POSTSYNAPTIC DEPOLARISATION ENHANCES TRANSMITTER RELEASE AND CAUSES THE APPEARANCE OF RESPONSES AT "SILENT" SYNAPSES IN RAT HIPPOCAMPUS

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Abstract—Recent data indicate that most “silent” synapses in the hippocampus are “presynaptically silent” due to low transmitter release rather than “postsynaptically silent” due to “latent” receptors of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid type (AMPARs). That synapses bearing only N-methyl-D-aspartate (NMDAR) receptors do exist is suggested by the decreased number of transmission failures during postsynaptic depolarisation and by the presence of NMDA-mediated excitatory postsynaptic currents (EPSCs) in synapses silent at rest. We tested whether these effects could be due to potentiated transmitter release at depolarised postsynaptic potentials rather than removal of Mg2+ block from NMDARs. Using whole-cell recordings of minimal EPSCs from CA1 and CA3 neurones of hippocampal slices we confirmed decreased incidence of failures at +40 mV as compared with −60 mV. This effect was associated with a gradual increase of EPSC amplitude after switching to +40 mV and with a decrease of paired-pulse facilitation. In initially silent synapses, potentiation of pharmacologically isolated AMPAR-mediated EPSCs was still observed at +40 mV and this persisted after stepping back to −60 mV. All above effects were blocked when the cell was dialysed with the Ca2+ chelator BAPTA (20 mM). These observations are difficult to reconcile with the “latent AMPAR” hypothesis and suggest an alternative explanation, namely that the reduction in failure rates at positive potentials is due to potentiation of transmitter release following Ca2+ influx through NMDARs. Our results suggest that silent synapses can be mainly “presynaptically” rather than “postsynaptically silent” and thus increased transmitter release rather than insertion of AMPARs is a major mechanism of early long-term potentiation maintenance. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: postsynaptically silent synapse, presynaptically silent synapse, AMPA receptor, NMDA receptor.

In the CNS, synaptic efficacy varies among different synapses. Certain synapses appear “silent.” “Presynaptically silent” synapses are defined as having either a low release probability or insufficient glutamate in one quantum to activate α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs; for review see Voronin and Cherubini, 2003). “Postsynaptically silent” synapses are defined as lacking functional subsynaptic AMPARs with N-methyl-D-aspartate receptors (NMDARs) non-conductive at rest because of the Mg2+ block (for reviews see Malinow and Malenka, 2002; Isaac, 2003).

"Presynaptically silent" synapses and their importance for long-term synaptic plasticities have been recognized long ago (Mendell, 1984; Redman, 1990; Voronin et al., 1988; Voronin, 1983). A popular experimental paradigm to study synaptic plasticity, learning and memory is long-term potentiation (LTP; Bliss and Collingridge, 1993). While the mechanisms of LTP induction are widely accepted, those for its maintenance are still under debate because of the relative contribution of presynaptic versus postsynaptic changes. Several forms of LTP are induced by postsynaptic depolarisation and by subsequent Ca2+ influx into the postsynaptic cell. Therefore, a retrograde message from the postsynaptic to presynaptic side is needed. Although initial evidence for such retrograde messengers has been obtained, this problem is still under investigation (Fitzsimonds and Poo, 1998; Volgushev et al., 2000).

Recently, it has been suggested that LTP is based on insertion of new AMPARs in "postsynaptically silent" (and "non-silent") synapses (Kullmann, 1994). This "latent AMPARs" hypothesis was based on findings of larger LTP of NMDAR- in comparison to AMPAR-mediated excitory postsynaptic current fluctuations (EPSCs) with smaller value of the inverse squared coefficient of variation (CV−2) of the latter in control conditions. The "latent AMPARs" hypothesis has been considered in numerous reviews (e.g. Luscher and Frerking, 2001; Malinow and Malenka, 2002; Isaac, 2003) that contain references to respective original publications. The hypothesis was strongly supported by recordings of "minimal" EPSCs that showed reduced rates of transmission failures at positive as compared with negative membrane potentials of the postsynaptic cell (Liao et al., 1995; Isaac et al., 1995; Durand et al., 1996). The reduction in failures was interpreted as due to the appearance of NMDAR-mediated EPSCs in postsynaptically silent (and...
non-silent) synapses following removal of voltage-dependent Mg\(^{2+}\) block. The "latent AMPARs" hypothesis interpreted changes in failure rate, CV\(^{-2}\) and quantal content commonly observed after LTP as resulting from the expression of additional AMPARs (Edwards, 1995) rather than from presynaptic modifications of transmitter release (Bliss and Collingridge, 1993; Larkman and Jack, 1995; Voronin, 1993).

However, this hypothesis has been challenged by the following observations: i. similar LTP of NMDAR- and AMPAR-mediated responses revealed in recent studies (Bashir et al., 1991; Berretta et al., 1991; Clark and Collingridge, 1995; Bayazitov and Kleschevnikov, 2000; Bayazitov et al., 2002) as opposed to earlier ones (e.g. Muller et al., 1988); ii. lack of changes in the ratio of spontaneous AMPAR-to NMDAR-mediated EPSC amplitudes over the first postnatal week (Groc et al., 2002) and after pharmacologically induced persistent Modifications (Watt et al., 2000; see also Hohnke et al., 2000); iii. the presence of active AMPARs in apparently silent synapses (Choi et al., 2000; Gasparini et al., 2000; Kimura et al., 1997; Maggi et al., 2003; Renger et al., 2001); iv. evidence for strong contribution of increased transmitter release to early LTP maintenance (Empagne et al., 2003; Voronin and Cherubini, 2003).

Moreover, both CV\(^{-2}\) (Niu et al., 1998) and paired-pulse facilitation (PPF) of NMDAR-mediated responses (Clark et al., 1994) were found to be potential-dependent. This may suggest changes in transmitter release at positive membrane potentials.

Our aim was to test the assumption that the reduction of EPSC failures at positive membrane potentials is due to a potentiation of transmitter release at depolarised postsynaptic potentials rather than difference in the number of functional \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid and NMDA receptors. To test this alternative hypothesis, we recorded minimal hippocampal EPSCs and compared failure rates, CV\(^{-2}\) and PPF at negative and positive membrane potentials.

