important to examine the pattern of their output connections, in addition to their visual inputs and processing characteristics. □

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Long-term potentiation of NMDA receptor-mediated synaptic transmission in the hippocampus

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NEUROTRANSMISSION at most excitatory synapses in the brain operates through two types of glutamate receptor termed α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors; these mediate the fast and slow components of excitatory postsynaptic potentials respectively[1–3]. Activation of NMDA receptors can also lead to a long-lasting modification in synaptic efficiency at glutamatergic synapses; this is exemplified in the CA1 region of the hippocampus, where NMDA receptors mediate the induction of long-term potentiation (LTP)[4]. It is believed that in this region LTP is maintained by a specific increase in the AMPA receptor-mediated component of synaptic transmission[5,6]. We now report, however, that a pharmacologically isolated NMDA receptor-mediated synaptic response can undergo robust, synapse-specific LTP. This finding has implications for neuropathologies such as epilepsy and neurodegeneration, in which excessive NMDA receptor activation has been implicated[7]. It adds fundamentally to theories of synaptic plasticity because NMDA receptor activation may, in addition to causing increased synaptic efficiency, directly alter the plasticity of synapses.

Synaptic responses evoked in area CA1 comprise an excitatory postsynaptic potential (e.p.s.p.), that is mediated by AMPA and NMDA receptors, and an inhibitory postsynaptic potential (i.p.s.p.), the first phase of which is mediated by $GABA_A$ receptors. To investigate whether the NMDA receptor-mediated component of the synaptic response is able to undergo LTP, we blocked the AMPA receptor-mediated component with 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and the $GABA_A$ receptor-mediated component with 50 μM picrotoxin. Two separate inputs were stimulated alternately (each at 0.033 Hz) so as to evoke small (0.4–0.9 mV) NMDA receptor-mediated field e.p.s.ps. After obtaining a stable baseline for about 15 min, a period of high-frequency (tetanic) stimulation was delivered (20–25 shocks at 100 Hz, test intensity) and responses were then followed for at least a further 45 min. In 6 out of 8 slices examined in this way, synapse-specific LTP (defined as at least a 20% increase in amplitude 45 min after the tetanus) was obtained (Fig. 1*a*, *b*). To ensure that the potentiated responses were mediated entirely through NMDA receptors, the specific NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (AP5; 20 μM) was applied; this reversibly blocked the response on each occasion ($n = 3$; Fig. 1*b*). We next investigated whether LTP of NMDA receptor-mediated responses required NMDA-receptor activation during the tetanus. We first delivered a tetanus in the presence of 50 μM AP5, a concentration of antagonist that blocks induction of conventional LTP, and then, after washout of AP5, applied a second identical tetanus to the same input. As shown in Fig. 1*c*, AP5 reversibly blocked the induction of LTP of the NMDA receptor-mediated response ($n = 3$).

Following the induction of LTP, the largest change in the synaptic waveform occurred several tens of milliseconds after the stimulus and was manifest as a burst of population spikes (Fig. 1*a*). From about 30 ms after the stimulus, a $GABA_B$ receptor-mediated i.p.s.p.[8] is coincident with the NMDA receptor-mediated synaptic component. The potentiation might therefore result, in part, from a long-lasting depression of $GABA_B$ receptor-mediated synaptic inhibition, rather than a direct potentiation of the NMDA receptor-mediated response. To preclude possible alterations in the $GABA_B$ receptor-mediated i.p.s.p., we applied the $GABA_B$ receptor antagonist 2-hydroxysaclofen (500 μM). Under these conditions, in 5 out of 7 slices, tetanic stimulation resulted in LTP like that induced in the absence of 2-hydroxysaclofen.

The most pronounced change following tetanization is in the ability of the response to elicit spike firing, reflected in the appearance of asynchronous population spikes (Fig. 1*a*). This is reminiscent of so-called E–S potentiation (e.p.s.p. to spike coupling) and could conceivably be due to modification of voltage-dependent conductances that are evoked by the NMDA receptor-mediated e.p.s.p., rather than a direct alteration in the NMDA receptor-mediated conductance *per se*. In the next series of experiments we investigated whether LTP of the NMDA receptor-mediated synaptic current occurred, using the perforated patch method of whole-cell recording from brain slices[9]; this provides a high-resolution voltage-clamp of the NMDA receptor-mediated response[10,11] without the problem of intracellular dialysis, which reduces the likelihood of inducing LTP with time[12]. Using this method, in the presence of CNQX and picrotoxin, we obtained LTP in each of 7 cells tested (Fig. 2). The mean amplitude of NMDA receptor-mediated synaptic currents, recorded at a membrane potential of −70 mV, 30 min following the tetanus, was 143 ± 13% of control ($P < 0.01$). In addition, we obtained similar LTP in each of two cells tetanized in two slices treated additonally with the $GABA_B$ antagonist CGP 35348 (500 μM; Fig. 3). The potentiated synaptic current was blocked by AP5 ($n = 2$) and increased in amplitude with membrane depolarization ($n = 2$), confirming that it represented an NMDA receptor-mediated synaptic current in the postsynaptic cell (Fig. 3).

