Can molecules explain long-term potentiation?

Joshua R. Sanes and Jeff W. Lichtman

Although over 100 molecules have been implicated in long-term potentiation and depression, no consensus on their underlying molecular mechanisms has emerged. Here we discuss the difficulties of providing molecular explanations for cellular neurobiological phenomena.

About ten years ago, cellular neurobiology—following hard on the heels of general cell biology—took a sharp turn toward the molecular. This shift produced various spectacular insights. For example, the formalisms that Hodgkin and Huxley devised to describe the action potential have been partially explained by conformational changes in a group of transmembrane proteins whose sequences we know and whose three-dimensional structures we will soon know. Likewise, many synaptic vesicle proteins have been identified, and these almost certainly will include the major players that underlie neurotransmitter release. Even neural development is yielding to molecular analysis—from the neurogenic genes that generate neurons to the caspases that kill them.

As these successes grew, it was natural to apply molecular tools to one of the most fascinating issues in all of biology: learning and memory. A principal strategy has been to focus on long-lasting alterations in the efficacy of synaptic transmission that are elicited by particular patterns of electrical stimulation. Two such forms of synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD), have been subjected to particularly intense scrutiny. The remarkable growth in interest is evident from publication records: the number of papers dealing at least in part with LTP rose exponentially from 12 during 1975–1979 to ~90 from 1980–1984, ~350 from 1985–1989, ~1000 from 1990–1994 and ~1800 from 1995–1999. (Numbers are minimum estimates based on key words indexed in Medline and include a conservative projection for 1999.) Because of this large-scale assault, over 100 molecules have been implicated in LTP and LTD[1–93]. Table 1 lists 117 of these molecules suggested to be causally associated with hippocampal LTP.

Despite this effort, a widely accepted and satisfying molecular explanation for LTP has not yet been discovered or, at least, been generally accepted. Rather, at least to outsiders, it appears that multiple hypotheses are still being generated and debated. Additionally, we hear varying degrees of frustration and embarrassment concerning their field from some of our LTP colleagues. Why? It may seem both presumptuous and peculiar for us, with absolutely no credentials in the area, to venture an opinion. We were motivated, however, to consider this issue for two reasons. First, LTP has become such a major feature of the scientific landscape that it looms large not only in journals but also in the minds of many cellular and molecular neurobiologists. Second, we regard LTP as a case study depicting the sorts of difficulties that might arise in other areas of study (including our own, vertebrate neural development) as cellular phenomena are reduced to molecular mechanisms. In pondering the general question of why LTP has seemed to resist molecular analysis, we came to believe that part of the answer might be found if we could answer another, more specific question: why do so many molecules seem to be involved in LTP?

The first few molecules

In the early 1970s, T.V.P. Bliss and T. Lomo recorded extracellular field potentials in the hippocampus evoked by stimulation of the perforant path of anesthetized rabbits. They found that the efficacy of synaptic transmission, as measured by the size of the field potentials, was potentiated for several hours following a short, high-frequency volley of stimuli[94]. Since then, this phenomenon, now called LTP, has captured the imagination of many scientists for three main reasons. First, it was discovered in the hippocampus, a region associated with learning and memory. Second, it occurs on a time scale long enough to be potentially useful for information storage. Third, it was soon shown that LTP had the Hebbian property believed to underlie associative learning: those inputs that fired at the same time as the stimulus were potentiated, whereas asynchronously activated inputs were not potentiated[95,96]. Interest in LTP increased as it was shown to occur at other synapses likely to mediate learning—for example, in the cerebral cortex[97]—and implicated in synaptic rearrangements that occur during normal development[98].

The discovery that one form involves NMDA-type glutamate receptors initiated the transition of LTP from a purely physiological phenomenon to a molecular one. Glutamate opens these calcium-permeable channels most effectively only when the membrane has been depolarized by a separate input, thus providing an attractive explanation for the associative feature of LTP[96]. Soon thereafter, biochemical analyses suggested that calcium entering through the NMDA receptor activated calcium-dependent protein kinases such as calcium/calmodulin-dependent (CaM) kinase, which then remained active even after the calcium level dropped. This enzyme, in a sense, 'remembered' that calcium had been elevated, thereby providing a means to maintain the synapse in a potentiated state for a period that substantially outlasted the inducing stimulus[99]. Thus, by the late 1980s, it seemed that a satisfying molecular explanation for LTP was in view (Fig. 1). Over the next decade, however, pharmacological, biochemical and genetic studies implicated an ever-expanding cadre of endogenous molecules in the process (Table 1), and this apparent clarity dissipated. What happened?

The authors are in the Department of Anatomy and Neurobiology, Washington University School of Medicine, 660 S. Euclid Avenue, Campus Box 8108, St. Louis, Missouri 63110, USA. Correspondence should be addressed to either J.W.L. or J.R.S.
email: jeff@thalamus.wustl.edu or sanesj@thalamus.wustl.edu

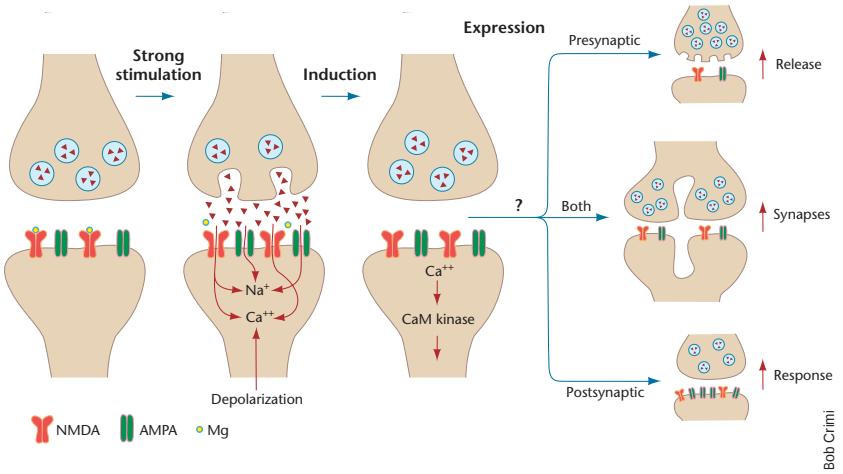

Fig. 1. Induction and expression of LTP. Strong repetitive stimulation of the input to a hippocampal neuron can activate AMPA and NMDA receptors. The opening of the NMDA channel allows Ca^{2+} entry, which is a critical step in the induction of LTP, as it activates a particular calcium-dependent protein kinase, calcium/calmodulin-dependent (CaM) kinase. The activation persists after the synaptic events that trigger it, and appears to be both necessary and sufficient for LTP induction in some neurons. The next series of steps that lead to the expression of LTP is still a matter of intense debate. There are three classes of explanation, and all have evidence to back them up. One possibility is that the potentiation is caused by an increase in the number of packets, or quanta, of glutamate released by presynaptic nerve terminals; alternatively, there is evidence that new AMPA receptors are recruited to synapses to increase the amplitude of the postsynaptic response to glutamate; lastly, several reports implicate structural changes that could include new synapse formation associated with LTP.

Why so many molecules?

