A REVERSIBLE SYNAPTIC DEPRESSION IN DEVELOPING RAT CA3 – CA1 SYNAPSES EXPLAINED BY A NOVEL CYCLE OF AMPA SILENCING - UNSILENCING

Abbreviated title: Inactivity produces AMPA unsilencing

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Abstract

In the developing hippocampus experiments using whole-cell recordings have shown that a small number of synaptic activations can convert many glutamate synapses to AMPA silent synapses. This depression of AMPA signaling is induced by low frequency (0.05 – 0.2 Hz) activation, does not require NMDA or metabotropic glutamate receptor activation for its induction, and does not readily reverse following stimulus interruption. Here we show, using field recordings and perforated patch-clamp recordings of transmission in developing CA3 - CA1 synapses, that this synaptic depression also can be observed under more non-invasive recording conditions. Moreover, under these conditions the synaptic depression spontaneously recovers within 20 minutes by the absence of synaptic activation alone, with a time constant of about 7 minutes as determined by field EPSP recordings. Thus, as for the expression of long-term potentiation, recovery from this depression is susceptible to whole-cell dialysis (“wash-out”). In contrast to LTP-induced unsilencing, the AMPA signaling following stimulus interruption was again labile, resumed stimulation resulted in renewed depression. The present study has thus identified a novel cycle for AMPA signaling in which the nascent glutamate synapse cycles between an AMPA silent state, induced by a small number of synaptic activations, and a labile AMPA signaling, induced by prolonged inactivity.
**Introduction**

The glutamate synapse has since long been found very plastic, altering its efficacy both in the short- and long-term in response to activity. Experimentally, the synapse is first activated at a low frequency (0.02 – 0.2 Hz), assumed to keep the synapse in its naïve state, thereafter it is subjected to various conditioning stimuli at higher frequencies to induce plasticity. The test frequency stimulation is then resumed to establish the short- or long-term alteration in the synaptic signaling. However, as found in the hippocampus of the neonatal rat, stimulation at test frequencies of 0.05 – 0.2 Hz can by itself lead to substantial alterations in synaptic signaling (Abrahamsson et al. 2005; Xiao et al. 2004). Thus, in response to such stimulation AMPA signaling at a previously (experimentally) non-stimulated set of synapses is substantially reduced. Based on variance and failure analysis as well as on stable NMDA receptor (NMDAR) mediated signaling this reduction was explained by removal of AMPA signaling (AMPA silencing) at a subset (about half) of these synapses (Xiao et al. 2004). This AMPA silencing does not require NMDAR or metabotropic glutamate receptor (mGluR) activation for its induction and is thus distinct from conventional forms of long-term depression (LTD) (Abrahamsson et al. 2005; Xiao et al. 2004). Nevertheless, since AMPA silencing, and its counterpart AMPA unsilencing, are considered important elements in the expression of LTD and long-term potentiation (LTP) (Malinow and Malenka 2002), respectively, such test frequency induced AMPA silencing will naturally have implications for the ability of the synapses to express LTP/LTD.

When examined in the neonatal rat AMPA silence appeared to be a consequence of test stimulation only (Abrahamsson et al. 2005; Groc et al. 2002; Xiao et al. 2004), i.e. not being present prior to stimulation, and LTP-inducing stimulation resulted merely in the recovery to the naïve state (TA, BG and EH, in preparation; Xiao et al. 2004). However, the signaling
state following LTP differed from the naive signaling state in that test frequency stimulation no longer reduced AMPA signaling. Thus, after LTP AMPA signaling may only cycle between silent/unsilent states via conventional plasticity mediated via NMDAR and/or mGluR activation. The question then arises whether AMPA signaling also prior to LTP can cycle between silent/unsilent states, or whether LTP is the sole cause for unsilencing. Considering that silencing prior to LTP is induced by such weak activity as a few synaptic activations, it would seem possible that synapses in the absence of such activity would tend to spontaneously recover its AMPA signaling. However, when test stimulation was interrupted for several minutes no such tendency was observed (Xiao et al. 2004), suggesting that the AMPA silencing is not readily reversible even in the absence of synaptic activation. In analogy with the difficulty in inducing LTP using whole-cell recordings (Malinow and Tsien 1990), an absence of experimentally observed recovery using whole-cell recordings may, however, be explained by “wash-out” of the biochemical processes underlying AMPA unsilencing. This possibility of course also raises the question to what extent the phenomenon of test frequency induced AMPA silencing might be a consequence of the whole-cell recording per se, this experimental procedure making AMPA signaling more labile.

