Postsynaptic conversion of silent synapses during LTP affects synaptic gain and transmission dynamics

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Synaptic transmission relies on both the gain and the dynamics of synapses. Activity-dependent changes in synaptic gain are well-documented at excitatory synapses and may represent a substrate for information storage in the brain. Here we examine the mechanisms of changes in transmission dynamics at excitatory synapses. We show that paired-pulse ratios (PPRs) of AMPAR and NMDAR EPSCs onto dentate gyrus granule cells are often different; this difference is reduced during LTP, reflecting PPR changes of AMPAR but not NMDAR EPSCs. Presynaptic manipulations, however, produce parallel changes in AMPAR and NMDAR EPSCs. LTP at these synapses reflects a reduction in the proportion of silent synapses lacking functional AMPARs. Changes in PPR during LTP therefore reflect the initial difference between PPRs of silent and functional synapses. Functional conversion of silent synapses permits postsynaptic sampling from additional release sites and thereby affects the dynamics and gain of signals conveyed between neurons.

Neuronal information in the brain is likely conveyed by spike trains rather than by discrete action potentials[1]. In the hippocampus as well as in other brain regions, principal cells spontaneously exhibit brief bursts of high-frequency firing[2,3]. Such repetitive firing leads to short-term plasticity of synaptic transmission, either facilitation or depression, both of presynaptic origin. Hippocampal excitatory synapses have traditionally been described as facilitating[4,5], especially under conditions in which large numbers of afferents are stimulated. However, more refined recordings from few or unitary afferents using paired recordings have revealed a more complex expression of use-dependent short-term plasticity. The same set of afferents may express either facilitation or depression depending on the quantal content of the first response[6–8]. The heterogeneity of release probabilities among excitatory inputs onto hippocampal neurons[9,10] is therefore likely to translate into a heterogeneity of short-term plastic properties of these inputs.

Short-term synaptic facilitation and depression may have a distinct role in integrating afferent signals. Synaptic facilitation may reinforce the reliability of transmission between synaptic partners[1], whereas synaptic depression tends to enhance the sensitivity of postsynaptic neurons to subtle variations of afferent firing patterns[11]. Both synaptic dynamics and synaptic gain therefore represent essential targets for long-term modifications of synaptic transmission.

Use-dependent long-term enhancement of synaptic gain (long-term potentiation, LTP) can be achieved by coincident pre- and postsynaptic activities, and has been extensively examined at hippocampal excitatory synapses[12–14]. The influence of such activities on release dynamics has also been examined, usually for the sake of determining the locus of expression of LTP. Given the well-established presynaptic origin of short-term plasticity, interactions with LTP have been interpreted as evidence supporting a presynaptic locus of expression of the latter[5]. Such interactions were usually observed for individual experiments[15,16] but were masked when results from several experiments were pooled[4,17].

If changes in short-term plasticity associated with LTP originated presynaptically, transmission mediated by both AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) and NMDA (*N*-methyl-D-aspartate) receptors should be affected similarly. However, several reports have demonstrated that the amplitude and quantal content of the AMPA receptor (AMPAR) but not the NMDAR EPSC are affected by LTP[18,19]. To address this apparent contradiction, we used perforated patch recordings from hippocampal granule cells to monitor both synaptic components before and after LTP induction. These small, electrically compact cells permit recordings of EPSCs of small amplitude with high signal-to-noise ratio, even in the perforated patch configuration. In addition, an electrode placed in either medial or lateral perforant path could separately stimulate depressing and facilitating inputs[20], allowing synapses with distinct initial dynamic properties to be examined. We report that short-term plasticity of AMPAR and NMDAR components of a synaptic response may differ and be differentially affected by LTP. PPRs of NMDAR EPSCs remained unchanged upon LTP induction, whereas PPRs of AMPAR EPSCs increased or decreased to minimize the difference between the PPRs of AMPAR and NMDAR components.