Our data demonstrate that NMDA receptor-mediated synaptic transmission is able to undergo a long-lasting modification. Previous reports have failed to observe significant potentiation of the isolated NMDA receptor-mediated synaptic response[5,6], possibly because it is more difficult to induce LTP of the isolated NMDA receptor-mediated component (compared with conventional LTP). A possible reason for this is that low-frequency activation of the NMDA receptor system, which is minimized under physiological conditions by synaptic inhibition[3,13], can reduce the probability of inducing LTP[14]. For this reason we used a stimulus intensity that evoked small NMDA receptor-mediated e.p.s.ps and performed the voltage-clamp experiments

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at −70 mV (a potential at which the NMDA receptor conductance is limited by its voltage-dependent block by Mg^{2+}; refs 15, 16). Because the conditions required to isolate the NMDA receptor-mediated response might have made it harder to induce LTP[14], it does not necessarily follow that under physiological conditions LTP of the NMDA receptor-mediated e.p.s.p. is a less robust phenomenon than that of conventional LTP.

Under experimental conditions in which both NMDA and AMPA receptors mediate synaptic responses, LTP is reported to involve a specific enhancement of the AMPA receptor component in the hippocampus[5,6] (but see refs 17 and 18 for LTP in the neocortex). This has been used to argue that LTP in the hippocampus is maintained solely by postsynaptic mechanisms[5,6]. Although we have not addressed, and therefore cannot

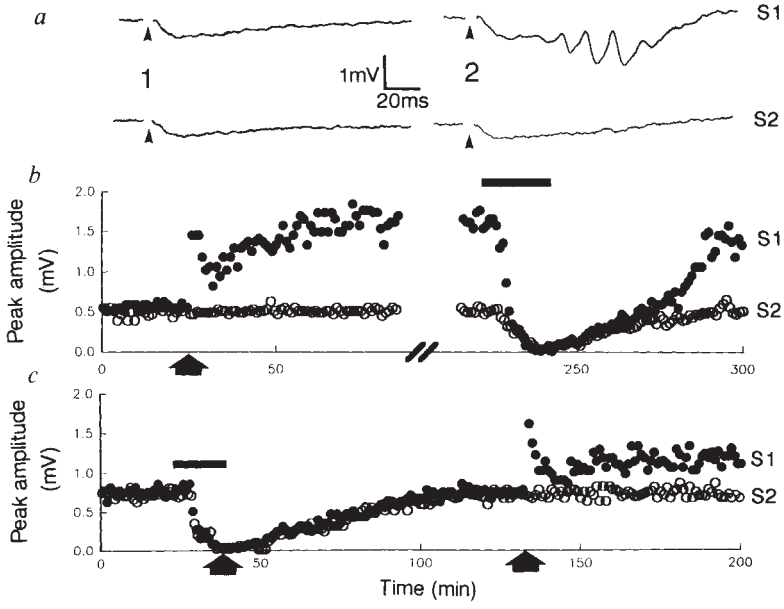

FIG. 1 Potentiation of NMDA receptor-mediated field e.p.s.ps. Field e.p.s.ps were recorded from stratum radiatum in response to alternate stimulation of two sets of Schaffer collateral-commissural fibres (S1 and S2; each pathway stimulated every 60 s). CNQX (10 μM) and picrotoxin (50 μM) were applied to block the AMPA and $GABA_A$ receptor-mediated components of synaptic transmission respectively. This virtually abolished synaptic transmission; the stimulus intensity was then increased by 33–200% to an intensity (test intensity) that evoked field e.p.s.ps of between 0.4 and 0.9 mV. *a*, A single tetanus (25 shocks, 100 Hz, test intensity) delivered to S1 resulted in rapid, robust and synapse-specific LTP. Traces are averages of 3 records obtained before and 120 min after induction of LTP. *b*, Both control and potentiated responses were reversibly blocked by bath application of 20 μM AP5 (applied for the duration of the bar). *c*, 50 μM AP5 (applied for the duration of the bar) blocked synaptic transmission in both inputs. A tetanus applied at this time did not induce LTP, whereas an identical tetanus delivered to the same input, after washout of AP5, induced synapse-specific LTP. Graphs plot peak amplitude of individual synaptic responses versus time. Because following LTP the peak response involves a population spike, the magnitude of the potentiation does not directly represent the size of the underlying synaptic potentiation. Arrows mark the time of tetanization (25 shocks, 100 Hz, test intensity). In this and subsequent traces arrowheads indicate the position of blanked stimulus artefacts.

