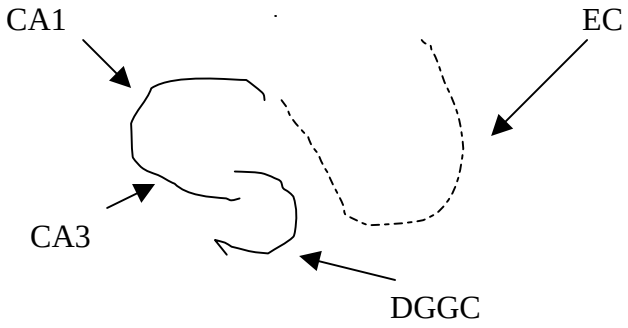
Hebbery

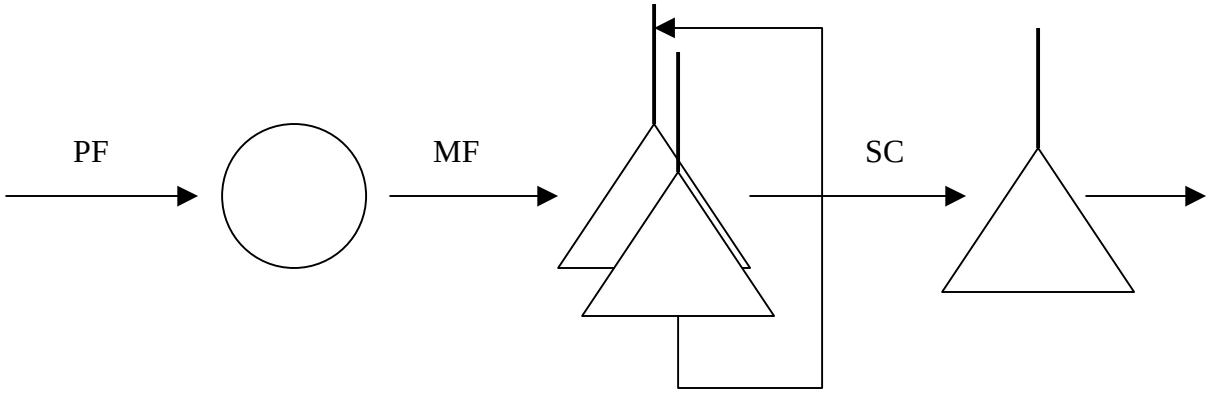
Do synapses with the properties postulated by Hebb exist? If so, what is the biophysical machinery implementing Hebb's suggestion?

Much of our knowledge of these issues comes from studies in the hippocampus. The hippocampus is a sausage-shaped structure (one on each side of the brain) which lies beneath the neocortical mantle. The sausages bend somewhat so the ends point forward, the front ("rostral") end lies near the septal nuclei (which are partly cholinergic and receive and send connections with the HC), and the back ("caudal") end lies near the temporal neocortex. The long axis of the sausage is thus called the septotemporal axis. The HC is called archicortex, because it is evolutionary older than neocortex (being found in reptiles and birds) and simpler, consisting of just one main neuron layer.



Transverse section through the HC sausage. The lines represent excitatory cell layers. EC = entorhinal cortex (only layer 2/3 of 6 layers shown); DGGC = dentate gyrus granule cells; CA3 – CA3 pyramidal cell region of HC; CA1 CA1 pyramidal cells

The main input to the hippocampus comes
from the adjacent entorhinal cortex, part of the neocortex. These incoming excitatory axons penetrate the superficial surfaces of EC and HC (which is why this is called the "perforant path" and synapse on the apical dendrites of the granule cells of the dentate gyrus. The axons of the granule cells then make rather larger (and strong) synapses on the apical dendrites of CA3 pyramidal cells of the HC proper. These synapses are located quite close to the cell body and are highly efficient in driving the CA3 cells. CA3 axons ramify extensively along the rostrocaudal axis and synapse widely onto the apical dendrites of other CA3 cells. This type of positive feedback arrangement within a cell layer is quite typical of cortical layers and is termed recurrent. A branch of the ca3 axons, known as the Schaffer collateral, travels further to form a few synapses on the apical dendrites of many (~5000) ca1 pyramidal cells. The output of ca1 cells is sent back to EC, either directly or via the subiculum, an intermediate type of cortex that lies between EC (neocortex) and HC(archicortex). Subicular fibers also innervate the mammillary bodies, which supply the anterior thalamic neocortex, and thence the neocortex. This simultaneous direct and transthalamic routes to neocortex are typical (see later lecture).