A simple answer might be that many molecules are required to mediate the process. However, we believe that there is a second, equally valid answer on which we want to focus: that several factors have thwarted attempts to pinpoint a minimal cadre of essential molecules among a larger group of candidates. Before enumerating these factors, we must present two caveats. First, we have not attempted to judge the quality of the data justifying inclusion of a molecule in Table 1, even though we fully realize that not all scientific data are equal. For example, synapsin is listed based on a correlative study[45], even though apparently stronger genetic data shows that at least two of the three synapsins are not critically involved[100]. As outsiders, we feel it imprudent to judge the merits of each data set. We note, however, that a substantial number of these papers have been published in top-ranked journals, giving us the impression that the quality of the data is good. Second, the factors itemized below probably do not contribute equally to the uncertainties that beset the field.

A rose is not a rose is not a rose

The terms 'potentiation' and 'depression' can be used to describe any enhancement or decrement in synaptic response, and 'long-term' is hardly more specific. It is no surprise that different neurons accomplish LTP in different ways, and that LTD is not a simple mirror image of LTP. We have been somewhat sensitive to this complexity in assembling Table 1, omitting the vast literature on LTP in structures other than hippocampus as well as all studies of LTD. However, even within the hippocampus, LTP as a physiological phenomenon exhibits at least five levels of heterogeneity. First, synaptic responses can be potentiated for a long time in several types of neurons—for example, granule cells of the dentate gyrus, pyramidal cells of CA1 and CA3 and interneurons—and each has distinct properties[5]. Second, multiple synaptic inputs can be potentiated on a single neuron, for example, stimulation of mossy fibers, associational fibers and entorhinal fibers can all evoke LTP in CA3 pyramidal cells, each with unique properties[101]. Third, at a single synapse, LTP can be evoked in various ways—for example, by 50 Hz, 100 Hz or theta burst stimulation—and each may have distinct mechanisms[27]. Fourth, LTP-inducing stimuli seem to initiate a multi-step process that has been divided into as many as four phases: initial, early, intermediate and late[95,102]. Each step might involve many molecules, and the ones that seem important probably depend on which phase is being measured. Finally, even with a standardized stimulation protocol, LTP at synapses on basal dendrites show different sensitivities to kinase inhibitors than do synapses on apical dendrites of the same neuron, suggesting involvement of distinct mechanisms in the two dendritic subfields[103].

In short, there may be a dozen or more forms of hippocampal LTP (with this number itself being a matter of considerable controversy), each mediated in different ways by different molecules. Some apparent controversies may be explained by failure to take this heterogeneity into account. In the vast majority of cases, however, investigators are well aware of these issues, and we therefore need to look further to understand why consensus has been elusive (Fig. 2).

Correlation does not prove causation

Numerous studies have compared levels of one molecule or another in hippocampi that have or have not undergone LTP[36,50]. Not uncommonly, the authors argue that a change in the level of molecule X implies that molecule X is involved in LTP itself. This reasoning is faulty in two respects. First, the biochemical change might be an indirect consequence of some process linked to LTP. For example, if a protease is activated as an integral part of the LTP process, it might proteolyze some substrates that have nothing to do with LTP. Second, experimentally, it is technically difficult to distinguish correlates of LTP from correlates of stimulation in general, or high-frequency stimulation in particular. In some investigations, the mere selective expression of a molecule in the hippocampus has been taken as evidence that it may be involved in LTP; we have not included such cases in Table 1. Nonetheless, some of the molecules implicated by the logic of correlation may not, in fact, be causally involved in LTP.

Modulators mistaken for mediators

For many neurobiological processes, a distinction is customarily made between mediators, which are required agents of the process, and modulators, which alter a process but are inessential for its occurrence. At the skeletal neuromuscular junction, for example, acetylcholine activates nicotinic acetylcholine receptors to mediate synaptic transmission, but other molecules, such as norepinephrine, CGRP, adenosine and muscarinic receptors, can modulate transmission by affecting the amount of transmitter released or the sensitivity of the postsynaptic receptors (see ref. 104). Activating or blocking receptors for these mod-

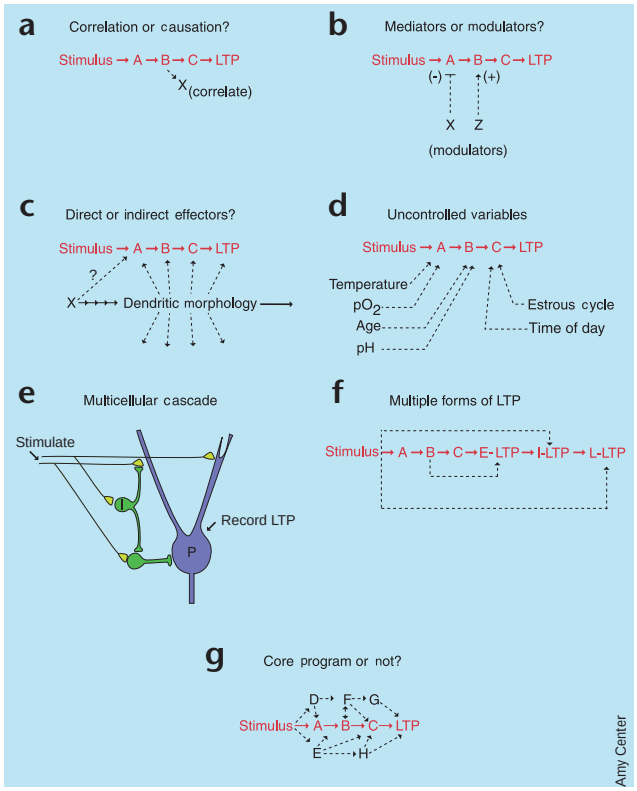

Fig. 2. Some of the reasons why so many molecules have been implicated in LTP. **(a)** Molecule X may be altered by some step in the LTP cascade (shown in bold) even though it has nothing to do with LTP. **(b)** Molecules may modulate the LTP cascade either directly (X or Z) or indirectly without having a central or required role in the LTP cascade. **(c)** Some factors can alter LTP by completely indirect means, as for example molecule X, which has a non-specific effect on dendritic morphology throughout the brain. **(d)** Molecules may be incorrectly implicated in part because the LTP assay can give variable results, due to the number of parameters that can affect the readout. **(e)** Some molecules are implicated because the actual circuit that underlies LTP extends to many more cells and synapses than the one being assayed by the extracellular electrode. **(f)** Investigators have argued that there are multiple forms of LTP: early (E-LTP), intermediate (I-LTP) and late (L-LTP). If the cascades that generate each of these are, in part, nonoverlapping, this will certainly increase the number of molecules involved. **(g)** Lastly, it is possible that the cellular cascade of LTP is not based on a linear molecular cascade. If there is no core molecular program, then there may be a very large number of molecules involved.

ulators can have dramatic effects on neurotransmission and could therefore be considered critical components of the mechanism. Nonetheless, there is very little confusion about the essential nature of acetylcholine or the dispensability of the others. Of course, most investigators understand that the same distinction is equally valid for LTP, but it is nonetheless sometimes insufficiently considered in interpreting results.

