AMP A Silencing Is a Prerequisite for Developmental Long-Term Potentiation in the Hippocampal CA1 Region

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Abrahamsson T, Gustafsson B, Hanse E. AMPA silencing is a prerequisite for developmental long-term potentiation in the hippocampal CA1 region. J Neurophysiol 100: 2605–2614, 2008. First published September 7, 2008; doi:10.1152/jn.90476.2008. AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) unsilencing is an often proposed expression mechanism both for developmental long-term potentiation (LTP), involved in circuitry refinement during brain development, and for mature LTP, involved in learning and memory. In the hippocampal CA3–CA1 connection naïve (non-stimulated) synapses are AMPA signaling and AMPA-silent synapses are created from naïve AMPA-signaling (AMPA-labile) synapses by test-pulse synaptic activation (AMPA silencing). To investigate to what extent LTPs at different developmental stages are explained by AMPA unsilencing, the amount of LTP obtained at these different developmental stages was related to the amount of AMPA silencing that preceded the induction of LTP. When examined in the second postnatal week Hebbian induction was found to produce no more stable potentiation than that causing a return to the naïve synaptic strength existing prior to the AMPA silencing. Moreover, in the absence of a preceding AMPA silencing Hebbian induction produced no stable potentiation above the naïve synaptic strength. Thus this early, or developmental, LTP is nothing more than an unsilencing (dedepression) and stabilization of the AMPA signaling that was lost by the prior AMPA silencing. This dedepression and stabilization of AMPA signaling was mimicked by the presence of the protein kinase A activator forskolin. As the relative degree of AMPA silencing decreased with development, LTP manifested itself more and more as a “genuine” potentiation (as opposed to a dedepression) not explained by unsilencing and stabilization of AMPA-labile synapses. This “genuine,” or mature, LTP rose from close to nothing of total LTP prior to postnatal day (P)13, to about 70% of total LTP at P16, and to about 90% of total LTP at P30. Developmental LTP, by stabilization of AMPA-labile synapses, thus seems adapted to select synaptic connections to the growing synaptic network. Mature LTP, by instead strengthening existing stable connections between cells, may then create functionally tightly connected cell assemblies within this network.

INTRODUCTION

N-Methyl-D-aspartate receptor (NMDAR)–dependent Hebbian induction of synaptic plasticity is thought to be involved both in activity-dependent refinement of neuronal circuitry during brain development (Constantine-Paton and Cline 1998; Katz and Shatz 1996) and in learning in the more mature brain (Gruart et al. 2006; Martin et al. 2000; Pastalkova et al. 2006; Whitlock et al. 2006). Brain development and learning both basically involve brain organization and reorganization, and synaptic plasticity in the more mature brain has been seen as a model for synaptic plasticity in the developing brain (Kandel and O’Dell 1992). However, to which extent Hebbian plasticity in the developing and in the mature brain is much the same, or fundamentally differs because of different requirements for synaptic reorganization during brain development and during learning, is unclear.

Although long-term potentiation (LTP) in the developing and in the mature brain has similar induction conditions (e.g., NMDAR dependence) qualitative discrepancies between them have been found. For example, whereas LTP at adult CA3–CA1 synapses relies on αCaMKII activation (Lisman et al. 2002), LTP examined at postnatal days 7–9 (P7–P9) does not rely on αCaMKII activation but rather on activation of protein kinase A (PKA) (Yasuda et al. 2003). Moreover, whereas for mature LTP the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) subunit glutamate receptor 1 (GluR1) seems critically involved (Hayashi et al. 2000; Jensen et al. 2003), GluR2long and GluR4 subunits seem involved in early NMDAR- and PKA-dependent synaptic AMPAR incorporation (Esteban et al. 2003; Kolleker et al. 2003; Qin et al. 2005; Zhu et al. 2000). Furthermore, although the conversion of AMPA-silent synapses into AMPA-signaling synapses is an often proposed expression mechanism for LTP (Atwood and Wojtowicz 1999; Busetto et al. 2008; Durand et al. 1996; Groc et al. 2006; Isaac et al. 1995; Liao et al. 1995; Montgomery and Madison 2004; Ward et al. 2006), the number of AMPA-silent synapses is thought to decline substantially with development (Busetto et al. 2008; Durand et al. 1996; Hsia et al. 1998; Liao and Månlin 1996)—implying that other expression mechanisms, at least eventually, must come into play to explain LTP. However, because it is technically more demanding to directly identify putative AMPA-silent synapses when the synaptic density increases with age, it is uncertain when and to what extent AMPA-silent synapses actually disappear.

