Title
Long-term potentiation is impaired in rat hippocampal slices that produce spontaneous sharp waves

Journal
Journal of Physiology-London, 558(3)

ISSN
0022-3751

Authors
Colgin, laura L
Kubota, D
Jia, Y S
et al.

Publication Date
2004-08-01

Peer reviewed

Long-term potentiation is impaired in rat hippocampal slices that produce spontaneous sharp waves

Laura Lee Colgin[1], Don Kubota[1], Yousheng Jia[2], Christopher S. Rex[3] and Gary Lynch[1]

[1] *101 Theory, No. 250, Department of Psychiatry and Human Behaviour, University of California, Irvine, CA 92612-1695, USA*
[2] *Department of Obstetrics and Gynecology, University of California Los Angeles School of Medicine, Harbour/UCLA Medical Center, Torrance, CA 90509, USA*
[3] *Department of Neurobiology and Behaviour, University of California, Irvine, CA 92697, USA*

Sharp waves (SPWs) occur in the hippocampal EEG during behaviours such as alert immobility and slow-wave sleep. Despite their widespread occurrence across brain regions and mammalian species, the functional importance of SPWs remains unknown. Experiments in the present study indicate that long-term potentiation (LTP) is significantly impaired in slices, prepared from the temporal aspect of rat hippocampus, that spontaneously generate SPW activity. This was probably not due to anatomical and/or biochemical abnormalities in temporal slices because stable LTP was uncovered in field CA1 when SPWs were eliminated by severing the projection from CA3. The same procedure did not alter LTP in slices lacking SPWs. Robust and stable LTP was obtained in the presence of SPWs in slices treated with an adenosine A1 receptor antagonist, a finding that links the present results to mechanisms related to the LTP reversal effect. In accord with this, single stimulation pulses delivered intermittently in a manner similar to the SPW pattern interfered with LTP to a similar degree as spontaneous SPWs. Taken together, these results suggest the possibility that SPWs in the hippocampus constitute a neural mechanism for forgetting.

(Resubmitted 13 May 2004; accepted 9 June 2004; first published online 11 June 2004)
Corresponding author L. L. Colgin: 101 Theory, No. 250, Department of Psychiatry and Human Behaviour, University of California, Irvine, CA 92612-1695, USA. Email: lcolgin@uci.edu

Long-term potentiation (LTP), a long-lasting increase in synaptic efficacy thought to be the cellular substrate of certain types of memory, is closely related to forebrain rhythms associated with learning. LTP is readily induced in rat hippocampus *in vitro* (Larson *et al.* 1986) and *in vivo* (Staubli & Lynch, 1987) by short bursts of stimulation, when the bursts are delivered at the frequency of the theta rhythm (i.e. ∼5 Hz). LTP can also be induced in anaesthetized as well as awake, behaving rats by delivering stimulation pulses time-locked to the positive phase of theta (Pavlides *et al.* 1988; Holscher *et al.* 1997; Hyman *et al.* 2003). The theta rhythm has been implicated by numerous studies in the encoding and retrieval of memory (see Vertes & Kocsis, 1997 for a review).

Sharp waves (SPWs) are a spontaneous EEG pattern intrinsically generated in hippocampus (i.e. without an external pacemaker). While their duration (∼30–120 ms) and average frequency (∼1–5 Hz) vary considerably from cycle to cycle (Buzsaki, 1986), SPWs are a characteristic and readily detected feature of the hippocampal EEG during behaviours (awake immobility, slow-wave sleep, drinking, grooming, and eating) in which input from the distant environment is assumed to be low or absent (Buzsaki *et al.* 1983; Buzsaki, 1986; Suzuki & Smith, 1987). The oscillations are reduced by cholinergic stimulation (Kubota *et al.* 2003) and enhanced by atropine (Buzsaki, 1986), suggesting that they are negatively regulated by activation of the septo-hippocampal pathways that generate theta.

In some respects, SPWs resemble stimulation patterns used in slice (Larson *et al.* 1993; Staubli & Chun, 1996) and chronic recording (Staubli & Lynch, 1990; Staubli & Scafidi, 1999) experiments to reverse recently induced LTP, an effect that is blocked by antagonists of the A1 receptor (Larson *et al.* 1993; Staubli & Chun, 1996). In the present studies, an *in vitro* SPW model (Papatheodoropoulos & Kostopoulos, 2002; Wu *et al.* 2002; Kubota *et al.* 2003; Maier *et al.* 2003) was used to test the possibility that the presence of SPWs influences the formation of LTP. The results suggest that SPWs, via an action on the adenosine A1 receptor, disrupt the cellular events that stabilize potentiation.