Molecules may act indirectly

LTP is influenced by factors extending beyond mediators and modulators. For example, alterations in intermediary metabolism, neural development and brain circuitry can affect the induction or expression of LTP. It is debatable whether these indirect effectors affect the basic mechanism, yet several have been studied in detail. Slightly more complicated are manipulations that may have both direct and indirect effects on LTP. In one of the studies that launched the molecular genetic analysis of behavior, defects in LTP were detected in hippocampi of mutant mice that lacked the tyrosine kinase fyn[66]. However, interpretation of this effect was complicated because these mice are severely myelin-deficient and their hippocampal neurons are morphologically aberrant[66,105]. In principle, such complexities could be resolved by conditional mutagenesis ('knocking out' a gene in a restricted subset of cells) or inducible transgene expression (turning a gene on or off at a specific time). Indeed, the related approach of transgenic rescue has been applied to fyn[106]. Nonetheless, these methods are presently imperfect and, to date, have not unequivocally separated direct from indirect effects.

Uncontrolled variables

LTP is an electrophysiological phenomenon, and it is usually detected and measured using intracellular or extracellular microelectrodes. Unfortunately, physiological assays are more technically demanding than many biochemical assays and can give maddeningly variable results, even in expert hands. For LTP, the situation is especially difficult because factors as diverse as temperature[107], time of day[108], time of month[108], age[109], gender[110] and social isolation[110] have been claimed to affect the outcome. For example, inhibitors of nitric oxide synthesis abolish LTP in hippocampal slices when the measurement is made at 25°C, but have no detectable effect on the same slices at 29°C (ref. 107). Even seemingly well-controlled studies might ignore some of these variables.

At least three additional complications are potentially pertinent for molecular studies. First, LTP can be either induced or blocked by transient anoxia, depending on whether the insult is delivered *in vivo* (before the slice is prepared) or *in vitro*[111,112]. Thus, not only can molecules that affect metabolism perturb LTP indirectly, but small changes in recording conditions (like perfusion rate) may alter the results. Second, inbred strains of mice apparently differ markedly in their ability to produce LTP. Because most transgenic and knockout mice are maintained on heterogeneous hybrid backgrounds, these strain differences have been reported to confound measurements[113]. Thus, even though competent labs are careful to control for untoward variables, it is possible that the sheer number of variables that can affect a physiological assay in a multicellular preparation like a brain slice has made it more difficult than in simpler systems to ferret out incorrect molecular claims. Third, it has recently been found that the mere act of preparing slices from a hippocampus can induce the formation of additional dendritic spines and synapses on the pyramidal cells in which LTP is usually recorded[114]. Because LTP may actually involve or be accompanied by formation of new spines[115], the state of the slice may therefore be particularly critical. Moreover, some synapses that undergo LTP *in vitro* may not have existed *in vivo*.

The brain is extremely complex

Hippocampal slices maintain, in organized form, many elements of the hippocampal circuit, which is, in turn, about as complicated as any other part of the brain. Hippocampi contain at least 12 types of interneurons, which use at least 6 different neurotransmitters, each of which may exert both localized (synaptic) and diffuse effects, and most of which affect each other's release[116]. Even in slices, much of this circuitry is activated by stimuli commonly used to elicit LTP, and much of it affects the output commonly used to measure LTP. Thus, although LTP is defined as an alteration in a monosynaptic current, some of the changes that lead to a potentiated response may not be occurring at the synapse (or even the type of synapse) under

Table I. Molecules implicated in hippocampal LTP

Glutamate receptors	Reference*
GluR1	1
GluR2	2
mGluR1	3
mGluR4	4
mGluR5	5
mGluR7	4
NMDA NR1	6
NMDA NR2A	7
NMDA NR2D	8
Other neurotransmitters, neuromodulators and their receptors	
norepinephrine and β-adrenergic receptors	9
adenosine and adenosine 2A receptors	10
dopamine and D1 dopamine receptors	11
μ opioid receptors	12
δ1 opioid receptors	12
acetylcholine and muscarinic receptors	13
GABA and GABA-B receptors	14
anandamine and CB1 cannabinoid receptors	15
orphanin NQ and nocioceptin receptors	16
serotonin and 5HT3 receptors	17
sn-2 arachidonylglycerol (2-AG)	18
endothelin-1	19
γ-hydroxybutynic acid (GHB) and GHB receptors	20
Intercellular messengers, their synthetic enzymes and their receptors	
CO	21
NO	21
EGF	22
basic FGF	22
superoxide	23
neuregulin	24
erbB4	24
NGF	25
BDNF	26
TrkB	27
nNOS	28
eNOS	29
arachidonic acid	30
platelet activating factor	31
interleukin 1β	32
H_2S	33
β-activin	34
Calcium/calmodulin binding proteins	
calmodulin	35
RC3/neurogranin	36
calretinin	37
GAP43/B50/neuromodulin	38
S100	39
Ion channels	
L-type calcium channels	40
olfactory cyclic nucleotide-gated channel	41
Vesicle- and synapse-associated proteins	
synaptophysin	42
α-SNAP	43
VAMP	43
rab3a	44
syntaxin 1B	45
Synapsin I	45
SNAP 25	46
PSD-95	47
Transcription factors	
Retinoic acid receptor β	48
CREB	49
Krox 20	50
Krox 24	50
Adhesion molecules	
ephA5	51
ephrinA5	51
NCAM	52
E-cadherin	53
N-cadherin	53
thy-1	54
telencephalin	55
L1/NgCAM	56
HB-GAM/pleitrophin	57
integrins	58
integrin-associated protein	59
tenascin-C	60
Carbohydrates	
Polysialic acid	61
Ganglioside GM1	62
Ganglioside GQ1B	62
Kinases	
inositol 1,4,5 -triphosphate-3-kinase	63
MAPK	64
src	65
fyn	66
ERK	67
protein kinase A Cβ1 subunit	68
protein kinase A RIβ subunit	69
protein kinase C-gamma	70
protein kinase G	71
protein kinase M-zeta	72
CaM kinase I	73
CaM kinase II	74
CaM kinase IV	73
ecto protein kinase	75
Proteases and their inhibitors	
calpain	76
calpastatin	77
protease nexin 1	78
tissue plasminogen activator	79
plasmin	80
E6-AP ubiquitin ligase	81
Other enzymes	
phospholipase A2	82
phospholipase Cβ	83
phospholipase Cγ	83
ADP ribosyl transferase	84
calcineurin	85
protein phosphatase I	86
acetylcholinesterase	87
adenylate cyclase	88
guanylate cyclase	71
Miscellaneous	
Spectrin/fodrin	76
GFAP	89
Stathmin RB3/XB3	90
EBI-1 G protein-coupled receptor	91
Mas G-protein coupled receptor	92
Vesl	93

*Because of space limitations, only a single reference is given for each molecule. In many cases, choices are arbitrary.

investigation. Moreover, some of the effects may not occur at synapses at all, but rather, may be occurring in axons or glia (for example, see ref. 89). Lastly, the common use of extracellular recording techniques to record LTP suggests that many different synapses contribute to the measured potential. In short, molecules that affect hippocampal circuitry in complex ways may sometimes be mistaken for mediators of LTP. In principle, this complexity could be circumvented by use of dissociated cells in culture, but it is apparently difficult to obtain robust LTP in such preparations.

