Reversal of hippocampal LTP by spontaneous seizure-like activity: role of group I mGluR and cell depolarization.

Bin Hu#, Sergei Karnup#, Lei Zhou, Armin Stelzer*
Department of Physiology and Pharmacology,
State University of New York
450 Clarkson Avenue, Box 29
Brooklyn, New York, 11203, U.S.A.

# Contributed equally

*Corresponding author
Tel: 718-270 3873
Fax: 718-270 2241
E-mail: armin.stelzer@downstate.edu

14 figures

Key words:
hippocampus, LTP, CA1, depotentiation, ictal activity, GABA_R, group I mGluR, depolarization

Abbreviations:
Bic: Bicuculline
PTX: picrotoxin
[Ca^{2+}]_e: extracellular calcium
LTP: long-term potentiation
LTD: long-term depression
SDP: spontaneous depotentiation
TBS: theta-burst stimulation
HFS: high-frequency stimulation
LFS: low-frequency stimulation
The terms ‘spontaneous depotentiation’ (‘SDP’) and ‘reversal of LTP’ were used interchangeably
Abstract. Memory impairment is a common consequence of epileptic seizures. The hippocampal formation is particularly prone to seizure-induced amnesia due to its prominent role in mnemonic processes. We used the isolated CA1 slice preparation to examine effects of seizure-like activity on hippocampal plasticity, long-term potentiation (LTP) and long-term depression (LTD). Repeated spontaneous ictal events, generated in the presence of antagonists of GABA<sub>A</sub> receptor function, led to a stepwise erasure of LTP (termed spontaneous depotentiation, SDP). SDP could be initiated at various stages of LTP consolidation (tested up to 120 min following the induction of LTP). Renewed tetanic stimulation re-established LTP. SDP was remarkably specific: baseline transmission and other forms of hippocampal plasticity, i.e., Ca<sup>2+</sup>-induced LTP and two forms of LTD (DHPG- and LFS-mediated) were not affected by the same type of seizure activity. Group I mGluR activation was necessary for the induction of SDP. SDP was blocked in the presence of the group I mGluR antagonist 4-CPG. The mGluR1 antagonist LY367385 blocked about 80%, the mGluR5-specific antagonist MPEP blocked about 30% of SDP. Most efficient implementation of SDP was observed during seizures in the combined presence of the group I mGluR agonist DHPG and the GABA<sub>A</sub> antagonist Bicuculline. However, similar ictal activity generated in the presence of DHPG alone, did not lead to SDP in the vast majority of recordings. Tonic cell depolarization of CA1 pyramidal cells was identified as a critical mechanism underlying SDP: (a) cells were significantly depolarized during the depotentiating pharmacological protocol DHPG+Bic; (b) hyperpolarization by negative current injection prevented intracellular SDP under depotentiating pharmacological conditions; (c) depolarization by positive current injection led to selective intracellular SDP in the non-depotentiating seizure protocol of DHPG.
Introduction

Memory loss is a common consequence of epileptic seizures (23; 36; 60) or therapeutic electroconvulsive stimulation (55). Although seizure-induced memory loss can persist for several months, it can occur without other neurological deficits or structural brain damage (66). Seizure-induced amnesia is especially frequent in patients with temporal lobe epilepsy and a particularly strong link between seizure activity and memory loss can be found in the hippocampal formation due to the hippocampus’ prominent role in memory consolidation processes (23; 42; 72).

Long-term potentiation (LTP) and its counterpart, long-term depression (LTD) are widely regarded as cellular models of learning mechanisms (7; 9; 27; 29; 44; 64). Given the prominent role attributed to LTP/LTD as cellular models of memory on the one hand and clinical observations of memory-impairing effects of seizures on the other, surprisingly few studies have examined seizure effects on synaptic plasticity. A reversible loss of LTP was seen in CA1 in vivo following stimulation-induced seizure activity (25). It was not clear, however, whether the loss of LTP was caused by seizures per se or spreading depression that followed seizures (25). The hippocampal slice (1) was the preparation of choice in more recent investigations of seizure effects on plasticity. LTP-like effects were observed in the disinhibited slice during interictal-type of epileptiform activity (8; 53). Both LTP- and LTD effects were reported using the potassium model of in-vitro epilepsy (14). The LTP induction process was impaired during post-ictal depression (3; 43).

In this study we used the recently described disinhibition model of electro-encephalographic seizure-like activity in the isolated CA1 slice preparation (30) to examine effects of seizures on hippocampal plasticity. The first objective was to examine whether seizures exerted specific effects: did seizures affect EPSPs in general
(including control EPSPs) or more specifically (i.e., only potentiated or depressed EPSPs)? Specific effects on synaptic plasticity are an important criterion for the validity of a given cellular model of seizure-induced amnesia. It was shown that the specific loss of LTP - by brief perfusion of high [K⁺] plus glutamate - was transient. In contrast, lasting (>1 h) depression by longer high-[K⁺]/glutamate perfusion was not specific as it was accompanied by a general failure of axonal responsiveness (24). A specific depression of potentiated EPSPs, however, was reported in an earlier in-vivo study in CA1: the seizure-induced complete depression of potentiated EPSPs recovered to or above pre-tetanization baseline suggesting a specific reversal of potentiated EPSPs (25). Another question concerning specificity is which type of plasticity would be affected by seizures. Besides stimulation-induced LTP (9) - which is most frequently linked to learning and memory mechanisms - several other forms of hippocampal plasticity have been described in recent years, e.g., Ca²⁺-induced LTP (65) and two forms of LTD, one evoked by low-frequency stimulation (19; 45), another by DHPG application (2; 32). The question whether ictal events affected hippocampal synaptic plasticity in general or more selectively was addressed by subjecting different forms of hippocampal plasticity to the same seizure protocol. We show that the reversal of stimulation-induced LTP, termed spontaneous depotentiation (SDP), was the only effect of seizures in this model: baseline EPSPs and other forms of hippocampal plasticity, i.e., Ca²⁺-induced LTP and two forms of LTD (DHPG- and LFS-mediated) were not affected by ictal activity. This remarkable specificity indicates that SDP may serve as a useful in-vitro model of seizure-induced amnesia.

The second objective was to examine cellular mechanisms of SDP. Although the pharmacological blockade of GABAₐ receptor function was the only and thus sufficient experimental means for the induction of SDP, a strong
activation of glutamate receptors (by tetanic stimulation and spontaneous ictal activity) was an integral part of the SDP protocol. We examined a possible role of group I mGluR in the induction of SDP. Group I mGluRs were shown to undergo long-term activation by seizure-like activity (22; 35; 69; 71) and LTP-inducing tetanization (10; 20; 52). In particular, a long-term synaptic activation of group I mGluR was shown under similar experimental conditions, i.e., during prolonged epileptiform discharges induced by Bicuculline and 4-AP (35). The diversity of mGluR subtypes and their different effects on neuronal excitability present a complex and often controversial picture, notably in the study of synaptic plasticity (2). The eight cloned subtypes of mammalian mGluRs are divided into three groups based on their respective primary structures, transduction pathways and pharmacological properties (47; 50). Hippocampal function is regulated by all three groups of mGluRs (2). We focused on group I mGluR. The two main subtypes of group I mGluR, mGluR1 and mGluR5 share similar transduction pathways - leading to the activation of phospholipase C and phosphoinositide hydrolysis - but their cellular effects are different (37). The rationale for focusing on group I mGluR was twofold. First, the pharmacological blockade of group I mGluR does not affect SDP-triggering ictal activity itself (frequency of events, ictal duration or any other parameter) in the applied seizure model (30). In contrast, the general blockade of mGluR by the broad-spectrum antagonist MCPG compromised ictal activity by reducing the frequency of epileptiform events and impairing the development of ictal components, notably the second and third burst component (30). The second reason to study effects of group I mGluR lies in its profound impact on CA1 neuronal excitability via cell depolarization and increased firing of CA1 neurons in both principal cells and interneurons (12; 15; 16; 37). We show that group I mGluR activation and its depolarizing effect on CA1 neurons played a critical role in the
implementation of SDP: depolarization of CA1 neurons promoted SDP in a non-depotentiating seizure model. In contrast, cell hyperpolarization prevented SDP in a depotentiating seizure model.
Methods

Slice preparation: Transverse hippocampal slices were obtained from adult guinea-pigs (Hartley, from Harlan Sprague Dawley, Inc., Indianapolis, IN; 150-200 g). Guinea-pigs were anesthetized by inhalation of halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) before decapitation with an animal guillotine (in conformation with the guidelines of the Institutional Animal Care and Use Committee (protocol 9808069)). After removal of the brain and isolation of the hippocampus, slices of 450 μm thickness were cut on a vibrotome (Series 1000, TPI, St.Louis, MO) in ice-cold ACSF. CA1 ‘mini’ slices were created by dissecting CA2/3 and the subiculum under microscopic control. Slices were superfused in an interface recording chamber (Fine Science Tools, Belmont, CA) with a solution saturated with 95% O2/5% CO2 (temperature 30-32°C) of the following composition (in mM): NaCl 118, KCl 3, NaHCO3 25, NaH2PO4 1.2, MgCl2 1.7, CaCl2 2.0, D-glucose 11.

Recordings. Recording electrodes (World Precision Instruments, Inc., Sarasota, FL.) were pulled by a Brown-Flaming electrode puller (Model P-87, Sutter Instrument Co., Novato, CA). Extracellular recordings were carried out in stratum radiatum of CA1. Sharp-electrode intracellular recordings were performed in CA1 pyramidal cell somata (n=41 and apical dendrites (n=18). Dendritic pyramidal cell recordings were identified by the recording site in stratum radiatum (100-250 μm perpendicular to stratum pyramidale) and the burst response to suprathreshold current injection (70). Tracking was performed using manually controlled hydraulic stepping micromanipulators (Narashige). Electrodes were filled with potassium acetate (2-3 M) yielding electrode resistances of 42-97 MΩ. Postsynaptic potentials (EPSPs) were elicited by single stimuli delivered to the Schaffer collateral-commissural pathway at 30-s intervals through a pair of insulated tungsten bipolar electrodes.
(stimulation range 15-50 µA). In most experiments, EPSPs were measured in response to stimulation of two independent afferent pathways (both in stratum radiatum, but opposite with respect to the recording electrode). Signals were recorded and amplified with an “Axoprobe-1A” (Axon Instruments), fed into an A/D converter (Digidata 1200, Axon Instruments) digitized, stored and analyzed off-line using “pCLAMP8” software from Axon Instruments in a Pentium PC computer.