Basic trisynaptic circuit of HC. Perforant path (PF) fibers from entorhinal cortex excite granule cells, which then excite CA3 cells (via Mossy Fibers). CA3 cells then excite CA1 cells (via Schaffer Collaterals). Note that CA3 cells also make recurrent synapses on each other.

These excitatory connections are complemented by an elaborate set of inhibitory neurons which target different parts of the excitatory cells.

There is considerable evidence that the HC plays an important role in “declarative” or “explicit” memory – memory of specific facts, places and stimulus combinations. Another type of memory is “procedural” or “implicit” – general relations, concepts, skills etc. These 2 types of memory depend on quite different brain structures. In the famous patient “HM” the HC on both sides was removed for the treatment of epilepsy. HM has severe anterograde amnesia – he cannot remember specific event for facts for more than a few seconds, even though he can acquire new skills. In monkeys HC lesions impair “delayed match to sample” – the ability to identify which of several images has recently been seen. We will see that the circuitry of the HC, together with its synaptic learning mechanisms, suit it to the declarative memory task.

A slice through the hippocampal sausage preserves much of this circuitry, in particular the trisynaptic PF-GC-MF-CA3-CA1 pathway. The preservation of the circuitry in a dish has made detailed study possible. However, these slices inevitably sever the vast majority of connections that run along the septotemporal axis, particularly the CA3-CA3 recurrent coonections, and the CA3-CA1 connections. Enough of these connections remain however.

Stimulating electrodes can be placed so as to activate fairly selectively a set PP, MF or Schaffer collateral actions, and the resulting epsps in the DG, CA3 or CA1 cells studied. This is called a compound epsp because it is composed of many epsps evoked concurrently in numerous axons. It is also possible to record from and stimulate individual pairs of pre- and postsynaptic cells, but this is quite tricky and has only been achieved occasionally, in ca3 – ca1 pairs (and also in pairs with 1 inhibitory neuron). Such “unitary” synaptic potentials are easier to interpret than compound epsps because there is no ambiguity about how many axons are involved. A technique called “minimal

stimulation" tries to isolate pairs by using very weak stimuli (which should only excite one axon) without the difficulties of pair recordings.
If the stimulus is strong, it will excite many axons and the resulting epsp will evoke a postsynaptic spike. The properties of the epsp are usually studied with weaker stimuli, to avoid the complications of the postsynaptic spike.

In all 3 pathways, the monosynaptic epsps are caused by glutamate release and activation of both ampa and nmda – receptors. The former stay open for only a millisecond, while the latter take 10 msecs to open and stay open for 30 msec (on average). These synapses are formed on spines, and both ampa and nmda Rs are found in the spine head subsynaptic membrane; the difference in kinetics reflects differences in the ampa and nmda Rs. The relative amount of excitatory current flowing through these 2 receptor types depends critically on the membrane potential prevailing at the time of synapse activation. If evoked at the normal resting potential of –70 mV, the nmdaR component of the epsc is very small. Thus the excitatory action of these synapses is almost entirely due to the ampars (and can therefore be temporally precisely sculpted to reflect the arrival times of presynaptic spikes). However, as the cell is progressively depolarized (either because of summation of incoming epsps, or because the postsynaptic cell fires spikes (because of summation of epsps) or because an experimenter injects depolarizing current), while the ampaR component of the epsc gets smaller (as expected from the decreased driving force : the reversal potential for both components is near 0 mV), the nmdaR component first gets larger, and only gets small for rather large depolarisations. This striking "negative conductance" behavior is due to voltage-dependent block by external magnesium ions of the open nmdaR pore, akin to, but much faster than, the local anesthetic block of nACHRs we already considered. Here however the blocker is not a drug but a normal (1 mM) component of extracellular fluids, and the block is particularly voltage-dependent because Mg carries a double positive charge. One consequence of the voltage-dependence of the nmdaR-epsc is a positive feedback cycle – depolarization via ampaRs can be reinforced and sustained by nmdarRs. This has been proposed as a model for short-term or working memory. It also plays a vital role in long-term memory.