What is a rose anyway?

LTP investigators view the process as involving two fundamental steps (Fig. 1). In one (induction), appropriate patterns of stimulation predispose the synapse to potentiation. In the other (expression), the synaptic response is actually potentiated. Elegant studies have led to the consensus view that induction occurs postsynaptically and that, at least for some forms of LTP, NMDA receptors, calcium and calcium-activated kinases are critical (see above). Astonishingly, however, the cellular locus of LTP expression remains a matter of controversy: the degree to which LTP results from an increase in transmitter release, an increased postsynaptic response or both is unclear[117]. Clearly, many LTP labs understand that determining the answer to this question is critical, but until a consensus is reached, it seems inevitable that the field

will continue to move in many unrelated directions. Those who believe that the potentiation is presynaptic debate whether increased levels of released transmitter activate underlying receptors at the stimulated synapse or 'spill over' to activate neighboring synapses[118]. Those who believe that potentiation is expressed postsynaptically debate whether receptor properties are changed during LTP (for example by phosphorylation) or whether new receptors are recruited to the postsynaptic membrane from intracellular stores[43]. Those who believe that the postsynaptic cell sends a retrograde signal to the presynaptic cell debate whether that signal is focally transmitted across the synaptic cleft or spreads to distant synapses[119].

These uncertainties complicate molecular analysis in two ways. First, many molecular studies are based on a prevailing cellular hypothesis, and their value—or, at least, their interpretation—is inextricably linked to that of the hypothesis. For example, intense interest in the nature of gaseous retrograde messengers has subsided recently in the wake of new evidence that LTP is expressed postsynaptically. Whether this evidence will dampen the swing of the pre- versus post- 'pendulum', or whether more presynaptic evidence will tilt the balance back again is beyond our expertise, but we have not yet read papers suggesting that the debate is over. Second, judging whether particular molecules are mediators, modulators or indirect effectors, as defined above, often requires knowledge of where and how the phenomenon takes place at a cellular level. For example, if LTP is expressed postsynaptically, molecules that affect transmitter release would almost automatically be relegated to modulator status. Lacking knowledge of the site of expression, it seems inevitable that indirect effectors or modulators will be more often mistaken for mediators than the other way around.

No rose goes unplucked

Finally, there are social factors that have contributed in complex ways. During the late 1980s, large numbers of molecular biologists saw the opportunity to address an issue of extraordinary fascination. In parallel, molecular biology techniques became so accessible that they could be adopted by groups previously restricted to physiological approaches. As a result, the field grew quickly. One might imagine that this plethora of activity would have a salutory effect: promising leads could be followed up quickly, and controversial findings would be put to the test just as quickly. However, perhaps because a unitary cell biological paradigm was lacking, investigators headed off in many directions. Moreover, the *Nature* of modern *Science* is that negative results are not valued as highly—that is, as deserving of publication in prestigious journals—as positive results. Therefore, the putative involvement of some candidate molecular mediators remains unchallenged. What may be worse, experts tell us that the prominence of some proponents may actually stifle critical analysis by less-well-known individuals. Therefore, molecules that some leading investigators privately feel to be only peripherally involved in the process remain under active scrutiny by others, who are not 'in the know'. Indeed, although there are numerous reviews on LTP, the lack of candid commentary by experts prevents outsiders from deciding the merits of the various models.

Why so few answers?

Readers could react to Table 1 in any of several ways. An optimist might conclude that we are well on our way to understanding how LTP works, separated from a coherent view by just a few insights or a few key molecules. Conversely, one might be inclined to view LTP as so complicated that it is not likely to be understood any time soon, at least at a molecular level. A third possibility is that molecular understanding is indeed attainable with currently available tools, but that these tools are often not applied in the best way. Here, we restrict ourselves to considering whether the second or third view has more merit.

In doing so, it may be instructive to return to the success stories with which we began this essay, and ask why the essentials of some cellular neurobiological phenomena seem to be 'explained' by a manageable number of key molecules, whereas LTP—which seems, *a priori*, to be no more complicated—has not. Three differences strike us as important.

First, molecular biologists and biochemists studying action potentials, vesicle release and so forth knew what they were trying to explain. In each case, there was a clear definition of the phenomenon in cell biological terms and a satisfying explanation of the mechanism at a cellular level. Importantly, one knew what the function of the process was: for example, conducting information along axons for the action potential, killing excess cells for apoptosis. For LTP, in contrast, it has remained a challenge to supply a clear definition, description or function. Because at least several phenomena share the name, one cannot specify exactly how much a synaptic response must be increased or how long the increase has to last to qualify as LTP. One way to construct a good working definition would be to take account of what roles LTP has in the nervous system or for the organism, but lacking this knowledge, any definition is necessarily arbitrary. It is therefore difficult to assess the biological, as opposed to statistical, significance of perturbations.

The greatest lack, in this regard, may be that the cellular underpinnings of LTP remain a matter of speculation. Because long-standing controversies (for example, over pre- and postsynaptic loci for expression) remain unresolved, there is no unitary cellular framework into which molecular mechanisms can be integrated. Therefore, some approaches being pursued are almost certainly blind alleys—but it is not easy to know which ones.

A second point is that the most satisfying molecular explanations of cellular phenomena set out a core program. So many intermolecular interactions occur within and between cells that it is not really possible to understand them all. It is therefore especially important to distinguish modulators and correlates from mediators. For the action potential, for example, one views the voltage-sensitive sodium and potassium channels as core mediators. Clearly, manipulations that affect the lipid composition of the membrane, the activity of the sodium pump or the phosphorylation state of the channels can affect the nature of the action potential. Yet, if we gave them equal emphasis in our hypotheses, we probably would not have the satisfying understanding of the action potential that we have today.

In short, a reductionist approach requires a difficult and sometimes painful willingness to separate the core mediators of an effect from its myriad modulators and indirect effectors. Physicists call this separating the first-order from the second- and third-order causes. Although a firm understanding of the cellular basis of a phenomenon is prerequisite to this, sadly, it may not be sufficient. It is conceivable that there are no core programs for some complex cellular phenomena; rather, they may emerge from a multitude of interactions in the same way that innumerable variables determine whether it rains or not.

Finally, elucidating cellular and then molecular mechanisms of important phenomena requires careful choice of experimental preparations and approaches. For neurobiologists, the choices made by giants such as Hodgkin, Huxley, Katz and Kuffler

are instructive. Hodgkin and Huxley used the squid giant axon to analyze the action potential[120], Katz used the frog neuromuscular junction to analyze quantal release[121], and Kuffler used the crayfish neuromuscular junction to analyze presynaptic inhibition[122] because, in each case, the preparation was simpler and more accessible than alternatives such as spinal cord or cerebellum, and therefore allowed more careful control of experimental conditions and more precise measurements. For LTP, by contrast, most studies have used hippocampal slices in which numerous synapses other than those nominally under investigation have the opportunity to enhance or attenuate LTP. Likewise, the experimental methods are critical. In assessing mechanisms of neurotransmitter release, for example, analysis of mutant mice lacking vesicle components has been valuable, but as an adjunct to, not a substitute for, detailed biochemical analysis. For LTP, enthusiasm for sophisticated genetic techniques has perhaps come at the expense of rigorous biochemical analysis.