We have previously demonstrated for synapses in the developing hippocampal dentate and CA1 regions that AMPA silencing is explained by the fact that many glutamate synapses are AMPA labile in that their AMPA signaling is lost as a consequence of merely sparse synaptic activation such as that evoked by test pulses during baseline stimulation (0.2–0.05 Hz) (Abrahamsson et al. 2005, 2007; Xiao et al. 2004). Thus in the absence of evoked stimulation (Groc et al. 2002) or following a prolonged absence of evoked stimulation (Abrahamsson et al. 2007) developing CA3–CA1 pyramidal cell synapses are essentially not AMPA silent. This AMPA-labile feature of glutamate synapses opens up the possibility to relate the

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amount of LTP to the amount of AMPA silence that can be created at the same age. Using this approach we have examined to what extent LTP in the developing and mature brain can be explained by a common expression mechanism: AMPA unsilencing. It will be described that up to P12 LTP is fully explained by AMPA unsilencing (i.e., is a dedepression of test-pulse depression). Thereafter, the relative degree of AMPA silencing (and, by implication, the relative number of AMPA-silent synapses) decreases with age and LTP manifests itself more and more as a genuine potentiation (i.e., not a dedepression) not explained by AMPA unsilencing.

**METHODS**

**Slice preparation and solutions**

Experiments were performed on hippocampal slices from 7- to 44-day-old Wistar rats. The animals were kept and killed in accordance with the guidelines of the Gothenburg ethical committee for animal research. The rats were anesthetized with isoflurane (Abbott) prior to decapitation. The brain was removed and placed in an ice-cold solution containing (in mM): 140 choline-Cl, 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 μm thick) were cut with a vibratome (Slicer HR 2, Sigmann Elektronik, Hüffenhardt, Germany) in the same ice-cold solution and they were subsequently stored in artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 3 KCl, 4 CaCl2, 4 MgCl2, 26 NaHCO3, 1.25 NaH2PO4, 0.5 ascorbic acid, 3 myoinositol, 4 D,L-lactic acid, and 10 D-glucose. After 1–8 h, typically 2–5 h, of storage at 25°C, a single slice was transferred to a recording chamber where it was kept submerged in a constant flow (~2 ml/min) at 30–32°C. We have previously shown that there was no relationship between the amount of test-pulse depression and the storage time (Abrahamsson et al. 2007). The perfusion ACSF contained (in mM): 124 NaCl, 3 KCl, 4 CaCl2, 4 MgCl2, 26 NaHCO3, 1.25 NaH2PO4, 0.5 ascorbic acid, 3 myoinositol, 4 D,L-lactic acid, and 10 D-glucose. After 1–8 h, typically 2–5 h, of storage at 25°C, a single slice was transferred to a recording chamber where it was kept submerged in a constant flow (~2 ml/min) at 30–32°C. We have previously shown that there was no relationship between the amount of test-pulse depression and the storage time (Abrahamsson et al. 2007). The perfusion ACSF contained (in mM): 124 NaCl, 3 KCl, 4 CaCl2, 4 MgCl2, 26 NaHCO3, 1.25 NaH2PO4, 0.5 ascorbic acid, 3 myoinositol, 4 D,L-lactic acid, and 10 D-glucose. Picrotoxin (100 μM) was always present in the perfusion ACSF to block γ-aminobutyric acid type A receptor (GABAAR)–mediated activity. All solutions were continuously bubbled with 95% O2-5% CO2 (pH = 7.4). A cut between CA3 and CA1 and the higher than normal Ca2+ concentration (10 mM) was always present in the perfusion ACSF to block N-methyl-D-aspartate receptors (NMDA–mediated) activity. The slice was continuously perfused (2 ml/min) at 30–32°C, and the recording was not included in the analysis. Fixed ACSF contained (in mM): 124 NaCl, 3 KCl, 4 CaCl2, 4 MgCl2, 26 NaHCO3, 1.25 NaH2PO4, 0.5 ascorbic acid, 3 myoinositol, 4 D,L-lactic acid, and 10 D-glucose. Picrotoxin (100 μM) was always present in the perfusion ACSF to block γ-aminobutyric acid type A receptor (GABAAR)–mediated activity. All solutions were continuously bubbled with 95% O2-5% CO2 (pH = 7.4). A cut between CA3 and CA1 and the higher than normal Ca2+ concentration (10 mM) was always present in the perfusion ACSF to block N-methyl-D-aspartate receptors (NMDA–mediated) activity. The slice was continuously perfused (2 ml/min) at 30–32°C, and the recording was not included in the analysis. Fixed ACSF contained (in mM): 124 NaCl, 3 KCl, 4 CaCl2, 4 MgCl2, 26 NaHCO3, 1.25 NaH2PO4, 0.5 ascorbic acid, 3 myoinositol, 4 D,L-lactic acid, and 10 D-glucose. Picrotoxin (100 μM) was always present in the perfusion ACSF to block γ-aminobutyric acid type A receptor (GABAAR)–mediated activity. All solutions were continuously bubbled with 95% O2-5% CO2 (pH = 7.4). A cut between CA3 and CA1 and the higher than normal Ca2+ concentration (10 mM) was always present in the perfusion ACSF to block N-methyl-D-aspartate receptors (NMDA–mediated) activity. The slice was continuously perfused (2 ml/min) at 30–32°C, and the recording was not included in the analysis. Fixed ACSF contained (in mM): 124 NaCl, 3 KCl, 4 CaCl2, 4 MgCl2, 26 NaHCO3, 1.25 NaH2PO4, 0.5 ascorbic acid, 3 myoinositol, 4 D,L-lactic acid, and 10 D-glucose. Picrotoxin (100 μM) was always present in the perfusion ACSF to block γ-aminobutyric acid type A receptor (GABAAR)–mediated activity. All solutions were continuously bubbled with 95% O2-5% CO2 (pH = 7.4). A cut between CA3 and CA1 and the higher than normal Ca2+ concentration (10 mM) was always present in the perfusion ACSF to block N-methyl-D-aspartate receptors (NMDA–mediated) activity. The slice was continuously perfused (2 ml/min) at 30–32°C, and the recording was not included in the analysis. Fix