LTP.

If the epsp in any of the 3 pathways discussed above is evoked every minute or so, it remains stable (apart from inevitable quantal fluctuations). However, if several stimuli are applied at high frequency (~ 100 Hz) , a "tetanus" (so called because in the disease lockjaw, or "tetanus", motoneurons fire briefly at high frequency because the toxin release by the bacteria blocks inhibitory glycine receptors in the spinal cord), the epsp increases to nearly double its previous value, and this increase can be sustained for many hours. This is called "long-term potentiation" or ltp. Under appropriate conditions (repetition of the tetani; release or application of serotonin; perhaps changes during sleep) this ltp can persist for weeks.

LTP is widely considered to be a form of synaptic memory, because it involves persistent changes in synaptic strength induced by brief synaptic activity. We will consider the

question of whether ltp actually underlies real learning later; here we consider its mechanisms. These fall into 2 categories:

1. Induction. What goes on in the tetanus that induces ltp?
2. expression. What components of the synapse change when the epsp shows enhancement?

The induction and expression of LTP occur in quite different ways in the MF pathway, and in the PP and Schaffer collateral pathway. The former does not require activation of nMdaRs, and is not prevented by competitive antagonists such as APV. It appears to involve entry of Ca into the MF boutons and changes in transmitter release. It is not Hebbian, because it does not depend on postsynaptic activity, and will not be considered further here. PP and SC LTP requires NMDAR activation and it is Hebbian, requiring activation of both presynaptic boutons (by the arriving spike) and postsynaptic activity (primarily, postsynaptic spiking). It is also "associative", since ltp of a weak pathway can be caused by simultaneous activation of that weak pathway (providing presynaptic activity) and another strong pathway (which causes postsynaptic firing). If ltp is triggered by a tetanus applied to a single pathway, temporal summation of epsps during the tetanus triggers postsynaptic spikes which overlap in time with presynaptic spikes.

The crucial feature of the NMDAR underlying LTP is the voltage-dependent block by Mg, together with significant Ca-permeability of the unblocked, open NMDAR. The AMPAR is normally Ca impermeant, because AMPAR usually contain a pair of AMPA-B type subunits. These subunits' P-region contains a crucial positively charged amino acid, arginine, which prevents Ca permeation through the pore. Curiously, the gene for the AMPA-B R subunit specifies asparagine (Q) rather than arginine (R) at this location. The Q-R switch is implemented by RNA –editing, an extremely unusual process in vertebrates. It is not understood why Ca-impermeability is conferred by RNA editing rather than gene alteration, but Ca impermeability of AMPARs is almost universal. This ensures that no significant Ca entry into the spine head occurs via AMPAR activation alone. However, if significant postsynaptic depolarization should occur while the NMDAR receptor is open, this will expel Mg from the open pore, and allow Ca entry. This spine head Ca signal triggers synapse strengthening. A number of lines of evidence support this conclusion:

1. Normal LTP requires normal levels of extracellular Mg.
2. As noted already, Hebbian ltp is blocked by nmda-r block, or by knockout of the gene.
3. Injecting Ca-chelators such as BAPTA into the postsynaptic cell prevents ltp.
4. Preventing postsynaptic-depolarisation (for example, by voltage-clamping the cell) during a tetanus prevents ltp. However, ltp can still be induced by pairing presynaptic activation (even at low frequency) with artificial postsynaptic depolarizing.
5. Local increases in postsynaptic calcium, produced by photolytic uncaging of sequestered Ca, can trigger ltp.
6. Approximately coincident pre- and postsynaptic spikes, which trigger ltp, also trigger spine calcium increases.

LTP expression.