In the present study we have therefore used field and perforated patch-clamp recordings to examine whether a test frequency induced synaptic depression corresponding to that observed using whole-cell recordings can be identified under more non-invasive conditions. It will be shown that such a synaptic depression exists and is reversed by inactivity within tens of minutes.
Methods

Slice preparation and solutions

Experiments were performed on hippocampal slices from 5 - 12 and 35 - 44 day–old Wistar rats. The animals were kept and killed in accordance with the guidelines of the Göteborg ethical committee for animal research. The rats were anaesthetized with isoflurane (Abbott) prior to decapitation. The brain was removed and placed in an ice–cold solution containing (in mM): 140 cholineCl, 2.5 KCl, 0.5 CaCl2, 7 MgCl2, 25 NaHCO3, 1.25 NaH2PO4, 1.3 ascorbic acid and 7 dextrose. Transverse hippocampal slices (300-400 \( \mu \)m thick) were cut with a vibratome (Slicer HR 2, Sigmann Elektronik, Germany) in the same ice–cold solution and they were subsequently stored in artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 3 KCl, 2 CaCl2, 4 MgCl2, 26 NaHCO3, 1.25 NaH2PO4, 0.5 ascorbic acid, 3 myo–inositol, 4 D,L–lactic acid, and 10 D–glucose. After 1 – 8 hours, typically 2 – 5 hours, of storage at 25ºC, a single slice was transferred to a recording chamber where it was kept submerged in a constant flow (~2 ml minutes\(^{-1}\)) at 30–32ºC. The slice was allowed to equilibrate in the recording chamber for about 10 minutes before the recording started. There was no relationship between the amount of depression and the storage time (\( r = -0.19, n = 16, p > 0.05 \)). The perfusion ACSF contained (in mM): 124 NaCl, 3 KCl, 4 CaCl2, 4 MgCl2, 26 NaHCO3, 1.25 NaH2PO4, and 10 D–glucose. Picrotoxin (100 \( \mu \)M) was always present in the perfusion ACSF to block GABA\(_A\) receptor–mediated activity. All solutions were continuously bubbled with 95% O\(_2\) and 5% CO\(_2\) (pH ~7.4). A cut between CA3 and CA1 and the higher than normal Ca\(^{2+}\) and Mg\(^{2+}\) concentrations were used to prevent spontaneous network activity. Under these conditions the spontaneous activity in the slice preparation is very low, spontaneous EPSCs occurring at a frequency of about 0.3 – 1 Hz (Groc et al. 2002; Hsia et al. 1998).
Recording and analysis