### EXPERIMENTAL PROCEDURES

#### Slice preparation and solutions

Experiments were performed on hippocampal slices obtained from Wistar rats aged 2–18 postnatal days (P2–P18). Hippocampal slices were prepared following the method already described (Gasparini et al., 2000). Animals were decapitated after being anesthetized with i.p. injection of urethane (2 g/kg). The procedure for killing the animals was performed in accordance with the regulations of the Italian Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimise the number of animals used and their suffering. The brain was quickly removed from the skull and placed in an ice-cold artificial cerebrospinal fluid containing (in mM): NaCl 130, KCl 3.5, NaH\(_2\)PO\(_4\) 1.2, NaHCO\(_3\) 25, MgCl\(_2\) 1.3, CaCl\(_2\) 2, glucose 11, saturated with 95% O\(_2\) and 5% CO\(_2\) (pH 7.3–7.4). Transverse hippocampal slices (300–400 \(\mu\)m thick) were cut with a vibratome and stored at room temperature in a holding bath containing the same saline solution as above. After a recovery period of at least 1 h, an individual slice was transferred to the recording chamber where it was continuously superfused with oxygenated artificial cerebrospinal fluid at a rate of 2–3 ml/min at 32 °C. EPSCs were recorded from single CA1 or CA3 pyramidal neurones by using the patch clamp technique in whole cell configuration. Patch pipettes were filled with a solution containing (in mM): Cs-methanesulphonate 125–130, CsCl 10, HEPES 10, EGTA 0.5–2, N-(2,6-dimethylphenylcarbamoylmethyl) triethylammoniumumbromide (GX-314; Alomone Laboratories, Jerusalem, Israel) 5, MgATP 2, NaGTP 0.3, NaCl 8 (pH 7.25, resistance 3–5 M\(\Omega\)). One series of recordings was performed with patch pipettes containing the calcium chelator 1,2-bis (2-amino- phenoxy) ethane-N,N,N',N'-tetraacetic acid (BAPTA; 10 or 20 mM; Sigma, Milan, Italy). Bicuculline methiodide (10 \(\mu\)M) or picrotinox (50–100 \(\mu\)M), both purchased from Sigma, were added to the bathing solution to block GABA\(_A\) receptors. In recordings from immature CA3 neurones a small amount of tetrodotoxin (TTX; 5–10 nM; Affiniti Research Products, Exeter, UK) was added to reduce polysynaptic activity and/or interictal discharges. At this concentration TTX did not significantly change the shape of action potential (Sokolov et al., 2003).

Bipolar twisted NiCr-insulated electrodes or glass pipettes were placed in stratum lucidum or radiatum to activate mossy fibers or Schaffer collaterals, respectively (Fig. 1A and B). The stimulus intensity was set to evoke minimal EPSCs. To this aim, paired-pulse (50 ms interval between pulses, 100 \(\mu\)s pulse duration) were used to induce PPF, a phenomenon well known for hippocampal synapses (see Voronin, 1993). During stimulus adjustment, the interval between stimulus pairs was kept long enough (>10–20 s) to avoid essential low-frequency depression (see e.g. Ananaya et al., 2003). The stimulus intensity was set to fulfill the following two apparently contradictory conditions: (1) maximum number of transmission failures to the first pulse and (2) minimum number in response to the second. Condition 1 indicates that only one (or a few) presynaptic fiber(s) was activated, while condition 2 suggests that at least one fiber was reliably activated. We believe that in the majority of cases, failures were true release failures and not failures in activating presynaptic fibers because on average the amplitude of the second response was similar whether it was preceded by a failure or a success (Voronin et al., 1992). In a sample of 10 CA3 neurones the mean amplitude of the second response occurring after failures or successes was 9.3±4.5 pA and 8.9±4.8 pA, respectively. In case of “axon failures” the amplitude of the second EPSC should be significantly smaller because transmitter release would not be facilitated in the absence of the presynaptic spike.

The experimental protocol was similar to that described in initial publications on “silent synapses” (Liao et al., 1995; Isaac et al., 1995) except that recordings were performed at 32 °C rather than room temperature, using paired stimuli and slower rates of test stimulation (0.05 or 0.5 Hz in CA3 and CA1 experiments, respectively). According to the protocol, the minimal intensity necessary to evoke a response at ~60 mV was found. After this, the stimulus intensity was slightly decreased in order to obtain only failures in response to the first pulse in the paired-pulse paradigm. The number of control trials varied from 20 to 200; at the testing frequency of 0.5 Hz at least 100 trials were delivered. Afterward the holding potential was changed to a positive value (usually +40 mV). We tried to fix every new holding potential as fast as possible. Therefore, even at testing frequency of 0.5 Hz only one to four initial trials were lost for accurate measurements and excluded from analysis. Similarly to recently published paper (Choi et al., 2000) inputs were considered silent when only failures of AMPAR-mediated responses were detected at ~60 mV whereas NMDAR-mediated synaptic currents were recorded at +40 mV.

Mossy fiber EPSCs were characterized by their fast rise time (~2 ms measured between 20 and 80% of their amplitude) and by their sensitivity to (2S,2'R,3'R)-2-(2,3'-dicarboxycyclopropyl)glycine (DCG i.v., 1 \(\mu\)M; Tocris Cookson, Bristol, UK), a selective agonist for metabotropic glutamate receptors 2/3, known to selectively block mossy fiber transmission (Kamiya et al., 1996; Berretta et al., 2000; Gasparini et al., 2000; Sokolov et al., 2003). It
should be stressed that in neonatal rats this agonist was less effective than in adults (Gasparini et al., 2000). It significantly reduced mossy fiber EPSCs in only 13 of 21 cells tested in slices obtained from P2–P7 animals. This can be attributed to an incomplete expression of metabotropic glutamate receptors on presynaptic terminals or to deficient transduction mechanisms. Therefore we cannot exclude that at least a portion of the EPSCs recorded from CA3 neurones at early postnatal ages were mediated by associative-commissural fibers.