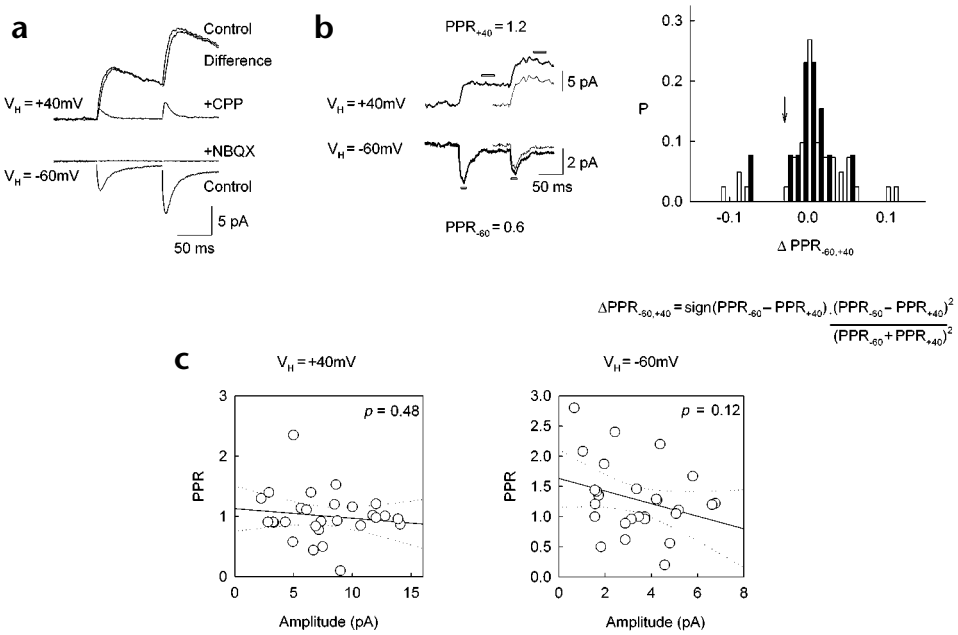

Fig. 1. Non-identical paired-pulse ratios of AMPAR- and NMDAR-mediated EPSCs in dentate gyrus granule cells. (**a**) EPSCs elicited in a granule cell by paired extracellular stimuli delivered 100 ms apart in stratum moleculare. EPSCs re-corded at a holding potential of –60 mV were entirely abolished upon application of 20 μM NBQX. EPSCs recorded at +40 mV were largely suppressed by 10 μM CPP. (**b**) Left, example showing that EPSCs recorded at –60 and +40 mV have different paired-pulse ratios. EPSC recorded at +40 mV showed paired-pulse facilitation at a 100-ms interval whereas the EPSC recorded at –60 mV showed marked depression at the same interval. Rectangles indicate the portion of the trace used for EPSC amplitude measurements. Thin line, second EPSC isolated after subtraction of interleaved episodes where only the first stimulus was delivered. Each trace represents the average of 100 consecutive episodes. Right, difference between paired pulse ratios of EPSCs recorded at –60 and +40 mV ($\Delta PPR_{-60,+40}$) was computed as described. In 41 recordings at the perforant path–granule cell synapse (white bars), $\Delta PPR_{-60,+40}$ took widely distributed values, both positive and negative. Arrow, $\Delta PPR_{-60,+40}$ value for the recording shown on the left panel. Similar dispersion of PPR_{-60} and PPR_{+40} was observed for EPSCs recorded from 13 CA1 pyramidal cells upon stimulation of Schaffer collateral/commissural afferents (black bars). (**c**) Paired-pulse ratios of EPSCs recorded at –60 or +40 mV were independent of EPSC amplitude (n = 28). Solid lines, linear regression. Dotted lines, 95% confidence intervals.

Such changes were always associated with a reduction in the proportion of synaptic failures of AMPAR but not NMDAR EPSCs. These results are consistent with a model of LTP expression based on uncovering of silent synapses initially lacking functional AMPARs[14,19,21,22]. We suggest that glutamate release at silent synapses does not always share identical dynamic properties with release at functional synapses. In these conditions, incorporation of AMPARs at previously silent synapses during LTP will affect not only the gain but also the dynamics of AMPAR transmission, by allowing these newly incorporated receptors to sample previously undetected release sites.

Results

Postsynaptic currents carried by either AMPA or NMDA receptors were distinguished by recording evoked synaptic responses at holding potentials of –60 or +40 mV (**Fig. 1a**). EPSCs recorded at –60 mV were entirely blocked by the selective AMPA receptor antagonist NBQX (2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f] quinoxaline7-sulphonamide, 20 μM; 5.6 ± 2.6% of control, n = 5). Conversely, EPSCs recorded at +40 mV were largely suppressed by the specific NMDAR antagonist CPP (3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid, 10 μM; –1.1 ± 2.1% of control, n = 6). The remaining AMPAR component decayed to a large extent by 50 ms, the point at which NMDAR response was measured.

We compared the PPRs of AMPAR and NMDAR EPSCs for an interstimulus interval of 100 ms. Surprisingly, those were not always identical (**Fig. 1b**, white bars; compare with ref. 23). Although, on average, the normalized difference between PPRs of EPSCs recorded at –60 and +40 mV ($\Delta PPR_{-60,+40}$, see Methods) was small (0.01 ± 0.01, n = 41), this difference for individual experiments was widely distributed and could take both positive and negative values (**Fig. 1b**). This difference was also observed at excitatory synapses onto CA1 pyramidal cells; in 13 recordings, AMPAR and NMDAR EPSCs evoked by stimulation of Schaffer collateral/commissural inputs showed widely distributed values of $\Delta PPR_{-60,+40}$ (**Fig. 1b**, black bars).

When two EPSCs occur in short succession, the local change in membrane resistance may lead to loss of control over transmembrane potential, thereby reducing the amplitude of the second EPSC. Such voltage clamp error was minimized by evoking EPSCs of small amplitudes (generally less than 20 pA). We asked whether a poorer voltage clamp of EPSCs recorded at depolarized potentials could explain the observed difference in PPRs of EPSCs evoked at –60 and +40 mV. PPRs of AMPAR EPSCs were not significantly different when measured at –60 or at +40 mV in the presence of CPP (**Fig. 1a**). In addition, PPRs of both AMPAR and NMDAR EPSCs were largely independent of EPSC amplitude (**Fig. 1c**; ANOVA, p = 0.12 and 0.48, respectively; n = 28). These results indicate that PPRs are neither affected by holding potential nor distorted by poor voltage clamp. The observed difference in PPRs of AMPAR and NMDAR EPSCs therefore suggests these receptors may 'sense' glutamate release from non-identical release sites with distinct temporal dynamics.

We examined how PPRs of AMPAR and NMDAR EPSCs are affected after LTP. A persistent, non-decremental increase in evoked EPSC amplitude was obtained by pairing 200–300 stimuli at 2 Hz with postsynaptic depolarization to 0 mV. In most cells, such pairing induced saturating LTP as a second pairing failed to increase EPSC amplitude further (4.26 ± 1.86 of control after first pairing, 4.06 ± 1.84 of control after a second pairing; p = 0.94, n = 4). In 15 cells examined, the absolute value of $\Delta PPR_{-60,+40}$ was significantly reduced after pairing (**Fig. 2a–c**; $p < 0.0002$). This reduction was largely explained by changes of PPR at –60 rather than +40 mV; the absolute values of the change in PPR_{-60} and PPR_{+40} were 0.32 ± 0.04 and 0.09 ± 0.03, respec-

tively ($p < 0.0001$). This effect strongly correlated with the initial difference between PPR_{-60} and PPR_{+40}. When PPR_{-60} was initially higher than PPR_{+40}, it decreased after pairing. Conversely, when PPR_{-60} was smaller than PPR_{+40}, it increased after pairing. When PPR_{-60} and PPR_{+40} were initially identical, PPR_{-60} did not change. In all cases, PPR_{+40} varied only little (**Fig. 2d**).