Electrical stimulation of Schaffer collateral afferents was carried out in the stratum radiatum. Stimuli consisted of biphasic constant current pulses (200 + 200 μs, STG 1004, Multi Channel Systems MCS GmbH, Reutlingen, Germany) delivered through either a glass pipette (resistance ~ 0.5 - 1 MΩ) or an insulated tungsten microelectrode (resistance ~ 0.3 - 0.5 MΩ). A stimulus intensity of 9 - 30 μA and 40 - 60 μA were used for slices from 35 - 44 and 5 - 12 day–old rats, respectively. Field EPSP recordings were made by means of a glass micropipette (~ 1 MΩ, filled with 1 M NaCl) in the stratum radiatum. Whole–cell patch–clamp recordings were performed on visually identified pyramidal cells, using infrared–differential interference contrast videomicroscopy mounted on a Nikon E600FN microscope (Nikon, Japan). The pipette solution contained (in mM): 130 Cs–methanesulfonate, 2 NaCl, 10 HEPES, 0.6 EGTA, 5 QX–314, 4 Mg–ATP and 0.4 GTP or 130 KCl, 2 NaCl, 20 HEPES, 0.2 EGTA, 4 Mg–ATP and 0.4 GTP (pH ~7.3 and osmolality 270–300 mOsm). Liquid junction potential was both measured and calculated to be about 8 mV (Cs-based solution) and 2 mV (K-based solution) and it was not corrected for. Patch pipette resistances were 2 – 6 MΩ. For perforated patch recordings, amphotericin B (240 μg/ml) was added to the pipette solution. Also, lucifer yellow (0.05%) was always present in the perforating solution to detect possible membrane leakage using a fluorescence camera. If lucifer yellow entered the cell the experiment was discarded. Field EPSPs and EPSCs were recorded at a sampling frequency of 10 kHz and filtered at 1 kHz, using an EPC–9 amplifier (HEKA Elektronik, Lambrecht, Germany). For AMPA EPSC recordings cells were held in voltage-clamp mode at −70 mV. Series resistance was monitored using a 5 ms 10 mV hyperpolarizing pulse and it was not allowed to change more than 15% in whole-cell and 20% in perforated patch-clamp experiments, otherwise the recording was not included in the analysis. Field EPSPs and EPSCs were analyzed off–line using custom–made IGOR Pro (WaveMetrics, Lake Oswego, OR) software. Field EPSPs and
EPSCs amplitudes were measured as the difference between the baseline level immediately preceding the stimulation artefact, and the mean amplitude during a 2 ms time window around the negative peak between 3 and 8 ms after the stimulation artefact. Field EPSPs were also estimated by linear regression of the initial slope. Since our experimental design precludes adjustment of stimulation intensity (cf. Results) experiments in which the field EPSPs exhibited signs of population spike activity were discarded. For the EPSPs not excluded by this criterion initial slope and amplitude measurements gave the same results. Since the amplitude measurements were less noisy these measurements were used for further analysis. Synaptic depression in the individual experiments was calculated as the percentage decrease between the 1st evoked EPSP/EPSC and the average EPSP/EPSC after 80-120 stimuli. Because of the large variation between individual EPSCs when recording the activity from a relatively small number of synapses, using whole-cell (Fig. 4) or perforated patch-clamp recordings (Fig. 5), recovery in those experiments was evaluated as the potentiation of the first six EPSCs following stimulus interruption. Recovery from the field EPSP depression (Fig. 3F) following stimulus interruption was calculated as percentage of the synaptic depression itself (i.e., depression as 100 %). To reduce the noise inherent in constructing such a ratio the average of the first three field EPSPs at the onset of stimulation and after stimulus interruption, respectively, was used. The presynaptic volley was measured as the amplitude of the initial positive–negative deflection and was not allowed to change more than 10%, otherwise the experiment was discarded. Data are expressed as means ± SEM. Statistical significance for independent and paired samples was evaluated using Student’s t – test.

Drugs

Chemicals were from Sigma–Aldrich (Stockholm, Sweden) except for D-AP5 and LY 341495 (Tocris Cookson, Bristol, UK) and QX–314 (Alomone Labs, Jerusalem, Israel).
Results

To examine the consequences on synaptic transmission in the neonatal rat (P5 – P12) of the initiation of low frequency activation, previously non-stimulated (naïve) CA3 - CA1 synapses have to be used. Thus, a pre-selected stimulation intensity (40 - 60 µA) was used, which in different experiments resulted in variable field EPSP magnitudes (the majority between 0.1 – 0.3 mV). As will be shown below, this variation in field EPSP magnitude had no consequence for the result obtained. The magnitude of the very first evoked field EPSP in each experiment was used as reference for the naïve synaptic strength, and the subsequently evoked responses were compared to this first reference EPSP.

Test frequency induced field EPSP depression

In conformity with the results obtained using whole-cell recordings (Xiao et al. 2004), stimulation of a naïve synaptic input in the neonatal rat at low frequency (0.2 Hz) led to a rapid and considerable decrease in field EPSP amplitude with no change in the presynaptic volley (Fig. 1A). In contrast to results obtained using whole-cell recordings, field EPSPs were always found to decrease, likely because of the larger number of synapses sampled using field than whole-cell recordings. Nevertheless, the amount of depression varied among the experiments. When measured after the first 80 – 120 stimuli, when the depression had started to display a plateau, the field EPSPs had decayed 25 – 60 % from the initial, naïve, value. On average the depression amounted to 37 ± 2.8 % (n = 16) (Fig. 1B). As indicated above, the depression could not be explained by fewer activated axons since the presynaptic volley remained unchanged in these experiments (-1.0 ± 1.2 %, n = 16) (Fig. 1B). As shown in Fig. 1C, the variation in depression magnitude could not be explained by variation in naïve field EPSP magnitude or animal age.
**Comparison between field EPSP and whole-cell EPSC depressions**

The whole-cell EPSC depression was identified as AMPA silencing based on the analysis of changes in EPSC variance, changes in failure rate, and the stability of the NMDA EPSC (Xiao et al. 2004). Such tools are not available for field recordings, and our identification of the field EPSP depression as similar to the EPSC depression observed in whole-cell recordings, and thus as being explained by AMPA silencing, thus rests on its similarity in properties to the depression observed for EPSCs. In agreement with the EPSC depression the field EPSP depression commenced immediately upon stimulation and displayed a similar development (compare e.g. Figs. 1 and 4). However, the average field EPSP depression observed (37 %) was smaller than the corresponding average EPSC depression previously (60%, Xiao et al. 2004), and presently (50 %, Fig. 2F, 4B) found following the same number of stimuli. It should be noted that this value of field EPSP depression (37 %) may not represent the full extent of this depression since further stimulation appears to produce further depression (Fig. 2B).