**Response measurements**

Responses were digitised at 5 or 10 kHz and stored on computer using LTP program (Andersen and Collingridge, 2001). The peak amplitude was measured between two windows using a standard procedure (Larkman et al., 1992) by averaging the current over two windows (approximately 3 ms width), one before the stimulus artifact and the other positioned at the peak of the response (Fig. 1D, bars). To improve the signal-to-noise ratio EPSCs were also measured using the standard statistical techniques of principal component analysis (PCA; Jackson, 1991) modified for application to minimal postsynaptic responses (Astrelin et al., 1998). The procedure has been described in details in previous publications (Astrelin et al., 1998; Berretta et al., 2000; Sokolov et al., 2003) and has been found to be appropriate for responses recorded from both CA1 or CA3 hippocampal areas and neocortex. Briefly, standard PCA scores were determined from a window covering

![Fig. 1. Recordings and measurements of minimal EPSCs from hippocampal CA3 and CA1 pyramidal neurones.](image-url)
the initial slope of the EPSC (Fig. 1C, bar). By its physical meaning
the scores of the first principal component reflect the correlation
between every single response and the average waveform. The
first principal component scores can be normalized to pA and
termed “PCA amplitude.” Fig. 1E illustrates that this measure
(ordinate) was strongly correlated to the peak amplitude of the
EPSC (abscissa). Fig. 1E also shows that the mean results do not
depend on whether they were derived from the peak amplitudes or
the amplitudes provided by the PCA (Voronin et al., 1999). How-
ever, data points marked by letters a and b in Fig. 1E strongly
differed between the two measures. Consideration of respective
responses (Fig. 1Fa and b) indicates that the major reason was
response contamination by occasional spontaneous activity. No-
tably, the “PCA amplitudes” gave reasonable results, namely
small values comparable to the noise level (Fig. 1E, ordinate)
unlike the peak amplitude measurement that gave too large val-
ues (Fig. 1E, abscissa) for these two cases where stimulus-locked
responses were apparently absent (Fig. 1Fa) or very small (Fig.
1Fb). Discrepancies in the opposite direction, namely with PCA
amplitude essentially larger than the peak amplitude measurement
we had to use a record from a different neurone (Fig. 1Fc). Note a clear EPSC is present in this
record and the PCA measurement gives about 13 pA amplitude
(Fig. 1Ec, ordinate). In contrast, the small peak amplitude (about
2 pA) would be misleading and suggest illusory absence of any
response (Fig. 1Ec, abscissa). In practice sweeps shown in Fig.
1F can be discarded following a visual inspection or measured
again with a different baseline window. However the visual inspec-
tion is impractical when hundreds of sweeps are recorded (see
e.g. Fig. 6C below). The comparison of the peak amplitudes and
PCA amplitude measurements (Fig. 1E) quickly reveals such

Fig. 2. Delayed appearance of NMDAR-mediated responses in apparently silent inputs after switching the membrane potential to +40 mV. (A–C)
Consecutive single traces (A), averages (B) and amplitude time course (C) from various periods (a–e) of the same experiment. EPSCs were recorded
from a CA3 neurone following minimal stimulation at 0.05 Hz to avoid low-frequency depression. Eight consecutive traces were superimposed in A;
averages from eight to 15 trials are given in B. The letters a–e in C mark the periods corresponding to the records in A and B. Postsynaptic holding
potential of −60 mV (a) was switched to +40 mV (b, c) and stepped back to −60 mV (d, e). (D) Average data for three similar apparently “silent”
synapses. Data points represent the mean (±S.E.M.) of at least 12 measurements (four consecutive responses for three experiments). The number
of trials during the depolarisation, application of the NMDAR blocker CPP (20 μM; D) and post-depolarisation period slightly varied in different cells
so that the final data points in each of these three experimental periods were calculated from larger samples (n = 19, 16 and 17, respectively). Because
of this variability in the number of trials the abscissa scale is conventional giving an approximate number of trials. The amplitudes were not significantly
different from 0 in control (0.31 ± 0.29 pA) and during the initial period of postsynaptic depolarisation (0.35 ± 0.35 pA). However they became non-zero
during the late depolarising period (2.2 ± 0.3 pA), the NMDAR antagonist application (3.1 ± 0.8 pA) and after switching back to −60 mV (3.8 ± 0.2 pA).
The absense of responses at −60 mV and the appearance of successes at +40 mV correspond to the common definition of a “postsynaptically silent”
synapse with only NMDAR-mediated responses. However the delayed effects of membrane depolarisation and the aftereffect are not compatable with the
removal of Mg2+ block from NMDARs.
"doubtful cases" that can be taken for visual inspection. Furthermore, when analyzing minimal responses, the PCA has the advantage of reducing the signal-to-noise ratio since it uses most of the information contained in the response waveform (Jackson, 1991). Fig. 1G illustrates this point by showing clear "quantal" peaks in the histogram based on the PCA amplitudes (thick line). We note, however, that the general shape and the relative position of the first non-zero histogram peak are very similar for both types of measurements (Fig. 1G, thick and thin lines, respectively). Both the peak amplitudes and "PCA amplitudes" were measured in most experiments, and they gave similar results in accordance with their high correlation and rare appearance of data points with strong divergence between the two measures at moderate spontaneous noise levels (Fig. 1E). However, because of the better signal-to-noise ratio the final data are presented as "PCA amplitudes." They were expressed in pA and termed "amplitudes" for simplicity (Berretta et al., 2000; Sokolov et al., 2003).

The number of transmission failures was estimated by two methods: by visual discrimination and by doubling the number of positive or negative amplitudes (at negative or positive membrane potentials, respectively). As a control, the visually selected failures were averaged and the selection was repeated if the average traces contained stimulus-locked deflections different from that for the interstimulus period. Typically it was not necessary: the averaging confirmed the adequacy of the visual selection and both methods gave very similar results. The mean number of failures determined by the two methods was not significantly different and the number of failures correlated highly significantly across differ-