**Recording and analysis**

Electrical stimulation of Schaffer collateral afferents was carried out in the stratum radiatum using a 0.2-Hz stimulation rate. Stimuli consisted of biphasic (negative + positive current) constant-current pulses (200 + 200 μs, STG 1004, Multi Channel Systems MCS, Reutlingen, Germany) delivered through an insulated tungsten microelectrode (resistance = 0.3–0.5 MΩ). Stimulation was not initiated until ≥10 min after the positioning of the stimulating electrodes. Field excitatory postsynaptic potential (fEPSP) 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 an infrared–differential interference contrast videomicroscope mounted on a Nikon E600FN microscope (Nikon, Tokyo, 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 for forskolin, D-2-amino-5-phosphonopentanoic acid (D-AP5), and (2S,1’S,2’S)-2-(2-carboxycyclopropyl)-2-(9H-xanthen-9-yl)glucine (LY341495, Tocris Cookson, Bristol, UK) and N-(2,6-dimethylphenyl carbamoylethyl)triethy lammonium bromide (QX-314, Alomone Labs, Jerusalem).

**RESULTS**

**LTP in the developing brain is a dedepression of test-pulse-induced depression and stabilizes naïve synaptic strength**

Stimulation in brain slices from developing rats (<P12) of previously nonstimulated (naïve) CA3–CA1 connections at low frequency (0.2–0.05 Hz) leads to a substantial reduction of the AMPAR-mediated synaptic response within about 50 stimuli, explained by AMPA silencing at a subset of the activated synapses (Abrahamsson et al. 2007; Xiao et al. 2004). Figure 1A shows an experiment using fEPSP recordings in which the activated synapses, after having been depressed from the naïve level by such low-frequency activation (120 stimuli at 0.2 Hz), were tetanized (3 × 20 impulses at 50 Hz, 20-s interval) to evoke LTP. After a transient enhancement above the naïve level, the fEPSP stabilized close to the naïve level. The fEPSP was depressed to 69.7 ± 13% (n = 13; P7–P12) of the naïve level by the test-pulse stimulation and did not differ from the naïve level 20 min after LTP induction (102 ± 0.4%, n = 13)
of the naïve synaptic strength we applied the LTP-inducing protocol at time points \(a, b, c, \) and \(d\) are shown on top. \(B:\) average (\(n = 13\)) time course of the 0.2-Hz-induced depression and subsequent HFS-induced LTP. \(C:\) same as in \(B,\) but the potentiation was followed for 50 min and the test stimulus frequency was 0.05 Hz (\(n = 5\)). \(D:\) tetanization in the combined presence of the \(N\)-methyl-\(D\)-aspartate receptor (NMDAR) antagonist \(d\)-2-amino-5-phosphonopentanoic acid (\(d\)-AP5, 50 \(\mu M\)) and the metabotropic glutamate receptor (mGluR) antagonist LY341495 (50 \(\mu M\)) does not result in any potentiation (\(n = 5\)). In 2 additional experiments, \(d\)-AP5 (50 \(\mu M\)) alone was sufficient to totally prevent the tetanus-induced potentiation.

(Fig. 1B). The stabilization of the naïve level induced by LTP-inducing stimulation could be followed for a longer period, at least up to about 1 h (Fig. 1C). The initial depression, but not LTP, was observed in the combined presence of the NMDAR antagonist \(d\)-AP5 (50 \(\mu M\)) and the mGluR antagonist LY341495 (50 \(\mu M\)) (Fig. 1D) (Abrahamsson et al. 2007). In agreement with Liao and Malinow (1996) \(d\)-AP5 (50 \(\mu M\)) alone was found sufficient to totally prevent the LTP (\(n = 2;\) data not shown).