General lessons

Is there going to be an intuitively satisfying molecular explanation for LTP, and would its elucidation help us to understand synaptic plasticity or memory more generally? We do not know, and indeed the two authors have different opinions on these questions. We both doubt, however, that an attempt to implicate additional molecules in the process is going to be useful at this stage. A fascinating paper[91] suggests why. This group assayed expression of 22 genes that had been tagged in a program of insertional mutagenesis in mice. Of these 22 randomly tagged genes, 8 (36%) were expressed at reasonably high levels in hippocampal neurons. Three of these mutants were bred to homozygosity, and hippocampal slices from the mice were tested physiologically. One of the three mutants had a striking abnormality (an increase) in the magnitude of hippocampal LTP. Extrapolating wildly from this small sample, one might guess that up to 10% of all genes—say 10,000 or so—when mutated, will have readily detectable effects on LTP. How many more would have subtle effects that resourceful investigators could uncover is anyone's guess. However, this molecular information may not provide much understanding until a clearer view of the cellular phenomenon can be agreed upon.

Finally, does the story of LTP provide insight into other emerging areas of molecular neurobiology? We think it does, because it illuminates the problematic nature of the interface between cellular and molecular explanations of biological phenomena. For topics like nerve conduction and synaptic transmission, cellular understanding was essentially complete before molecular techniques were brought to bear. The reason was historical: penetrating investigations began before sophisticated molecular techniques were available. Likewise, many components of the core programs of neurogenesis and apoptosis were elucidated genetically, providing cellular and even organismic understanding in invertebrates before molecular analysis was attempted. As a result, molecular biology was able to have its accustomed reductionist role, in which a molecular description provides a satisfying explanation of a cellular phenomenon. For LTP, in contrast, cellular and molecular studies have proceeded in parallel. Therefore, the rules of biological reductionism seem to have been violated. Rather than seeking a molecular description/explanation of a cellular phenomenon, LTP investigators sometimes seem to be seeking cellular frameworks to make sense of their molecular findings.

Now that molecular techniques are so powerful and widely available, other less advanced areas of neurobiology—including those studied in our laboratories—are beginning to face similar challenges. No one would want to slow the pace of molecular discovery; the challenge will be to provide continuity between molecular facts and the biological understanding.

Note added in proof: After submission of this manuscript, the following additional molecules have been reported to be implicated in LTP: actin (Kim, C. H., Lisman, J.E. A role of actin filament in synaptic transmission and long-term potentiation. *J. Neurosci.* 19, 4314–4324, 1999); amyloid precursor protein (Seabrook, G. R. *et al.* Mechanisms contributing to the deficits in hippocampal synaptic plasticity in mice lacking amyloid precursor protein. *Neuropharmacology* 38, 349–359, 1999); N-syndecan (Lauri, S. E. *et al.* Regulatory role and molecular interactions of a cell-surface heparan sulfate proteoglycan (N-syndecan) in hippocampal long-term potentiation. *J. Neurosci.* 19, 1226–1235, 1999); and Kv1.4 (Meiri, N., Sun, M. K., Segal, Z. & Alkon, D. L. Memory and long-term potentiation (LTP) dissociated: normal spatial memory despite CA1 LTP elimination with Kv1.4 antisense. *Proc. Natl. Acad. Sci. USA* 95, 15037–15042, 1998).

RECEIVED 31 MARCH; ACCEPTED 26 MAY 1999

1. Barria, A., Muller, D., Derkach, V., Griffith, L. C. & Soderling, T. R. Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. *Science* 276, 2042–2045 (1997).
2. Jia, Z. *et al.* Enhanced LTP in mice deficient in the AMPA receptor GluR2. *Neuron* 17, 945–956 (1996).
3. Aiba, A. *et al.* Reduced hippocampal long-term potentiation and context-specific deficit in associative learning in mGluR1 mutant mice. *Cell* 79, 365–375 (1994).
4. Manahan-Vaughan, D. & Reymann, K. G. Regional and developmental profile of modulation of hippocampal synaptic transmission and LTP by AP4-sensitive mGluRs *in vivo*. *Neuropharmacology* 34, 991–1001 (1995).
5. Lu, Y. M. *et al.* Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. *J. Neurosci.* 17, 5196–5205 (1997).
6. Tsien, J. Z., Huerta, P. T. & Tonegawa, S. The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell* 87, 1327–1338 (1996).
7. Kiyama, Y. *et al.* Increased thresholds for long-term potentiation and contextual learning in mice lacking the NMDA-type glutamate receptor epsilon1 subunit. *J. Neurosci.* 18, 6704–6712 (1998).
8. Okabe, S. *et al.* Hippocampal synaptic plasticity in mice overexpressing an embryonic subunit of the NMDA receptor. *J. Neurosci* 18, 4177–4188 (1998).
9. Bramham, C. R., Bacher-Svendsen, K. & Sarvey, J. M. LTP in the lateral perforant path is beta-adrenergic receptor-dependent. *Neuroreport* 8, 719–724 (1997).
10. Kessey, K., Trommer, B. L., Overstreet, L. S., Ji, T. & Mogul, D. J. A role for adenosine A2 receptors in the induction of long-term potentiation in the CA1 region of rat hippocampus. *Brain Res.* 756, 184–190 (1997).
11. Matthies, H. *et al.* Dopamine D1-deficient mutant mice do not express the late phase of hippocampal long-term potentiation. *Neuroreport* 8, 3533–3535 (1997).
12. Bramham, C. R. & Sarvey, J. M. Endogenous activation of mu and delta-1 opioid receptors is required for long-term potentiation induction in the lateral perforant path: dependence on GABAergic inhibition. *J. Neurosci.* 16, 8123–8131 (1996).
13. Auerbach, J. M. & Segal, M. Muscarinic receptors mediating depression and long-term potentiation in rat hippocampus. *J. Physiol. (Lond.)* 492, 479–493 (1996).
14. Davies, C. H., Starkey, S. J., Pozza, M. F. & Collingridge, G. L. GABA autoreceptors regulate the induction of LTP. *Nature* 349, 609–611 (1991).
15. Terranova, J. P., Michaud, J. C., Le Fur, G. & Soubrie, P. Inhibition of long-term potentiation in rat hippocampal slices by anandamide and WIN55212-2: reversal by SR141716 A, a selective antagonist of CB1 cannabinoid receptors. *Naunyn-Schmiedebergs Arch. Pharmacol.* 352, 576–579 (1995).
16. Manabe, T. *et al.* Facilitation of long-term potentiation and memory in mice lacking nociceptin receptors. *Nature* 394, 577–581 (1998).
17. Staubli, U. & Xu, F. B. Effects of 5-HT3 receptor antagonism on hippocampal theta rhythm, memory, and LTP induction in the freely moving rat. *J. Neurosci.*15, 2445–2452 (1995).
18. Stella, N., Schweitzer, P. & Piomelli, D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* 388, 773–778 (1997).
19. Drew, G. M., Coussens, C. M. & Abraham, W. C. Effects of endothelin-1 on hippocampal

synaptic plasticity. *Neuroreport* 9, 1827–1830 (1998).