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Fig. 3. Summary of changes in response amplitudes and in indices of transmitter release at positive holding potentials in CA3 neurones. (A) A series of 10 consecutive synaptic responses recorded during four periods (a–d) of a representative experiment. Initial holding potential of −60 mV (a) was changed to +40 mV (b) and the NMDAR antagonist CPP (20 μM) was applied (c) to isolate AMPAR-mediated EPSCs. Afterward the NMDAR antagonist was washed out and the holding potential was switched back to −60 mV (d). At the hyperpolarized holding potential, the cell responded to both stimuli in the paired-pulse paradigm ("non-silent" input), but showed numerous transmission failures in response to the first pulse (a). The absence of failures at +40 mV suggests potentiation of both NMDAR-mediated (b) and AMPAR-mediated responses (c). The first response remained potentiated after switching back to −60 mV (d). (B) Summary plot of the EPSC amplitudes at different periods for all similar experiments. Closed circles represent data from all nine inputs (three "silent" and six "non-silent") recorded from CA3 neurones. Open squares represent data from "non-silent" inputs (n=6). The "silent" inputs were defined as those that showed only failures in responses to the first pulse in the paired-pulse paradigm at −60 mV (as illustrated in Fig. 2Aa, b). Measurements from four consecutive trials were averaged for each input. Afterward, the general means were calculated for all inputs so that each point represents the mean of at least 24 or 36 measurements (open and closed symbols, respectively). See Fig. 2 for other explanations and for illustration of similar effects in apparently silent cells from the same series of experiments. Notice a slow increase in EPSC amplitude at +40 mV so that eventually the means became significantly larger (asterisks) as compared with the initial data points at +40 mV. (C) Comparison of indices of transmitter release for the same nine experiments. White, black, dotted and hatched columns represent data obtained at −60 mV, at +40 mV, at +40 mV with NMDAR block by CPP and after stepping back to −60 mV, respectively. Significant changes in failure rate, reversed squared coefficient of variation of response amplitudes (CV−2) and the paired-pulse ratio persisting under NMDAR block are compatible with increased transmitter release at +40 mV. Only data from "non-silent" inputs are included in the calculations of the paired-pulse ratio because the ratio is unreliable ("indefinitely large") in silent inputs with only failures after first pulses in the paired-pulse paradigm. However, appearance of measurable paired-pulse ratio at +40 mV signifies its changes in the same direction as for non-silent inputs.
ent experiments \((r=0.87, P<0.0001, n=72)\) so that the general result did not depend on the method used. The final data are based on visually determined failures. PPF ratio was calculated as mean EPSC amplitude (2nd pulse)/mean EPSC amplitude (1st pulse). Values are given as means±S.E.M. Significance was assessed using the Student’s t-test, Mann-Whitney rank sum test or Fisher exact probability test for discrete data, as appropriate. The differences were considered significant at \(P<0.05\).

RESULTS

Delayed appearance of NMDAR-mediated EPSCs at positive membrane potentials in apparently silent inputs

In the first series of experiments we attempted to identify “postsynaptically silent” synapses in immature (P2–P7) CA3 pyramidal neurones using a protocol similar to that employed by others in the CA1 area (Liao et al., 1995; Isaac et al., 1995; Durand et al., 1996). However, we used a higher temperature (32 °C) and lower (1/20 s) stimulation frequencies to avoid amplitude depression common at higher testing frequencies (Gasparini et al., 2000). According to this protocol we lowered the stimulus strength until only response failures were evoked at \(-60 \text{ mV}\) when stimuli were delivered at electrodes S-I or S-II (Fig. 1A) located to stimulate the mossy fiber tract. Afterward, the presence of responses was tested at \(+40 \text{ mV}\) in several trials. If no responses could be detected at \(+40 \text{ mV}\), the stimulation intensity was slightly increased until a response could be detected (Gasparini et al., 2000). Synapses were considered “silent” if, after resetting the potential at \(-60 \text{ mV}\), only transmission failures were recorded in response to the first pulse for 20–60 consecutive trials delivered at 0.05 Hz. This was found in three of nine recorded CA3 pyramidal neurones (Fig. 2Aa and 2Ba).

Fig. 4. Potentiation of NMDAR- and AMPAR-mediated responses in a silent Schaffer collateral-CA1 synapse during and following depolarisation to positive potential. Consecutive traces (A), averages over respective periods (B) and amplitude time course (C) are shown. Ten to 20 consecutive traces were superimposed in A; 60, 28, 16 and 250 trials were averaged in B over periods a–d, respectively (see C for the same periods). The protocol was similar to that in Fig. 3. The initial membrane potential of \(-60 \text{ mV}\) (a) was changed to \(+40 \text{ mV}\) (b), an antagonist of NMDA receptors APV (50 μM) was applied to isolate AMPAR-mediated EPSCs (c) and the holding potential was stepped back to \(-60 \text{ mV}\) (d). The cell can be considered “silent” because it showed no significant responses to single pulses at the hyperpolarized potential (Aa, Ba) but showed clear responses at \(+40 \text{ mV}\) (Ab, Bb). Notice strong EPSC potentiation at \(+40 \text{ mV}\) in A and B (b, c) and persistent potentiation after stepping back to \(-60 \text{ mV}\) (A–C, d).
and below for data of Figs. 2, 4 and 7). Second, after stepping back to −60 mV this potentiation persisted for at least 10 trials (about 4 min, Fig. 2Ad–Cd; P < 0.001; compare also Fig. 2Ad and Bd with 2Aa and Ba, respectively). The amplitude potentiation was not persistent in this particular neuron because the number of successes during the last 10 trials (Fig. 2A–C, e) was not significantly different from that before depolarisation (P > 0.1).

A similar increase in EPSC amplitude at depolarised potentials was observed in three cells apparently silent, at −60 mV. Their average behavior is shown in Fig. 2D. It is clear from the figure that responses at +40 mV persisted also during the block of the NMDARs with α-3-(2-carboxy-piperazin-4-yl)-propyl-1-phosphonic acid (CPP; 20 μM; Fig. 2D) indicating that they were mediated also by AMPARs (see below also data for non-silent inputs recorded from CA3 cells).

Potentiation of pharmacologically isolated AMPAR-mediated EPSCs at positive membrane potentials

In agreement with previous observations (Liao et al., 1995; Durand et al., 1996), we found that in non-silent CA3 cells failure rates were reduced or failures disappeared altogether when the membrane potential was switched from −60 mV to +40 mV (Fig. 3Aa and b). This observation has been interpreted as the appearance of NMDAR-mediated EPSCs due to removal of the Mg2+ block at positive membrane potential. To further test the presence of the NMDAR-mediated EPSC at +40 mV, we applied the NMDAR antagonist (Fig. 3Ac, CPP, 20 μM). As expected, the decay of the EPSC became faster showing major suppression of NMDAR-mediated responses. Appearance of failures would be expected if at least part of successes at +40 mV were due to pure NMDAR-mediated EPSCs. However no failures appeared (Fig. 4Ac, CPP) indicating that AMPAR-mediated EPSCs were clearly potentiated at +40 mV as compared with control. The responses possessed the same fast rising times and showed similar paired-pulse ratio as before CPP application. Moreover, the potentiation of the first EPSC persisted after switching the membrane potential back to −60 mV as shown in Fig. 3Ad. The second EPSC did not change in comparison with the control at −60 mV (compare Fig. 3Ad and a) so that the PPF decreased.