Data analysis. The strength of synaptic excitatory responses was assessed by measuring the slope (20-80%) of the EPSP rising phase. Data were pooled through averaging and normalization. Control values were recorded for 20 to 40 min prior to tetanization. Comparisons of synaptic strength at stated points of time following tetanic stimulation (e.g., 120 min following tetanization) are based on 10 measurements over 5 min periods (5 measurements were obtained before and 5 measurements were obtained after the stated point of time). Values are depicted as mean and standard error (mean ± SE). Statistical comparisons of EPSPs were performed by Student’s t test (two groups) or ANOVA (three or more groups). Statistical significance was accepted for all p < 0.05.

Seizures: Seizure-like activity in the CA1 minislice preparation was triggered by the competitive GABA<sub>A</sub> receptor antagonist Bicuculline-methiodide (Bic, 50-100 µM) or the chloride channel blocker picrotoxin (PTX, 100-200 µM) or the group I mGluR stimulator (RS)-3,5-dihydroxyphenylglycine ((RS)-3,5-DHPG), 30-60 µM) as recently described in detail (30). Extracellular calcium ([Ca<sup>2+</sup>]<sub>e</sub>) was used to control epileptiform activity. Ictal events were reliably observed in the presence of control [Ca<sup>2+</sup>]<sub>e</sub> (2 mM), but completely blocked during elevated [Ca<sup>2+</sup>]<sub>e</sub> (6 mM).

LTP. LTP was normally triggered by theta burst stimulation (TBS, 3-4 trains of 4 pulses at 100 Hz separated by 200 ms
repeated 2-4 times in 30 s intervals (5; 33). In some recordings (figs. 3Bb,6), high-frequency stimulation (HFS, 1-2 trains, 1 s, 100 Hz, at test pulse strength) was applied to induce LTP. The term ‘tetanic stimulation’ was used for both TBS and HFS. Experiments were designed to ensure proper induction of LTP (or LTD) uninfluenced by impairing effects of post-ictal depression (3; 43). For example, when ictal activity was present during pre-tetanization controls, tetanic stimulation was only applied after full recovery from post-ictal depression. Ca^{2+}-induced LTP was implemented by increasing [Ca^{2+}]_{e} from 2 mM controls to 6 mM (fig. 4D).

LTD. LTD was induced by low-frequency stimulation (LFS, 1 Hz for 10 min at test pulse strength)(fig. 7B). mGluR-LTD was induced through transient application of DHPG (30 - 60 µM)(fig. 7A).

**Drugs:** Bicuculline-methiodide (Bic), picrotoxin (PTX) (from Sigma, St. Louis, MO), (RS)-3,5-dihydroxyphenyglycine ((RS)-3,5-DHPG)), (S)-4-carboxyphenylglycine (4-CPG), (S)-(+)-α-amino-methylbenzeneacetic acid (LY367385) and 2-methyl-6-(phenylethynyl)-pyridine (MPEP)(from Tocris Cookson, Inc.; Ballwin, MO) were applied by bath perfusion.
Results.

Spontaneous ictal events result in depotentiation.

Individual recordings in figures 1 and 2 illustrate the basic finding of this study: seizure-like events which occurred spontaneously during the blockade of GABA<sub>A</sub>-receptor function in the isolated CA1 subfield (30) caused a stepwise reversal of LTP. LTP was elicited at the Schaffer collateral/CA1 synapse by theta-burst stimulation (if not otherwise stated). The most frequently observed pattern of depotentiation (in > 90% of recordings) is illustrated in figure 1: each ictal event was followed by a large, but transient (1-5 min) post-ictal depression (in some cases below pre-tetanization controls). The recovery from post-ictal depression was not complete, however, leaving a small, but long-lasting depression. Only these lasting decreases of fEPSPs after recovery from transient post-ictal depression are referred to as spontaneous depotentiation (“SDP”) or ictal-induced LTP reversal in the following. EPSP peaks (fig. 1Aa) and slopes (fig. 1Ab) exhibited the same time courses of depotentiation.
Figure 1: Stepwise depression of potentiated EPSPs following seizure-like events. A, orthodromically evoked fEPSPs in individual recording, peaks (a) and slopes (b). Test pulses were delivered every 30 s. Theta-burst stimulation (TBS) was applied at t = 0 min. Positive values of “t” denote the times after LTP induction, negative values the times before. Ictal events are marked by arrows (the third is displayed in inset). 100 µM Bic and 2 mM [Ca\(^{2+}\)]\(_e\) were present throughout. c, representative fEPSP responses (average of n = 5) before (1), shortly after TBS (2) and after complete depotentiation (3). B, depression of potentiated fEPSPs depicted as a function of the nth ictal event. Top graph illustrates the calculation of depotentiation steps (exemplified for the first three ictal events after the induction of LTP using the same data as in Ab). SDP was evaluated by averaging EPSPs between ictal events after complete recovery from transient post-ictal depression (averages are marked by horizontal bars). Bottom (b), histogram of averaged fEPSPs
depicted as a function of the $n^{\text{th}}$ ictal event (based on data shown in Ab).

The observation that EPSPs remained at a somewhat lower level after each ictal event is illustrated in more detail in figure 1Ba for the first three ictal events following the induction of LTP. The histogram in figure 1Bb depicts the averaged value of fEPSPs between ictal events as a function of the $n^{\text{th}}$ ictal event. It illustrates that the accumulation of small depression steps after each ictal event (10.1 ± 1.3 % on average in this particular recording) resulted in a complete depotentiation after the 10$^{\text{th}}$ ictal event.

A different depotentiation pattern is shown in figure 2. Fewer seizures caused larger depotentiation steps. Although less frequently observed (in only 5 of 67 recordings), this depotentiation pattern highlights three important properties of SDP. First, it illustrates more clearly the temporal link between ictal events and individual depotentiation steps. Only two ictal events (marked by arrows at $t = 40$ min and 47 min, resp.) led to a combined 64% reversal of potentiated fEPSPs. After each event, fEPSP remained at the partially depotentiated levels (in the absence of further ictal events). The coincidence of seizures and depotentiation steps indicates that seizures were instrumental in the reversal of LTP. Second, transient post-ictal depression was absent in the recording in figure 2. Thus, lasting depotentiation was not contingent upon effects of post-ictal depression or even spreading depression as suggested in an earlier in-vivo study (25). A third property featured in figure 2 is that seizure-induced depotentiation could be reversed by renewed tetanic stimulation in agreement with previous reports (14; 24; 25; 43). Reversal of SDP was seen after partial depotentiation (figs. 2 and 4C), but also after complete depotentiation (not shown). Renewed LTP exhibited the same behavior as LTP established by the first TBS, i.e., it was maintained in the
absence of ictal activity, but exhibited depotentiation during ictal activity (not shown).

Figure 2: Ictal-induced depotentiation in the absence of transient post-ictal depression. Individual recording of orthodromically evoked fEPSPs (50 µM Bic was present throughout). The first TBS was applied at t = 0 min. Ictal events at t = 40 min (shown as inset) and at t = 47 min are marked by arrows. A second TBS was applied at t = 60 min. Bottom, representative fEPSPs (average of n = five responses) at marked points of time (denoted "1 - 3").
Specificity of SDP. Two-pathway experiments demonstrate that only potentiated EPSPs were affected by seizures (see summary graphs in figure 3A): EPSPs were evoked at two independent stimulation sites (both in stratum radiatum, but opposite with respect to the recording electrode (termed paths 1 and 2, respectively). Only path 1 was tetanized. Similar to the individual recordings of figures 1 and 2, a lasting decrease of potentiated EPSPs was observed following each ictal event. On average, depotentiation after each ictal event was 5.9 ± 1.2 % (based on n = 281 ictal events in 16 different recordings). Depotentiation steps accumulated until pre-TBS baseline values were reached and remained at pre-tetanization baseline regardless of whether seizure activity was present (e.g., fig. 3Aa) or discontinued (fig. 4Ab after t = 160 min). Ictal activity had no long-term effect on all controls, i.e., EPSPs in both pathways before tetanization and also EPSPs of the non-tetanized paths 2 throughout (fig. 3A). Depotentiated EPSPs of paths 1 became statistically equal with those of the non-potentiated paths 2 at t = 82 ± 9 min. At t = 120 min, averaged and normalized EPSP slopes were 1.05 ± 0.08 in paths 1 and 1.04 ± 0.06 in path 2 (p = 0.34, n = 16). The strength of afferent input was unchanged as shown by the amplitude of afferent volleys in the tetanized paths 1 (fig. 3Ad). In summary, we demonstrate one component of specificity - arguably the most critical one - in that seizures affected potentiated EPSPs, but not baseline EPSPs or EPSPs after complete depotentiation.

Tetanization had increased the overall duration of ictal activity by 46% on average (fig. 3Ac). This increase was due to an increase of the frequency of ictal events as the average duration of a given ictal event was the same before and after TBS (6.3 ± 1.8 s, n = 379 episodes in 16 recordings, pre- and post-TBS ictal events lumped together; fig. 3Ab). As a function of the n\textsuperscript{th} ictal event, SDP was completed after 17 ictal events on average (fig. 5B).
Figure 3: Properties of SDP. Aa, summary graphs of orthodromically evoked fEPSP (slopes, averaged, normalized, n=16) recorded in stratum radiatum of the CA1 minislice (50-100 µM Bic, 2 mM [Ca²⁺]₀). EPSPs were evoked at two independent stimulation sites, both in stratum radiatum, but opposite with respect to the recording electrode (paths 1 and 2, resp.). Here and in the following, pooled data of path-1 EPSPs and ictal activity are shown in red. Distances between stimulation and recording electrodes were between 0.7 and 1 mm. Test pulses were alternately delivered every 15 sec to paths 1 and 2, resp. Stimulation intensity of test pulses was adjusted to generate between 30% and 50% of the maximal response during control recordings (before TBS). After fEPSPs in both pathways were stable for at least 20 min (control responses from -20 to 0 min),
TBS was applied (at t = 0 min) to path 1: in 10 recordings at the CA2/3 site, in 6 recordings at the subicular site. Afferents of the respective second pathways (path 2) were not tetanized. b, averaged duration of single ictal events within 5 min bins. c, averaged overall duration of ictal activity (number of events times their duration within 5 min bins). d, afferent volleys (amplitudes; averaged, normalized). B, maintained potentiation in the absence of ictal activity: a, summary graphs of averaged, normalized fEPSPs (n = 14, filled squares) recorded in the presence of 6 mM [Ca$^{2+}$]$_o$ and 50-100 µM Bic (present throughout). Graph of depotentiating EPSPs (path 1 in figure 3A) is superimposed for comparison. b, The same protocols were applied as in a, except that potentiation was induced by high-frequency stimulation (3 tetani, each 1 s, 100 Hz, 20 s apart) instead of TBS.