We asked whether these changes could be due to prolonged 2-Hz stimulation, independent of LTP expression. We repeated a similar pairing protocol but kept the recorded cell hyperpolarized to –60 mV. Such 'hyperpolarized' pairing failed to produce any lasting increase in synaptic transmission (**Fig. 2e**). In addition, no change in either PPR_{-60} or PPR_{+40} was apparent (**Fig. 2f–h**, $p = 0.70$, $n = 7$). We conclude that LTP expression is associated with changes in PPRs of EPSCs carried by AMPA but not NMDA receptors. These changes tend to reduce the difference between PPRs of AMPAR and NMDAR EPSCs, independent of the initial sign of this difference.

If changes in PPRs associated with LTP reflect changes in the probability of transmitter release[5,24,25] then PPRs of AMPAR and NMDAR EPSCs should vary similarly during LTP. However, changes in PPRs of only AMPAR EPSCs may be observed if our measure of PPRs of NMDAR EPSCs were somewhat biased or less sensitive to actual variations in charge transfer. We tested this issue by comparing EPSCs recorded –60 and +40 mV before and during pharmacological manipulations affecting presynaptic function. Activation of adenosine A1 receptors depresses excitatory synapses by a presynaptic mechanism[26,27]. A1 receptor antagonists, in contrast, enhance synaptic transmission, probably by competing with endogenous adenosine[26]. Bath application of the A1 antagonist SPT (8-(p-sulfophenyl)theophylline, 20 μM,