We next examined whether the presently found EPSP depression exhibited stimulation dependence, input specificity, age dependence, and NMDA and mGluR independence, characteristic hallmarks of the EPSC depression (Abrahamsson et al. 2005; Xiao et al. 2004). In agreement with the EPSC depression, the field EPSP depression was found to be independent of stimulus frequency in that it was evoked to the same extent (per stimulus) by 0.05 Hz as by 0.2 Hz (36 ± 4.3 %, n = 7 vs 37 ± 2.8 %, n = 16) (Fig. 2A). To test for input specificity, field EPSP depression was first elicited in one pathway by 240 stimuli at 0.2 Hz after which stimulation of a second pathway was started. In agreement with input specificity, the depression elicited in this second pathway (41 ± 3.1 %, n = 7) was indistinguishable from
that in the first (42 ± 1.7 %, n = 7) (Fig. 2B). The field EPSP depression was also unaffected by the combined presence of the NMDAR antagonist D-AP5 (50 μM) and the broad spectrum mGluR antagonist LY 341495 (20 - 100 μM) (35 ± 2.4 %, n = 16) (Fig. 2C). In whole-cell experiments, EPSC depression was not observed in slices from rats older than one month (Xiao et al. 2004). When examined in slices from 35 – 44 day-old rats using field EPSPs a small depression was found (7.9 ± 3.1 %, n = 10) (Fig. 2D), which was also observed in the combined presence of D-AP5 and LY 341495 (10 ± 1.8 %, n = 11, not shown). However, in general agreement with the whole-cell result, the depression in these older animals was substantially smaller than that observed in slices from the young rats (p < 0.0001).

**Occlusion between field EPSP and whole-cell EPSC depressions**

To further examine equivalence between the synaptic depression observed in intact cells using field recordings and that observed using whole-cell recordings we examined their interaction. 120 stimuli at 0.2 Hz were first given prior to the initiation of the whole-cell recording while the recording electrode was kept in a cell-attached position. The cell membrane was then ruptured to obtain the whole-cell recording, and stimulation was resumed within 2 - 3 minutes with an additional 120 stimuli. The EPSC depression was occluded following this pre-stimulation (11 ± 11 %, average of binned values (three consecutive EPSCs), n = 5) (Fig. 2E) and it was significantly different from that observed in interleaved control experiments (53 ± 8.8 %, average of binned values (three consecutive EPSCs), n = 5, p = 0.02) when no stimulation was given prior to the whole-cell recording (Fig. 2F). Whereas the same stimulation strengths were used for the interleaved experiments shown in E and F, the EPSC magnitudes in the pre-stimulated cells were much smaller (122 ± 42 pA, n = 5), than those in the interleaved control cells (326 ± 95 pA, n = 5). This is to be expected because of the depression caused by the pre-stimulation, and this amplitude difference likely accounts for the
larger variability in $E$ than in $F$. Taken together, these results establish that the field EPSP depression is the same process as the EPSC depression observed using whole-cell recording.

**Recovery of the field EPSP depression following inactivity**

To examine whether the maintenance of the field EPSP depression requires continued synaptic activation stimulation was interrupted for various time periods. Fig. 3A shows an experiment in which stimulation was interrupted for 40 minutes, demonstrating a complete recovery of the depression. On the other hand, a 1 minute stimulus interruption resulted in no recovery (-0.8 ± 6.7 %, $n = 6$). The average recoveries following stimulus interruption for 2, 5, 20 and 40 minutes, respectively, are shown in Fig. 3B-E. When plotting the relative amount of recovery against time of stimulus interruption, the recovery could be approximated by a single exponential function with a time constant of 7 minutes (Fig. 3F). Although not statistically significant, there was a tendency for the recovery not to be complete, even after 40 minutes of stimulus interruption. Following this long stimulus interruption (Fig. 3E) the volley was 96 ± 2.6 % ($n = 6$) of the initial value, which would explain about half the lack of full recovery, suggesting that the recovery is likely to be a complete one. Following the resumption of stimulation the field EPSP decreased back to the level of depression obtained prior to the stimulus interruption (Figs. 3A-E) with a similar time course as the initial depression (Fig. 3E, grey line). It can be noted that the recovery depended on stimulus interruption, and not on time, since continued stimulation at 0.2 Hz did not result in any recovery, rather some further depression (Fig. 2B).