Fig. 3B shows the mean amplitudes of EPSCs recorded from “non-silent” cells (open squares, n = 6) and from all cells tested at 0.05 Hz (silent and non-silent, closed circles, n = 9). The results for the two samples were similar and showed a slow increase in EPSC amplitude at +40 mV similar to that observed for “silent” inputs plotted separately in Fig. 2D. The increase in EPSC amplitude persisted for at least 3 min after stepping back to −60 mV when the mean EPSC amplitude was significantly larger than control values (9.2 ± 2.1 and 4.6 ± 1.5 pA, respectively; n = 9 cells; P < 0.05).

There was a significant correlation between initial failure rates and percent change of the mean EPSC amplitude after depolarisation (r = 0.62; P < 0.05; n = 9 cells) indicating a strong trend for “more silent” inputs to be potentiated and for more active inputs to be depressed. Because of this variable direction of the “post-depolarisation” effect, the overall mean amplitudes before and after depolarisation did not differ significantly (4.6 ± 1.5 and 6.1 ± 3.2 pA, respectively; P > 0.32; n = 9 cells; Fig. 3B).

Fig. 3C (left graph) illustrates summary data on the reduction in failure rate at depolarised potentials (black column) as compared with control (empty column). It also shows that at +40 mV the reduction in failure rate persisted when pharmacologically isolated AMPAR-mediated EPSCs were recorded in the presence of NMDARs antagonists (Fig. 3C, dotted bars, CPP).

To test whether the reduction in failure rates at +40 mV is due to an increased transmitter release, two other known indices of transmitter release were evaluated: the amplitude variance measured as CV−2 and PPF ratio (Fig. 3C). Compatible with response potentiation, both indices significantly changed during depolarisation. The PPF ratio is given only for “non-silent” inputs (n = 6) because calculated ratios are unreliable (“infinitely large”) when the mean amplitude of the first EPSC is close to zero (accordingly, CV−2 was also close to zero in these cases compatible with the lack of essential quantal release). However in these initially silent inputs, the PPF ratio became measurable at +40 mV indicating that PPF has changed in the same direction as for “non-silent” inputs, i.e. strongly decreased during postsynaptic depolarisation. Changes of all three indices of transmitter release were similar for mixed and pharmacologically isolated AMPAR-mediated EPSCs (Fig. 3C, black and dotted columns, respectively) compatible with an increased release probability.

Minimal EPSCs of CA1 neurones are potentiated by switching to the positive postsynaptic potential

Most of the data regarding silent synapses have been obtained with recordings of minimal EPSCs from CA1 cells (Liao et al., 1995; Isaac et al., 1995; Durand et al., 1996). Therefore, in the next series of experiments we checked whether the described effects of membrane potential shifts could also be detected in CA1 neurones. In this set of experiments, we used a protocol similar to that described above for selecting apparently silent synapses. The differences were that slices were prepared from young rats (P10–P21) similar to the age group used in previous studies (Liao et al., 1995; Isaac et al., 1995) and that the stimulation frequency was 0.5 Hz. This value was close to that used in previous studies (Liao et al., 1995; Isaac et al., 1995; Choi et al., 2000). This relatively high testing frequency could not be used in previous experiments because of strong response depression in immature CA3 synapses (Gasparini et al., 2000). However, we modified the experimental protocol to follow more closely the known studies on “silent synapses.”

Fig. 4Aa shows that the first pulse in the paired-pulse paradigm produced only transmission failures while the second evoked occasional successes due to PPF. Accordingly, the average responses showed no EPSCs following the first pulse, but a clear EPSC following the second one (Fig. 4Ba). The responses to the first pulse were apparently potentiated
at +40 mV as indicated by the appearance of short-latency EPSCs in single and averaged recordings (Fig. 4A and B, traces b). The potentiation persisted during application of the NMDAR blocker D-2-amino-5-phosphonovalerate (APV; 50 μM; Fig. 4Ac, Bc) and after resetting the membrane potential to −60 mV (Fig. 4Ad, Bd). Accordingly, the plot of single amplitudes (Fig. 4C) showed only failures at −60 mV (a), appearance of occasional successes with >10 pA amplitudes at +40 mV (b, c) and much larger negative amplitudes after stepping back to the “resting” membrane potential (d). To evaluate statistical significance of the aftereffect and its persistence we compared the number of successes after the first pulse over periods of 2.5 min (corresponding to 75 trials) at −60 mV before and after membrane depolarisation. While no clear successes were observed before depolarisation (Fig. 4Aa) from six to 20 successes appeared after depolarisation. This corresponds to a statistically significant difference from 0 at least at P<0.04 level (Fisher exact probability test) and to a significant LTP-like after effect in a cell apparently silent before postsynaptic depolarisation.

Fig. 5 summarizes data obtained from nine CA1 neurones recorded before, during and after postsynaptic depolarisation. The upper plot (Fig. 5A) demonstrates a slowly developing amplitude increase after switching to +40 mV, a result similar to that obtained in CA3 neurones (see Fig. 3B). After stepping back to −60 mV, the successes persisted in the CA1 neurone that was initially silent to the first pulse (Fig. 4) and in three other (non-silent) cells. However, a persistent post-depolarisation depression was observed in three neurones and no significant after-effect was found in two more cells. As a result the average data showed no significant changes in EPSC amplitude after stepping back to −60 mV (Fig. 5A).

As for CA3 pyramidal cells, in CA1 neurones the classical indices of transmitter release (failure rate, CV² and PPF ratio) changed at +40 mV as compared with control (Fig. 5B, black and white columns, respectively).

Altogether, these results (Figs. 4 and 5) are similar to those obtained from CA3 neurones in the first experimental series (Figs. 2 and 3) and are compatible with an increased...
probability of transmitter release at positive membrane potentials.