The question of what underlies the increased epsp during ltp expression has been much more controversial. An obvious first question is, is the increased epsp due to presynaptic processes (such as increased transmitter release) or due to postsynaptic processes? At first glance quantal analysis seems well suited to answer this. Recall that epsps fluctuate because they are composed of variable numbers (x) of quanta . The variance of x, var, and the mean number of quanta released, m , are related by the formula var /m^2 = CV^2 where CV is the coefficient of variation of x. Therefore the CV of the epsp amplitude will be given by q (root var)/q m, where q is the size of the epsp generated by an individual quantum. If ltp is postsynaptically expressed, only q should change, and thus there would be no change in the CV. The variance for the Poisson distribution is m, so if ltp is expressed presynaptically, CV should decrease; the variance for the Binomial distribution is less by the factor 1-p, so the CV should also decrease, but (if ltp affects p) by a smaller amount. Furthermore, for the Poisson distribution the failure (x=0) rate is given by ln m, so if ltp were presynaptic, there should be less failures. When quantal analysis was performed all these tests suggested that ltp was mainly presynaptic (there was a decrease in both CV and failure rates).
Nevertheless, a large number of more direct experiments failed to find any change in transmitter release:

1. During ltp, only the ampaR component of the epsp increases, which would not be expected for an increase in transmitter release, which would also increase the nmdaR component (unless the nmdaRs were already completely saturated by glutamate before ltp; this is not the case).
2. Uptake of glutamate into glia cells is electrogenic, and glial potentials due to glutamate release can be recorded; these do not change in ltp
3. released glutamate can also be detected using a “sniffer” electrode which carries at its tip a membrane patch carrying outward-facing glutamate receptors. No change in release during ltp is seen.
4. Certain drugs, such as “angel dust”, phencyclidine, act as slowly reversible open channel blockers of the NMDAR. Thus following a series of epsps, the nmda r component of the epsp gets gradually smaller, at a rate that depends on how much transmitter is released. During ltp this rundown is unaffected, showing that release is unaffected.

These 2 contradictory results on the expression of ltp provoked great controversy, but it is now clear that the quantal analysis work was misleading, because at central synapses its assumptions may be wrong. In particular, it is now though that many central synapses are *silent*: they completely lack ampar receptors, and since nmdaRs do not contribute to the epsp at the resting potential (because of Mg block), presynaptic stimulation may produce no postsynaptic epsp even though a functioning synapse is present. LTP induction leads to the appearance of ampa Rs at the synapse (either by unmasking of pre-existing receptors, or insertion of new receptors into the membrane), and unsilencing of the synapse. Several lines of evidence for silent synapses:

1. In some cases, where presynaptic stimuli elicit no responses in cells held at –70 mV, there are responses at +30 mV. These epsps are reversed in sign (outward), small and slow, and are due to nmdaR activation. Following ltp, AMPA-R dependent epsps appear at –70 mV.
2. In developing HC and in cell cultures, many synapses do not label with gold-marked antibodies to AMPARs, while the same synapses do label for NMDARs. But synapses that do not exhibit NMDAR-dependent always label fro AMPARs.
3. Activation of NMDARs causes movement of fluorescently-labelled AMPARs into spines.
4. NMDARs and AMPARs are anchored in the subsynaptic membrane by different proteins (eg PSD-95 and GRIP), suggesting they are regulated independently.
5. Chemicals that interfere with exocytosis prevent ltp when injected postsynaptically, suggesting that during ltp expression ampaRs are delivered to the spine head membrane by a fusion step.

Although there is evidence that postsynaptic exocytosis, and subsequent incorporation into the spine head membrane of AMPARs embedded in membranes of postsynaptic vesicles, may be important, there is also evidence that phosphorylation of existing AMPARs may increase the conductance of existing AMPARs.

LTD

LTP alone would lead to progressive increase in all synaptic strengths. Also, when we considered the linear associator network, we said that

$\Delta w_{i,j} = g_i f_j$

i.e. the Hebbian change in strength of a connection depends on the product of the pre- and post-synaptic firing rates, and can decrease as well as increase. This led to a (successful) search for Long Term Depression (LTD). In the HC ltd is induced by low frequency stimulation (LFS, at around 1 Hz) without accompanying postsynaptic firing or depolarization. There are 2 types, which can both be seen at Ca3-Ca1 synapses. The first type is NMDAR dependent; the second type is not. However, both types are “Hebbian” (i.e. they depend on postsynaptic activity: in this case the relative *absence* of postsynaptic activity; theoretically postsynaptic firing unaccompanied by presynaptic firing might also be expected to produce ltd, although this would not be synapse specific; there is evidence that overactivity of cultured neurons, produced by blocking inhibition, can lead to a general decrease in the size of epsps.)
It may seem odd that if LTD is caused by presynaptic activity in the absence of postsynaptic activity it could be NMDAR-dependent. However, if LFS is performed when the postsynaptic cell is artificially hyperpolarised, ltd does not occur. It is thought that the NMDAR-dependent component of LTD is caused by a relatively prolonged but low level increase in spine Ca (via the NMDAR). The low level Ca increase is thought to selectively activate Ca-dependent phosphatases such as calcineurin. These phosphatases remove phosphates from the AMPAR, reducing its conductance, as well as triggering the removal of AMPARs from the subsynaptic membrane. It seems likely that under some