**Maintained potentiation in the absence of ictal activity.**

Data in figure 3B show that potentiation was maintained (> 2 hours) when ictal activity was blocked (in the presence of 6 mM [Ca$^{2+}$]$_o$; see Methods): fEPSPs slopes were 1.87 ± 0.14 of pre-TBS controls at t = 120 min (n = 8; p < 0.0001 compared with depotentiated values obtained in the presence of 2 mM [Ca$^{2+}$]$_o$; fig. 3Ba). Figure 3Bb illustrates the same behavior for LTP induced by HFS (1 or 2 trains at 100 Hz, each 1 s duration, 20 s apart, test-pulse strength). Similar to TBS-induced potentiation (fig. 3Ba), HFS-induced potentiation was reversed during ictal activity (2 mM [Ca$^{2+}$]$_o$, 50-100 µM Bic present throughout), but maintained (> 2 hours) in the absence of ictal activity (6 mM [Ca$^{2+}$]$_o$, 50-100 µM Bic, present throughout; fig. 3Bb). EPSPs were 1.96 ± 0.06 at t = 120 min (n=5) in the absence of ictal activity and 0.83 ± 0.12 in the presence of ictal activity (n = 7; p < 0.00001). No further attempts were made to examine possible mechanisms of the small depression after complete SDP in the HFS protocol.
Initiation of SDP at different stages of LTP consolidation.
The question was asked whether seizures would impact different phases of LTP consolidation. Clinical observations show that memories occurring in close proximity to seizures are disproportionately impaired. Analogously, it can be expected that earlier phases in the LTP consolidation process would be more vulnerable to seizures. Previous studies had demonstrated that seizures interfered with the LTP induction process (3; 43). But reversal of LTP following seizures - shown in vivo - was also effective, at least, partially, at various intervals (measured up to 60 min) following the initial induction of LTP (25).

To examine whether different stages of LTP consolidation were vulnerable to seizure activity, ictal activity was initiated at various points of time after LTP induction (fig. 4). Control and potentiated EPSPs were initially recorded in the absence of ictal activity (in 6 mM [Ca\(^{2+}\)]\(_o\)) during which LTP was maintained (as shown in figure 3B). Ictal activity was then initiated at different points of time following TBS, i.e., 30 min (in PTX, fig. 5A), 40 min (fig. 4C), 60 min (fig. 4A) and 120 min (fig. 4B) by lowering [Ca\(^{2+}\)]\(_o\) to 2 mM. Such delayed initiation of ictal activity led to SDP in 17 of 19 recordings. In these 17 recordings, SDP occurred in close temporal correlation with the delayed onset of ictal activity (in 5/5 recordings after 30 min of potentiation as in fig. 5A, in 7/8 recordings after 60 min potentiation as in fig. 4A and in 5/6 recordings after 120 min potentiation as in fig. 4B). The properties of delayed SDP initiated after maintained potentiation (figs. 4,5A) were similar to those of immediate SDP (figs. 1-3): small depotentiation steps after each ictal event accumulated leading to complete depotentiation. Figure 4C illustrates that - similar to immediate SDP - tetanic stimulation after delayed SDP led to maintained LTP in the absence of ictal activity. The times from seizure onset to SDP completion, i.e., when EPSPs of the potentiated paths 1
and the non-tetanized paths 2 became statistically equal, were 61 ± 9 min when seizure onset was delayed by 30 min (n=5, data not shown), 64 ± 11 min when seizures were delayed by 60 min (n=7, fig. 4A) and 62 ± 13 min when seizures were initiated 120 min after LTP induction (n=5, fig. 4B). These data demonstrate that seizures were capable of disrupting different phases of LTP consolidation with equal efficacy. This observation corresponds to stimulation studies using strong stimulation paradigms: theta bursts at high stimulation intensities which were shown to reverse LTP at later stages of consolidation (4) whereas depotentiation by weaker low-frequency stimulation protocols were only effective within a very narrow time window following LTP induction (see (26)).
Figure 4: Delayed SDP after maintained potentiation. A, normalized, averaged fEPSPs (b, n=7) and averaged duration of ictal activity (in 5 min bins) (a). Maintained potentiation (in the absence of ictal activity during 6 mM [Ca^{2+}]_e, path 1) was reversed by ictal activity introduced at t = 60 min by 2 mM [Ca^{2+}]_e. Bic (50-100 µM) was present throughout. Discontinuation of ictal activity (by raising [Ca^{2+}]_e to 6 mM at t = 160 min) did not reverse SDP. Path 2 was not tetanized. B, same experimental protocol as in A except that ictal activity was initiated at t = 120 min (n = 5) and was not discontinued after completion of SDP. C, individual recording of fEPSPs: maintained potentiation during 6 mM [Ca^{2+}]_e in the absence of ictal events was disrupted following ictal activity (marked by arrows) in the presence of 2 mM [Ca^{2+}]_e. The re-application of 6 mM [Ca^{2+}]_e at t = 108 min blocked ictal activity and resulted in stabilization of partially depotentiated fEPSPs. A second TBS led to maintained potentiation in the absence of ictal activity (at t > 125 min). Db, Ca^{2+}-induced LTP is not affected by ictal activity. Elevation from [Ca^{2+}]_e from 2 to 6 mM led to maintained potentiation of fEPSPs ("Ca^{2+}-induced LTP"). Duration of ictal activity is depicted in upper panel (a). Renewed onset of ictal activity (at t = 50 min; by lowering [Ca^{2+}]_e from 6 mM back to 2 mM) did not affect Ca^{2+}-potentiated EPSPs.

The pattern of reversibility was the same after immediate and delayed SDP. Renewed tetanic stimulation led to a reversal of SDP (compare figs. 2 and 4C), but SDP was not reversed in the absence of tetanic intervention regardless of whether ictal activity was present or discontinued. Figure 4A shows that fEPSPs remained at the depotentiated baseline after ictal activity was discontinued (from t = 160 min to t = 245 min). Similarly, depotentiated EPSPs did not recover when ictal activity was discontinued after partial SDP (as shown in figure 4C).

Reversal of LTP during PTX-induced ictal activity. Bic-methiodide-containing solution was routinely used because it
prompted ictal activity reliably and more frequently than other antagonists of GABA<sub>A</sub> receptor function (30). But Bic-methiodide was linked to several GABA<sub>A</sub>R-unrelated effects, notably block of Ca<sup>2+</sup>-activated K<sup>+</sup> conductances (54). To examine whether seizures caused by the blockade of GABA<sub>A</sub> receptor function were responsible for SDP, stimulation-evoked LTP was subjected to ictal activity generated by the chloride-channel blocker PTX (instead of Bic; (30); fig. 5). The delayed SDP protocol (as in figs. 4A-C) was applied. Figure 5Ab illustrates that PTX-induced ictal activity (initiated at t = 30 min) led to progressive SDP similar to previously featured experiments during Bic-induced seizures. The time course towards the completion of SDP, however, was considerably longer during PTX compared with recordings during Bic: fEPSPs of the potentiated and non-tetanized paths became statistically indistinguishable for at t = 140 min, i.e., 110 min following ictal onset as opposed to t = 71 min following ictal onset in Bic (n = 28; this number is based on lumped recordings following immediate and delayed SDP; see figs. 2A,4A,B). But when depicted as function of the n<sup>th</sup> ictal event, EPSPs were statistically the same after each ictal event, (except the 17<sup>th</sup>) in the presence of Bic and PTX, respectively (fig. 5B). On average, complete reversal of LTP in PTX was seen after the 18<sup>th</sup> event. The most probable explanation for the longer time course of SDP during PTX-induced ictal activity can be found in the lower frequency of ictal activity (compared with Bic-containing solution; fig. 5Aa). The depotentiating efficacy of a given ictal event, however, was the same in the presence of the competitive GABA<sub>A</sub>R blocker Bic and the chloride-channel blocker picrotoxin. These data support the notion that SDP was triggered by disinhibition-induced ictal activity. In addition, these data demonstrate that SDP was implemented by seizures occurring at considerably lower frequency (compared with the Bic protocol).
Figure 5: LTP is reversed by PTX-induced ictal activity. A, summary graphs of orthodromically evoked fEPSPs (slopes, averaged, normalized, n = 7) in the presence of picrotoxin (PTX, 100-200 µM, applied throughout). TBS was applied to path 1 at t = 0 min. Ictal activity was initiated at t = 30 min lowering [Ca\(^{2+}\)]\(_e\) to 2 mM (from 6 mM). Ictal duration (averaged over 5 min intervals) is shown in top panel (a). B, fEPSPs of the potentiated path 1 (averaged, normalized) as a function of the \(n\)\(^{th}\) ictal event before (negative integers) and after TBS (positive integers): "PTX" denotes fEPSP measurements (averaged between ictal events, normalized) from path-1 recordings in A, "Bic" denotes recordings in the presence of 50 - 100 µM Bic (n = 16; see fig. 8A).