The naïve synaptic strength thus seems to represent an upper limit for LTP at these developing synapses. To examine whether this finding was merely a chance consequence of the specific protocol used we tested other LTP-inducing protocols.

A weak LTP induction protocol (a single 10-impulse, 50 Hz train; cf. Wigstrom and Gustafsson 1983) resulted in a peak potentiation that almost reached that produced by the 3 \(\times\) 20-impulse stimulation (Fig. 2A). However, this potentiation thereafter decayed below the naïve level, reaching 79.0 \(\pm\) 8.4\% (\(n = 5\)) at 20 min posttetanus (Fig. 2A). Nevertheless, the fact that a single 10-impulse tetanus gives an LTP about one third of that given by a 3 \(\times\) 20-impulse tetanization suggests that LTP is rather easily induced in these slices with the tetanization procedure used (see METHODS). Consistent with this, a protocol stronger than 3 \(\times\) 20 impulses (6 \(\times\) 20 impulses) did not result in larger, or more stable, potentiation, in which the fEPSP was 104 \(\pm\) 3\% (\(n = 13\)) of the naïve level 20 min posttetanus (Fig. 2B). We next tested a theta burst protocol (10-four-impulse, 100-Hz bursts at 5 Hz; cf. Hoffman et al. 2002; Larson et al. 1986) that produced an LTP virtually indistinguishable from that produced by the 3 \(\times\) 20-impulse protocol, the potentiation of which was 94.0 \(\pm\) 6.3\% (\(n = 7\)) of the naïve level 20 min posttetanus (Fig. 2C). To possibly induce LTP beyond the level of the naïve synaptic strength we applied the LTP-inducing stimulation (3 \(\times\) 20 impulses) thrice with 20-min interval (Fig. 2D). This repetition reinduced the transient potentiation, but it did not produce any LTP beyond the level of the naïve synaptic strength, in which the fEPSP was 97.9 \(\pm\) 7.8\% (\(n = 5\)) of the naïve level 20 min after the last tetanization. It would thus appear that LTP in these developing synapses is nothing but a dedepression of the test-pulse depression.

These results would then predict that tetanization elicited from the naïve level should not produce any LTP. Thus the synapses were activated only twice (0.2 Hz) to establish the naïve level and were thereafter tetanized (3 \(\times\) 20 impulses). As shown in Fig. 3A, there was indeed no stable potentiation beyond the naïve level, in which the fEPSP was 100 \(\pm\) 6\% (\(n = 6\)) of the naïve level 20 min posttetanus. It can be noted that when this tetanization of a naïve input was evoked in the absence of NMDAR/mGluR activation [in \(d\)-AP5 (50–100 \(\mu M\)) and LY341495 (20–50 \(\mu M\))] to isolate NMDA/mGluR-independent plasticity (such as test-pulse depression), there was an immediate (<5 s after the last tetanus) depression to 60.6 \(\pm\) 2.1\% (\(n = 6\)) of the naïve level (Fig. 3B). This depression likely represents an immediate induction of the test-pulse depression, considering that \(I\) the level of depression reached after the 60 stimuli involved in the tetanization

![Fig. 1. Long-term potentiation (LTP) at developing CA3–CA1 synapses following test-pulse depression. A: experiment showing the field excitatory postsynaptic potential (fEPSP) depression that develops when stimulating a naïve synaptic input at 0.2 Hz, and the subsequent LTP induced by high-frequency stimulation (HFS = 20 impulses at 50 Hz repeated three times 20 s apart) applied to this test input and, simultaneously, to a separate conditioning input. Average fEPSPs taken at time points a, b, c, and d are shown on top. B: average (n = 13) time course of the 0.2-Hz-induced depression and subsequent HFS-induced LTP. C: same as in B, but the potentiation was followed for 50 min and the test stimulus frequency was 0.05 Hz (n = 5). D: tetanization in the combined presence of the N-methyl-D-aspartate receptor (NMDAR) antagonist d-2-amino-5-phosphonopentanoic acid (d-AP5, 50 µM) and the metabotropic glutamate receptor (mGluR) antagonist LY341495 (50 µM) does not result in any potentiation (n = 5). In 2 additional experiments, d-AP5 (50 µM) alone was sufficient to totally prevent the tetanus-induced potentiation.](http://jn.physiology.org/).
corresponded well to that given by 60 impulses at low frequency, 2) this depression obviously occludes further test-pulse depression, and 3) this tetanus-induced depression is less obvious when tetanizing a nonnaïve synaptic input (Fig. 1D). It would thus appear that high-frequency activation in itself is a strongly depressing stimulus to a naïve synaptic input. Conversely, the NMDAR activation occurring during high-frequency activation not only can produce dedepression of a nonnaïve synaptic input but also can prevent the inception of this depression in a naïve synaptic input. Figure 3C summarizes the results obtained using the different LTP induction protocols.

