et al 1995) created mice with a mutation of the GluRε subunit of the NMDA-receptor channel. No obvious morphological brain abnormalities were observed, probably because this gene is expressed after development. However, the mutants appeared jumpy and had an apparently enhanced startle response. LTP could be induced in the mutants but at a reduced magnitude (smaller percentage increase from baseline). As in the case of the PKC_γ mutants, low-frequency stimulation prior to LTP restored some function but not to the level of the wild-type control. During training in the Morris water maze the mutants showed an initial latency deficit that disappeared by the end of training. During the transfer test the mutants searched the previously correct quadrant, crossed the trained site—though not at the same level of efficiency as the wild-type mice—and were less precise in their crossings. The authors consider

their findings positive evidence for the participation of the GluRε subunit of the NMDA receptor in both LTP and the acquisition of spatial learning. Yet, as in the other studies reviewed, the gene mutation did not abolish either LTP or spatial learning, in which case this gene cannot be necessary for either.

The metabotropic glutamate receptor (mGlu) is implicated in LTP induction, though this conclusion remains controversial (Bashir et al 1993, Manzoni et al 1994). Activation of the metabotropic glutamate receptor 1 (mGluR1) may activate G-protein-coupled second messenger processes, and these processes may play an important role in LTP induction, acting like a metabolic switch that enables the induction of LTP. Recently one research group created an mGluR1 mutant to test involvement of mGluR1 in LTP and contextual-fear conditioning (Aiba et al 1993). This receptor subtype is plentiful in the dentate gyrus and CA3 areas and is apparently restricted to the presynaptic side of the Schaffer collateral projection to area CA1. These mGluR1 mutants had ataxia and were poor breeders but had brains that appeared normal. Synaptic transmission, STP, and paired-pulse potentiation were normal. LTP was observed in the mGluR1 mutants, but as in the GluRε mutants its magnitude was reduced. Low-frequency priming had no effect. The mGluR1 mutants were impaired in the hippocampus-dependent contextual-fear conditioning task and exhibited less freezing than did the wild-type controls in the cage where they were shocked. By contrast, the mutants learned as well as the wild-type animals to freeze in response to the tone and thus showed normal learning in response to this hippocampus-independent form of fear conditioning. The authors concluded that the mGluR1 receptor is not necessary for induction of LTP but that it modulates neural plasticity, apparently expressed as the magnitude of LTP. Because the mutant animals were moderately impaired in their learning, Aiba et al posited that the mGluR1 receptor is not necessary for learning of the contextual-fear response but perhaps participates in some way.

A quite different set of results was found by another group who created an mGluR1 mutant (Conquet et al 1994). These mutants exhibited ataxia as well. A neurological exam of the mutants revealed a complete loss of the righting reflex and reduced locomotor activity. LTD in cerebellar slices was severely reduced. Synaptic transmission appeared normal in the Schaffer collateral-commissural pathway to CA1, medial and lateral perforant pathways to dentate, and mossy-fiber and associational pathways in CA3. LTP was normal in all pathways except the mossy-fiber CA3 pathway, where it was greatly reduced. In the visible platform version of the Morris water maze the mutant mice were initially slower than the wild-type mice, but after three sessions they were indistinguishable from controls. However, in the hidden-platform version of the maze, the mGluR1 mutants could not find the platform and evidenced no learning. Because the mutant mice did learn the visually guided maze, the authors concluded that the deficit observed with respect to the

hidden platform was due to an impairment of spatial ability mediated by mGluR1 receptors and probably in the mossy-fiber CA3 system, because LTP was reduced only in the mossy fiber-CA3 system. If the authors' interpretation of the data is correct, then deficits in the mossy-fiber system cannot be compensated by correctly functioning NMDA-receptor-dependent systems in other hippocampal pathways. This suggests an important role for both the dentate gyrus and LTP_{μ} in its mossy-fiber projections to area CA3 in learning (Marr 1971, McNaughton et al 1989).