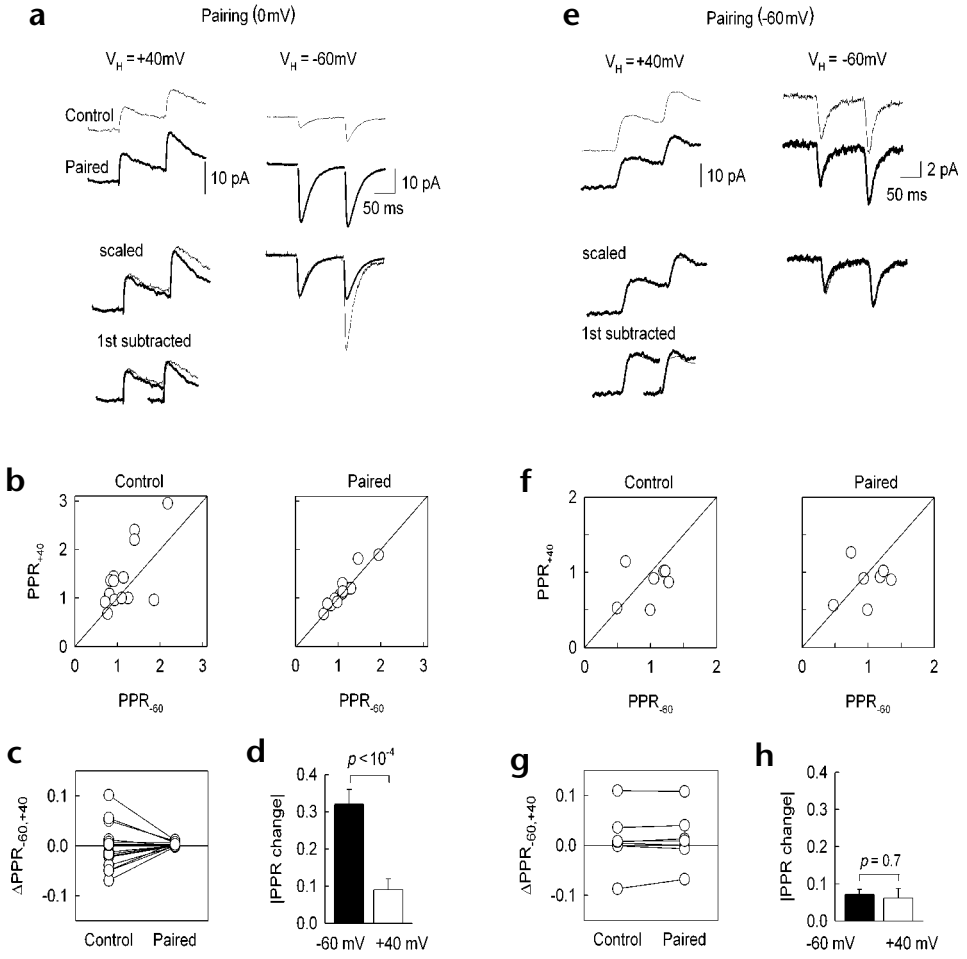

Fig. 2. Pairing-induced LTP is associated with changes in paired-pulse ratios of AMPAR but not NMDAR EPSCs. (**a**) LTP was induced by pairing 200 stimuli at 2 Hz with depolarization of the cell to 0 mV. This led to a marked potentiation of the AMPAR EPSC recorded at –60 mV without much effect on the EPSC recorded at +40 mV. Seventy-five consecutive episodes were averaged before and after pairing. Traces were scaled to their first peak to show changes in paired-pulse ratio. For EPSCs recorded at +40 mV, traces are shown (1st subtracted) where the second EPSC was isolated after subtraction of interleaved episodes where only the first stimulus was delivered. PPRs of EPSC recorded at –60 mV and +40 mV were differently affected by pairing (–50.0 and –7.1%, respectively). (**b**) PPR_{+40} was plotted against PPR_{-60} for 15 independent experiments. Dispersion is shown by deviation from the $y = x$ relationship (thin line) and was clearly reduced after pairing. (**c**) Summary plot from 15 experiments. In all experiments, $|\Delta PPR_{-60,+40}|$ was decreased after pairing. (**d**) This decrease largely reflected a change in PPR of AMPAR rather than NMDAR EPSC (32 ± 4 and 9 ± 3%, respectively). (**e**) The same 2-Hz stimulation pattern delivered to a granule cell voltage-clamped at –60 mV failed to produce any persistent change in the amplitude of either AMPAR or NMDAR EPSCs. PPRs of both EPSCs were also little affected (+6.0 and +3.6%, respectively). (**f**, **g**) Summary plots from seven experiments. The difference between PPR_{-60} and PPR_{+40} was unaffected by pairing delivered at a hyperpolarized potential, as shown by the lack of variation of $|\Delta PPR_{-60,+40}|$ in all experiments. (**h**) Accordingly, little change in PPR of AMPAR and NMDAR EPSCs was observed (7 ± 1 and 6 ± 3%, respectively).

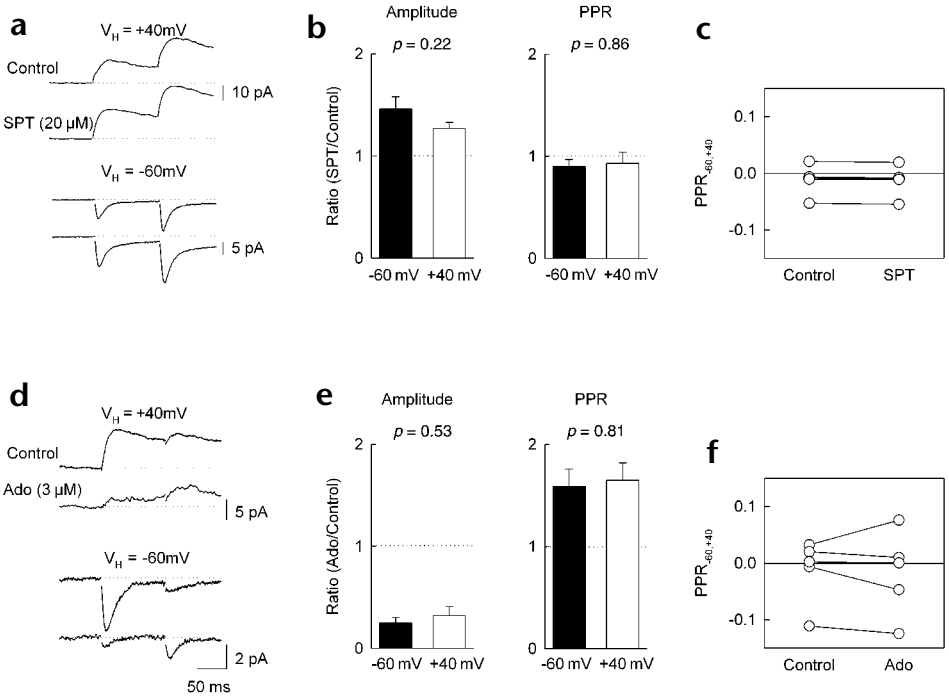

Fig. 3. The amplitude and paired-pulse ratios of AMPAR and NMDAR EPSCs are similarly affected by presynaptic manipulations. The effects of adenosine A1 receptor antagonist (**a–c**) and agonist (**d–f**) were compared on EPSCs recorded at –60 and +40 mV. (**a**) The A1 receptor antagonist SPT (20 μM) increased the amplitude and reduced PPF of both AMPAR and NMDAR EPSCs. Each trace represents the average of 75 consecutive EPSCs. (**b**) Summary data from five experiments. Left, ratio of EPSC amplitudes before and after application of 20 μM SPT. SPT increased AMPAR and NMDAR EPSCs by 46 ± 12 and 27 ± 6%, respectively (p = 0.22). Right, ratio of PPRs before and after application of SPT. SPT decreased PPR of both AMPAR and NMDAR EPSCs by 90 ± 7 and 93 ± 11%, respectively (p = 0.86). (**c**) Accordingly, $\Delta PPR_{-60,+40}$ was unaffected upon application of SPT (p = 0.94). (**d**) Application of 3 μM Ado reduced the amplitude and increased PPRs of EPSCs recorded at both –60 and +40 mV. (**e**) Summary data from six experiments. The amplitude of EPSCs recorded at –60 and +40 mV was reduced by 75 ± 5 and 68 ± 9%, respectively (p = 0.53). PPRs were increased by 59 ± 17 and 65 ± 17%, respectively (p = 0.81). (**f**) Again, Ado did not affect $\Delta PPR_{-60,+40}$ significantly (p = 0.92).

Fig. 3a) increased the amplitude and decreased the PPR of both AMPAR and NMDAR EPSCs similarly (1.46 ± 0.12 versus 1.27 ± 0.06 of control for EPSC amplitude, 0.90 ± 0.07 versus 0.93 ± 0.11 of control for PPR; n = 5). Accordingly, $\Delta PPR_{-60,+40}$ was unaffected by SPT (**Fig. 3c**, p = 0.94). Conversely, application of the A1 agonist Ado (2-chloro-adenosine, 3 μM, **Fig. 3d**) also reduced the amplitude and increased the PPR of AMPAR and NMDAR EPSCs to the same extent (0.25 ± 0.05 versus 0.32 ± 0.09 of control for EPSC amplitude, 1.59 ± 0.17 versus 1.65 ± 0.17 of control for PPR; n = 6). Again, $\Delta PPR_{-60,+40}$ was not significantly affected by Ado (**Fig. 3f**, p = 0.92). We conclude that synaptic transmission affected from purely presynaptic mechanisms can be detected by parallel changes of both AMPAR and NMDAR EPSCs.