This relatively rapid recovery presently observed would suggest that significant recovery should have been observed previously, using whole-cell recordings and an eight minutes stimulus interruption (Xiao et al. 2004). Nevertheless, we re-examined recovery in whole-cell
recordings using a substantially longer (20 minutes) period of stimulus interruption (Fig. 4A).

In these experiments the initial EPSC depression amounted to 45 ± 3.8 % of the initial, naïve, value (Fig. 4B), but there was no significant recovery (n = 5, p = 0.43) after the 20 minutes stimulus interruption. However, using the perforated patch-clamp technique and stimulus interruptions for 2 and 15 minutes, respectively, a recovery in agreement with the data obtained using field EPSP recordings was found. Thus, while the 2 minutes stimulus interruption was not sufficient to produce any significant recovery (n = 5, p = 0.19) of the initial EPSC depression (44 ± 12 %, Fig. 5C), the 15 minutes stimulus interruption produced significant recovery (n = 8, p = 0.015) of the initial depression (31 ± 12 %, Fig. 5A). This result suggests that the lack of recovery using whole-cell recordings is explained by wash-out. As shown in Fig. 5B, both the initial and the recovered AMPA EPSC depression were associated with corresponding changes in EPSC variance (1/CV^2). This result confirms previous studies using whole-cell patch-clamp recordings that have associated the AMPA EPSC depression with a proportional decrease in the 1/CV^2 value, a finding that links the AMPA EPSC depression to AMPA silencing in a subset of the activated synapses (Abrahamsson et al. 2005; Xiao et al. 2004).

A feature of the test frequency induced EPSC depression was that it was not associated with any change in paired-pulse facilitation (Abrahamsson et al. 2005; Xiao et al. 2004). Moreover, the depression produced by single or paired stimulation did not differ in magnitude (Abrahamsson et al. 2005; Xiao et al. 2004). These features could not be examined using field recordings since paired pulse stimulation in this unclamped condition produces LTD whose induction relies on NMDAR and T-type voltage-gated calcium channels, and which is associated with an increase in paired-pulse facilitation (Wasling et al. 2002). However, using voltage-clamp in the perforated patch-clamp mode, both single stimulation and paired pulse
stimulation were used and there was no distinguishable difference in depression magnitude (single: 39 ± 4.3 %, n = 9; paired: 42 ± 6.1 %, n = 10). Moreover, the depression was not associated with any change in paired-pulse facilitation (Fig. 5D). The average paired-pulse ratio in the beginning and at the end of the depression protocol (first and last five binned data points) were 1.48 ± 0.17 and 1.46 ± 0.14 (n = 10), respectively. Thus, using perforated patch recordings, the depression not only resembles that observed using field recordings (magnitude and recovery) but also that observed in whole-cell recordings (with respect to paired-pulse stimulation and facilitation), further establishing the equivalence between the field EPSP and EPSC depression.
Discussion

The present study using field and perforated patch-clamp recordings shows that AMPA signaling in the developing CA3 – CA1 hippocampal synapse can rapidly depress by low frequency (0.05 – 0.2 Hz) activation alone and that this depression then reverses within some 10 – 20 min by stimulus interruption alone. This synaptic depression bears close similarity to a depression previously described using whole-cell recordings explained by AMPA silencing. It is known that the glutamate synapses can exist in AMPA signaling or AMPA silent states, and the transition between these states is thought to be governed by NMDAR and/or mGluR activation given by specific patterned activity (Bredt and Nicoll 2003; Malinow and Malenka 2002; Montgomery and Madison 2004). The present results suggest that AMPA signaling in the developing synapse can also transit between silent/unsilent states in a manner not involving NMDAR and/or mGluR activation. It thus appears that glutamate released by single synaptic activations can cause rapid dispersal of AMPARs from the synaptic membrane, these receptors then slowly reappearing in the absence of glutamate release. Internalization and recycling of receptors in response to exogenous agonist exposure is a well established phenomenon (Perez and Karnik 2005). For example, prolonged (> 5 minutes) AMPA application (in culture) leads to a reversible AMPAR dispersion (and internalization) and to the transient creation of NMDAR-only synapses where none existed before (Lissin et al. 1999). The present study is, to our knowledge, the first in which a brief physiological stimulus has been suggested to evoke such cycling of ionotropic receptors.