Developmental profile of LTP

The above-cited results indicate that test-pulse depression is a prerequisite for LTP at developing (≤P12) CA3–CA1 synapses. The number of AMPA-silent synapses is thought to decline with age (Durand et al. 1996; Hsia et al. 1998; Liao and Malinow 1996) and test-pulse depression appears also largely absent in adults (Abrahamsson et al. 2007; Xiao et al. 2004). LTP should then gradually (with age) be expressed as a potentiation beyond the naïve synaptic strength. To test this prediction the experimental protocol was applied to more mature synapses (P13–P25 and >P30). Figure 4, A and B shows that the test-pulse depression indeed decreased progressively with age, in which the fEPSP averaged 81.1 ± 2.9% (n = 18) and 92.1 ± 3.1% (n = 10) of the naïve level in the P13–P25 and >P30 groups, respectively. Moreover, LTP became more and more overshooting with age, being 137 ± 7.2% (n = 18) and 176 ± 11.6% (n = 10) of the naïve fEPSP in the P13–P25 and >P30 groups, respectively, compared with the 102 ± 4.0% (n = 13) in the P7–P12 group (Fig. 4, A and C). These results indicate that with increasing age LTP relies increasingly less on dedepression. The relative contribution of dedepression and of overshooting potentiation, respectively, for the LTP presently observed was calculated for various age groups and plotted in Fig. 4D. It can be seen that the contribution from dedepression (developmental LTP; see DISCUSSION) decreases from close to 100% at ≤P12, to about 30% at P15–P16 and to about 10% at P30. Conversely, overshooting LTP (mature LTP; see DISCUSSION) increases rapidly from close to nothing at ≤P12 to about 70% at P16.

Stable forskolin-induced potentiation at developing synapses also requires prior test-pulse depression

The preceding results thus suggest that LTP up to P12 is a distinct process from the LTP appearing later on, this early LTP being only a recovery of what is lost during the baseline stimulation. It has been described previously that at about this developmental stage (P7–P9) forskolin-induced PKA activation induces a potentiation of these synapses that largely occludes LTP and that becomes smaller with age (Yasuda et al. 2003). The present results would then predict that this forskolin-induced potentiation also should relate to the degree of prior test-pulse depression. We tested this prediction by applying forskolin under conditions of various degrees of induced depression. In general agreement with the results by Yasuda et al. (2003), we found that forskolin induced a potentiation in developing (Fig. 5A), but not in mature, CA3–CA1 synapses (Fig. 5A, inset). Evaluated from the baseline just preceding the application of forskolin there was a significant potentiation in developing (P7–P11) synapses (to 133 ± 3.7% of baseline, n = 15, P < 0.001; Fig. 5B), but no potentiation in mature (P30–P37) synapses (to 98 ± 4.3% of baseline, n = 3). Moreover, when applied to a naïve synaptic input (Fig. 5C), similar to LTP-inducing stimulation (cf. Fig. 3A), forskolin did not cause any stable potentiation but only a transient enhancement above the naïve level.

Forskolin-induced potentiation thus also appears to relate to the degree of prior depression and may thus be explained mainly by dedepression. Using whole cell recording both LTP and recovery from test-pulse depression (by stimulus interruption) are susceptible to washout (Abrahamsson et al. 2007; Malinow and Tsien 1990). In conformity with those results,
using whole cell recordings forskolin (20–50 μM) had only a small, statistically nonsignificant, effect on evoked EPSCs (to 113 ± 12% of the preceding baseline, n = 5, P = 0.34) (Fig. 5D) at developing synapses. We also found occlusion (at least partial) between forskolin-induced potentiation and LTP, LTP being reduced from 144 ± 5.1% (n = 13) to 120 ± 3.9% (n = 9) of the pretetanus level (20 min posttetanus, P = 0.002) (Fig. 5E).

Similar to LTP, the forskolin (20 μM) induced potentiation at developing synapses seemed limited to the naïve level, in which the fEPSP (in forskolin) was 105 ± 3% (n = 11) of the naïve level (cf. Fig. 5B). Increasing the forskolin concentration (to 40 μM) did not produce any larger potentiation (to 88 ± 5% of the naïve level, n = 4). These experiments with forskolin were performed on slices from rats between P7 and P11, but during this time period there was no trend in the amount of forskolin potentiation (r = −0.07, n = 15). In this context it should be noted that the forskolin-induced potentiation was not secondary to NMDAR activation (Otmakhov et al. 2004) since application of forskolin (20 μM) in the presence of 50 μM D-AP5 also resulted in a potentiation to about the naïve level (to 106 ± 6.9% of the naïve level, n = 3; data not shown).