LTP may be associated with a functional recruitment of AMPARs at previously silent synapses, that is, those that produce only NMDAR responses[21,22,28,29]. If synapses lacking functional AMPARs exist and are recruited during LTP, newly added AMPARs could detect presynaptic properties of previously silent synapses. Therefore, if release dynamics at silent synapses were initially different from those of synapses expressing both AMPA and NMDA receptors (**Fig. 1b**), this difference in PPRs of AMPAR and NMDAR responses would be expected to decrease during LTP (**Fig. 2c**).

We first tested whether LTP at perforant path synapses onto granule cells is indeed associated with recruitment of previously silent synapses[30]. Medial (MPP) and lateral (LPP) perforant path inputs were examined independently by stimulating either in the outer or medial stratum moleculare (**Fig. 4**). We recorded EPSCs at –60 and +40 mV before and after pairing. EPSC amplitude density estimates were then plotted and synaptic failure rates were estimated as previously described[31]. We observed that failure rates of AMPAR and NMDAR EPSCs are initially different at both MPP and LPP, with fewer failures recorded at +40 than –60 mV, as described at Schaffer collateral synapses onto CA1 pyramidal neurons[21,22]. This suggests that some perforant path synapses initially lack functional AMPA receptors. Pairing-induced LTP was associated with a marked reduction of synaptic failure rate of AMPAR EPSCs ($p < 0.0001$, n = 14). However, the failure rate of NMDAR EPSCs was not significantly changed after pairing (p = 0.70). These results suggest that pairing-induced LTP at both MPP and LPP synapses reflects, at least in part, the functional recruitment of AMPA receptors at previously silent synapses.

If the variation of PPR_{-60} during LTP reflects AMPA receptors 'sensing' transmitter release at synapses that were previously probed by NMDA receptors only, then this variation should depend on both the initial difference between PPR_{-60} and PPR_{+40} (designated as $\Delta PPR^{Ct}_{-60,+40}$) and the proportion of silent synapses. We plotted the absolute change in PPR_{-60} produced by LTP (computed by $|(PPR^{LTP}_{-60} - PPR^{Ct}_{-60})/PPR^{Ct}_{-60}|$) as a function of $\Delta PPR^{Ct}_{-60,+40}$ (**Fig. 5a**). As predicted by our model, a significant correlation was found between these two variables (ANOVA, $p < 2.10^{-5}$). In addition, we plotted $|(PPR^{LTP}_{-60} - PPR^{Ct}_{-60})/PPR^{Ct}_{-60}|$ versus the initial difference in failure rates at +40 and –60 mV. The latter was used as an index of the proportion of silent synapses. Again, we found a significant correlation (**Fig. 5b**; ANOVA, $p < 0.05$). The LTP-induced change in PPR_{-60} tended to be smaller when both the initial difference between PPR_{-60} and PPR_{+40} and the proportion of synapses lacking functional AMPA receptors were initially small. Conversely, PPR_{-60} changed most when these two initial parameters were the largest. Unlike previous reports[5,15], we found a rather weak correlation between the magnitude of LTP and the