The knockout strategy has provided some evidence that LTP and LTD are substrates of learning. What the knockout gains in specificity of elimination is lessened by the complexity of the mutant creature that develops without a particular gene. For example, is synaptic transmission in the mutant normal? In both the knockout studies and studies using selective drugs, it is assumed that if low-frequency synaptic transmission is not altered, then synaptic transmission is normal. However, there is no reason to believe that normal hippocampal function involves exclusively low-frequency activity; rather, high-frequency information is important for aspects of hippocampal function independent of its potential involvement in LTP induction. Such activity may be greatly influenced by the absence of a gene, as evidenced by the alterations in facilitation in one study (Silva et al 1992b). Other basic questions concern whether an animal's motor system is competent to perform what is required and whether the animal can see the elevated platform. We find it curious in these mutant studies that learning is measured in vivo and induction of LTP is measured in vitro in the hippocampal slice. This strategy is based on the as-yet-uncertain assumption that LTP observed in the slice is identical to that observed in vivo.

The most striking study, the last in this review, is undoubtedly that by Conquet et al (1994), in which five pathways in the hippocampus were characterized for normal synaptic transmission and induction of LTP. The learning deficit, which was impressive, may be related in an unexpected manner to malfunctioning in the mossy-fiber system, a pathway known to exhibit NMDA-receptor-independent, opioid-receptor-dependent LTP_{μ} (Derrick et al 1991, Harris & Cotman 1986). Prior to this study, most researchers assumed NMDA-receptor-independent LTP had a relatively unimportant role and attached primary importance to NMDA-receptor-dependent LTP in spatial learning.

To be fair, however, the knockout studies do demonstrate deficits in hippocampal LTP that mirror deficits in hippocampus-dependent learning, and if we apply the same explanation of graceful degradation as we have previously, then it is not surprising that some memory is evident in a distributed neural system.

However, it remains disquieting that, even within a discrete afferent system, no single specific kinase appears essential for the induction of NMDA-re-

ceptor-dependent LTP. Such findings, suggesting as they do the existence of parallel intracellular cascades, are problematic for reductionists trying to delineate the essential components of a successive molecular cascade. From a larger view, these results emphasize that no single approach will be sufficient to elucidate the role of LTP in memory, even though the knockout approach is powerful and increases our understanding of the relationship between LTP and learning.

CONCLUSION

The rationale for considering LTP a memory mechanism is strong. The absence of proof that LTP is involved in memory results from our current uncertainties about what memory is and how we should observe it. The occurrence of multiple forms of LTP, together with the distributed nature of hippocampal information storage, makes it difficult to identify the processes necessary to hippocampal memory and to implicate specific LTP processes in memory. Thus we should proceed cautiously in interpreting negative findings. Might LTP emerge as an epiphenomenon unrelated to learning or memory? If it does, then the focus of research would shift to such other potential neural mechanisms of memory storage as LTD, population spike potentiation, and presynaptic facilitation. After 20 years under scrutiny, however, LTP remains the best single candidate for the primary cellular process of synaptic change that underlies learning and memory in the vertebrate brain.

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Literature Cited

Abeliovich A, Paylor R, Chen C, Kim JJ, Wehner JM, Tonegawa S. 1993. PKC gamma mutant mice exhibit mild deficits in spatial and contextual learning. *Cell* 75(7):1263–71

Aiba A, Chen C, Herrup K, Rosenmund C, Stevens CF, Tonegawa S. 1993. Reduced hippocampal long-term potentiation and context-specific deficit in associative learning in mGluR1 mutant mice. *Cell* 79(2): 365–75

Barnes CA. 1979. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *J. Comp. Physiol. Psychol.* 93:74–104

Barnes CA. 1988. Spatial learning and memory processes: the search for their neurobiological mechanisms in the rat. *Trends Neurosci.* 11:163–69

Barnes CA, Jung MW, McNaughton BL, Korol DL, Andreasson K, Worley PF. 1994. LTP saturation and spatial learning

disruption: effects of task variables and saturation levels. *J. Neurosci.* 14(10): 5793–5806

Bashir ZI, Bortolotto ZA, Davies CH, Berretta N, Irving AJ, et al. 1993. Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. *Nature* 363(6427):347–50

Bear MF, Kirkwood A. 1993. Neocortical long-term potentiation. *Curr. Opin. Neurobiol.* 3:197–202