20. Aizawa, M., Ito, Y. & Fukuda, H. Roles of gamma-aminobutyric acid B (GABA B) and gamma-hydroxybutyric acid receptors in hippocampal long-term potentiation and pathogenesis of absence seizures. *Biol. Pharmaceutical Bull.* 20, 1066–1070 (1997).
21. Zhuo, M., Small, S. A., Kandel, E. R. & Hawkins, R. D. Nitric oxide and carbon monoxide produce activity-dependent long-term synaptic enhancement in hippocampus. *Science* 260, 1946–1950 (1993).
22. Ishiyama, J., Saito, H. & Abe, K. Epidermal growth factor and basic fibroblast growth factor promote the generation of long-term potentiation in the dentate gyrus of anaesthetized rats. *Neuroscience Res.* 12, 403–411 (1991).
23. Klann, E., Roberson, E. D., Knapp, L. T. & Sweatt, J. D. A role for superoxide in protein kinase C activation and induction of long-term potentiation. *J. Biol. Chem.* 273, 4516–4522 (1998).
24. Eilam, R., Pinkas-Kramarski, R., Ratzkin, B. J., Segal, M. & Yarden, Y. Activity-dependent regulation of Neu differentiation factor/neuregulin expression in rat brain. *Proc. Natl. Acad. Sci. USA* 95, 1888–1893 (1998).
25. Tancredi, V., D'Arcangelo, G., Mercanti, D. & Calissano, P. Nerve growth factor inhibits the expression of long-term potentiation in hippocampal slices. *Neuroreport* 4, 147–150 (1993).
26. Korte, M. *et al.* Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc. Natl. Acad. Sci. USA* 92, 8856–8860 (1995).
27. Kang, H., Welcher, A. A., Shelton, D. & Schuman, E. M. Neurotrophins and time: different roles for TrkB signaling in hippocampal long-term potentiation. *Neuron* 19, 653–664 (1997).
28. Wu, J., Wang, Y., Rowan, M. J. & Anwyl, R. Evidence for involvement of the neuronal isoform of nitric oxide synthase during induction of long-term potentiation and long-term depression in the rat dentate gyrus *in vitro*. *Neuroscience* 78, 393–398 (1997).
29. Kantor, D. B. *et al.* A role for endothelial NO synthase in LTP revealed by adenovirus-mediated inhibition and rescue. *Science* 274, 1744–1748 (1996).
30. Miller, B., Sarantis, M., Traynelis, S. F. & Attwell, D. Potentiation of NMDA receptor currents by arachidonic acid. *Nature* 355, 722–725 (1992).
31. Kato, K., Clark, G. D., Bazan, N. G. & Zorumski, C. F. Platelet-activating factor as a potential retrograde messenger in CA1 hippocampal long-term potentiation. *Nature* 367, 175–179 (1994).
32. Schneider, H. *et al.* A neuromodulatory role of interleukin-1beta in the hippocampus. *Proc. Natl. Acad. Sci. USA* 95, 7778–7783 (1998).
33. Abe, K. & Kimura, H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J. Neurosci.* 16, 1066–1071 (1996).
34. Andreasson, K. & Worley, P. F. Induction of beta-A activin expression by synaptic activity and during neocortical development. *Neuroscience* 69, 781–796 (1995).
35. Wang, J. H. & Kelly, P. T. Postsynaptic injection of Ca^{2+}/CaM induces synaptic potentiation requiring CaMKII and PKC activity. *Neuron* 15, 443–452 (1995).
36. Chen, S. J., Sweatt, J. D. & Klann, E. Enhanced phosphorylation of the postsynaptic protein kinase C substrate RC3/neurogranin during long-term potentiation. *Brain Res.* 749, 181–187 (1997).
37. Schurmans, S. *et al.* Impaired long-term potentiation induction in dentate gyrus of calretinin-deficient mice. *Proc. Natl. Acad. Sci. USA* 94, 10415–10420 (1997).
38. Benowitz, L. I. & Routtenberg, A. GAP-43: an intrinsic determinant of neuronal development and plasticity. *Trends Neurosci.* 20, 84–91 (1997).
39. Roder, J. K., Roder, J. C. & Gerlai, R. Memory and the effect of cold shock in the water maze in S100 beta transgenic mice. *Physiol. Behav.* 60, 611–615 (1996).
40. Kapur, A., Yeckel, M. F., Gray, R. & Johnston, D. L-type calcium channels are required for one form of hippocampal mossy fiber LTP. *J. Neurophysiol.* 79, 2181–2190 (1998).
41. Parent, A. *et al.* Synaptic transmission and hippocampal long-term potential in olfactory cyclic nucleotide-gated channel type 1 null mouse. *J. Neurophysiol.* 79, 3295–3301 (1998).
42. Mullany, P. M. & Lynch, M. A. Evidence for a role for synaptophysin in expression of long-term potentiation in rat dentate gyrus. *Neuroreport* 9, 2489–2494 (1998).
43. Lledo, P. M., Zhang, X., Sudhof, T. C., Malenka, R. C. & Nicoll, R. A. Postsynaptic membrane fusion and long-term potentiation. *Science* 279, 399–403 (1998).
44. Castillo, P. E. *et al.* Rab3A is essential for mossy fibre long-term potentiation in the hippocampus. *Nature* 388, 590–593 (1997).