circumstances AMPARs can be completely removed, silencing the synapse – this probably accounts for early reports that during LTD failures increase, which was originally interpreted as reflecting decreased transmitter release via an unknown retrograde messenger (see also discussion below of spike-timing dependent ltp).
The non-NMDAR component of ltd involves both activation of mGluRs and of voltage-dependent ca channels; this will be discussed in the Cerebellum lecture.
Finally a third form of LTD should be mentioned – "depotentiation". If LFS is given immediately after an ltp protocol (within 5 minutes) it can prevent the establishment of stable ltp. This seems to be due to reversal of the phosphorylation steps that initiate ltp.

GluR Trafficking

The initial changes in synaptic strength in ltp and ltd seem to be mainly due to addition or subtraction of AMPARs from the subsynaptic membrane. Since ltp and ltd are the main ways information is stored in the brain, its machinery has as much importance in neuroscience as do polynucleotide replication, transcription and translation in molecular biology, and is being intensively studied. One aspect is the "trafficking" of AMPARs to and from the subsynaptic membrane (NMDRs seem to be much more stable). This seems to be controlled by the nature of the cytoplasmic C- terminals of the AMPArs. GluR1 receptors have long tails, and glurR2 and 3 receptors have short tails (all AMPARs have 2 AMPAR2 subunits; usually the remaining 2 are glur1 (long-tail) or gluR 3 (short tail). Short tail receptors constitutively cycle in and out on a time scale of days, probably by exocytosis/endocytosis; long-tail receptors are exocytosed into the extrasynaptic membrane, as a result of ltp, via steps involving the membrane protein *stargazing* (which is also involved in the subsequent translocation into the subsynaptic membrane); they are removed by ltd-triggered endocytosis. It seems likely that some further process can exchange subsynaptic long tailed for short tailed forms. The net effect would be (1) the quantity of AMPARs inserted into the synapse would depend on ltp, but (2) once the quantity has been so determined, the constitutive cycling process keeps this fixed (in the absnce of further ltp/ltd) amount "fresh" by renewal. One possibility is that ltp adds not just long-tailed receptors but also "slot molecules" – specific anchoring molecules that correspond to, and register, the number of inserted AMPARs.

Backpropagation.

According to Hebb's rule, ltp should depend not m just on substantial postsynaptic depolarization, but on whether the postsynaptic cell fires an action potential. The simplest way to ensure this would be (1) to ensure that the spike fired in the initial segment backpropagates along the dendrite to the synapse where ltp is to occur and (2) ensure that relief of Mg block of the open NMDAR requires a depolarization of magnitude and time course comparable to this backpropagating spike. Of course, this requirements could be met most simply if the ltp – inducing epsps themselves could directly fire a local spike, however, this would destroy the summating properties of the neuron (which would fire spikes in response to any input, not to a weighted combination of many inputs).
Dendrites do have sodium channels (though at a lower density than the initial segment, which therefore has the lowest threshold. Intradendritic recordings show that dendritic

epsps first trigger a spike in the IS, which then backpropagates retrogradely along the dendrites (though it becomes somewhat slower and smaller as it does so). That this backpropagation is active is shown by an ingenious voltage clamp (VC) experiment. First somatic and dendritic spikes generated by a somatic current pulse are recorded. Then TTX is added (which of course blocks both spikes), and the VC switched on. The recorded somatic spike is then used to command the somatic membrane potential to follow exactly the time course of an active somatic spike. It is found that the voltage waveform of the dendritic recording in these conditions, which reflects the passive cable properties of the dendrite, is much smaller and slower than the dendritic spike recorded without TTX, which therefore must be due to opening of dendritic Na channels.
Why does the dendrite respond asymmetrically, to the somatic spike triggered by the attenuated somatic epsp, but not to the larger and faster dendritic epsp? One reason is that dendrites have lots of A-channels, which open during the initial epsp. The resulting brief potassium current makes it more difficult to reach threshold. These A channels have largely inactivated by the time the backpropagating action potential arrives.