**Increased postsynaptic calcium concentration accounts for depolarisation-induced potentiation of EPSCs**

A reduced number of transmission failures at positive as compared with negative membrane potential was initially shown using recording electrodes filled with Ca\(^{2+}\) chelators (10 mM EGTA or 10 mM BAPTA) in the attempt to prevent Ca\(^{2+}\)-dependent plasticity (Liao et al., 1995). To further check the "potentiation hypothesis," we performed recordings from CA1 pyramidal neurones with electrodes filled with BAPTA. The time period from formation of the whole-cell configuration to the beginning of depolarisation was 3–4 min. Fig. 6A–C illustrates a cell that was "almost silent" at −60 mV. It showed 95% transmission failures following the first pulse in the paired-pulse paradigm (Fig. 6A–C, parts a). Moreover, only failures were observed during the trials immediately preceding the shift to +40 mV holding potential (Fig. 6Ab, Bb). This cell exhibited slow (apparently NMDAR-mediated) responses at +40 mV. However, as in previous observations from recordings obtained in the absence of BAPTA the appearance of the
successes was delayed. Immediately after shifting to \(+40\) mV only a few clear stimulus-locked responses were observed (Fig. 6Ac, Bc). These became more pronounced later (Fig. 6Ad, Bd). After switching the holding potential back to \(-60\) mV, the failure rate remained low for the rest of the recording period, and EPSC amplitude was potentiated (Fig. 6A–C, parts e).

Similar to Liao et al. (1995), differences in failure rates at negative and positive holding potentials were found in four of five cells. The summary data from these cells show a significant decrease in failure rate during postsynaptic depolarisation despite of dialyses with BAPTA (20 mM). Eight consecutive single traces are shown in A to exemplify responses before (a) during (b, c) and after postsynaptic depolarisation (d). B, averages of all 198 trials before membrane depolarisation (a) and after switching back to \(-60\) mV (d); eight trials were averaged immediately (b) and about 1 min after beginning of membrane depolarisation (c) to demonstrate a delayed increase in EPSC amplitude. Note also only one clear success after stepping back to \(-60\) mV (Ad, Cd) indicating post-depolarisation depression. (D) Mean indices of transmitter release calculated from all six cells dialysed with BAPTA (20 mM). White, black and hatched columns represent periods before, during and after postsynaptic depolarisation. The absence of significant depolarisation-induced effects or aftereffects suggests importance of changes in postsynaptic Ca\(^{2+}\) concentration for the effects of membrane depolarisation found in previous experiments.

Our experiments with BAPTA (10 mM) show clear signs of EPSC potentiation at positive potentials. These observations are difficult to reconcile with a Ca\(^{2+}\)-dependent potentiation. However, it is possible that the concentration of BAPTA used was not sufficient to completely block the depolarisation-induced increase in postsynaptic Ca\(^{2+}\). Another critical variable could be the dialysis time (e.g. Mellor and Nicoll, 2001). We repeated the experiments using a higher (20 mM) concentration of BAPTA in the patch pi-
pete and longer time periods between the formation of the whole-cell configuration and postsynaptic depolarisation (from 5 to 31 min with the mean 12±2 min, n=6 cells).

In one out of the six cells recorded in these conditions, failure rate decreased during depolarisation in comparison with control at −60 mV (from 88% to 45%). This was accompanied by a slowly developing amplitude potentiation as illustrated by EPSCs recorded immediately after switching to +40 mV (Fig. 7A and B, traces b) and about 20 trials (about 1 min) afterward (Fig. 7Ac, Bc). Note also the strong decrease in PPF at +40 mV as compared with −60 mV evident from the fact that at +40 mV the first and second EPSCs in the pair became almost identical (Fig. 7B, compare traces a and c). However, after stepping back to −60 mV synaptic responses appeared depressed (Fig. 7Bd) so that the input became almost silent showing only one clear success to the first pulse in the paired-pulse paradigm (Fig. 7A–C, parts d). Such post-depolarisation depression could be due to incomplete chelation of Ca²⁺ entering during postsynaptic depolarisation. In this case, postsynaptic Ca²⁺ concentration could be sufficient for development of short-term potentiation (STP) during depolarisation and long-term depression (LTD) afterward (Cho et al., 2001; Lisman, 2001; see Discussion).

In the remaining five cells recorded with 20 mM BAPTA electrodes, failure rates showed no significant reduction during membrane depolarisation. Accordingly, summary data from all six cells showed no significant changes in failure rate, CV⁻² and PPF ratio either during or after depolarisation (Fig. 7D). These data suggest that the reduction in failure rate and the amplitude potentiation during postsynaptic depolarisation depend on the increase in intracellular Ca²⁺ concentration that could in turn influence probability of transmitter release (presumably via a retrograde signal originating from the postsynaptic cell). The blocking action of BAPTA makes unlikely alternative explanations of depolarisation effects based on changes in excitability at the nerve terminal, e.g. due to Cs⁺ accumulation in the subsynaptic cleft that could increase the number of failures to activate (invade) presynaptic axon. Also the number of the “axon failures” is likely to be negligible under our experimental conditions as indicated by the paired-pulse tests as described in Experimental Procedures.

The increase in intracellular Ca²⁺ concentration could be due to Ca²⁺ influx via NMDAR channels as suggested by Liao et al. (1995) who found that NMDAR antagonists prevented changes in failure rate with changes in holding potentials. To test whether also in our experimental conditions the depolarisation-induced potentiation was NMDAR-dependent similar experiments were performed in the presence of the NMDAR antagonist CPP (20 μM). The results were qualitatively similar to those of Liao et al. (1995) showing no significant decrease in the mean failure rate during depolarisation (74±12% to 54±15%, P>0.1; n=5) and no significant changes in the PPF ratio (from 11.6±5.7–10.3±9.3, P>0.4).