45. Hicks, A. *et al.* Synapsin I and syntaxin 1B: key elements in the control of neurotransmitter release are regulated by neuronal activation and long-term potentiation *in vivo*. *Neuroscience* 79, 329–340 (1997).
46. Roberts, L. A., Morris, B. J. & O'Shaughnessy, C. T. Involvement of two isoforms of SNAP-25 in the expression of long-term potentiation in the rat hippocampus. *Neuroreport* 9, 33–36 (1998).
47. Migaud, M. *et al.* Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* 396, 433–439 (1998).
48. Chiang, M. Y. *et al.* An essential role for retinoid receptors RARb and RXRg in long-term potentiation and depression. *Neuron* 21, 1353–1361 (1998).
49. Bourtchuladze, R. *et al.* Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell* 79, 59–68 (1994).
50. Williams, J. *et al.* Krox20 may play a key role in the stabilization of long-term potentiation. *Mol. Brain Res.* 28, 87–93 (1995).
51. Gao, W. Q. *et al.* Regulation of hippocampal synaptic plasticity by the tyrosine kinase receptor, REK7/EphA5, and its ligand, AL-1/Ephrin-A5. *Mol. Cell. Neurosci.* 11, 247–259 (1998).
52. Cremer, H. *et al.* Long-term but not short-term plasticity at mossy fiber synapses is impaired in neural cell adhesion molecule-deficient mice. *Proc. Natl. Acad. Sci. USA* 95, 13242–13247 (1998).
53. Tang, L., Hung, C. P. & Schuman, E. M. A role for the cadherin family of cell adhesion molecules in hippocampal long-term potentiation. *Neuron* 20, 1165–1175 (1998).
54. Nosten-Bertrand, M. *et al.* Normal spatial learning despite regional inhibition of LTP in mice lacking Thy-1. *Nature* 379, 826–829 (1996).
55. Sakurai, E. *et al.* Involvement of dendritic adhesion molecule telencephalin in hippocampal long-term potentiation. *Neuroreport* 9, 881–886 (1998).
56. Luthi, A., Mohajeri, H., Schachner, M. & Laurent, J. P. Reduction of hippocampal long-term potentiation in transgenic mice ectopically expressing the neural cell adhesion molecule L1 in astrocytes. *J. Neurosci. Res.* 46, 1–6 (1996).
57. Lauri, S. E., Rauvala, H., Kaila, K. & Taira, T. Effect of heparin-binding growth-associated molecule (HB-GAM) on synaptic transmission and early LTP in rat hippocampal slices. *Eur. J. Neurosci.* 10, 188–194 (1998).
58. Bahr, B. A. *et al.* Arg-Gly-Asp-Ser-selective adhesion and the stabilization of long-term potentiation: pharmacological studies and the characterization of a candidate matrix receptor. *J. Neurosci.* 17, 1320–1329 (1997).
59. Huang, A. M., Wang, H. L., Tang, Y. P. & Lee, E. H. Expression of integrin-associated protein gene associated with memory formation in rats. *J. Neurosci.*18, 4305–4313 (1998).
60. Nakic, M., Manahanvaughan, D., Reymann, K. G. & Schachner, M. Long-term potentiation *in vivo* increases rat hippocampal Tenascin-C expression. *J. Neurobiol.* 37, 393–404 (1998).
61. Becker, C. G. *et al.* The polysialic acid modification of the neural cell adhesion molecule is involved in spatial learning and hippocampal long-term potentiation. *J. Neurosci. Res.* 45, 143–152 (1996).
62. Furuse, H. *et al.* Effect of the mono- and tetra-sialogangliosides, GM1 and GQ1B, on long-term potentiation in the CA1 hippocampal neurons of the guinea pig. *Exp. Brain. Res.* 123, 307–314 (1998).
63. Jun, K. S. *et al.* Enhanced hippocampal CA1 LTP but normal spatial learning in inositol 1,4,5-trisphophate 3-kinase(A)-deficient mice. *Learn. Mem.* 5, 317–330 (1998).
64. English, J. D. & Sweatt, J. D. A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. *J. Biol. Chem.* 272, 19103–19106 (1997).
65. Lu, Y. M., Roder, J. C., Davidow, J. & Salter, M. W. Src activation in the induction of long-term potentiation in CA1 hippocampal neurons. *Science* 279, 1363–1367 (1998).
66. Grant, S. G. *et al.* Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. *Science* 258, 1903–1910 (1992).
67. Impey, S. *et al.* Cross talk between ERK and PKA is required for Ca^{2+} stimulation of CREB-dependent transcription and ERK nuclear translocation. *Neuron* 21, 869–883 (1998).
68. Qi, M. *et al.* Impaired hippocampal plasticity in mice lacking the Cβ1 catalytic subunit of cAMP-dependent protein kinase. *Proc. Natl. Acad. Sci.* 93, 1571–1576 (1996).
69. Huang, Y. Y. *et al.* A genetic test of the effects of mutations in PKA on mossy fiber LTP and its relation to spatial and contextual learning. *Cell* 83, 1211–1222 (1995).
70. Abeliovich, A. *et al.* Modified hippocampal long-term potentiation in PKC gamma-mutant mice. *Cell* 75, 1253–1262 (1993).
71. Zhuo, M., Hu, Y., Schultz, C., Kandel, E. R. & Hawkins, R. D. Role of guanylyl cyclase and cGMP-dependent protein kinase in long-term