METHODS. Rat hippocampal slices (400 μm thick) were prepared using standard techniques. The CA3 regions were discarded and slices were perfused with medium containing: (mM) NaCl, 124; $NaHCO_3$, 26; KCl, 3; NaH_2PO_4, 1.25; $CaCl_2$, 2; $MgSO_4$, 1; D-glucose, 10 (bubbled with 95% O_2/5% CO_2). The Schaffer collateral-commissural pathway was stimulated with bipolar electrodes placed in stratum radiatum. Data are presented as means ± s.e.m. and probabilities were estimated using Student's paired *t*-tests.

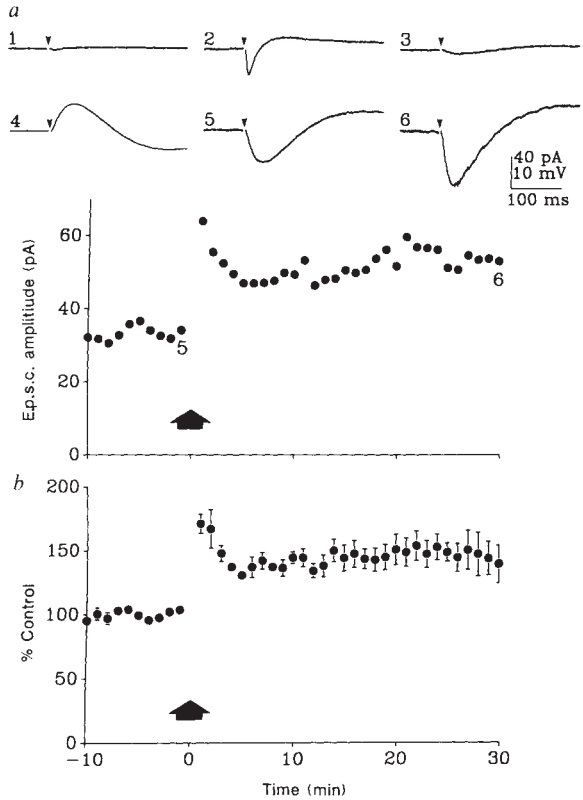

FIG. 2 LTP of NMDA receptor-mediated synaptic currents recorded with the perforated whole-cell patch technique. Cell-attached patch-clamp recordings were made using the 'blind' patch technique[30] with electrodes containing (mM): caesium methanesulphonate, 130; NaCl, 5; $CaCl_2$, 1; HEPES, 5; EGTA, 10, pH 7.3. Nystatin (100 μg ml^{-1}) was added to the electrode solution immediately before the experiment. Nystatin causes the formation of ion-permeant pores in the patch, enabling recordings to be made in the whole-cell mode without dialysis of the cell contents[31]. Seal resistances measured immediately after achieving cell-attached recordings ranged from 1 to 8 GΩ. The formation of the perforated whole-cell patch recording was monitored by development of whole-cell capacitance transients and emergence of synaptic currents, *a*, Immediately after attachment of the pipette to the cell membrane, the synaptic currents were small (1), but increased in size and stabilized after 30 min (2). Addition of 10 μM CNQX and 50 μM picrotoxin to the bathing medium left an NMDA receptor-mediated inward current followed by a $GABA_B$ receptor-mediated outward current (3). The stimuus intensity was then increased to elicit a larger synaptic current (5). (4 shows the equivalent synaptic potential recorded in current-clamp.) The potentiated synaptic current, 30 min after tetanus (20 shocks at 100 Hz, test intensity) is shown (6). The graph plots the peak amplitudes of the inward current before and following the tetanus for this cell. *b*, Graph showing normalized data from all 7 neurons (from 7 slices) from which such recordings were made, under these conditions. The mean values immediately before and 30 min following the tetanus were 16 ± 4 and 24 ± 7 pA, respectively. In all experiments, stimuli were delivered at 15-s intervals and the holding potential was −70 mV throughout (including during the tetanus). All traces and data points are averages of 4 successive records. Mean input resistance was 270 ± 18 MΩ and resting membrane potential was −52 ± 2 mV.