Reversal of potentiation induced in ACSF. Experiments were carried out to test whether LTP induced under physiological
conditions (i.e., with inhibition intact) would be reversed by ictal activity (fig. 6). Controls were established in two pathways in the absence of pharmacological treatment. HFS (2 trains, 100 Hz, 1 s each, 20 sec apart, test pulse strength) were applied to path 1. The stronger HFS paradigm was used to compensate for the absence of disinhibition-mediated facilitation of LTP induction (67). Tetanization resulted in fEPSPs increases to 173 ± 2 over controls (n = 6; measured at t = 50 min; fig. 6Ab, path 1). At t = 50 min, Bic (50-100 µM) was applied which prompted ictal activity shortly thereafter (fig. 6Aa). EPSP slopes in both paths were enhanced by Bic (31). The Bic-induced potentiation of path 1 was transient followed by a progressive decline leading to the complete erasure of LTP. This conclusion is based on the comparison with EPSPs of the non-tetanized pathway 2 which remained elevated after Bic: EPSPs of path 1 stabilized at 123 ± 8 (n = 6; p = 0.09 compared with 134 ± 7 of path 2, measured at t = 170 min).
Figure 6: Reversal of LTP induced in ACSF. A, Averaged, normalized fEPSPs recorded in ACSF (n = 6). 2 tetani (100 Hz, 20 sec apart) were administered to path 1 at t = 0 min. Path 2 was not tetanized. Bic (50 - 100 µM) was applied at t = 50 min, triggering ictal activity (Aa). B, individual recording: same protocol as in (a) until t = 150 min. Washout of Bic (at t = 140 min) resulted in a return to pre-Bic control values in both the tetanized path 1 and non-tetanized path 2. Averages of 10 original fEPSP recordings at various points of time (marked 1-4) are shown in the bottom panel. In all recordings (A and B), [Ca²⁺]e was 2 mM throughout.
The same protocol as in figure 6A was used in the individual recording shown in figure 6B. In addition, this recording shows the return to pre-tetanus/pre-disinhibition values following Bic washout (at t = 140 min). EPSP values measured after Bic washout were 91 ± 8 in path 1, and 102 ± 8 in path 2 (values from t = 180 to 200 min following HFS). In both pathways, values were statistically the same compared with respective pre-HFS controls; p = 0.22 and p = 0.19, respectively). In summary, based on the comparison between the tetanized (path 1) and non-tetanized (control) pathway 2, it is shown that ictal events were effective in reversing LTP induced by tetanic stimulation under physiological conditions.

**Ca²⁺-induced LTP is not affected by ictal activity.** The question was asked whether SDP-generating ictal activity affected other forms of plasticity. We examined Ca²⁺-induced LTP (see fig. 4D) and two types of long-term depression (LTD; see below, fig. 7). Data depicted in figure 4D illustrate that Ca²⁺-induced LTP was not affected by ictal activity. Switching \([\text{Ca}^{2+}]_e\) from 2 to 6 mM (at t = 0 min) led to potentiation of fEPSPs to 1.55±0.03 (measured from t = 40 min to t = 80 min; p < 0.001 compared with controls in 2 mM \([\text{Ca}^{2+}]_e\)). EPSPs remained potentiated during ictal activity following the re-introduction of 2 mM control \([\text{Ca}^{2+}]_e\) at t = 80 min (1.52±0.06, measured from t = 100 min to 220 min, p = 0.12 compared with values during 6 mM \([\text{Ca}^{2+}]_e\)) (fig. 4Da).

The observation that Ca²⁺-induced LTP was not affected by ictal activity is critical for our interpretation of delayed SDP given that the pre-tetanization controls in figures 4A-C (obtained in the presence of 6 mM \([\text{Ca}^{2+}]_e\)) represented Ca²⁺-potentiated EPSPs. In these recordings, TBS-induced potentiation was implemented on top of Ca²⁺-mediated potentiation. The ictal-induced depression of EPSPs potentiated by TBS (figs. 4A-C) ended at the pre-TBS
baseline. If Ca$^{2+}$-induced potentiation had been affected by ictal activity, the ictal-induced depression in figures 4A-C would have continued below the pre-tetanization baseline to the lower values recorded in the presence of 2mM [Ca$^{2+}$]$_e$. These data allow the conclusion that Ca$^{2+}$-potentiated fEPSPs were not affected by ictal activity. The comparison demonstrates that ictal activity had - specifically - affected the stimulation-induced form of LTP.

**Two types of LTD were not reversed by ictal activity.** The question was asked whether ictal activity would affect the opposite type of plasticity, i.e., long-term depression (LTD). Two LTD forms can be distinguished at the CA3-CA1 synaptic junction, homosynaptic LTD induced by low-frequency stimulation (LFS) (19; 45) and LTD induced by the group I mGlur agonist (RS)-3,5-dihydroxyphenylglycine ((RS)-3,5-DHPG) (2; 32). Ictal activity did not reverse either form of LTD. Data shown in figure 7A illustrate that ictal activity did not change the DHPG-induced form of LTD. LTD was induced by a 40 min application of DHPG (30-60 µM, applied from t = 40 min to 80 min) in the absence of ictal activity (6 mM [Ca$^{2+}$]$_e$; Bic, 50-100 µM, was present throughout). The onset of ictal activity (after switching to ictal promoting 2 mM [Ca$^{2+}$]$_e$) did not change DHPG-depressed EPSPs: fEPSPs during DHPG-LTD were 53 ± 2 before ictal activity (measured between 90 and 120 min, n = 8 recordings) and 54 ± 4 during ictal activity (measured between 160 and 240 min; p = 0.53).
Figure 7: DHPG-induced LTD (A) and LFS-induced homosynaptic LTD (B) were not affected by ictal activity. Ab, control fEPSPs (pooled data, n = 8) were recorded in the presence of 6 mM [Ca\textsuperscript{2+}]\textsubscript{e}. Bic (50-100 µM) was present throughout. mGluR-LTD was induced by DHPG (50 - 100 µM), applied from t = 40 to 80 min. Ictal activity (sampled in top panel), initiated at t = 120 min by lowering [Ca\textsuperscript{2+}]\textsubscript{e} to 2 mM, did not reverse the depression of fEPSPs. Bb, fEPSPs recordings in the presence of 100 µM Bic (pooled data, n = 6). Downward arrow denotes the onset of LFS (10 min at 1 Hz). Pathway 2 was not conditioned. Ictal activity was introduced by lowering [Ca\textsuperscript{2+}]\textsubscript{e} from 6 to 2 mM at t = 60 min. Averaged ictal duration (per 5 min bins) is depicted in top panel.

LFS-induced LTD was also not reversed by Bic-induced ictal activity (fig. 7B). Controls were established in the presence of Bic (50-100 µM) and 6 mM [Ca\textsuperscript{2+}]\textsubscript{e} (to block ictal
activity) in two-pathway experiments. LTD was induced by LFS in pathway 1 (1 Hz for 10 min; marked by downward arrow in fig. 7Bb). It is inferred that the LTD-inducing LFS stimulation pattern (1 Hz for 10 min) induced the homosynaptic, NMDA-dependent, possibly postsynaptic form of LTD (see (51), but no efforts were made to characterize this LTD type further. The second pathway was not conditioned. LFS-induced depression (68 ± 11, n = 6, measured at t = 60 min) was not affected by ictal activity (introduced by 2 mM containing [Ca^{2+}]_e at t = 60 min). At t = 160 min (i.e., 100 min after ictal onset), EPSP slopes were 71 ± 6 in path 1 and 101 ± 5 in path 2 (p > 0.05, n = 6, compared with respective values before ictal onset). It would have been desirable to use a potentiated control (path 2) in combination with LFS-induced LTD to demonstrate the specificity of SDP. However, such potentiation was shown to induce (heterosynaptic) reversal of the LFS-treated fiber pathway (46) thus precluding the experimental objective.

**SDP is blocked by group I mGluR antagonists.** Although the blockade of GABA_{A}-receptor function was sufficient to generate ictal-like events (30) and subsequently SDP in the isolated CA1 slice (figs. 1-6), a strong activation of glutamate receptors by tetanic stimulation or ictal activity can be inferred (10; 20; 22; 35; 52; 69). We examined whether the activation of group I metabotropic glutamate receptors (mGluR) had contributed to SDP. A possible role of group I mGluR was examined by adding group I mGluR antagonists to the standard, ictal- and SDP-generating solution containing high concentrations of Bic (50-100 µM; fig. 8). We had shown previously (30) that the pharmacological blockade of group I mGluR had no impact on Bic-induced ictal activity itself (frequency of events, ictal duration, shape or burst components).
Figure 8: Pharmacological blockade of group I mGluR reduces the depotentiating efficacy of seizures. A, control experiments (n = 16) show delayed SDP initiated at t = 30 min by lowering \([\text{Ca}^2+]_o\) from 6 mM to 2 mM. Bic (50-100 µM) was present throughout. The three fEPSP traces, shown as insets underneath the summary graphs were taken from one typical experiment (path 1, average of n = 5 recorded before TBS, 20 min after TBS and the third after complete SDP at t = 100 min). B, same protocol as in A, except that the group I mGluR antagonist 4-CPG (100 µM, n = 9) was introduced together with ictal promoting 2 mM \([\text{Ca}^2+]_o\) at t = 30 min. C, same protocol as in A except that the mGluR1-specific antagonist LY367385 (100 µM; n = 8) was added at t = 30 min. D, same protocol as in A except that the mGluR5-specific antagonist MPEP (50 µM; n=5) was added at t = 30 min. E, EPSP summary graphs of tetanized pathways 1 in the presence of Bic and various group
I mGluR antagonists (normalized, averaged, n = 6 - 16; error bars are omitted for clarity) as function of time (a) and as function of the n\textsuperscript{th} ictal event (b). Values in Eb represent the average of orthodromically evoked fEPSPs between respective ictal events (see method in figure 1B).