Quantitative Aspects of LTP

Although the general features of ltp described so far correspond quite well to Hebb's postulate, we still need to see if this correspondence is quantitative as well as qualitative. On obvious question is, does ltp produced by *repeated* correlated activity add up, in the way suggested by the above equation? A second question is, if correlated pre-and post-synaptic spikes are required for ltp, exactly what timing relations between these spikes is optimal?
The answer to the first question is, the ltp due to repeated pre-post pairing does not add linearly, at least in the first hour or so. Indeed, after initially inducing ltp in a pathway that pathway may become completely refractory to further pairing, unless a period of an hour or more elapses (and during this refractory period serotonin must be present, and protein synthesis occur, to allow further ltp).
This has been investigated in a "minimum stimulation" protocol, in which probably only a single synapse is activated. It is found that ltp occurs in a stochastic all-or-none manner : either pairing triggers ltp or it doesn't, and the stronger the pairing the more likely the response. Once this "single unit" of potentiation has occurred, the synapse is refractory to further potentiation for at least an hour.
These observations make sense. First, if synaptic weight changes are to be stable over long periods of time, they should be digital rather than analog. Second, since potentiation may occur quite rarely, it might be efficient to only stockpile enough receptors for 1 strengthening event. Third, because both the volume of the spine head and the number of NMDARs are extremely small, the actual numbers of free calcium ions released during ltp will be quite variable, and it might be difficult to produce a graded, rather than an all-or-none, AMPAR increase. However, we do not know the actual mechanism underlying this all-or-none microscopic ltp effect.
The answer to the second question has been found by experiments in which pairs of cells were studied, and single spikes caused in both pre- and post-synaptic neurons, but with varied relative timing. It is found that maximum ltp is produced when the presynaptic spike precedes the postsynaptic spike by about 10 milliseconds. If the delay increases to

100 msec no ltp occurs. If instead the postsynaptic spike preceeds the presynaptic spike by 10 msec, ltd results; ltd deceases if the post-pre delay increases to 100 msec. If both spikes fire at exactly the same time, no change in strength results. The overall curve therefore resembles a differentiated Gaussian. These spike-timing dependent ltp/ltd effects follow a curve that one might call the "Hebb function".
Again these results make sense. Hebb suggested that ltp should occur if the presynaptic spike contributed to the firing of the postsynaptic spike. To a first approximation this "contribution" follows (and is due to) time course of the epsp caused by a presynaptic spike. Of course this epsp time course depends on the location of the synapse. If it were on the soma, the peak of the epsp should occur at a time equal to the time constant of decay of the epsc (why?), which is under 1 msec. The epsp lasts about 3 membrane time constants. The observed spike delay time dependence of ltp follows roughly this prediction, except the peak is somewhat slower, possibly because of backpropagation delays.

If the presynaptic spike is simultaneous with or after the postsynaptic spike, it cannot possibly have contributed to its firing, so there should be no ltp, as observed. If there was no systematic relationship between the pre- and post-synaptic spikes, then Hebb's rule would suggest that there should be no net change in the strength of the synapse. However, if both neurons are firing randomly at rates like those typically seen in the brain (~10 Hz), then just by chance it will quite often happen that presynaptic spikes will be followed, within 100 msec, by postsynaptic spikes. This would lead to undesireable progressive "spontaneous" ltp. This could be eliminated if the Hebb function were antisymmetrical around zero time delay, as observed.

Specificity of LTP

Early experiments showed that if 2 separate pathways synapsing on the same postsynaptic cell are studied, inducing ltp in one does not produce ltp in the other. However more recent work suggests that if 2 synapses are anatomically very close, then ltp at one can "spillover" to cause ltp in another. This has been shown both by recording from 2 different but close-by CA1 pyramidal cells which both receive a common synaptic input

Late LTP