Bear MF, Malenka RC. 1994. Synaptic plasticity: LTP and LTD. *Curr. Opin. Neurobiol.* 4(3):389–99

Bennett EL, Diamond MC, Krech D, Rosenzweig MR. 1964. Chemical and anatomical plasticity of brain. *Science* 146:610–19

Berger TW. 1984. Long-term potentiation of hippocampal synaptic transmission affects rate of behavioral learning. *Science* 224(4649):627–30

Berger TW, Orr WB. 1983. Hippocampectomy selectively disrupts discrimination reversal conditioning of the rabbit nictitating membrane response. *Behav. Brain Res.* 8(1): 49–68

Berger TW, Rinaldi PC, Weisz DJ, Thompson RF. 1983. Single-unit analysis of different hippocampal cell types during classical conditioning of rabbit nictitating membrane response. *J. Neurophysiol.* 50(5): 1197–1219

Blazis DE, Fischer TM, Carew TJ. 1993. A neural network model of inhibitory information processing in Aplysia. *Neural Comput.* 5(2):213–27

Bliss TVP, Collingridge GL. 1993. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361 :31–39

Bliss TVP, Lomo T. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. *J. Physiol.* 232:331–56

Bliss TVP, Lynch MA. 1988. Long-term potentiation of synaptic transmission in the hippocampus: properties and mechanisms. In *LTP: From Biophysics to Behavior*, ed. P Landfield, SA Deadwyler, pp. 3–72. New York: Liss

Bonhoeffer T, Staiger V, Aertsen A. 1989. Synaptic plasticity in rat hippocampal slice cultures: local "Hebbian" conjunction of pre- and postsynaptic stimulation leads to distributed synaptic enhancement. *Proc. Natl. Acad. Sci. USA* 86(20):8113–17

Bramham CR. 1992. Opioid receptor–dependent long-term potentiation: peptidergic regulation of synaptic plasticity in the hippocampus. *Neurochem. Int.* 20:441–55

Bramham CR, Milgram NW, Srebro B. 1991a. Delta opioid receptor activation is required to induce LTP of synaptic transmission in the lateral perforant path in vivo. *Brain Res.* 567(1):42–50

Bramham CR, Milgram NW, Srebro B. 1991b. Activation of AP5-sensitive NMDA receptors is not required to induce LTP of synaptic transmission in the lateral perforant path. *Eur. J. Neurosci.* 3:1300–8

Breindl AB, Derrick BE, Rodriguez SB, Martinez JL Jr. 1994. Opioid receptor–dependent long-term potentiation at the lateral perforant path–CA3 synapse in rat hippocampus. *Brain Res. Bull.* 33(1): 17–24

Brinton RE. 1991. Biochemical correlates of learning and memory. See Martinez & Kesner 1991, pp. 199–257

Butcher SP, Davis S, Morris RGM. 1990. A dose-related impairment of spatial learning by the NMDA receptor antagonist, 2-amino-5-phosphonovalerate (AP5). *Eur. Neuropsychopharmacol.* 1(1):15–20

Butcher SP, Hamberger A, Morris RGM. 1991. Intracerebral distribution of DL-2-aminophosphonopentanoic acid (AP5) and the dissociation of different types of learning. *Exp. Brain Res.* 83(3):521–26

Castro CA, Silbert LH, McNaughton BL, Barnes CA. 1989. Recovery of learning following decay of experimental saturation of LTE at perforant path synapses. *Nature* 342:545–48

Chattarji S, Stanton PK, Sejnowski TJ. 1989. Commissural synapses, but not mossy fiber synapses, in hippocampal field CA3 exhibit associative long-term potentiation and depression. *Brain Res.* 495(1):145–50

Collingridge GL, Kehl SJ, McLennan H. 1983. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *J. Physiol.* 334:33–46

Conquet F, Bashir ZI, Davies CH, Daniel H, Ferraguti F, et al. 1994. Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1. *Nature* 372(6503): 237–43

Dailey ME, Buchanan J, Bergles DE, Smith SJ. 1994. Mossy fiber growth and synaptogenesis in rat hippocampal slices in vitro. *J. Neurosci.* 14(3):1060–78