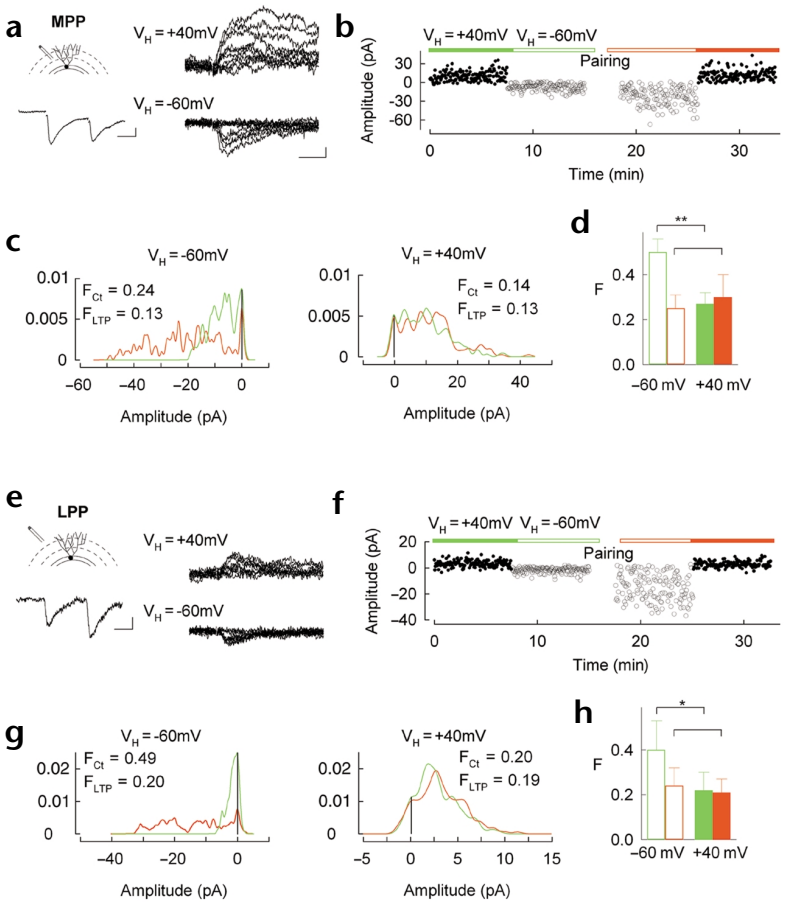

Fig. 4. Pairing-induced LTP at perforant path synapses leads to a reduction of failure rate of AMPAR but not NMDAR EPSCs. (**a**) An electrode placed in the inner stratum moleculare was used to stimulate medial perforant path inputs. Left, paired stimuli delivered 100 ms apart induced short-term depression of the EPSC. Scale bars, 1 pA, 50 ms. Right, 10 consecutive episodes are presented for each potential showing a mixture of EPSCs and synaptic failures. Scale bars, 5 pA, 25 ms. (**b**) The responses to 150 stimuli were recorded at +40 and –60 mV before and after pairing (200 stimulations at 2 Hz, V_H = 0 mV). Pairing produced a persistent, ~2-fold increase in the amplitude of the AMPAR EPSC. (**c**) Peak amplitude was measured for all responses and used to construct density estimates histograms. The area under the first peak of the histogram, centered on 0 pA, was used as an estimate of the proportion of failures (see Methods). The proportion of synaptic failures for control responses (F_{Ct}) recorded at –60 mV was higher than for responses recorded at +40 mV. Pairing induced a ~46% reduction in the proportion of failures (F_{LTP}) observed at –60 but not +40 mV. (**e**, **f**) Similar experiment using a stimulating electrode placed in the outer stratum moleculare to stimulate lateral perforant path inputs. Paired stimulation caused a facilitation of the second EPSC. Scale bars, 1 pA, 50 ms. (**g**, **h**) Pairing induced similar changes as in (**c**). $^{**}p < 0.001$; $^{*}p < 0.01$.

change in PPR of the AMPAR EPSC (ANOVA, $p = 0.13$, data not shown), supporting the idea that LTP was not a consequence of presynaptic modifications. Instead, we found a strong correlation between the magnitude of LTP and the absolute value of the change in PPRs of AMPAR EPSCs (ANOVA $p < 0.01$, **Fig. 5c**). This observation is predicted by a model in which LTP is maximally expressed when a larger number of silent synapses have been recruited, which should be apparent as a larger change (independent of its sign) in PPR.

Signals between neurons are usually conveyed by spike trains rather than by discrete action potentials[32–34]. It has been suggested that LTP at neocortical excitatory synapses involves redistribution of available synaptic efficacy rather than an increase in synaptic potency, and that redistribution could affect EPSCs differentially according to their frequency range[16]. This observation could be explained if silent and non-silent synapses initially had different dynamic properties and silent synapses were converted to functional synapses by incorporation of AMPA receptors. We compared the transmission dynamics of EPSCs carried by either NMDA or AMPA receptors during trains of afferent stimuli (**Fig. 6**). We computed a ratio of the amplitude of each EPSC in the train over that of the first and compared this ratio for AMPAR and NMDAR EPSCs ($|\Delta PPR_{-60,+40}|$). Again, the dynamic profiles of EPSCs mediated by either receptor subtype were initially different in all six cells examined. Pairing-induced LTP was accompanied by a potentiation of AMPAR but not NMDAR transmission. The difference in PPRs of AMPAR and NMDAR EPSCs was reduced after pairing for all EPSCs in the train.

Discussion

We report that synaptic transmission dynamics as detected by AMPA and NMDA receptors are not always identical at central synapses. In addition, we show that paired-pulse ratios of AMPAR and NMDAR EPSCs are differentially affected by LTP at the perforant path–granule cell synapse. The PPR of NMDAR EPSCs remains unchanged after LTP, whereas the PPR of AMPAR EPSCs increases or decreases according to the initial difference between the PPR of NMDAR and AMPAR EPSCs. LTP at these synapses is always associated with a reduction in the proportion of synaptic failures of AMPAR but not NMDAR EPSCs. These results support a model in which LTP at perforant path–granule cell synapses reflects incorporation of functional AMPARs at previously silent synapses with presynaptic dynamic properties distinct from those of non-silent synapses.

Although on average, PPRs of AMPAR and NMDAR EPSCs were similar, as previously described[23], they could take different values in individual experiments when a small number of synapses were activated (**Fig. 1b**). Because the amplitude of the postsynaptic response did not significantly affect PPRs, this difference is unlikely to reflect a voltage-clamp error in our recordings. Instead, we suggest AMPARs and NMDARs were activated by glutamate released from non-identical sites with distinct release dynamics. Release sites facing postsynaptic densities devoid of functional AMPA receptors and showing release probabilities distinct from those of other synapses could represent a substrate for this scenario. The existence of such release sites is supported by evidence that a significant proportion of hippocampal excitatory synapses express postsynaptic NMDARs but not AMPARs[28,29,35,36]. The postsynaptic content in AMPARs is correlated with synaptic size[36], which itself may affect synaptic function, including the probability of transmitter release[37,38]. Heterogeneity of release probabilities among hippocampal excitatory synapses was also found in studies[9,10] based on use-