Despite the small effect of forskolin on evoked EPSCs at developing synapses using whole cell recordings (see earlier text) we found a substantial increase in spontaneous EPSC frequency (to 171 ± 37% of the predrug control), without any effect on spontaneous EPSC amplitude (to 112 ± 9% of the predrug control, n = 4; data not shown). Such discrepancy between the effect on evoked and on spontaneous EPSCs might be explained by the finding that PKA-dependent phosphorylation of synaptotagmin-12 can increase spontaneous vesicle fusion without affecting evoked release (Maximov et al. 2007).

**DISCUSSION**

In contrast to other studies of LTP the present study has not examined LTP only against a background of a stable baseline of test-pulse-evoked synaptic activity. Instead, the present study of developing CA3–CA1 hippocampal synapses has also examined LTP against the background of the synaptic activity evoked by the very first test-pulse-evoked stimulation of these synapses, i.e., against a naïve (nonstimulated) level of synaptic transmission. This novel procedure is based on our previous finding that test-pulse stimulation of these developing synapses leads to a synaptic depression that may well be thought to interact with LTP, being based on AMPA silencing (Abrahamsson et al. 2007; Xiao et al. 2004). In fact, when evaluated against this naïve background it emerges from our results that prior to P13, Hebbian induction produces no stable potentiation above this naïve level. Thus when LTP-inducing tetanization is applied prior to the induction of the test-pulse depression (i.e., just after the very first few test-pulse stimulations of these synapses), no LTP, but only a stabilization of this naïve background level, is observed (Fig. 3). Furthermore, when tetanizing against a background of test-pulse-induced depression the resulting prolonged potentiation (conventionally an LTP) could not be made to exceed the naïve level of synaptic transmission (Figs. 1 and 2). These results would then suggest that what is seen as LTP in developing CA3–CA1 synapses (P7–P12) is nothing but a dedepression of test-pulse-induced synaptic depression. Nonetheless, since LTP is generally evaluated from a preceding stable baseline obtained using test-pulse stimulation, it still seems reasonable to describe this potentiation as an LTP rather than as a depression (which conventionally requires a preceding long-term depression).

Previous studies on developing hippocampus have suggested that the naïve level of synaptic transmission corresponds to a state in which CA3–CA1 pyramidal cell synapses are essentially not AMPA silent (Gröc et al. 2002; Xiao et al. 2004) and from which test-pulse stimulation creates AMPA silence in a subset of the synapses, the AMPA-labile synapses (Abrahamsson et al. 2007; Xiao et al. 2004) (Fig. 6). Following 10–20 min of interruption of test-pulse stimulation AMPA signaling largely returns to its naïve state, renewed test-pulse
stimulation again depressing it (Abrahamsson et al. 2007). That is, in the absence of evoked activity the AMPA-silenced synapses become unsilenced, although only to the AMPA-labile state (Fig. 6). The present results, together with these previous results, would then suggest that these test-pulse AMPA-silenced synapses can also be unsilenced by Hebbian induction, although to a different state—an AMPA-stable state (Fig. 6), from which they cannot become AMPA silenced by test-pulse stimulation, at least not within 1 h following the Hebbian induction. In fact, the present results suggest that LTP in the second postnatal week (before P13) is nothing but this unsilencing and stabilization of the silenced synapses. In conformity with this suggestion, this LTP was associated with a corresponding change in quantal content compatible with unsilencing (Xiao et al. 2004). In other words, prior to P13 Hebbian induction (and NMDAR activation) only initiates processes that help to stabilize AMPA signaling, i.e., convert AMPA-labile/AMPA-silenced synapses to AMPA-stable synapses (Fig. 6).

Developmental LTP

As noted in the introduction, LTP is thought to participate both in activity-dependent refinement of neuronal circuitry during brain development and in learning in the more mature brain. For the rat hippocampus-dependent learning seems to gradually emerge during the second half of the first postnatal month (Dumas 2005), suggesting that hippocampal LTP during the first two postnatal weeks is mainly an instrument for activity-dependent circuitry refinement. Such a refinement should be a selection of which synapses to keep and which synapses to eliminate rather than a strengthening of each individual synapse. The CA3–CA1 pyramidal cell connections during this time period are also single release site connections to keep or to eliminate (Groc et al. 2002; Hanse and Gustafsson 2001; Hsia et al. 1998). It would then seem that an LTP that via Hebbian induction conditions tends to stabilize AMPA-labile synapses, and thereby prevents their elimination, would be a suitable expression mechanism. In fact, based on our results we
suggest that this LTP, delimited to the early development, based on dedepression (unsilencing) of test-pulse-induced depression (AMPA silencing), is a specific form of LTP that could be referred to as developmental LTP. This LTP should then, according to our results, constitute virtually all of LTP up to P12, the fraction then decreasing to about 30% at P16 and to about 10% at >P30 (Fig. 4D). Such a decreased reliance with age on AMPA unsilencing as an LTP mechanism would also seem to comply with the situation in other cortical regions where AMPA-silent synapses are also scarce in the mature brain (Busetto et al. 2008; Isaac et al. 1997; Rumpel et al. 2004). Conversely, after P12, LTP must rely increasingly less on AMPA unsilencing as an expression mechanism, other mechanisms already being responsible for about 70% of LTP at P16. This overshooting LTP not based on unsilencing of AMPA-silent synapses is subsequently referred to as mature LTP.