Pooled data in figure 8 demonstrate that SDP was blocked or considerably impaired during the pharmacological blockade of group I mGluR. Group-I mGluR Antagonists were introduced together with seizure-generating 2 mM [Ca\textsuperscript{2+}]\textsubscript{e} 30 min after LTP induction to allow for proper induction and consolidation of LTP (“delayed SDP” as shown in figure 4). In the presence of the specific group I mGluR antagonist 4-CPG (100 µM) (fig. 8Bb), path 1 EPSPs remained potentiated at 1.56 ± 0.07 (n = 9) at t = 120 min, i.e., 90 min after onset of ictal activity. In contrast, the control recordings in which ictal activity was generated at t = 30 min in the absence of mGluR antagonists (fig. 8Ab) exhibited a complete depotentiation of path 1 EPSPs at t = 87 min, i.e., 53 min after ictal onset. At t = 120 min, these path-1 control EPSPs were 0.99 ± 0.06 (n = 16; p < 0.0005 comparing EPSPs during Bic alone and during Bic+4-CPG). In the presence of the mGluR1-specific antagonist LY367385 (100 µM), path 1 EPSPs remained at 1.42 ± 0.04 at t = 120 min (i.e., 90 min after ictal onset; n=8; p < 0.0001 compared with Bic alone; fig. 8Db). In the presence of the mGluR5-specific antagonist MPEP (50 µM), path 1 EPSPs remained at 1.25 ± 0.09 at t = 120 min (n=5, p < 0.05 compared with Bic alone; fig. 8Cb). The superimposed summary graphs in figure 8E illustrate the SDP-preventing efficacies of various group I mGluR antagonists in direct comparison, as function of time (fig. 8Ea) and as function of the n\textsuperscript{th} ictal event (fig. 8Eb).

Properties of ictal activity, i.e., overall duration (fig. 8Aa-Da) and frequency of ictal activity, duration of individual ictal events, burst duration etc., were similar in the depotentiating controls of Bic alone and in the
presence of different group I mGluR antagonists (30). Data in figure 8Eb (potentiated EPSPs depicted as a function of the nᵗʰ ictal event) and table 1 (percentage of depotentiation mediated by a single ictal event) show that the depotentiating strength of a given ictal event was reduced in the presence of group I mGluR antagonists. After 17 ictal events - at which SDP was completed in the presence of Bic alone - EPSPs remained at 1.57 ± 0.07 potentiation in the presence of 4-CPG (p < 0.0005 compared with Bic alone), 1.54 ± 0.04 in the presence of the mGluR1 antagonist LY367385 (p < 0.001 compared with Bic alone) and 1.27 ± 0.13 in the presence of the mGluR5 antagonist MPEP (p < 0.05 compared with Bic alone).

**DHPG-induced ictal activity did not trigger depotentiation.** The results shown in figure 8 and table 1 demonstrate that the synaptic activation of group I mGluR was a critical step in the implementation of SDP. Based on these observations, we then hypothesized that ictal activity triggered by the pharmacological stimulation of group I mGluR (22; 30; 35; 69) would be most efficient in the induction of SDP. This hypothesis was tested by the same basic experimental approach as shown in figure 8A, except that (RS)-3,5-DHPG (30-60 µM) was used as the source of ictal activity (30). Contrary to the working hypothesis, however, DHPG-induced ictal activity did not result in significant depotentiation (fig. 9A). During DHPG-induced ictal activity (initiated at t = 30 min following TBS), potentiated EPSPs remained at 1.58 ± 0.07 at t = 120 min, i.e., 90 min after ictal onset (n = 14; p < 0.001 compared with Bic alone; see superimposed graphs in fig. 9B). After 17 DHPG-induced ictal events following tetanic stimulation, fEPSPs remained at 1.60 ± 0.06 of pre-tetanized controls (p < 0.001 compared with experiments during Bic; fig. 10D).
Figure 9: DHPG-induced ictal activity did not lead to SDP. A, fEPSPs (averaged, normalized, n = 14 recordings) recorded in the presence of DHPG (30-60 µM; applied throughout). Tetanization was applied to path 1 afferents at t = 0 min, path 2 was not tetanized. Ictal activity was induced at t = 30 min by switching to 2 mM [Ca²⁺]e from ictal preventing 6 mM [Ca²⁺]e. Ictal activity is depicted as overall, averaged duration in 5 min intervals in top panel. Bb, superimposition of EPSPs of tetanized pathways in the presence of Bic (50-100 µM; n=16) and DHPG (red color; path 1 in A). Bb, superimposed overall ictal durations in Bic and DHPG (in red), resp. The "*" symbol denotes the 5-min period in which the duration of Bic-induced ictal activity was significantly higher than the duration of DHPG-induced ictal activity.
The difference in the efficacy of SDP cannot be explained by ictal properties: the overall duration of DHPG-induced ictal activity was the same or higher in all but one (out of 18) 5-min intervals in comparison with experiments during Bic alone (fig. 9Ba). Moreover, all ictal parameters, e.g., duration of individual episodes, expression and duration of the three burst components, were similar during DHPG and Bic, respectively (see reference 30). A breakdown of individual recordings indicates that SDP was possible, but far less probable during DHPG-induced ictal activity: potentiation was completely maintained in 8 of 14 recordings (fEPSPs were 1.87 ± 0.09 at t = 120 min; p < 0.001 compared with Bic). Gradual SDP (with variable time courses) was observed in 6 of 14 recordings: EPSPs in these 6 recordings were 1.27 ± 0.09 at t = 120 min (p < 0.05 compared with Bic alone).

SDP was most effective when disinhibition was combined with group I mGluR stimulation. Two explanations are conceivable as to why DHPG-induced ictal events were less effective in mediating SDP than those generated during Bic. First, it could be argued that DHPG-mediated LTD (2; 32; 37) had occluded SDP: the pre-tetanization controls obtained in the presence of DHPG (fig. 9A) represented DHPG-depressed EPSPs as illustrated in the individual recording of figure 10B. An alternative explanation would be that both disinhibition and group I mGluR activation were required for the successful implementation of SDP. Data shown in figure 10 clearly demonstrate that the latter hypothesis is correct. SDP was readily implemented in the combined presence of DHPG+Bic (fig. 10). Since the DHPG+Bic protocol also relied on DHPG-depressed EPSPs as controls, it can be ruled out that DHPG-mediated LTD had occluded SDP. In the combined presence of Bic and DHPG, ictal events not only reversed LTP, but did so far more effectively compared with other seizure protocols, Bic alone and - even more so - DHPG alone. This is best
illustrated by the depiction of SDP as a function of the nth ictal event (fig. 10D). The potentiation of the tetanized path 1 was completely reversed after the 9th ictal event in the presence of DHPG+Bic (compared with an average of 17 events required for complete depotentiation during Bic alone) (fig. 10D). The time to completion of SDP (figs. 10A-C,E) is another indicator of the higher depotentiating efficacy of the DHPG+Bic protocol: on average, SDP was complete 27 ± 6 min after ictal onset in the presence of DHPG+Bic (n = 11; fig. 10A) compared with 63 ± 10 min in the presence of Bic alone.

Figure 10: Most effective SDP in the presence of DHPG and Bic is accompanied by tonic cell depolarization. Ab, summary graphs of
fEPSPs (n = 11) recorded in two afferent pathways (path 1 and 2, resp.) in the combined presence of Bic (50-100 µM) and DHPG (30-60 µM). TBS was applied to pathway 1 at t = 0 min, pathway 2 was not tetanized. Ictal activity was induced at t = 30 min by switching to 2 mM [Ca²⁺]e from ictal preventing 6 mM [Ca²⁺]e. B, individual recording of fEPSPs. Same protocol as in A except that the initial DHPG-induced depression of EPSPs (induced at t = -60 min) is shown. EPSPs were normalized in reference to pre-tetanus controls recorded from t = -20 to 0 min. TBS was applied at t = 0 min to path 1. Ictal activity was induced at t = 50 min. C, summary graphs of intracellularly recorded EPSPs (slopes, averaged, normalized; n = 9; n = 4 in proximal apical dendrites, n = 5 in somata of CA1 pyramidal cells). Ictal activity in the presence of DHPG alone (30-60 µM; [Ca²⁺]e 2 mM throughout) did not lead to depression of potentiated EPSPs. Addition of Bic (100 µM) at t = 40 min led to rapid LTP reversal. The top panel (a) depicts the ictal activity (overall duration, averaged over 5 min intervals). D, EPSPs (averaged, norm) depicted as function of the n'th ictal event during DHPG+Bic, Bic alone and DHPG alone. E, Individual intracellular recording using the same protocol as in figure 10C: DHPG (50 µM) was present throughout, Bic (100 µM) was added at t = 40 min. Top panel depicts the duration of individual ictal events, the middle panel lists slope measurements of intracellular EPSPs, the bottom trace shows representative ictal events during DHPG alone (at t = 21 min) and during SDP following Bic application (at t = 55 min). Vm shifted from -66 mV in DHPG to -58 mV after Bic was added. F, SDP efficacy (%) plotted against the membrane potential (Vm) measured at the onset of each ictal event (r = 0.89, p < 0.0001). Data are based on a total of 111 individual ictal events and resultant depotentiation steps (obtained from the recordings in figure 10C): empty circles represent measurements in the presence of DHPG alone, full circles in red after Bic was added.

The experimental protocol in figures 10C (pooled data) and 10E (individual recording) illustrates the critical role of disinhibition most directly: maintained potentiation of intracellular EPSPs during DHPG-induced ictal activity was
followed by fast depotentiation (in path 1) after Bic was added to the DHPG-containing solution (at $t = 40$ min). EPSPs of the potentiated path 1 and non-tetanized path 2 became statistically equal (at Bic-induced elevated levels) for all $t > 64$ min, i.e., 24 min after Bic application (fig. 10Cb). A similarly fast time course of SDP completion can be seen in the individual recording (fig. 10Eb). In sum, most expedient SDP was observed when disinhibition was combined with pharmacological group I mGluR stimulation (table 1).