Davis S, Butcher SP, Morris RGM. 1992. The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP in vivo at intracerebral concentrations comparable to those that block LTP in vitro. *J. Neurosci.* 12(1): 21–34

Decker MW, Introini-Collison IB, McGaugh JL. 1989. Effects of naloxone on Morris water maze learning in the rat: enhanced acquisition with pretraining but not posttraining administration. *Psychobiology* 17:270–75

Derrick BE, Martinez JL Jr. 1989. A unique,

opioid peptide–dependent form of long-term potentiation is found in the CA3 region of the rat hippocampus. *Adv. Biosci.* 75:213–16

Derrick BE, Martinez JL Jr. 1994a. Opioid receptors underlie the frequency-dependence of mossy fiber LTP induction. *J. Neurosci.* 14(7):4359–67

Derrick BE, Martinez JL Jr. 1994b. Frequency-dependent associative LTP at the mossy fiber-CA3 synapse. *Proc. Natl. Acad. Sci. USA* 91(22):10290–94

Derrick BE, Martinez JL Jr. 1995. Associative LTD at the Hippocampal Mossy Fiber–CA3 Synapse. *Soc. Neurosci. Abstr.* 21:603

Derrick BE, Rodriguez SB, Lieberman DN, Martinez JL Jr. 1992. Mu opioid receptors are associated with the induction of LTP at hippocampal mossy fiber synapses. *J. Pharmacol. Exp. Ther.* 263: 725–33

Derrick BE, Weinberger SB, Martinez JL Jr. 1991. Opioid receptors are involved in an NMDA receptor–independent mechanism of LTP induction at hippocampal mossy fiber–CA3 synapses. *Brain Res. Bull.* 27: 219–23

Deutsch JA. 1993. Spatial learning in mutant mice. *Science* 262(5134):760–63

Diamond DM, Rose GM. 1994. Does associative LTP underlie classical conditioning? *Psychobiology* 22(4):263–69

Dudek SM, Bear MF. 1993. Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. *J. Neurosci.* 13:2910–18

Erickson CA, McNaughton BL, Barnes CA. 1993. Comparison of long-term enhancement and short-term exploratory modulation of perforant path synaptic transmission. *Brain Res.* 615(2):275–80

Erickson CA, McNaughton BL, Barnes CA. 1990. Exploration-dependent enhancement of synaptic responses in rat fascia dentata is blocked by MK801. *Soc. Neurosci. Abstr.* 16:442

Gallistel R. 1994. Interview with Randy Gallistel. *J. Cogn. Neurosci.* 6(2): 174–79

Gallistel R. 1995. Is long-term potentiation a plausible basis for memory? In *Brain and Memory: Modulation and Mediation of Plasticity*, ed. JL McGaugh, NMWeinberger, G Lynch, pp. 328–37. New York: Oxford Univ. Press

Goddard GV. 1986. A step nearer a neural substrate. *Nature* 319:721–22

Grant SG, O'Dell TJ, Karl KA, Stein PL, Soriano P, Kandel ER. 1992. Impaired long-term potentiation, spatial learning, and hippocampal development in *fyn* mutant mice. *Science* 258(5090):1903–10

Green EJ, Greenough WT. 1986. Altered synaptic transmission in dentate gyrus of rats reared in complex environments: evidence from hippocampal slices maintained in vitro. *J. Neurophysiol.* 55(4):739–50

Greenough EJ, McNaughton BL, Barnes CA. 1990. Exploration-dependent modulation of evoked responses in fascia dentata: dissociation of motor, EEG, and sensory factors, and evidence for a synaptic efficacy change. *J. Neurosci.* 10(5):1455–71

Greenough WT, Volkmar FR, Juraska JM. 1973. Effects of rearing complexity on dendritic branching in frontolateral and temporal cortex of rat. *Exp. Neurol.* 41: 371–78

Harris EW, Cotman CW. 1986. Long-term potentiation of guinea pig mossy fiber responses is not blocked by *N*-methyl D-aspartate antagonists. *Neurosci. Lett.* 70: 132–37