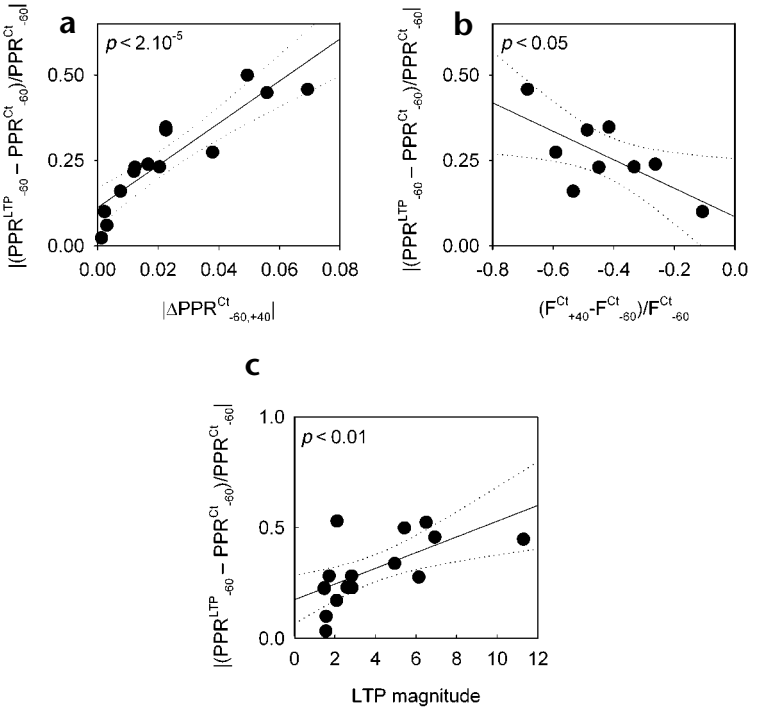

Fig. 5. LTP-induced changes in paired-pulse ratios depend on the initial differences of PPR and failure rates between AMPAR and NMDAR EPSCs. (**a**) In 14 independent experiments, the absolute value of the change in PPR of AMPAR EPSCs $|(PPR^{LTP}_{-60} - PPR^{Ct}_{-60})/PPR^{Ct}_{-60}|$ was plotted against the initial difference in PPR of EPSCs recorded at –60 and +40 mV, $\Delta PPR^{Ct}_{-60,+40}$. A strong correlation was found between these variables as indicated by r^2 (ANOVA, $p < 0.001$). (**b**) In 9 of these 14 experiments, failure rates of evoked EPSCs recorded at –60 and +40 mV could be reliably estimated. A significant correlation was also found between $|(PPR^{LTP}_{-60} - PPR^{Ct}_{-60})/PPR^{Ct}_{-60}|$ and the initial difference in the failure rates $(F^{Ct}_{+40} - F^{Ct}_{-60})/F^{Ct}_{-60}$ (ANOVA, $p < 0.05$). (**c**) The absolute change in PPR_{-60} was also significantly correlated with the magnitude of LTP (proportion of control EPSC amplitude) in 16 independent recordings (ANOVA, $p < 0.01$). Solid line, regression line. Dotted lines, 95% confidence intervals.

dependent block of NMDARs by MK-801 (see also ref. 39). Similarly, variability of transmitter release dynamics at different sites along the same axon has been reported at numerous central and peripheral synapses[40–42].

One may consider alternative hypotheses for asymmetrical changes in PPRs of AMPAR and NMDAR EPSCs during LTP. As previously reported[18,19], we observed no change in NMDAR EPSC amplitude associated with LTP. If LTP reflected the recruitment of presynaptically silent synapses[39], those should therefore contain only AMPARs. The PPR of AMPAR EPSCs could then increase or decrease during LTP whereas that of NMDAR EPSCs would remain constant. However, this change should depend only on the dynamic properties of the previously silent release sites, and should therefore be independent of the initial difference between the PPRs of AMPAR and NMDAR EPSCs. If, instead, LTP reflected the recruitment of new synapses with lower release probabilities[5], the PPRs of AMPAR EPSCs would be expected to always increase, independent of the initial difference between PPRs of AMPAR and NMDAR EPSCs. If LTP were due to increased release at existing synapses, PPRs of AMPAR EPSCs would be expected to always decrease. Alternatively, although NMDARs are probably not saturated by cleft glutamate[43], such a mechanism might be invoked to explain the lack of increase of PPRs of EPSCs mediated by these receptors when PPRs of AMPAR EPSCs increased. However, we show that the latter could either increase or decrease after LTP, and PPRs of NMDAR EPSCs remained unchanged in either case. The predictions of these models are therefore inconsistent with our observations. Finally, PPF of AMPAR EPSCs has been suggested to decrease upon LTP induction by a parallel mechanism involv-

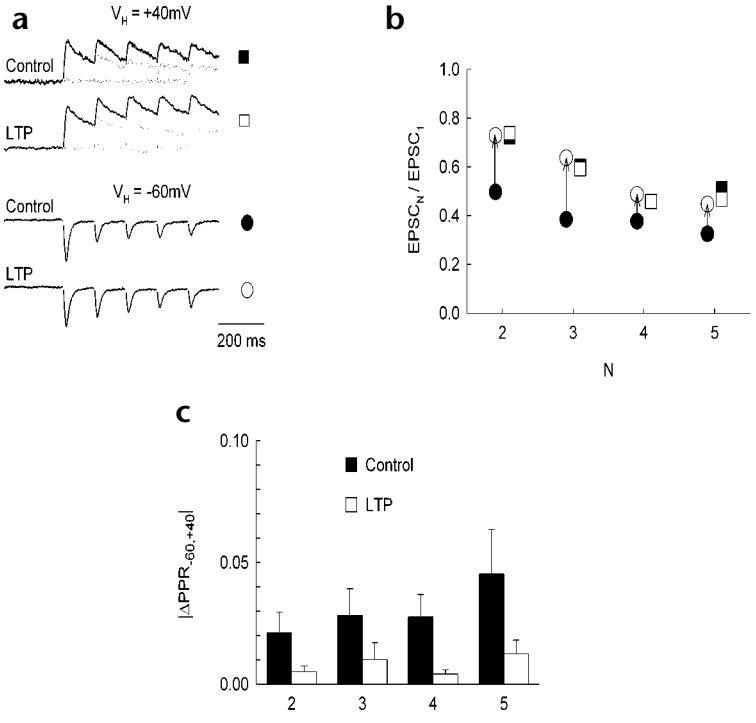

Fig. 6. LTP-induced changes in transmission dynamics during repetitive stimulation. (**a**) Synaptic responses evoked in a granule cell during extracellular stimulation of medial perforant path at 8 Hz before and after pairing (200 stimuli delivered at 2 Hz, V_H = 0 mV). Top, each response recorded at +40 mV during the train was fit by a dual exponential and subtracted from the original trace (thick line). Thin lines represent the result of the subtraction. (**b**) Ratio of the amplitude of each EPSC in the train to that of the first, for the experiment shown in (**a**). Black symbols, control. White symbols, after pairing. Amplitude ratios of EPSCs recorded at +40 mV (squares) were largely unchanged after pairing. In contrast, amplitude ratios of all EPSCs recorded at –60 mV (circles) were increased so that for each stimulus, the difference between the ratios measured at –60 and +40 mV was reduced (arrows). (**c**) The normalized difference of amplitude ratios measured at –60 and +40 mV, $|\Delta PPR_{-60,+40}|$, was compared before and after pairing. This difference was significantly reduced for all EPSCs in the train ($p < 0.05$, except for EPSC 3, $p < 0.1$). Summary data from seven independent experiments.

ing postsynaptic phosphorylation[44]. Again, such model cannot account for the bidirectional changes we report.