**DISCUSSION**

The present experiments confirmed the appearance of EPSCs in apparently silent cells or reduced failure rates in non-silent synapses during postsynaptic depolarisation and the block of these effects by NMDA antagonists applied before the depolarisation (Liao et al., 1995; Isaac et al., 1995; Durand et al., 1996). In addition we provided the following novel observations: i. a delayed reduction of failure rates in initially silent cells associated with a gradual increase in EPSC amplitudes; ii. persistence of the depolarisation-induced responses after resetting the membrane potential to resting values; iii. slow changes in EPSC amplitudes and failure rates in non-silent cells; iv. changes in both PPF and CV⁻² of minimal EPSCs; v. persistence of changes in failure rate, CV⁻² and PPF after pharmacological isolation of AMPAR-mediated responses at depolarising potentials; vi. block of these effects in the presence of BAPTA (20 mM). The easiest explanation for these observations is that the decreased failure rate is due to an increased probability of glutamate release at positive membrane potentials that results from Ca²⁺ entry mainly via NMDAR channels. These observations are difficult to reconcile with the “latent AMPARs” model according to which differences in failure rate between hyperpolarised and depolarised potentials depend on the removal of Mg²⁺ from NMDAR channels in synapses without functional AMPARs. We cannot exclude the possibility of insertion of new AMPARs or their phosphorylation (Soderling and Derkach, 2000) do not match the time course of synapse “unsilencing” and LTP induction that occur in seconds (Bliss and Collingridge, 1993).

In comparison with related studies, in the present experiments lower testing frequency was used because even a stimulation frequency of 0.2–0.1 Hz can lead to complete “silencing” (Akaneya et al., 2003; Gasparini et al., 2000) due to presynaptic frequency depression (Christoffersen, 1997; Hawkins et al., 1993; Voronin, 1993). In hippocampal slice culture, this was often accompanied by the conversion of PPF into paired-pulse depression (Saviane et al., 2002). This explains why Montgomery et al. (2001) who used 0.2 Hz testing stimulation could not reproduce the appearance of successes to second pulses in apparently silent cells (Gasparini et al., 2000; present data). In addition, other approaches used to change transmitter release suggest that “synapse silencing” is due to low transmitter release rather than the lack of functional AMPARs and that the vast majority of apparently silent synapses are presynaptically rather than postsynaptically silent (Choi et al., 2000; Gasparini et al., 2000; Magni et al., 2003; Renger et al., 2001; see Voronin and Cherubini, 2003 for a recent review). The presence of postsynaptically silent synapses
has been supported by immunohistochemical data (Petraila et al., 1999; Takumi et al., 1999). However, the synapses without AMPARs may represent non-functional structures. Accordingly, these studies have been recently challenged with the demonstration that nascent hippocampal glutamatergic synapses express functional AMPARs and NMDARs (Friedman et al., 2000; Groc et al., 2002).

Mechanisms of changes in EPSC amplitude and failure rates at depolarised potentials

The progressive increase in EPSCs amplitude at depolarised potentials is hard to reconcile with the latent AMPARs hypothesis because the removal of Mg$^{2+}$ from NMDARs is fast. In contrast, it is known that a certain (threshold) number of afferent volleys are necessary to induce STP or LTP and that their final “saturated” magnitude depends on the number of repeated stimuli. One common way to induce LTP is pairing afferent stimulation with postsynaptic depolarisation (to approximately 0 mV). Postsynaptic depolarisation alone can also induce STP (Kullmann et al., 1992), LTP (Kühnt et al., 1994) or LTD (Vickery and Bindman, 1997). The direction and magnitude of the depolarisation-induced effects depend on “pairing” (Kullmann et al., 1992), number of depolarising pulses, postsynaptic Ca$^{2+}$ concentration (Vickery and Bindman, 1997; Cho et al., 2001; Lisman, 2001). Calcium influx at +40 mV has been suggested to be insufficient to support LTP (Isaac et al., 1995) because of the reduced driving force for Ca$^{2+}$ as compared with 0 mV used in the “pairing protocol.” This could explain why often we observed STP rather than LTP. However, the exact driving force for Ca$^{2+}$ is not easy to evaluate. Therefore, concomitant Ca$^{2+}$ influx via voltage dependent channels and NMDARs may ensure enough Ca$^{2+}$ to trigger STP, LTP or LTD-like plasticities depending on the initial state of the synapse. This is an important variable for the direction and magnitudes of aftereffects of unpaired postsynaptic depolarisation (Berretta et al., 1999; Volgushev et al., 1997). It is conceivable that the magnitude and/or time course of intracellular Ca$^{2+}$ elevation was not adequate to further increase probability of release in “non-silent” inputs under conditions of relatively low driving force for Ca$^{2+}$ ions but adequate for the induction of LTD-like aftereffects.

As already mentioned, in the present experiments, the use of paired pulses revealed the presence of AMPARs in apparently silent synapses because occasional responses occurred after the second pulse (Gasparini et al., 2000). Therefore, the question arises how potentiation could appear at silent synapses when single pulses are used so that actual “pairings” with postsynaptic depolarisation are absent. First of all, it should be stressed that many experiments related to the “latent AMPARs” hypothesis, have been performed in “non-silent” cells (Liao et al., 1995). However, several reasons can account for potentiation of apparently silent inputs as well. i. The absence of responses in apparently silent cells has been rarely quantified. Therefore, at least in a subset of presynaptically silent synapses with very low release probability (but different from 0) or with very small EPSCs (not well detected) occasional successes could appear in the course of prolonged depolarisation and these could further boost pairing-dependent potentiation. Notably, not all initially silent cells showed successes during postsynaptic depolarisation (e.g. approximately 40%; Isaac et al., 1995). In line with this, we failed to reproduce the appearance of successes at +40 mV in inputs silent to both stimuli in the paired-pulse paradigm at −60 mV (unpublished observations). ii. Pairing with spontaneous activity and spillover from adjacent synapses (Kullmann, 2000) could contribute to STP/LTP induction. iii. Factors not depending on “pairing” procedure could also participate. One possibility is modification of ionic concentration in the subsynaptic space/cleft during depolarisation. Specifically, Cs$^{+}$ could accumulate around the synaptic terminal and affect presynaptic release. The blocking action of BAPTA and NMDAR antagonists suggests that such mechanism is not critical for the basic depolarisation effects but it could contribute, especially if supported by other factors. Note that BAPTA was not effective at 10 mM (Fig. 6) and sometimes even at 20 mM concentration (Fig. 7A–C). Most importantly, unpaired postsynaptic depolarisation can activate voltage dependent Ca$^{2+}$ channels, increase postsynaptic Ca$^{2+}$ concentration and thus trigger a cascade of events that induce STP or LTP-like modifications presumably depending on changes in transmitter release due to a retrograde messenger (Volgushev et al., 1997, 2000 with refs).