Hebb DO. 1949. *The Organization of Behavior*. New York: Wiley

Ishihara K, Katsuki H, Sugimura M, Kaneko S, Satoh M. 1990. Different drug-susceptibilities of long-term potentiation in three input systems to the CA3 region of the guinea pig hippocampus in vitro. *Neuropharmacology* 29(5):487–92

Jaffe D, Johnston D. 1990. Induction of long-term potentiation at hippocampal mossy fibers follows a Hebbian rule. *J. Neurophysiol.* 64:948–60

Jarrard JE. 1986. Selective hippocampal lesions and behavior. In *The Hippocampus*, ed. RL Isaacson, KH Pribram, 4:93–122. New York: Plenum

Jeffery KJ, Morris RGM. 1993. Cumulative long-term potentiation in the rat dentate gyrus correlates with, but does not modify, performance in the water maze. *Hippocampus* 3(2):133–40

Johnston D, Williams S, Jaffe D, Gray R. 1992. NMDA-receptor-independent long-term potentiation. *Annu. Rev. Physiol.* 54: 489–505

Jung MW, Larson J, Lynch G. 1990. Long-term potentiation of monosynaptic EPSPs in rat piriform cortex in vitro. *Synapse* 6(3):279–83

Kanter ED, Haberly LB. 1993. Associative long-term potentiation in piriform cortex slices requires GABAA blockade. *J. Neurosci.* 13(6):2477–82

Keith JR, Rudy JW. 1990. Why NMDA receptor–dependent long-term potentiation may not be a mechanism of learning and memory: a reappraisal of the NMDA receptor blockade strategy. *Psychobiology* 18(3): 251–57

Kelso SR, Brown TH. 1986. Differential conditioning of associative synaptic enhancement in hippocampal brain slices. *Science* 232(4746):85–87