Several reports suggest a presynaptic locus of LTP expression based on the observation that paired-pulse facilitation, a mechanism of well-established presynaptic origin[6,45], is affected by LTP[5,25,46–48]. This conclusion stands only if all synapses are assumed initially homogeneous with respect to release probability and hence synaptic facilitation. Instead, our results and others[9,10,39] show that this may not be the case for hippocampal excitatory synapses. Postsynaptic incorporation of AMPARs at silent synapses makes detectable release sites that were previously undetectable. LTP may thereby affect the short-term plasticity of AMPAR EPSCs by a purely postsynaptic mechanism. Our results therefore suggest that paired-pulse plasticity may not be a reliable index for elucidating the locus of expression of long-term synaptic changes.

Sequences of action potentials may encode information based on their timing and translation into release events at synaptic terminals[34]. Persistent modifications of synaptic function may therefore concern both the gain and the dynamics of synaptic transmission at individual synapses. We have shown that conversion of silent synapses during LTP not only enhances the amplitude of the postsynaptic response at rest but may also affect short-term release dynamics (**Fig. 6**). Similar observations were reported previously[16] for LTP of cortical excitatory synapses, and interpreted as evidence for redistribution of synaptic strength within the sequence of action potentials. In contrast, LTP seemed to preserve the fidelity of synaptic transmission at CA3–CA1 and CA1–CA1 synapses[17]. Although this discrepancy may reflect fundamental differences in the mechanisms of LTP expression at these synapses, our model suggests it could instead reflect a difference in the initial dynamics of silent and non-silent synapses. We would predict that the dynamics of silent and non-silent synapses differ more in cortex than at CA1 synapses. Comparisons of short-term plastic properties of silent and non-silent synapses in different cortical areas may allow predictions regarding the dynamic changes associated with long-term synaptic plasticity.

Methods

Hippocampal slices were prepared from 10–15-day-old rats decapitated after deep anesthesia induced by intraperitoneal injection of ketamine/xylazine. The brain was removed and submerged in ice-cold artificial cerebrospinal fluid (ACSF) containing 250 mM sucrose, 26 mM $NaHCO_3$, 10 mM glucose, 4 mM KCl, 1 mM $CaCl_2$ and 5 mM $MgCl_2$. Horizontal slices from ventral hippocampus were prepared with a Vibratome and held at room temperature. For recording, slices were transferred to a submerged chamber maintained at 29–31°C and superfused with ACSF containing 118 mM NaCl, 26 mM $NaHCO_3$, 10 mM glucose, 1 mM NaH_2PO_4, 2.5 mM KCl, 3 mM $CaCl_2$ and 2 mM $MgCl_2$, bubbled with 95% O_2 and 5% CO_2.

Perforated-patch recordings from dentate gyrus granule cells were obtained using borosilicate electrodes containing 90 mM Cs-methylsulfonate, 10 mM CsCl, 20 mM HEPES, 10 mM EGTA, 4 mM $MgCl_2$ and 4 μg/ml amphotericin-B in DMSO. Tight seals (>2 GΩ) were made with visually identified cells and recordings were started only when series resistance reached less than ~40 MΩ and the ratio of input to series resistance was greater than 100. This ratio was continuously monitored while recording.

Excitatory postsynaptic currents were evoked in the presence of bicuculline methochloride (20 μM) by extracellular stimulation delivered either in the outer or medial stratum moleculare for stimulation of lateral or medial perforant path synapses, respectively. Cells were held at –60 or +40 mV, and stimuli were delivered at 0.3 Hz.

Signals were filtered at 1 kHz and sampled at 5 kHz using programs written in Axobasic. Data were analyzed subsequently using macros written under Visual Basic for Microsoft Excel. For display, stimulation artifacts were digitally subtracted. For PPR calculation, the second EPSC was isolated after digital subtraction of the first, scaled from interleaved trials with only one stimulus. PPR was calculated as $(EPSC_2 - EPSC_1)/EPSC_1$, where $EPSC_1$ and $EPSC_2$ represent the amplitude of the first and the second EPSC, respectively. Difference of paired-pulse ratios of EPSCs recorded at –60 and +40 mV ($\Delta PPR_{-60,+40}$) was computed as $sign(PPR_{-60} - PPR_{+40})((PPR_{-60} - PPR_{+40})^2/(PPR_{-60} + PPR_{+40})^2)$. For the estimation of failure rates, 200 to 300 EPSCs were collected at +40 and then –60 mV. Density estimates of amplitude distributions were computed using a Gaussian kernel of about half the variance of baseline noise[31]. Failure rates were estimated as twice the integral of the negative half of the peak centered on 0 pA. Values are expressed as mean ± s.e.m. D,L-APV and NBQX were purchased from Tocris Cookson (Bristol, UK); bicuculline methochloride, 2-chloro-adenosine, 8-(p-sulphophenyl)-theophylline and amphotericin-B, from Sigma (St. Louis, Missouri).

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