A transient potentiation of synaptic transmission following a prolonged stimulus interruption has previously been described for the CA3 – CA1 synapses (Niu et al. 1999). However, that potentiation seems distinct from the presently described inactivity-induced AMPA unsilencing in that it largely develops following the resumption of the stimulation (and not by
the inactivity per se). Moreover, the experiments by Niu et al used composite EPSPs (AMPA/NMDA) in a low (0.1 mM) Mg\(^{2+}\) solution and both the potentiation and its subsequent decay were largely NMDAR dependent.

The field recordings used in this study can not by themselves reveal what underlies the activity-induced depression of AMPA signaling. However, in conformity with the AMPA silencing previously observed using whole-cell recordings (Abrahamsson et al. 2005; Xiao et al. 2004); the field EPSP depression was input specific, commenced immediately upon stimulation (and developed with a similar time course with stimulation), developed to the same extent by 0.2 Hz and 0.05 Hz stimulation, was unaffected by NMDAR- and mGluR antagonists, and was down-regulated with age (Fig. 2 A-D). Moreover, prior induction of field EPSP depression largely occluded later induction of depression in the whole-cell recording mode (Fig. 2 E-F). In contrast to the field EPSP depression, the AMPA silencing observed using whole-cell recordings was not reversed by stimulus interruption, as also verified in the present study (Fig. 4). However, using perforated patch recordings, reversal was observed (Fig. 5), suggesting that inactivity-induced AMPA unsilencing is susceptible to “wash-out”. This susceptibility is shared with the induction of LTP in the whole-cell configuration (Malinow and Tsien 1990), although the diffusible factors might not necessarily be the same. It thus seems safe to conclude that the field EPSP depression presently observed is the same process as that previously observed using whole-cell recordings, and thus explained by AMPA silencing.

The fact that the inactivity-induced unsilencing is blocked by “wash-out” suggests that the AMPA silencing/unsilencing takes place in the postsynaptic cell rather than being explained by kiss-and run fusion (Choi et al. 2003), or by glutamate spill-over (Kullmann and Asztely 2003).
A postsynaptic AMPA receptor based explanation is consistent with experimental data from glutamate-induced internalization of AMPA receptors, these receptors reappearing into the synaptic membrane during a time period comparable to the presently observed inactivity-induced unsilencing (Ehlers 2000; Passafaro et al. 2001). Moreover, following irreversible blockade of synaptic NMDARs (Tovar and Westbrook 2002) and GABA$_A$Rs (Thomas et al. 2005) most of these non-functional receptors are replaced by naïve receptors within ten minutes. It thus seems plausible that the cycling of the nascent glutamate synapse between an AMPA signaling (but labile) state and an AMPA silent one is explained by a rapid glutamate-induced AMPA receptor dispersion (and internalization) followed by a slow recovery given by AMPA receptor insertion.

In the above scenario for cycling between an AMPA labile and an AMPA silent state the kinetics of AMPA receptor re-insertion is explained by constitutive exocytosis of receptors into the membrane or, possibly, by lateral diffusion of pre-existing extrasynaptic receptors. However, none of these explanations may hold. Receptor insertion via constitutive exocytosis has been suggested to take a much longer time (hours) (Adesnik et al. 2005) than the tens of minutes observed, while a lateral diffusion by itself would be much faster (Choquet and Triller 2003). Moreover, in contrast to exocytosis, and the presently observed recovery, lateral diffusion of AMPARs is not affected by whole-cell related “wash-out” (Adesnik et al. 2005). A possible explanation could then be that the re-insertion kinetics is determined not by the supply of AMPARs but by the AMPAR binding capacity of the synaptic membrane. The inactivity-induced unsilencing should then reflect the rebuilding of this binding capacity whereas silencing should reflect the disruption of this binding capacity.
Interestingly in this context key proteins involved in the reversible anchoring of AMPARs to the postsynaptic density change substantially during development (Tomita et al. 2003), raising the possibility that there is an immature expression pattern that could be more conducive towards the silencing/unsilencing cycle now described. Thus, TARP γ8, a major TARP (transmembrane AMPA receptor regulatory protein) in the hippocampus that allow AMPARs to interact with various scaffolding proteins in the postsynaptic density (Rouach et al. 2005), starts to be expressed towards the end of the 2nd postnatal week and may be absent in AMPA silent synapses (Tomita et al. 2003). TARPs interact with MAGUK (membrane associated guanylate kinase) proteins in the postsynaptic density (Chen et al. 2000), and the expression pattern of these proteins also change. Thus, before postnatal day 10 SAP-102 is the dominating MAGUK (Elias et al. 2006; Sans et al. 2000) and between postnatal day 10 and 35 there is a dramatic increase in the expression of PSD-95 and PSD-93 (Elias et al. 2006; Sans et al. 2000). The expression of PSD-95 has been suggested to drive synaptic maturation and stabilization (Beique et al. 2006; Ehrlich et al. 2007; El-Husseini et al. 2000; Elias et al. 2006) and knock-out of PSD-95 results in an increased proportion of AMPA silent synapses (Beique et al. 2006). However, since stabilization of AMPA signaling in the developing synapse, at least in the short-term, can be achieved via LTP in the neonatal rat, the immature expression pattern of TARPs and MAGUKs does not preclude AMPA stable synaptic transmission. On the other hand, the presence of the mature expression pattern of TARPs and MAGUKs in the synapse seems inconsistent with AMPA labile / silent transmission.