Kim JJ, DeCola JP, Landeira-Fernandez J, Fanselow MS. 1991. N-methyl-D-aspartate

receptor antagonist APV blocks acquisition but not expression of fear conditioning. *Behav. Neurosci.* 105(1):126–33
Kim JJ, Fanselow MS. 1992. Modality-specific retrograde amnesia of fear. *Science* 256: 675–77
Kim JJ, Fanselow MS, DeCola JP, Landeira-Fernandez J. 1992. Selective impairment of long-term but not short-term conditional fear by the *N*-methyl-D-aspartate antagonist APV. *Behav. Neurosci.* 106(4):591–96
Laroche S, Doyere V, Bloch V. 1989. Linear relation between the magnitude of long-term potentiation in the dentate gyrus and associative learning in the rat: a demonstration using commissural inhibition and local infusion of an N-methyl-D-aspartate receptor antagonist. *Neuroscience* 28(2): 375–86
Laroche S, Doyere V, Redini Del Negro C. 1991. What role for LTP in learning and the maintenance of memories? In *LTP: A Debate of the Current Issues*, ed. M Baudry, JL Davis, pp. 301–16. London: MIT Press
Levy WB, Steward O. 1979. Synapses as associative memory elements in the hippocampal formation. *Brain Res.* 175(2):233–45
Linden DJ, Connor JA. 1995. Long-term synaptic depression. *Annu. Rev. Neurosci.* 8: 319–57
Lisman J. 1989. A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. *Proc. Natl. Acad. Sci. USA* 86(23):9574–78
Lynch G, Granger R. 1986. Variations in synaptic plasticity and types of memory in corticohippocampal networks *J. Cogn. Neurosci.* 4(3):189–99
Makintosh NJ. 1974. *The Psychology of Animal Learning*. London: Academic
Malenka RC, Kauer JA, Perkel DJ, Mauk MD, Kelly PT, et al. 1989. An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation. *Nature* 340(6234):554–57
Malinow R, Schulman H, Tsien RW. 1989. Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. *Science* 245(4920):862–66
Manzoni OJ, Weisskopf MG, Nicoll RA. 1994. MCPG antagonizes metabotropic glutamate receptors but not long-term potentiation in the hippocampus. *Eur. J. Neurosci.* 6(6):1050–54
Marr D. 1971. Simple memory: a theory of archicortex. *Philos. Trans. R. Soc. London Ser. B* 262:23–81
Martin MR. 1983. Naloxone and long-term potentiation of hippocampal CA3 field potentials in vitro. *Neuropeptides* 4:45–50
Martinez JL Jr, Derrick BE. 1994. Opioid receptors contribute to lateral perforant path–CA3 responses days, but not hours, following LTP induction. *Soc. Neurosci. Abstr.* 20:897
Martinez JL Jr, Kesner RP. 1991. *Learning and Memory: A Biological View*. San Diego: Academic
Martinez JL Jr, Schulteis G, Weinberger SB. 1991. How to increase and decrease the strength of memory traces: the effects of drugs and hormones. See Martinez & Kesner 1991, pp. 149–287
Matthies H, Ruethrich H, Ott T, Matthies HK, Matthies R. 1986. Low-frequency perforant path stimulation as a conditioned stimulus demonstrates correlations between long-term synaptic potentiation and learning. *Physiol. Behav.* 36(5):811–21
McDaniel KL, Mundy WR, Tilson HA. 1990. Microinjection of dynorphin into the hippocampus impairs spatial learning in rats. *Pharmacol. Biochem. Behav.* 35(2): 429–35
McLean S, Rothman RB, Jacobson AE, Rice KC, Herkenham M. 1987. Distribution of opiate receptor subtypes and enkephalin and dynorphin immunoreactivity in the hippocampus of the squirrel, guinea pig, rat and hamster. *J. Comp. Neurol.* 255: 497–510
McNaughton BL, Barnes CA, Meltzer J, Sutherland RJ. 1989. Hippocampal granule cells are necessary for normal spatial learning but not for spatially-selective pyramidal cell discharge. *Exp. Brain Res.* 76(3): 485–96
McNaughton BL, Barnes CA, Rao G, Baldwin J, Rasmussen M. 1986. Long-term enhancement of hippocampal synaptic transmission and the acquisition of spatial information. *J. Neurosci.* 6(2):563–71
McNaughton BL, Douglas RM, Goddard GV. 1978. Synaptic enhancement in fascia dentata: cooperativity among coactive afferents. *Brain Res.* 157:277–93
McNaughton BL, Morris RGM. 1987. Hippocampal synaptic enhancement and information storage within a distributed memory system. *Trends Neurosci.* 10:408–15
Mitsuno K, Sasa M, Ishihara K, Ishikawa M, Kikuchi H. 1994. LTP of mossy fiber-stimulated potentials in CA3 during learning in rats. *Physiol. Behav.* 55(4):633–38
Mondadori C, Weiskrantz L, Buerki H, Petschke F, Fagg GE. 1989. NMDA receptor antagonists can enhance or impair learning performance in animals. *Exp. Brain Res.* 75(3):449–56
Montague PR, Sejnowski TJ. 1994. The predictive brain: temporal coincidence and temporal order in synaptic learning mechanisms. *Learn. Mem.* 1:1–33
Morris RGM. 1989a. Synaptic plasticity and learning: selective impairment of learning rats and blockade of long-term potentiation in vivo by the *N*-methyl-D-aspartate recep-