Molecular Mechanisms. During the second and third postnatal weeks there is a shift in the kinase dependence of LTP (Li et al. 2006; Yasuda et al. 2003) and in the expression of AMPAR subunits, GluR4 and GluR2 long subunits decreasing their expression (Kolleker et al. 2003; Zhu et al. 2000). It may be presumed that such developmental changes may underlie the shift from developmental to mature LTP. With respect to kinase dependence Yasuda et al. (2003) found that LTP at about P7–P9, in contrast to mature LTP, did not rely at all on CaMKII activation, PKA activation being both necessary, and sufficient, for LTP. PKA activation appears to affect AMPAR trafficking such that PKA phosphorylation of GluR4 subunits (at ser842) and GluR2 long subunits (at ser841) is sufficient for synaptic insertion of AMPARs containing such subunits (Esteban et al. 2003; Qin et al. 2005). The present findings that forskolin application, like Hebbian induction, produced a stable potentiation, limited to the early development (P7–P11) illustrating the effect of forskolin (20–40 µM) on a test pulse depressed fEPSP. Average fEPSPs taken at time points a, b, c, and d are shown on top. The inset is from an experiment using a P41-old rat showing no effect of forskolin (50 µM). B: forskolin (20–40 µM) causes a complete dedepression in developing (P7–P11) CA3–CA1 synapses (n = 15). C: forskolin (20–40 µM) produces no stable potentiation above the naive synaptic strength. The synapses were activated only twice prior to 20 min of stimulus interruption during which forskolin was applied (P7–P11, n = 7). D: effect of forskolin on developing synapses measured using whole cell patch-clamp recordings (P7–P9, n = 5). E: interaction between forskolin-induced and tetanus-induced potentiation. Control LTP (closed circles, n = 13) and LTP obtained on top of forskolin-induced potentiation (open circles, P8–P11, n = 9).
increase in cAMP and PKA activity. Considering that GluR4 subunit expression peaks in the first postnatal week and is largely absent at P12 (Zhu et al. 2000), GluR2_\text{long} subunits are more likely to be involved in such a scenario (Kolleker et al. 2003). These subunits show peak expression in the second postnatal week, AMPARs containing such subunits can be synaptically inserted by spontaneous activity (and NMDAR activation), and such AMPARs participate in about a third of the synaptic response as late as P14 (Kolleker et al. 2003; Qin et al. 2005; Zhu et al. 2000). In fact, Kolleker et al. (2003) showed that AMPARs containing such subunits are responsible for about half of the LTP observed at P14, a proportion that agrees well with our estimated fraction of developmental LTP at that age (Fig. 4D). A PKA-mediated insertion of GluR2_\text{long} subunit-containing AMPARs has also been suggested to explain the LTP observed in GluR1-lacking mice (Jensen et al. 2003; Qin et al. 2005). This LTP was more than half the magnitude of wild-type LTP at P14, thereafter diminishing to some 30% of wild-type LTP at P35 (Jensen et al. 2003). This agreement in age dependence (compare with Fig. 4D) indicates that this LTP may well correspond to the developmental LTP presently described.

Conversely, the requirement for CamKII activation for synaptic insertion of GluR1-containing AMPARs (Hayashi et al. 2000), the low level of CamKII at this early time period (Kelly et al. 1987), and the lack of effect of CamKII inhibition on early LTP (Yasuda et al. 2003) would suggest that GluR1 subunit-containing AMPARs should be scarcely involved in developmental LTP. In fact, even as late as P14 the LTP observed in GluR1 knockouts is not much less than that in wild-type (Jensen et al. 2003).

This scenario predicts that LTP up to P12 would be fully blocked by PKA inhibition and not be affected by CamKII inhibition. However, although Yasuda et al. (2003) found no effect of CamKII inhibition at P7–P9, they noted some partial blockade by CamKII inhibition already at P9–P10; however, another study reported no effect at all by CamKII inhibition at P12–P16 unless combined with PKA or protein kinase C (PKC) inhibition (Wikstrom et al. 2003). The exact date for transition in the kinase dependence of LTP can be modulated, e.g., by neonatal stress (Huang et al. 2005), which may explain why we observe only developmental LTP (dedepression) when others observe a CamKII-dependent LTP component. Alternatively, dedepression may also shift in its kinase dependence with age. Obviously, further studies are needed to clarify this issue.