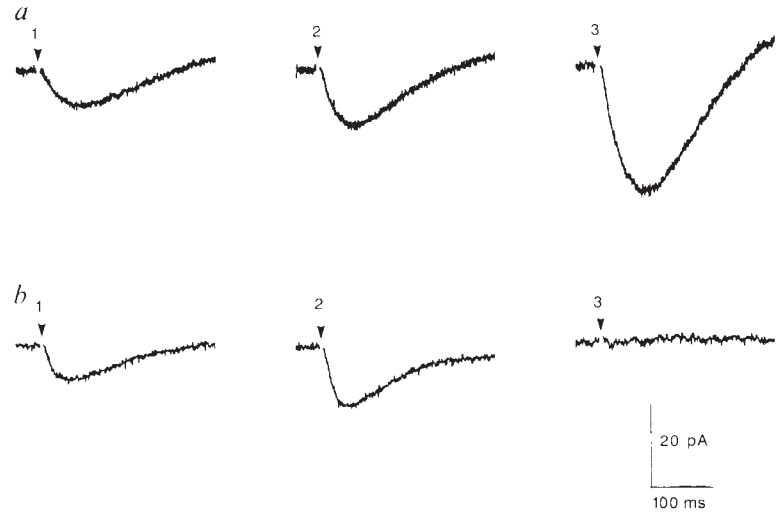

FIG. 3 LTP of NMDA receptor-mediated synaptic currents. *a*, This cell was recorded as described in Fig. 2. Responses were obtained (at −70 mV) before (1) and 30 min following (2) the induction of LTP. The cell was then depolarized to −50 mV (3). *b*, This cell was recorded as described in Fig. 2, except that the perfusate also contained 500 μM CGP 35348 to block the $GABA_B$ receptor-mediated i.p.s.c. Responses were obtained (at −70 mV) before (1) and 30 min following (2) the induction of LTP. The potentiated synaptic current was blocked by 40 μM AP5 (3).

exclude, differential expression of the two synaptic components during LTP, our positive finding that LTP of the NMDA receptor-mediated response can occur refutes the argument that presynaptic mechanisms are not involved in the maintenance of LTP. Indeed, other lines of evidence strongly suggest that presynaptic mechanisms contribute to the expression of LTP in the hippocampus[12,19–22]. With respect to LTP of the NMDA receptor component, this could be mediated by a persistent presynaptic change and/or a postsynaptic alteration, such as modification of the NMDA receptor channel complex. It may be relevant that activation of protein kinase C can upregulate NMDA currents[23].

As NMDA receptor gated channels are permeable to Ca^{2+} (refs 24–26), a consequence of LTP of this component is that a given synaptic input should provide increased Ca^{2+} entry by this route. This is likely to have implications for both physiological and pathological function. In terms of LTP, it means that a tetanus, in addition to increasing the efficiency of synaptic transmission, could directly alter the plasticity in the pathway for long periods. Subsequent tetani could result in correspondingly greater enhancements of synaptic transmission, leading to the genesis of epileptiform activity[27,28] and, taken to extreme, cell death[29]. □

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Polarized sorting of glypiated proteins in hippocampal neurons

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OUR recent studies suggested that neurons and epithelial cells sort viral glycoproteins in a similar manner. The apical influenza virus haemagglutinin was preferentially delivered to the axon of hippocampal neurons in culture, whereas the basolateral vesicular stomatitis virus glycoprotein was sorted to the dendrites.[1] To investigate whether other membrane proteins showed similar sorting in neurons and epithelial cells, we have analysed the localization of a glypiated (glycosylphosphatidylinositol-anchored) protein, Thy-1, in hippocampal neurons in culture. In MDCK and other epithelial cells, endogenous glycosylphosphatidylinositol (GPI)-anchored proteins, as well as mutated exogenous proteins containing the GPI-attachment signal, undergo preferential delivery to the apical surface[2–4]. This polarized sorting of GPI-anchored proteins has been proposed to occur by the same mechanisms as the sorting of glycolipids to the apical surface[5,6]. We report here that the neuronal GPI-protein Thy-1 is present in hippocampal neurons in culture and is exclusively located on the axonal surface. This finding further strengthens our hypothesis that the mechanisms of sorting of surface components may be similar in neurons and epithelial cells.

In a first series of experiments we investigated whether Thy-1 existed as a GPI-anchored protein on the surface of hippocampal cells in culture. To do this we took advantage of the fact that on treatment of the detergent phase of extracted cells with phosphatidylinositol-specific phospholipase C (PI-PLC), GPI-anchored proteins undergo a conversion from a hydrophobic to a hydrophilic state, thus partitioning into the aqueous phase[2,7].

The detergent phase of Triton X-114-extracted stage 5 hippocampal cells[8] was incubated with PI-PLC and the shift of proteins from the detergent into the aqueous phase was monitored by polyacrylamide gel electrophoresis (Fig. 1). After PI-PLC treatment several proteins present in the detergent fraction