tor antagonist AP5. *J. Neurosci.* 9(9): 3040–57
Morris RGM. 1989b. Does synaptic plasticity play a role in information storage in the vertebrate brain? In *Parallel Distributed Processing: Implications for Psychology and Neurobiology*, ed. RGM Morris, pp. 248–85. Oxford: Clarendon
Morris RGM. 1990. It's heads they win, tails I lose! *Psychobiology* 18:261–66
Morris RGM, Anderson E, Lynch GS, Baudry M. 1986. Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist AP5. *Nature* 319(6056):774–76
Morris RGM, Garrud P, Rawlins JN, O'Keefe J. 1982. Place navigation impaired in rats with hippocampal lesions. *Nature* 297(5868):681–83
Moser E, Mathiesen I, Andersen P. 1993a. Association between brain temperature and dentate field potentials in exploring and swimming rats. *Science* 259(5099): 1324–26
Moser E, Moser MB, Andersen P. 1993b. Synaptic potentiation in the rat dentate gyrus during exploratory learning. *NeuroReport* 5(3):317–20
Nadel L. 1968. Dorsal and ventral hippocampal lesions and behavior. *Physiol. Behav.* 3(6):891–900
O'Dell TJ, Kandel ER, Grant SG. 1991. Long-term potentiation in the hippocampus is blocked by tyrosine kinase inhibitors. *Nature* 353(6344):558–60
Ohki Y. 1982. The effects of hippocampal lesions on two types of avoidance learning in rats: effects on learning to be active or to be inactive. *Jpn. J. Psychol.* 53(2): 65–71
Ott T, Ruthrich K, Reymann L, Lindenau L, Matthies H. 1982. Direct evidence for the participation of changes in synaptic efficacy in the development of behavioral plasticity. In *Neuronal Plasticity and Memory Formation*, ed. CA Marsan, H Matthies, pp. 441–52. New York: Raven
Pavlov IP. 1927. *Conditioned Reflexes.* London: Oxford Univ. Press
Peng Y, Horn JP. 1991. Continuous repetitive stimuli are more effective than bursts for evoking LHRH release in bullfrog sympathetic ganglia. *J. Neurosci.* 11:85–95
Port RL, Beggs AL, Patterson MM. 1987. Hippocampal substrate of sensory associations. *Physiol. Behav.* 39(5):643–47
Racine RJ, Milgram NW, Hafner S. 1983. Long-term potentiation phenomena in the rat limbic forebrain. *Brain Res.* 260(2): 217–31
Reymann KG, Ruthrich H, Lindenau L, Ott T, Matthies H. 1982. Monosynaptic activation of the hippocampus as a conditioned stimulus: behavioral effects. *Physiol. Behav.* 29(6):1007–12
Richter-Levin G, Errington ML, Maegawa H, Bliss TV. 1994. Activation of metabotropic glutamate receptors is necessary for long-term potentiation in the dentate gyrus and for spatial learning. *Neuropharmacology* 33(7):853–57
Robinson GS, Crooks GB, Shinkman PG, Gallagher M. 1990. Behavioral effects of MK-801 mimic deficits associated with hippocampal damage. *Psychobiology* 17(2): 156–64
Robinson GB. 1992. Maintained saturation of hippocampal long-term potentiation does not disrupt acquisition of the eight-arm radial maze. *Hippocampus* 2(4):389–95
Rolls ET. 1989. Parallel distributed processing in the brain: implications of the functional architecture of neuronal networks in the hippocampus. In *Parallel Distributed Processing: Implications for Psychology and Neurobiology*, ed. RGM Morris, pp. 286–307. Oxford: Clarendon
Roman FS, Chaillan FA, Soumireu-Mourat B. 1993. Long-term potentiation in rat piriform cortex following discrimination learning. *Brain Res.* 601(1–2):265–72
Roman FS, Staubli U, Lynch G. 1987. Evidence for synaptic potentiation in a cortical network during learning. *Brain Res.* 418(2):221–26
Rosenzweig MR, Krech D, Bennett EL, Diamond MC. 1962. Effects of environmental complexity and training on brain chemistry and anatomy: a replication and extension. *J. Comp. Physiol. Psychol.* 55:429–37
Rumelhart D, McClelland J. 1986. *Parallel Distributed Processing*, Vol. 1. Cambridge: MIT Press
Ruthrich H, Dorochow W, Pohle W, Ruthrich HL, Matthies H. 1987. Colchicine-induced lesion of rat hippocampal granular cells prevents conditioned active avoidance with perforant path stimulation as conditioned stimulus, but not conditioned emotion. *Physiol. Behav.* 40(2):147–54
Sakimura K, Kutsuwada T, Ito I, Manabe T, Takayama C, et al. 1995. Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor epsilon 1 subunit. *Nature* 373(6510):151–55
Schacter DL, Tulving E. 1994. What are the memory systems of 1994? In *Memory Systems 1994*, ed. DL Schacter, E Tulving, pp. 1–38. Cambridge: MIT Press
Schuman EM, Madison DV. 1991. A requirement for the intercellular messenger nitric oxide in long-term potentiation. *Science* 254(5037):1503–6
Sejnowski TJ. 1977. Storing covariance with nonlinearly interacting neurons. *J. Math. Biol.* 4(4):303–21