Table 1:

<table>
<thead>
<tr>
<th></th>
<th>EPSPs after 10(^{th}) post-tetanic ictal event</th>
<th>SDP (%) per ictal event</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHPG+Bic (n=11)</td>
<td>1.02 ± 0.10</td>
<td>10.1 ± 1.4</td>
</tr>
<tr>
<td>Bic (n=16)</td>
<td>1.26 ± 0.08</td>
<td>6.9 ± 0.6</td>
</tr>
<tr>
<td>Bic + LY367385 (n=8)</td>
<td>1.53 ± 0.12</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>Bic + 4-CPG (n=9)</td>
<td>1.58 ± 0.14</td>
<td>1.7 ± 0.7</td>
</tr>
<tr>
<td>Bic + MPEP (n=5)</td>
<td>1.38 ± 0.21</td>
<td>5.6 ± 1.0</td>
</tr>
<tr>
<td>DHPG (n=14)</td>
<td>1.74 ± 0.13</td>
<td>0.9 ± 0.3</td>
</tr>
</tbody>
</table>

Table 1: SDP efficacies in various pharmacological seizure protocols. EPSPs (slopes, normalized, average of 10) were measured after the 10\(^{th}\) ictal event (following TBS). The 10\(^{th}\) ictal event was the earliest point of complete depotentiation in the most efficient protocol of DHPG+Bic. SDP (% per ictal event; third column) represents the average depotentiation after a given ictal event. The SDP percentage measurement is based on the average of at least 5 measurements of EPSPs before and after a given ictal event (EPSP measurements during transient post-ictal depression were discarded). The “100% SDP” value was equivalent to pre-tetanization control EPSP values, the “0%” value was derived from the potentiated EPSPs prior to the first ictal event after TBS (average of 10 EPSPs).
Cell depolarization during depotentiating pharmacological conditions (DHPG+Bic). Examination of ictal activity did not reveal properties that could have accounted for the observed differences in SDP efficacy during various pharmacological models (DHPG+Bic >> Bic >> DHPG; table 1). Neither the overall duration (fig. 10Ca) nor the duration of individual ictal events (fig. 10Ea) was changed after Bic was added to the DHPG-containing solution (30). On average, the duration of a given ictal event was 5.1 ± 0.7 s (n = 22) during DHPG and 5.3 ± 0.9 after Bic was added (n = 35; p = 0.1). Other properties of the individual ictal event (shape and amplitude, duration of the entire episode, duration of burst components (41) were similar in the three seizure models used in this study.

Intracellular recordings revealed tonic cell depolarization as a main difference between the non-depotentiating seizure model of DHPG alone and the depotentiating seizure model of DHPG+Bic (figs. 10Ec,11). The rapid implementation of SDP in the combined presence of DHPG+Bic was accompanied by an average depolarization shift of +7.8 ± 1.1 mV (from -64.8 ± 1.1 mV to -57.0 ± 1.9 mV; p < 0.001, t test; compared with maintained potentiation during DHPG alone; n = 9 pyramidal cell recordings: somatic (n = 5) and dendritic (n = 4) lumped together). In the individual recording shown in figure 10E, the cell’s membrane potential was tonically depolarized by +8 mV to -58 mV during rapid SDP in the combined presence of DHPG+Bic (from -66 mV before onset of SDP, i.e., during maintained potentiation in the presence of DHPG alone; fig. 10Ec).

The plot of depotentiation steps versus holding potentiation (V_m; fig. 10F) reveals a clear separation of two populations: in the presence of DHPG alone, ictal events - generated near physiological membrane potentials (between -70 and -60 mV, -64.4 ± 1.1 mV on average; empty circles) - were followed by small depotentiation steps (1.3 ± 0.2% on average, n = 60). In contrast, after Bic was added to the
DHPG-containing solutions, ictal events were generated in depolarized cells (between -62 and -50 mV, -57 ± 1.7 mV on average; full circles in red). Depotentiation steps were 8.5 times larger on average (11.0 ± 0.42%, n = 51). Correlation between SDP efficacy and \( V_m \) was highly significant (r < 0.0001).

The application of DHPG alone did not lead to significant depolarization (+0.7 ± 0.5 mV; from -64.3 ± 1.3 mV in the untreated slice to -63.6 ± 1.5 mV; p = 0.12, n = 8). Our data suggest that - although DHPG was responsible for tonic cell depolarization as previously shown (12; 15; 16; 37) - significant depolarization in pyramidal cells embedded in the CA1 network was only observed when fast synaptic inhibition was completely blocked. The concomitant intra- and extracellular recordings shown in figure 11A depict the depolarization shift - caused by adding Bic to a DHPG-containing solution - in a complete cycle. Ictal activity - initially in the presence of DHPG alone - occurred in a remarkably regular rhythm in this particular recording (every 1.2 min). Intracellularly recorded ictal events exhibited long AHPs and pre-ictal plateau phases. \( V_{rest} \) (determined during pre-ictal plateaus) was about -65 mV. The addition of Bic induced a tonic depolarization shift of roughly +9 mV. The depolarization persisted as long as Bic was present, but was reversed after washout. The addition of Bic did not affect ictal properties, e.g., expression of burst components, frequency of occurrence, duration or periodicity.
Figure 11: Reversible cell depolarization in the presence of DHPG+Bic. A, Two segments of continuous intra- (top) and extracellular (bottom) recordings. The cell was recorded in CA1 stratum pyramidale, field potentials were recorded in stratum radiatum. Rhythmic occurrence (0.0025 Hz) of ictal activity was established in the presence of DHPG (60 μM, applied throughout). Adding Bic (100 μM) led to a slow, progressive depolarization shift of ca. +9 mV. The cell remained at the tonically depolarized level as long as DHPG and Bic were present (about 45 min; 20 min of recordings - marked by gaps - are omitted). The depolarization shift was reversed after Bic washout. B, representative traces containing a single ictal event before (a) and after addition of Bic (b) depicted at extended time scale.
Cell depolarization promoted ictal-induced SDP. Was cell depolarization a critical mechanism in the implementation of SDP? The correlation of SDP efficacy and $V_m$ (fig. 10F) - albeit highly significant - does not establish a causal effect. Two series of concomitant extra/intracellular recordings were performed to examine whether the tonic cell depolarization during depotentiating pharmacological conditions (figs. 10,11) was an essential mechanism in the induction of SDP or merely a byproduct. First, it was asked whether experimental depolarization of the recorded cell (via positive current injection) would result in selective intracellular SDP during the non-depotentiating seizure protocol of DHPG alone (fig. 12). Similar to fEPSPs, intracellular EPSPs remained potentiated during the DHPG-induced ictal activity as long as the cells were held at respective $V_{rest}$ (-64.5 ± 1.4 mV, n=6) (fig. 12Bb from $t = 0$ to 60 min). Starting at $t = 60$ min, positive current (between +0.2 and +0.5 nA) was injected between test pulses for 28 s (out of 30 s as illustrated in fig. 12A). Test EPSPs were recorded at $V_{rest}$ as before. The experimental objective was to keep the recorded cells at depolarized $V_m$ as long as possible to ensure that random ictal events were initiated at depolarized levels. The rationale of this experimental protocol was to mimic the condition of tonic cell depolarization observed during the depotentiating pharmacological protocol of DHPG+Bic (figs. 10E,11). Positive current injection between test pulses led to an average depolarization shift of +6.7 ± 1.4 mV (from -64.5 ± 1.4 mV to 58.2 ± 1.1 mV). Figure 12Bb illustrates that the injection of positive DC between test pulses led to fast SDP: potentiated intracellular EPSPs (1.64 ± 0.02, n = 6; recorded before cell depolarization at $t = 60$ min) became completely depotentiated within 8 and 19 min following experimental cell depolarization: pooled EPSPs were 1.02 ± 0.03 measured at $t = 80$ min, i.e., 20 min after tonic cell
depolarization in these 6 intracellular recordings. In contrast, concomitantly recorded fEPSPs remained potentiated (1.70 ± 0.05; p < 0.0001 comparing intra- and extracellular EPSPs at t = 80 min). The cells’ input resistances (measured at respective V_{rest}, fig. 12Bc) were unchanged during SDP indicating that the depression of intracellular EPSPs was not due to cell deterioration. Depotentiation was brought to a halt when current injection between test pulses was discontinued (not shown).
Figure 12: Cell depolarization promotes SDP. A, experimental protocol of tonic depolarization: positive current was injected through sharp electrodes into the recorded CA1 pyramidal cell for 28 sec between test pulses. EPSP responses to test pulse stimulation were recorded at $V_{rest}$ in the absence of DC. B, concomitantly recorded extra and intracellular EPSPs (slopes, averaged, normalized, n=7) in the presence of DHPG (30-60 µM) and 2 mM $[Ca^{2+}]_e$. Field EPSPs were recorded in stratum radiatum, intracellular EPSPs (red symbols) in stratum pyramidale. TBS was applied at $t = 0$ min. Starting at $t = 60$ min, positive current (between +0.2 and +0.5 nA) was injected between test pulses for 28 out of 30 s (as illustrated in fig. 12A). Test pulse EPSPs were measured at $V_{rest}$. C, input resistance measured by hyperpolarizing current pulses (200 ms, 0.2 nA, response not shown).

The basic properties of depolarization-induced intracellular SDP were identical to pharmacologically induced SDP: (a) control EPSPs were not affected by ictal events: when the cell was held at similarly depolarized potential, but in the absence of tetanic stimulation, orthodromic EPSPs were not affected by ictal events (regardless of the pharmacological seizure model; not shown); (b) after complete depotentiation, EPSPs stabilized at pre-TBS controls (fig. 12B); (c) depolarization-induced SDP did not spontaneously recover: EPSPs remained at the respective depotentiated levels upon discontinuation of tonic depolarization (see fig. 12Bb for $t > 100$ min); (d) renewed tetanization (in two recordings) led to a partial restitution of potentiation as shown in figures 2 and 4C (not shown here); (e) in the absence of ictal activity (n = 4, DHPG 30-60 µM, 6 mM $[Ca^{2+}]_e$), cell depolarization did not lead to depotentiation (not shown).