Effects of NMDAR antagonists and BAPTA

In agreement with the potentiation model, fast rising apparently AMPAR-mediated successes persisted during application of NMDAR antagonists. However, the latter prevented the decrease in failure rate if applied before depolarising the membrane. Similar NMDAR dependence is expressed also in the gradual reduction in EPSC amplitude (Fig. 3B) and eventual disappearance of the effects of postsynaptic depolarisation following a large number of trials (e.g. 200–400 at 0.5–1 Hz) in the presence of NMDAR antagonists (Isaac et al., 1995; our unpublished observations). The possibility that changes in failure rate may depend on intracellular Ca$^{2+}$ rise has been denied on the basis of the persistence of this effect with 10 mM of BAPTA (Liao et al., 1995), a condition that blocks tetanus-induced LTP in the CA1 area (Bliss and Collingridge, 1993). We confirmed that intracellular dialysis with 10 mM BAPTA for 3–4 min did not prevent major depolarisation effects (Liao et al., 1995). However, postsynaptic dialyses with 20 mM BAPTA for 5–30 min was more effective suggesting that the depolarisation-induced effects are similar to the “classical” Ca$^{2+}$-dependent STP/LTP in the CA1 area. The lack of the effects of 10 mM BAPTA could be attributed to combination of its insufficient concentration and relatively short dialyses time: both variables are crucial for BAPTA effects (Melor and Nicoll, 2001). It should be stressed that BAPTA effects can vary in different cells, e.g. being influenced by the spatial location of respective synapses that is generally unknown. The effects of BAPTA and NMDAR blockers are compatible with changes in...
probability of transmitter release rather than with a decrease in the number of “axon failures” that in our experimental conditions is negligible (see Experimental Procedures).

**Decreased PPF and amplitude variability during postsynaptic depolarisation**

The decrease in PPF supports an increased probability of transmitter release during postsynaptic depolarisation. Similar reduction of PPF has been observed during LTP (Kleschevnikov et al., 1997, 2002; Schulz, 1997; Sokolov et al., 1998) or LTP-like phenomena induced by unpaired postsynaptic depolarisation (Volgushev et al., 1997; Berretta et al., 1999). Although initial publications showed no decrease in the PPF ratio measured from multifibre field potentials (Schulz, 1997 with references) already first recordings of minimal EPSCs revealed a clear reduction that was correlated with LTP magnitude (Voronin and Kuhn, 1990). It should be stressed that, when LTP was small or release probability high (Schulz, 1997; Kleschevnikov et al., 1997; Sokolov et al., 1998; Li et al., 2000) on average PPF did not change. However PPF could either increase or decrease if correlated with the initial value. In the same line we have found large changes in inputs with initially high PPF ratios and failure rates. In agreement with our data, large (multifibre) NMDAR-mediated EPSCs (but not pharmacologically isolated AMPAR-mediated EPSCs) have also shown similar potential dependence of PPF (Clark et al., 1994). As for LTP, changes in PPF were associated with an increase in CV² (e.g. Kleschevnikov et al., 2002; Sokolov et al., 2002) thought to reflect an increased amount of quanta released. Difference in CV² of AMPAR and NMDAR-mediated responses have been considered as an important argument in favor of the “latent AMPARs” hypothesis (Kullmann, 1994). However, such differences have not been found when AMPAR- and NMDAR-mediated responses were measured at the same membrane potential (~75 mV) or when NMDAR-mediated responses were measured at ~30 mV rather than at +30 mV (Niu et al., 1998). These results are compatible with the “potentiation hypothesis” because no significant potentiation is expected either at ~75 or ~30 mV. These data stress that measurements of AMPAR- and NMDAR-mediated responses should be done under the same experimental conditions (Bayazitov and Kleschevnikov, 2000; Bayazitov et al., 2002).

Taking together, our and literature data obtained with measurements of response amplitudes, failure rates, PPF and CV² are more compatible with an increased probability of transmitter release at positive membrane potentials and therefore with the potentiation model rather than with the latent AMPAR model.

**Implications for LTP mechanisms**

The observation that apparently silent synapses are presynaptically rather than postsynaptically silent is compatible with a strong contribution of increased transmitter release to early LTP maintenance (Voronin and Cherubini, 2003; Emptage et al., 2003). Morphological data indicate increases in the size of the synapses, appearance of additional active zones and perhaps appearance of new spines during LTP with time courses that could vary depending on the induction conditions (e.g. Edwards, 1995). These postsynaptic rearrangements should involve also NMDARs (Rostas et al., 1996; Luscher and Frerking, 2001; Skeberdis et al., 2001) and should be accompanied by respective changes in transmitter release, a mechanism neglected by the “latent AMPAR” hypothesis that assumes already high initial release (Isaac, 2003). Altogether, we favor the hypothesis that a crosstalk between post- and presynaptic elements is crucial for co-ordinating pre- and postsynaptic modifications (Lisman and Harris, 1993; Soderling and Derkach, 2000; Antonova et al., 2001). According to this scheme increased transmitter release induced by conditioning tetanus would cause a postsynaptic influx of Ca²⁺ that would trigger the cascade of events leading to LTP induction. As a next step, a retrograde signal would produce a further enhancement in transmitter release. In turn, this would induce a rearrangement of subsynaptic receptors (phosphorylation and clustering) but typically with a time course of minutes (e.g. Davies et al., 1989). At later phases, neurotransmitter release can change again (also because of increased number of active zones or/and growth of new boutons) to match delayed modifications in receptor functioning.

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**REFERENCES**


Lisman JE (2001) Three Ca2+ levels affect plasticity differently: the LTP zone, the LTD zone and no man's land. J Physiol (Lond) 532:285.


Redman SV (1990) Quantal analysis of synaptic potentials in neurons of the central nervous system. Physiol Rev 70:122–165.


Skeberdis VA, Lan J, Opitz T, Zheng X, Bennett MV, Zukin RS (2001) mGluR1-mediated potentiation of NMDA receptors involves a rise in intracellular calcium and activation of protein kinase C. Neuropharmacology 40:856–865.


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