**EXPRESSION MECHANISMS.** As noted earlier, it seems reasonable that developmental plasticity should be a selection of which synapses to keep and which synapses to eliminate rather than a strengthening of an individual synapse. Therefore an LTP based on unsilencing and stabilization of AMPAR-mediated transmission seems suitable. Indeed, the pioneering studies proposing AMPA unsilencing as the main mechanism for LTP at the CA3–CA1 synapse were all performed on developing synapses (younger than 3 wk) (Durand et al. 1996; Isaac et al. 1995; Liao and Malinow 1996; Liao et al. 1995). Nevertheless, other mechanisms for expression of LTP at developing CA3–CA1 synapses have been shown. Lauri et al. (2006) showed that LTP induction results in increased release probability due to removal of tonic kainate receptor-mediated presynaptic inhibition. However, this LTP was restricted to low release probability synapses and to the first postnatal week (P3–P6). Benke et al. (1998) reported that LTP examined at P13–P15 (see also Palmer et al. 2004) was explained mostly by an increased potency, possibly mediated via a CamKII-medi-
ated phosphorylation of GluR1 subunits at ser831, producing a larger single-channel conductance. Our data would not suggest such an expression mechanism either to dominate at P13–P15 or to be conspicuous already at P12 (Palmer et al. 2004). However, as discussed earlier, the exact age for transition may depend on experimental conditions. Moreover, the use of EPSCs evoked by minimal stimulation in whole cell experiments may introduce sampling problems compared with using field recordings.

**Short-term potentiation above the naı\-ve level**

In addition to producing depression Hebbian induction led to a short-term potentiation (STP) decaying within 10–15 min. This STP exceeded the naïve level and should then not be explained by AMPA unsilencing. Interestingly, an STP was also observed when forskolin was applied, although only when test-pulse stimulation was begun some time after the start of forskolin application (Fig. 5C). When examined in somewhat older animals (≥P14) STP was not present in GluR1-lacking mutants, suggesting a role for GluR1 subunit-containing AMPARs in STP (Jensen et al. 2003). Such AMPARs are expressed to a high degree in the second postnatal week, but require not only PKA- but also PKC/CamKII-mediated phosphorylation for their stable synaptic insertion (Esteban et al. 2003). The question then arises whether PKA activation may be sufficient for synaptic insertion of GluR1 subunit-containing AMPARs in an unstable manner, these AMPARs then being removed by test-pulse stimulation. Indeed, the decay of tetr

**Developmental versus mature LTP**

Our results suggest that a distinct new LTP process is added at about P12 that within a few days becomes the dominating LTP component (Fig. 4D). As noted earlier, one possibility is that this added component involves a phosphorylation of AMPARs increasing AMPAR single-channel conductance, thus increasing the strength of the single release site connection. Nonetheless, mature LTP in the CA3–CA1 connection is also thought to involve the incorporation of postsynaptic AMPARs (Malinow and Malenka 2002) and be associated with changes in quantal content, rather than quantal size (for references see Lisman and Raghavachari 2006). The present analysis then shows that such an AMPAR incorporation (associated with change in quantal content) is not explained by unsilencing of test-pulse AMPA-silenced synapses. However, we cannot exclude an AMPAR incorporation based on the creation of new independent AMPA modules within a synapse (Fig. 6), as suggested by Lisman and Raghavachari (2006) (see also Edwards 1995). In fact, the emergence of mature LTP after P12 (Fig. 4D) coincides in time with CA3–CA1 connections starting to exhibit more than one release site (Hsia et al. 1998). Moreover, although multivesicular release is not found electrophysiologically at the CA3–CA1 connection at early developmental stages (Hanse and Gustafsson 2001), it has been found, using imaging of NMDA calcium transients, in large synapses at later stages (>P16) (Conti and Lisman 2003; Oertner et al. 2002). The change in LTP at ages >P12 also coincides in time with developmental changes in the expression of scaffolding proteins that have been implicated in synaptic maturation and LTP, such as PSD-95/PSD-97 instead of SAP-102 (Elias et al. 2006; Sans et al. 2000). It may be presumed that such developmental changes may create conditions for the addition of independent AMPAR modules at existing synapses.

Thus although our results suggest the addition of a distinctly new LTP process at about P12, this LTP does not have to be fundamentally different from that prior to P12, both developmental and mature LTP being expressed by the creation of an AMPA-stable module. Nonetheless, the developmental LTP would be suited to refine the activity-independent formation of synaptic contacts by selective stabilization of appropriate single release site connections, resulting in a network of uniformly strong connectivity. The mature LTP, on the other hand—not being expressed by just unsilencing/stabilization of an already existing connection but by a stronger connection between cells with correlated activity—could create functionally tightly connected cell assemblies, as a basis for information storage, within this network.

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