Cell hyperpolarization prevented ictal-induced SDP. Experiments in which the reverse protocol was applied (fig. 13) confirmed the notion that cell depolarization was a
sufficient condition for seizure-induced reversal of LTP. It was asked whether cell hyperpolarization would prevent SDP under depotentiating pharmacological conditions. Concomitant intra/extracellular recordings (n = 8) were carried out. Depotentiating conditions were provided at t = 30 min by adding Bic (n = 6) or PTX (n = 2) to a DHPG-containing solution (as shown before, see fig. 10C). SDP of intracellular SDP was prevented by negative current injection (as schematically shown in figure 13A) from t = 30 min to t = 90 min. Intracellular EPSPs were 1.96 ± 0.03 at 60 min and 1.94 ± 0.05 at t= 90 min (p = 0.09). In contrast, fEPSPs exhibited rapid SDP after a brief Bic or PTX-induced potentiation. SDP of fEPSPs was complete within 30 min of Bic or PTX application: fEPSPs were 1.29 ± 0.02 at t = 60 and 1.30 ± 0.02 at t = 90 min (p = 0.14). The discontinuation of negative current injection (for t > 90 min) led to rapid SDP of intracellular EPSPs. SDP was complete within 26 min: intracellular EPSPs between t = 116 and t = 120 min were 1.33 ± 0.03 and fEPSPs were 1.29 ± 0.03 (p = 0.35; fig. 13Bb). These data demonstrate that hyperpolarizing the recorded cell resulted in selective (intracellular) protection from depotentiation during the depotentiating pharmacological protocol of DHPG+Bic.
Figure 13: Hyperpolarization prevents SDP. A, experimental scheme of tonic cell hyperpolarization: negative current was injected into the recorded CA1 pyramidal cell for 28 sec between test pulses. Orthodromic responses to test pulses were recorded at respective V_{rest}. B, concomitantly recorded extracellular EPSP slopes (averaged, normalized) in the presence of DHPG (30-60 µM). TBS was applied at t = 0 min. Bic (100 µM, n=6) or PTX (100 µM, n=2) were added to the DHPG containing solution at t = 30 min leading to enhanced EPSPs in both paths. Negative current (-0.1 to -0.6 nA) was injected for 28 sec during test pulses (as shown in A) between t = 30 and t = 90 min (marked “ΔV_{m}(·)”). The amount of DC was adjusted to keep V_{m} around the membrane potential measured during DHPG alone.
**Somatic – dendritic depolarization.** Tonic cell depolarization was measured in both somatic and apical dendritic CA1 pyramidal cell recordings during the depotentiating seizure protocol of DHPG+Bic. In apical dendritic recordings (n = 4, recorded at 100 to 200 µm distances from soma), $V_m$ in the combined presence of DHPG and Bic was $-58.3 \pm 2.7$ mV (up from $-65.0 \pm 1.9$ mV in the same recordings in the native slice, p < 0.001). In somatic recordings (n = 5), $V_m$ during DHPG + Bic was $-56.3 \pm 2.3$ mV (compared with $-64.1 \pm 1.8$ mV, p < 0.001). In contrast to the rather homogeneous membrane potential distributions in the pharmacological protocols of depotentiation, considerable passive membrane potential gradients - away from the respective current injection sites - may have existed in the current injection protocols (figs. 12,13). Passive responses to local current injections illustrate the gradients in concomitant somatic and dendritic recordings in the same CA1 pyramidal cell. Only 37% of the voltage response was preserved in the passively coupled recording site over a ca. 150 µm distance: current pulses (-0.4 nA) injected through the somatic recording electrode led to a membrane potential change of -8 mV at the somatic injection site (fig. 14Aa), whereas the membrane deflection in the passively coupled dendritic recording was only -3 mV (fig. 14Aa). The same numbers of membrane deflections, i.e., $\Delta V(m) = -8$ mV at the dendritic recording site and $\Delta V(m) = -3$ mV at the passively coupled somatic recording site were obtained through the reverse current pulse application (i.e., - 0.4 nA through the dendritic recording electrode; fig. 14Ab). Similar gradients were in effect in response to subthreshold positive current injections. The larger resistances to axial current flow in smaller-diameter branches yields considerably smaller passive $\Delta V(m)$ in more distant dendritic branches. Thus, passive responses per se were unlikely a factor in the control of SDP during the current injection protocols (figs. 12,13). Instead, local changes of $V_m$ could
have been involved in the control of the propagation and amplification of active, suprathreshold responses. Figure 14B illustrates this notion: the somatic responses to the same dendritic input were recorded at two different holding potentials. A +0.3 nA current pulse through the dendritic electrode led to a modest burst response (68) containing a spiklet followed by a full action potential (top panel in fig. 14B). The passive somatic response at \( V_{\text{rest}} \) (-60 mV, "soma 1", fig. 14B) was a single AP in close temporal vicinity to the dendritic AP. In contrast, a relatively modest tonic depolarization (+4mV; "soma 2", fig. 14B) of the cell soma led to AP doublets riding on a much larger depolarizing envelope. These data illustrate the control exerted by local membrane potential changes over propagating responses. But it remains to be seen if the local control of active responses was indeed instrumental in the promotion (fig. 12) or prevention of SDP (fig. 13).
Figure 14: Passive responses in somato-dendritic recording in CA1 pyramidal cell. Concomitant somatic and apical dendritic recordings in a single CA1 pyramidal cell: the apical dendritic recording site was about 150 µm apart away from the somatic site in stratum pyramidale. $V_m$ was -60 mV. A, responses ($\Delta V(m)$) to a current pulse (-0.4 nA): a, injected through the somatic recording electrode; b, through the dendritic recording electrode. $\Delta V(m)$ were -8 mV at the respective injection sites and -3 mV in the passively coupled sites. B, depolarizing current pulses (0.3 nA, top) led to a small spike, followed by a full action potential at the dendritic injection site. The passive responses in the cell soma were a single action potential (middle trace, “soma 1”), but two action potentials on top of larger depolarization envelope during +4mV tonic depolarization (bottom trace, “soma 2”).
Discussion.

Summary of basic properties of SDP. We show that spontaneous ictal events – caused by the removal of GABA\(_A\)-mediated inhibition – led to a stepwise depression of potentiated fEPSPs in the isolated CA1 slice. The ictal-induced depression of EPSPs ended at the pre-tetanization control level (figs. 1-5) or in few cases slightly below control levels (figs.3Bb, 6B). Ictal activity had no long-term effects on baseline EPSPs (figs. 1-6). Based on criteria established in stimulation studies (4; 6; 21; 28; 34; 48; 56), it can be concluded that the depression of EPSPs by ictal activity shown here represents a clear form of depotentiation as opposed to forms of long-term depression of EPSPs at naïve synapses (7; 49). The efficacy of spontaneous depotentiation did not decrease over time (shown up to 120 min following the induction of LTP; see fig. 4A,B). In analogy to stimulation-induced depotentiation, SDP resembles depotentiation achieved by high-intensity stimulation (4; 6). In contrast, weaker stimulation protocols were only effective within a short window of LTP induction (see 26). Spontaneous seizure-like events were causal in triggering depotentiation steps as evidenced by the close temporal correlation of the two phenomena (figs. 1,2). SDP was also observed in cases where transient post-ictal depression was minimal or completely absent (see fig. 2). Spreading depression – which was suggested as the cause of seizure-induced loss of LTP in-vivo (25) - was not involved in SDP. Taken together, our data suggest that seizures per se led to the reversal of LTP.

A common feature of seizure-induced forms of depotentiation is their heterosynaptic nature (24; 25; 43). The strongest evidence that ictal events in our model activated a different – although partially overlapping - set of excitatory synapses than the ones potentiated by tetanic stimulation derives from the finding that the stimulation of the Schaffer collaterals (even at highest intensities) not
only failed to trigger ictal activity, but in fact blocked it (30). Similar to CA3 (40), recurrent excitatory connections between CA1 principal cells (18) may have become functional after their inhibitory control was removed. This notion is also supported by the properties of developing synchronization in the CA1 disinhibition model of epilepsy (30) which closely resembled the synchronization mechanisms in the recurrently connected CA3 network (63). Dependent on the subcellular sites of recurrent excitatory synapses, ictal events could have affected sites of potentiation through direct activation of glutamatergic synapses at pyramidal cell dendrites (18) or through back-propagating action potentials after suprathreshold activation of somatic or proximal dendritic glutamatergic receptors (59). In either case, SDP was mediated by a heterosynaptic mechanism.

**Specificity of SDP.** Seizure effects on excitatory transmission in this model were remarkably specific. We demonstrate the reversal of stimulation-induced potentiation whereas baseline EPSPs and other forms of plasticity, Ca$^{2+}$-induced LTP and both forms of LTD (figs. 4D, 7), were not affected by ictal activity. Types of plasticity spared included those with postulated postsynaptic loci of expression (e.g., Ca$^{2+}$-induced LTP (65) or LFS-induced LTD (51) (fig. 7), but also with a likely presynaptic locus of expression (e.g., DHPG-mediated LTD (2)). In addition, we did not observe LTP-like effects by seizures seen in the disinhibition model (8; 53) nor a dual expression of both LTP- and LTD effects observed for example in the potassium model of in-vitro epilepsy (14) or in several protocols of electrical stimulation (4; 6; 24; 28; 48). The lack of some forms of plasticity - such as LTP-like effects seen in the disinhibited slice during interictal-type of epileptiform activity from the CA3 subfield (8; 53) - can be explained by the specific experimental conditions applied in this study: the isolation of the CA1 region and application of high concentrations of GABA$_A$ antagonists (Bic: 50-100 µM, PTX
≥100 µM) precluded CA3-derived interictal events and led to a state of exclusive ictal activity as shown previously (30). Similarly, the (pharmacological) conditions for bidirectional plasticity contingent upon the phase angle of an underlying theta wave (28) were absent in this study. In analogy to theta burst stimulation which induced LTP at low-intensity stimulation, but reversed LTP when applied at 10 times higher intensity (4), it might be inferred that ictal activity was consistently strong enough to reverse, but not generate LTP. Taken together, ictal events in this study - despite their complex composition of three distinct burst components and wide range of firing frequencies (from about 100 to 3 Hz) (61) - were extremely consistent in their exclusive action of reversing stimulation-induced LTP.

Our data show that LTP-inducing tetanic stimulation had increased the frequency of ictal events and thus the overall duration of ictal activity by 46% on average (fig. 3Ac). Was the higher volume of seizure activity responsible for the specific effects on EPSPs after tetanization? SDP in the presence of PTX (fig. 5) indicates that this was not the case. During PTX, the frequency of events and thus the overall duration of ictal activity was even lower than the seizure duration during controls and other forms of plasticity which were not affected by seizures (compare figures 5Aa and 5D, 7Aa, Ba). No differences were seen between Bic- and PTX-induced SDP as a function of the nth ictal event (fig. 5). Thus, the lower frequency of ictal events per se was unlikely responsible for the lack of seizure effects on baseline excitation, Ca²⁺-induced LTP (fig. 4D) and both forms of LTD during ictal activity (fig. 7). But a higher ictal volume is only one consequence of the different network dynamics introduced by tetanic stimulation. In addition to its augmenting effect on seizure frequency, the LTP-inducing tetanic stimulation - absent during baseline transmission and during Ca²⁺-induced LTP and both forms of LTD - could have generated other cellular conditions which
were responsible for the specific erasure of stimulation-
induced LTP by seizures. An example is the critical role of
synaptic activation of group I mGluR, possibly through
tetanic stimulation, in the induction of SDP (fig. 8). This
notion is also not valid: when tetanic stimulation was
applied prior to the induction of Ca\(^{2+}\)-LTP or both forms of
LTD, ictal events were equally ineffective in reversing
these forms of plasticity (not shown). In addition, the fact
that EPSPs returned to - and remained at - pre-tetanization
baseline levels after completion of SDP is further evidence
that the specific reversal of potentiated EPSPs following
tetanic stimulation was not contingent upon network dynamics
introduced by LTP-inducing stimulation.