The synaptic depression described here can of course be seen as an extreme variant of novelty detector mechanism, only providing for at most a few activations before a several minutes silence. However, considering that it largely exists only in the developing brain it may seem more useful to view it from a developmental perspective and as the behavior of a glutamate
synapse not yet stabilized within a neuronal network. It is a common notion that the glutamate synapse is born without AMPA receptors, this state being maintained until the synapse eventually is exposed to the appropriate correlated pre- and postsynaptic activity, and AMPA signaling is added (Durand et al. 1996; Isaac et al. 1995; Liao et al. 1995). Since this addition of AMPA receptors occurs essentially momentarily it seems reasonable that, as indicated by present and previous results (Xiao et al. 2004), the whole machinery for AMPA signaling, including AMPA receptors, is rather present beforehand, the AMPA signaling however being labile until the synapse is exposed to the proper activity (Groc et al. 2006). Sporadic and non-correlated activity will cause such synapses to temporarily lose its AMPA signaling but will regain it either momentarily by correlated pre- and postsynaptic activity, or more slowly via inactivity.

Thus, besides the fact that this test frequency induced synaptic plasticity is a pre-requisite for the ability of the developing synapse to demonstrate LTP (TA, BG and EH, in preparation), it should perhaps not be seen as having a functional role as for example short-term plasticity in neural computation. In fact, it does not conform to conventional categorizations of synaptic plasticity. The depression part, though appearing prolonged, distinctly differs from LTD in that it needs to be maintained by synaptic activity albeit at low frequency. A potentiation part induced by the absence of synaptic activation is obviously remote from LTP. And since it is specific for a given synapse, not scaling the efficacy of a population of synapses in response to activity, it can not be categorized as homeostatic plasticity. Nevertheless, since it involves expression mechanisms (AMPA silencing/unsilencing), albeit in a more labile form, thought to be involved in LTP/LTD its interaction/non-interaction with these processes may be helpful in further understanding of the machinery underlying LTP/LTD.
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References


**Figure legends**

**Figure 1.** Field EPSP depression induced by test frequency stimulation at previously non-stimulated CA3 - CA1 synapses. 

*Figure A.* An experiment illustrating depression of the EPSP that occurs when stimulating a naive input at 0.2 Hz. Average EPSPs taken at time points a and b are shown on top. Lower panel shows measurements of the presynaptic volley. 

*Figure B.* Average time course of field EPSP depression (n = 16). Lower panel shows the average values of the presynaptic volley. 

*Figure C.* Relationship between the naive field EPSP amplitude and the amount of field EPSP depression (n = 16). The line represents a linear regression to the data points. Filled and open circles are from experiments on 5-8 and 10-12 day-old rats, respectively.

**Figure 2.** Comparison between field EPSP depression and EPSC depression. 

*Figure A.* Field EPSP depression elicited by stimulation at 0.05 Hz (open circles, n = 7). Field EPSP depression elicited by stimulation at 0.2 Hz is shown for comparison (filled circles, same as in Fig. 1B). 

*Figure B.* Field EPSP depression elicited by stimulation at 0.2 Hz (filled circles) in one pathway followed by a field EPSP depression elicited in another pathway (open circles) 20 minutes later (n = 7). The depression obtained in the second pathway is also shown as a superimposed grey line. 