potentiation. *Nature* 368, 635-639 (1994).

72. Osten, P., Valsamis, L., Harris, A. & Sacktor, T. C. Protein synthesis-dependent formation of protein kinase Mζ in long-term potentiation. *J. Neurosci.* 16, 2444-2451 (1996).
73. Tokuda, M. *et al.* Involvement of calmodulin-dependent protein kinases-I and -IV in long-term potentiation. *Brain Res.* 755, 162–166 (1997).
74. Mayford, M. *et al.* Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274, 1678–1683 (1996).
75. Chen, W. *et al.* Surface protein phosphorylation by ecto-protein kinase is required for the maintenance of hippocampal long-term potentiation. *Proc. Natl. Acad. Sci. USA* 93, 8688–8693 (1996).
76. Vanderklish, P., Saido, T. C., Gall, C., Arai, A. & Lynch, G. Proteolysis of spectrin by calpain accompanies theta-burst stimulation in cultured hippocampal slices. *Mol. Brain Res.* 32, 25–35 (1995).
77. Muller, D., Molinari, I., Soldati, L. & Bianchi, G. A genetic deficiency in calpastatin and isovalerylcarnitine treatment is associated with enhanced hippocampal long-term potentiation. *Synapse* 19, 37–45 (1995).
78. Luthi, A. *et al.* Endogenous serine protease inhibitor modulates epileptic activity and hippocampal long-term potentiation. *J. Neurosci.* 17, 4688–4699 (1997).
79. Huang, Y. Y. *et al.* Mice lacking the gene encoding tissue-type plasminogen activator show a selective interference with late-phase long-term potentiation in both Schaffer collateral and mossy fiber pathways. *Proc. Natl. Acad. Sci. USA* 93, 8699–8704 (1996).
80. Mizutani, A., Saito, H. & Matsuki, N. Possible involvement of plasmin in long-term potentiation of rat hippocampal slices. *Brain Res.* 739, 276–281 (1996).
81. Jiang, Y. H. *et al.* Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic P53 and deficits of contextual learning and long-term potentiation. *Neuron* 21, 799–811 (1998).
82. Wolf, M. J., Izumi, Y., Zorumski, C. F. & Gross, R. W. Long-term potentiation requires activation of calcium-independent phospholipase A2. *FEBS Lett.* 377, 358–362 (1995).
83. McGahon, B. & Lynch, M. A. Analysis of the interaction between arachidonic acid and metabotropic glutamate receptor activation reveals that phospholipase C acts as a coincidence detector in the expression of long-term potentiation in the rat dentate gyrus. *Hippocampus* 8, 48–56 (1998).
84. Schuman, E. M., Meffert, M. K., Schulman, H. & Madison, D. V. An ADP-ribosyltransferase as a potential target for nitric oxide action in hippocampal long-term potentiation. *Proc. Natl. Acad. Sci. USA* 91, 11958–11962 (1994).
85. Wang, J. H. & Kelly, P. T. Postsynaptic calcineurin activity downregulates synaptic transmission by weakening intracellular Ca^{2+} signaling mechanisms in hippocampal CA1 neurons. *J. Neurosci.* 17, 4600–4611 (1997).
86. Blitzer, R. D. *et al.* Gating of CaMKII by cAMP-regulated protein phosphatase activity during LTP. *Science* 280, 1940–1942 (1998).
87. Appleyard, M. E. Acetylcholinesterase induces long-term potentiation in CA1 pyramidal cells by a mechanism dependent on metabotropic glutamate receptors. *Neurosci. Lett.* 190, 25–28 (1995).
88. Wu, Z. L. *et al.* Altered behavior and long-term potentiation in type I adenylyl cyclase mutant mice. *Proc. Natl. Acad. Sci. USA* 92, 220–224 (1995).
89. McCall, M. A. *et al.* Targeted deletion in astrocyte intermediate filament (GFAP) alters neuronal physiology. *Proc. Natl. Acad. Sci. USA* 93, 6361–6366 (1996).
90. Beilharz, E. J. *et al.* Neuronal activity induction of the stathmin-like gene RB3 in the rat hippocampus — possible role in neuronal plasticity. *J. Neurosci.* 18, 9780–9789 (1998).
91. Steel, M. *et al.* Gene-trapping to identify and analyze genes expressed in the mouse hippocampus. *Hippocampus* 8, 444–457 (1998).
92. Walther, T. *et al.* Sustained long term potentiation and anxiety in mice lacking the Mas protooncogene. *J. Biol. Chem.* 273, 11867–11873 (1998).
93. Kato, A., Ozawa, F., Saitoh, Y., Hirai, K. & Inokuchi, K. Vesl, a gene encoding VASP/Ena family related protein, is upregulated during seizure, long-term potentiation and synaptogenesis. *FEBS Lett.* 412, 183–189 (1997).
94. Bliss, T. V. & Lomo, T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol. (Lond.)* 232, 331–356 (1973).
95. Bliss, T. V. & Collingridge, G. L. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361, 31–39 (1993).
96. Cotman, C. W. & Monaghan, D. T. Excitatory amino acid neurotransmission: NMDA receptors and Hebb-type synaptic plasticity. *Annu. Rev. Neurosci.* 11, 61–80 (1988).
97. Bear, M. F. & Kirkwood, A. Neocortical long-term potentiation. *Curr. Opin. Neurobiol.* 3, 197–202 (1993).
98. Katz, L. C. & Shatz, C. J. Synaptic activity and the construction of cortical circuits. *Science* 274, 1133–1138 (1996).
99. Lisman, J. The CaM kinase II hypothesis for the storage of synaptic memory. *Trends Neurosci.* 17, 406–412 (1994).
100. Spillane, D. M., Rosahl, T. W., Sudhof, T. C. & Malenka, R. C. Long-term potentiation in mice lacking synapsins. *Neuropharmacology* 34, 1573–1579 (1995).
101. Claiborne, B. J., Xiang, Z. & Brown, T. H. Hippocampal circuitry complicates analysis of long-term potentiation in mossy fiber synapses. *Hippocampus* 3, 115–121 (1993).
102. Winder, D. G., Mansuy, I. M., Osman, M., Moallem, T. M. & Kandel, E. R. Genetic and pharmacological evidence for a novel, intermediate phase of long-term potentiation suppressed by calcineurin. *Cell* 92, 25–37 (1998).
103. Cavus, I. & Teyler, T. J. NMDA receptor-independent LTP in basal versus apical dendrites of CA1 pyramidal cells in rat hippocampal slice. *Hippocampus* 8, 373–379 (1998).
104. Henning, R. H. Purinoceptors in neuromuscular transmission. *Pharmacol. Therapeutics* 74, 115–128 (1997).
105. Umemori, H., Sato, S., Yagi, T., Aizawa, S. & Yamamoto, T. Initial events of myelination involve Fyn tyrosine kinase signalling. *Nature* 367, 572–576 (1994).
106. Kojima, N. *et al.* Rescuing impairment of long-term potentiation in fyn-deficient mice by introducing fyn transgene. *Proc. Natl. Acad. Sci. USA* 94, 4761–4765 (1997).
107. Williams, J. H. *et al.* The suppression of long-term potentiation in rat hippocampus by inhibitors of nitric oxide synthase is temperature and age dependent. *Neuron* 11, 877–884 (1993).
108. Warren, S. G., Humphreys, A. G., Juraska, J. M. & Greenough, W. T. LTP varies across the estrous cycle: enhanced synaptic plasticity in proestrus rats. *Brain Res.* 703, 26–30 (1995).
109. Shankar, S., Teyler, T. J. & Robbins, N. Aging differentially alters forms of long-term potentiation in rat hippocampal area CA1. *J. Neurophysiol.* 79, 334–341 (1998).
110. Bronzino, J. D., Kehoe, P., Austin-LaFrance, R. J., Rushmore, R. J. & Kurdian, J. Neonatal isolation alters LTP in freely moving juvenile rats: sex differences. *Brain Res. Bull.* 41, 175–183 (1996).
111. Crepel, V., Hammond, C., Krnjevic, K., Chinestra, P. & Ben-Ari, Y. Anoxia-induced LTP of isolated NMDA receptor-mediated synaptic responses. *J. Neurophysiol.* 69, 1774–1778 (1993).
112. Izumi, Y., Katsuki, H. & Zorumski, C. F. Monocarboxylates (pyruvate and lactate) as alternative energy substrates for the induction of long-term potentiation in rat hippocampal slices. *Neurosci. Lett.* 232, 17–20 (1997).
113. Gerlai, R. Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? *Trends Neurosci.* 19, 177–181 (1996).
114. Kirov, S. E., Sorra, K. E. & Harris, K. M. Slices have more synapses than perfusion-fixed hippocampus from both young and mature rats. *J. Neurosci.* 19, 2876–2886 (1999).
115. Maletic-Savatic, M., Malinow, R. & Svoboda, K. Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* 283, 1923–1927 (1999).
116. Vizi, E. S. & Kiss, J. P. Neurochemistry and pharmacology of the major hippocampal transmitter systems: Synaptic and nonsynaptic interactions. *Hippocampus* 8, 566–607 (1998).
117. Kullman, D. M. & Siegelbaum, S. A. The site of expression of NMDA receptor-dependent LTP: new fuel for an old fire. Neuron 15, 997–1002 (1995).
118. Kullmann, D. M. & Asztely, F. Extrasynaptic glutamate spillover in the hippocampus — evidence and implications. *Trends Neurosci.* 21, 8–14 (1998).
119. Bonhoeffer, T., Kossel, A., Bolz, J. & Aertsen, A. Modified Hebbian rule for synaptic enhancement in the hippocampus and the visual cortex. *Cold Spring Harb. Symp. Quant. Biol.* 55, 137–146 (1990).
120. Hodgkin, A. L. Chance and design in electrophysiology: an informal account of certain experiments on nerve carried out between 1934 and 1952. *J. Physiol. (Lond.)* 263, 1–21 (1976).
121. Katz, B. Neural transmitter release: from quantal secretion to exocytosis and beyond. The Fenn Lecture. *J. Neurocytol.* 25, 677–686 (1996).
122. Dudel, J. & Kuffler, S. W. Presynaptic inhibition at the crayfish neuromuscular junction. *J. Physiol. (Lond.)* 155, 543–562 (1961).