Sharp PE, McNaughton BL, Barnes CA. 1985. Enhancement of hippocampal field potentials in rats exposed to a novel, complex environment. *Brain Res*. 339(2):361–65

Shimai S, Ohki Y. 1980. Facilitation of discriminated rearing-avoidance in rats with hippocampal lesions. *Percept. Mot. Skills* 50(1):56–8

Silva AJ, Paylor R, Wehner JM, Tonegawa S. 1992a. Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. *Science* 257(5067):206–11

Silva AJ, Stevens CF, Tonegawa S, Wang Y. 1992b. Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. *Science* 257(5067): 201–6

Squire LR. 1987. *Memory and Brain*. New York: Oxford Univ. Press

Staubli U. 1992. A peculiar form of potentiation in mossy fiber synapses. *Epilepsy Res. Suppl*. 7:151–57

Staubli U, Larson J, Lynch G. 1990. Mossy fiber potentiation and long-term potentiation involve different expression mechanisms. *Synapse* 5(4):333–35

Staubli U, Lynch G. 1987. Stable hippocampal long-term potentiation elicited by 'theta' pattern stimulation. *Brain Res*. 435:227–34

Staubli U, Thibault O, Lynch G. 1986. Antagonism of NMDA receptors impairs acquisition, but not retention, of olfactory memory. *Behav. Neurosci*. 103:54–60

Stent G. 1973. A physiological mechanism for Hebb's postulate of learning. *Proc. Natl. Acad. Sci. USA* 70:997–1001

Stewart CA, Jeffery K, Reid I. 1994. LTP-like synaptic efficacy changes following electroconvulsive stimulation. *NeuroReport* 5(9):1041–44

Stewart CA, Reid IC. 1994. Ketamine prevents ECS-induced synaptic enhancement in rat hippocampus. *Neurosci. Lett*. 178(1):11–14

Stripling JS, Patneau DK, Gramlich CA. 1991. Characterization and anatomical distribution of selective long-term potentiation in the olfactory forebrain. *Brain Res.* 542(1): 107–22

Sutherland RJ, Dringenberg HC, Hoesing JM. 1993. Induction of long-term potentiation at perforant path dentate synapses does not affect place learning or memory. *Hippocampus* 3(2):141–47

Teyler TJ, Grover L. 1993. In *Synaptic Plasticity: Molecular, Cellular and Functional Aspects*, ed. M Baudry, RF Thompson, JL Davis, pp. 73–86. Cambridge: MIT Press

Thompson RF. 1992. Memory. *Curr. Opin. Neurobiol.* 2(2):203–8

Thompson RF, Spencer WA. 1966. Habituation: a model phenomenon for the study of the neuronal substrates of behavior. *Psych. Rev*. 173:16–43

Wagner JJ, Terman GW, Chaukin C. 1993. Endogenous dynorphins inhibit excitatory neurotransmission and block LTP induction in hippocampus. *Nature* 36:(6428): 451–54

Walker DL, Gold PE. 1991. Effects of the novel NMDA antagonist NPC 12626, on long-term potentiation, learning and memory. *Brain Res*. 549(2):213–21

Weisskopf MG, Zalutsky RA, Nicoll RA. 1993. The opioid peptide dynorphin mediates heterosynaptic depression of hippocampal mossy fibre synapses and modulates long-term potentiation. *Nature* 362: 423–27

Williams S, Johnston D. 1989. Long-term potentiation of hippocampal mossy fibers is blocked by postsynaptic injection of calcium chelators. *Neuron* 3:583–88

Winson J. 1978. Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. *Science* 201(4351):160–63

Zalutsky RA, Nicoll RA. 1990. Comparison of two forms of long-term potentiation in single hippocampal neurons. *Science* 248: 1619–24

Zalutsky RA, Nicoll RA. 1992. Mossy fiber long-term potentiation shows specificity but no apparent cooperativity. *Neurosci. Lett.* 138:193–97

Zhang DX, Levy WB. 1992. Ketamine blocks the induction of LTP at the lateral entorhinal cortex-dentate gyrus synapses. *Brain Res*. 593(1):124–27

Zola-Morgan S, Squire LR. 1993. Neuroanatomy of memory. *Annu. Rev. Neurosci*. 16:547–63