**Depolarization promoted seizure-induced depotentiation.**

Results from two experimental protocols show that cell
depolarization was a pivotal mechanism for the induction of
SDP: (a) current injection promoted or prevented SDP on a
single-cell level (figs. 12,13); (b) depotentiating
pharmacological protocols were accompanied by tonic
depolarization shifts (fig. 11C; table 1). The current
injection protocols demonstrate a postsynaptic induction
mechanism. Correlation analysis (fig. 10F) suggests that
ictal-induced depotentiation was not an all-or-none, but a
graded event: the size of depotentiating steps was
functionally linked to the amount of cell depolarization
(fig. 11Cc). These data can explain observations that
seemingly identical ictal events triggered individual
depotentiation steps of vastly variable sizes ranging from
no depotentiation (e.g., in the presence of DHPG alone,
figs. 9, 10F) to 50% depotentiation mediated by a single
ictal event (see fig. 2).

It is inferred that group I mGluR activation was
responsible for the tonic depolarization (37) seen in
depotentiating pharmacological protocols. Group I mGluR-
mediated depolarization can be traced to several conductance
mechanisms, e.g., the impairment of (diverse) I\(_k\) and
resulting membrane resistance increases or activation of cationic conductance increases (for review see the introduction in Chuang et al. (13)). However, our data demonstrate that group I mGluR-mediated depolarization - and subsequently SDP - required the complete block of GABA_A-mediated inhibition (figs. 10E,F; 11). Previous reports have shown changes of the inhibitory efficacy by group I mGluR activation (16; 37-39). Our data point to a reverse interaction in that fast synaptic inhibition controlled DHPG-mediated depolarization in CA1 pyramidal cells. We demonstrate one particular functional consequence of the inhibitory control of group I mGluR in that seizure-induced reversal of LTP was prevented unless inhibition was completely blocked. Partial disinhibition did not suffice. The DHPG protocol of seizure activity exemplifies this notion. During DHPG, only minimal cell depolarization (< 2 mV on average) was expressed and SDP was absent in the vast majority of recordings (fig. 9). In these recordings, fast synaptic inhibition was at least partially blocked by the action of DHPG itself (16; 37). After LTP-inducing tetanic stimulation, an additional component of disinhibition was added (17; 57; 58). Even the addition of lower, submaximal concentrations of Bic (1-5 µM) to a DHPG containing solution did not reveal significant cell depolarization or SDP (data not shown). In contrast, only saturating concentrations of Bic (50-100 µM) - added to the DHPG-containing solution - led to significant depolarization and most expedient SDP (fig. 10, table 1). Depotentiating efficacies during various pharmacological protocols (DHPG+Bic >> Bic alone >> DHPG alone; table 1) were a reflection of the expression of the cellular conditions leading to cell depolarization, i.e., complete disinhibition and - at least partial - group I mGluR stimulation. In the presence of Bic alone, complete disinhibition was paired with (synaptic) activation of mGluR1 (fig. 8, table 1) which was arguably weaker than the pharmacological stimulation by DHPG. The largest
depolarization and the highest depotentiation efficacy (figs. 4,5, table 1) was obtained under saturating pharmacological conditions in the presence of DHPG plus Bic.

The question is whether such stringent conditions, notably the complete block of inhibition, were to occur in vivo. Two main scenarios are feasible. First, depotentiating cellular conditions need to be implemented in very small parts of brain tissue: we have previously shown that spontaneous ictal events could be generated in ca. 1 mm³ CA1 tissue (about 80% of the CA1 slice(30)). SDP can occur in far smaller neuronal units, in fact in a single neuron (figs. 12,13). Second, far more permissible circumstances for cell depolarization are conceivable. Tonic depolarization protocols in this study were applied out of experimental necessity. Since seizure activity in most recordings occurred infrequently and randomly in time (except in very few recordings; see fig. 10), tonic depolarization protocols best ensured the presence of depolarization at the time of ictal onset. But it is possible that transient depolarization shifts coinciding with ictal onset may suffice to reverse LTP by seizures.

We cannot conclusively comment on how tonic (or possibly phasic) cell depolarization preceding ictal events promoted SDP. Ictal firing - for several seconds at various frequencies from 3 to 100 Hz on top of a large depolarization envelope (62) may have led to the same phasic activation of voltage-gated conductances (e.g., NMDAR) regardless of the presence or absence of underlying tonic depolarization. NMDAR were also activated by LTP-inducing/reversing tetanic stimulation (4; 9) and possibly potentiated by the stimulation of mGluR5 (20; 37). But a role of NMDAR in SDP remains to be established. The simple approach of using antagonists is precluded by the fact that NMDAR antagonists (even at low concentrations) blocked SDP-triggering ictal activity (30). A more likely contribution of depolarization prior to ictal activity may be found in
activity before the ictal event especially if the pre-ictal depolarization shift was above firing threshold (see fig. 11Bb). Priming effects by voltage-gated conductances are conceivable. But exact mechanisms remain to be elucidated.

**SDP as a model of seizure-induced amnesia.** In contrast to stimulation-induced forms of depotentiation which are generally discussed within the context of a physiological mechanism, akin to “forgetting”, seizure-induced depotentiation represents undoubtedly a cellular model of a pathological form of “memory loss”. A number of properties of SDP conforming with clinical observations - its clear distinction from the transient, general post-ictal depression of excitation, its specificity, renewed LTP after partial or complete depotentiation - point to a useful model of seizure-induced amnesia. LTP, however, is not equivalent to learning as LTP-inducing stimulation does not create, but rather eliminate the particular spatial pattern of synaptic weights of memory engrams (11). The LTP/memory analogy presumes that memory processes and LTP share the same cellular mechanisms. In this context, both the reversal, but also the creation of plasticity by seizures (8; 14; 53) represent forms of interference with memory processes.

Several questions emerge from our findings that SDP could be implemented in a single neuron (figs. 12,13) - possibly even at subsets of synapses in which potential gradients were maintained (fig. 14) - but also in neuronal populations (during the pharmacological protocols; figs. 1-6, 10). Considering that information is stored in distributed patterns of synaptic weights in the hippocampal formation (11), how did depotentiation in a single cell influence or even erase the particular spatial pattern of synaptic weights created by tetanic stimulation? In contrast to single-cell SDP, SDP under pharmacological conditions was more likely to engage populations of neurons. How small were independently depotentiated subpopulations and what determined their size? Assuming that the stability of the
newly consolidated (potentiated) state was preserved by cross-talk between neurons, how did SDP interfere with the binding of neuronal assemblies? It is conceivable that the elucidation of the mechanisms underlying selective depotentiation independently in individual cells or small subpopulations may also bear relevance for the physiological erasure of information stored in the hippocampal network.

**Acknowledgement:** This study was supported by a grant from NINDS. We thank T.C. Sacktor, M. Stewart and R.K.S. Wong for reading the manuscript.
Reference List


17. Desai MA, McBain CJ, Kauer JA and Conn PJ. Metabotropic glutamate receptor-induced disinhibition is mediated by


22. **Galoyan SM and Merlin LR.** Long-lasting potentiation of epileptiform bursts by group I mGluRs is NMDA receptor independent. *J Neurophysiol* 83: 2463-2467, 2000.


24. **Harrison CM and Alger BE.** Perfusion with high potassium plus glutamate can cause LTP erasure or persistent loss of neuronal responsiveness in the CA1 region of the hippocampal slice. *Brain Res* 602: 175-179, 1993.


27. **Huang YY and Kandel ER.** Recruitment of long-lasting and protein kinase A-dependent long-term potentiation in
the CA1 region of hippocampus requires repeated

28. Huerta PT and Lisman JE. Bidirectional synaptic
plasticity induced by a single burst during
cholingergeric theta oscillation in CA1 in vitro. Neuron

29. Kandel ER, Klein M, Castellucci VF, Schacher S and
Goelet P. Some principles emerging from the study of
short- and long-term memory. Neurosci Res 3: 498-520,
1986.

30. Karnup S and Stelzer A. Seizure-like activity in the
disinhibited CA1 minislice of adult guinea- pigs. J

31. Karnup SV and Stelzer A. Temporal overlap of excitatory
and inhibitory afferent input in guinea-pig CA1

32. Kemp N and Bashir ZI. Long-term depression: a cascade
of induction and expression mechanisms. Prog Neurobiol

33. Larson J, Wong D and Lynch G. Patterned stimulation at
the theta frequency is optimal for the induction of


40. **Miles R and Wong RKS.** Excitatory synaptic interactions between CA3 neurones in the guinea pig hippocampus. *J Physiol* 373: 397-418, 1986.


51. **Rammes G, Palmer M, Eder M, Dodt HU, Zieglgansberger W and Collingridge GL.** Activation of mGlu receptors


64. **Tsien JZ, Huerta PT and Tonegawa S.** The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell* 87: 1327-1338, 1996.

65. **Turner RW, Baimbridge KG and Miller JJ.** Calcium-induced long-term potentiation in the hippocampus. *Neuroscience* 7: 1411-1416, 1982.


69. **Wong RKS, Bianchi R, Taylor GW and Merlin LR.** Role of metabotropic glutamate receptors in epilepsy. In: *Mechanisms of the Epilepsies*, edited by Delgado-

70. Wong RKS, Prince DA and Basbaum AI. Intradendritic recordings from hippocampal neurons. Proceedings of the National Academy of Science of the USA 76: 986-990, 1979.