*Figure C.* Field EPSP depression in the presence of 50 μM D-AP5 and 20 – 100 μM LY 341495 from 5 – 12 day-old rats (n = 16). 

*Figure D.* Field EPSP depression in 35 – 44 day-old rats (n = 10). 

*Figure E.* Occlusion of EPSC depression following pre-stimulation with 120 stimuli at 0.2 Hz in cell-attached mode (n = 5). Insets show example average EPSCs taken at the beginning and at the end of the whole-cell recording. 

*Figure F.* Control experiments without pre-stimulation interleaved with those shown in E (n = 5). Insets show example average EPSCs taken at the beginning and at the end of the whole-cell recording.
Figure 3. Recovery of EPSP depression by inactivity. A, An experiment illustrating recovery of field EPSP depression after stimulus interruption for 40 minutes. Average EPSPs taken at indicated time points are shown on top. B – E, Effect of stimulus interruption for 2 (n = 7), 5 (n = 6), 20 (n = 7) and 40 (n = 6) minutes, respectively, following depression. The average time course of depression observed when stimulation was resumed after the stimulus interruption is shown as a superimposed grey line in E. F, Time constant of recovery from EPSP depression by inactivity. The data points for 1 (n = 6), 2 (n = 7), 5 (n = 6), 20 (n = 7) and 40 (n = 6) minutes of stimulus interruption were fitted to a single exponential function (solid line, $\tau = 7$ minutes).

Figure 4. Lack of recovery of EPSC depression in whole-cell recordings. A, An experiment illustrating depression of naïve AMPA EPSCs followed by a stimulus interruption for 20 minutes, resulting in no recovery. Average EPSCs taken at indicated time points are shown on top. B, Summary graph showing the result of stimulus interruption for 20 minutes in whole-cell recordings (n = 5).

Figure 5. Recovery of EPSC depression in perforated patch-clamp recordings. A, Recovery of the AMPA EPSC depression after stimulus interruption for 15 minutes (n = 8). B, EPSC depression in perforated patch-clamp recordings is associated with a decrease in $1/CV^2$. The left and right bar diagrams refer to the initial (Initial) and recovered (Recovery) depression, respectively. C, No recovery of the AMPA EPSC depression after stimulus interruption for 2 minutes (n = 5). D, AMPA EPSC depression is not associated with any change in the paired-pulse ratio. Upper graph shows the depression of naïve AMPA EPSCs produced by paired-pulses (50 ms) evoked at 0.2 Hz (n = 10). Lower graph shows the average paired-pulse ratio
from these experiments. In these two graphs data points were binned in groups of three before averaging.
Figure 1. Field EPSP depression induced by test frequency stimulation at previously non-stimulated CA3 - CA1 synapses. A, An experiment illustrating depression of the EPSP that occurs when stimulating a naive input at 0.2 Hz. Average EPSPs taken at time points a and b are shown on top. Lower panel shows measurements of the presynaptic volley. B, Average time course of field EPSP depression (n = 16). Lower panel shows the average values of the presynaptic volley. C, Relationship between the naive field EPSP amplitude and the amount of field EPSP depression (n = 16). The line represents a linear regression to the data points. Filled and open circles are from experiments on 5-8 and 10-12 day-old rats, respectively.

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Figure 2. Comparison between field EPSP depression and EPSC depression. A, Field EPSP depression elicited by stimulation at 0.05 Hz (open circles, n = 7). Field EPSP depression elicited by stimulation at 0.2 Hz is shown for comparison (filled circles, same as in Fig. 1B). B, Field EPSP depression elicited by stimulation at 0.2 Hz (filled circles) in one pathway followed by a field EPSP depression elicited in another pathway (open circles) 20 minutes later (n = 7). The depression obtained in the second pathway is also shown as a superimposed grey line. C, Field EPSP depression in the presence of 50 µM D-AP5 and 20 - 100 µM LY 341495 from 5 - 12 day-old rats (n = 16). D, Field EPSP depression in 35 - 44 day-old rats (n = 10). E, Occlusion of EPSC depression following pre-stimulation with 120 stimuli at 0.2 Hz in cell-attached mode (n = 5). Insets show example average EPSCs taken at the beginning and at the end of the whole-cell recording. F, Control experiments without pre-stimulation interleaved with those shown in E (n = 5). Insets show example average EPSCs taken at the beginning and at the end of the whole-cell recording.
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