 DOI: 10.1113/jphysiol.2004.068080

Methods

Slice preparation

Male Sprague-Dawley rats, 4–5 weeks of age, were anaesthetized with halothane and killed via decapitation under a protocol approved by the University of California Institutional Animal Care and Use Committee. The brain was then quickly removed and submerged in icy, oxygenated artificial cerebrospinal fluid (ACSF) containing the following (mM): NaCl 124, KCl 3, KH_2PO_4 1.25, $MgSO_4$ 5, $CaCl_2$ 3.4, D-glucose 10, $NaHCO_3$ 26. A tissue block was then prepared and glued to the stage of a vibrating tissue slicer (Leica VT1000; Bannockburn, IL, USA). Slices were cut roughly perpendicular to the longitudinal axis of the hippocampus at a thickness of 350 μm. As previously described (Papatheodoropoulos & Kostopoulos, 2002; Kubota *et al.* 2003), slices prepared from the ventral portion of the rat hippocampus exhibited spontaneous SPW activity. Little to no SPW activity was observed in slices from the mid-to-dorsal hippocampus, consistent with the majority of reports of EEG activity in hippocampal slices. Slices were placed on an interface recording chamber and allowed to recover for at least 1 h prior to commencement of recording.

Field potential recording

Throughout recording, oxygenated ACSF (composition (mM): NaCl 124, KCl 3, KH_2PO_4 1.25, $MgSO_4$ 3, $CaCl_2$ 1, D-glucose 10, $NaHCO_3$ 26) was infused at a rate of approximately 60 ml h^{-1}. Additionally, warmed and humidified 95% O_2–5% CO_2 was blown into the chamber over the tissue. Slices were maintained at $32 \pm 1^{\circ}$C. Glass electrodes filled with 2 M NaCl were used to record field activity. Extracellular signals were amplified with a differential AC amplifier (Model 1700, A-M Systems; Carlsborg, WA, USA) and digitized at 10 kHz using NAC 2.0 Neurodata Acquisition System (Theta Burst Corp.; Irvine, CA, USA). Stimulation pulses were generated using a Grass-Telefactor Model S88 Stimulator and Model PSIU6 photoelectric stimulus isolation unit (Grass-Telefactor; West Warwick, RI, USA) and delivered through bipolar electrodes made of twisted nichrome wire. Stable field excitatory postsynaptic potentials (EPSPs) were evoked by adjusting stimulation current such that responses were approximately 1–2 mV in amplitude and $\leq 50\%$ of the maximal monophasic response.

Intracellular recording

Whole cell recordings were made with 3–5 MΩ recording pipettes filled with pipette solution of the following composition (mM): 130 caesium gluconate, 10 CsCl, 0.2 EGTA, 8 NaCl, 2 ATP, 0.3 GTP, and 10 Hepes (pH 7.35, 290–300 mosmol l^{-1}). The liquid junction potential of the pipette solution was −6 mV with respect to the Ringer solution. Holding potentials were −70 mV after correcting for the junction potential. Synaptic currents were recorded with a patch amplifier (AxoPatch-200A, Axon Instruments, Burlingame, CA, USA) with a 4-pole low-pass Bessel filter at 2 kHz and digitized at 5 kHz.

Induction of LTP

A stimulation protocol similar to that used in an earlier study of LTP reversal (Larson *et al.* 1993) was employed here for LTP induction. Theta burst triplets, consisting of three bursts (100 Hz, 4 pulses each) separated by 200 ms, were delivered simultaneously to two stimulation pathways. This pattern was repeated four times at 1 min intervals (i.e. for a total of 12 bursts). The duration of stimulus pulses was not changed during burst delivery.

Reagents

8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) was purchased from Tocris (Ellisville, MO, USA) and dissolved each day in DMSO. Prior to infusion, DPCPX stock solution (10 mM) was diluted to a final working concentration (250–500 nM) in ACSF containing $\leq 0.005\%$ DMSO. CNQX was purchased from Sigma (St Louis, MO, USA) and applied to the infusion line using a syringe pump (Syringe Pump Model 341B, Sage Instruments, Boston, MA, USA).

Data analysis

Data are illustrated and reported as mean ± S.E.M., unless indicated otherwise. To assess statistical significance, two-way ANOVA with repeated measures tests were employed. Spectral power and peak frequency of spontaneous activity were estimated using the Fast Fourier Transformation function in MATLAB (MathWorks, Natick, MA, USA). Correlation coefficients and corresponding lag times were estimated using the cross-correlation function in MATLAB.

Results

Figure 1*A* (top) illustrates a representative SPW trace recorded from the pyramidal cell layer in field CA3b of ventral hippocampal slices under baseline conditions, as previously described (Kubota *et al.* 2003). SPWs recorded

from CA3 occurred with a frequency of 5.3 ± 3.9 Hz (mean $\pm$ S.D., $n = 9$), while SPWs recorded from CA1 occurred with a slightly lower frequency of 2.0 ± 0.6 Hz (mean $\pm$ S.D., $n = 5$). Values fell within the same frequency range as has been reported for CA1 SPWs *in vivo*; i.e. 0.01–3 Hz, on average (Buzsaki 1986). Antagonists of the AMPA-type glutamate receptors that mediate fast excitatory synaptic currents have been shown previously to block SPWs in slices from mouse hippocampus (Maier *et al.* 2003). A similar result was obtained for the SPWs recorded in temporal hippocampal slices from rats ($n = 10$ slices): Infusion of the antagonist CNQX eliminated the SPW activity (Fig. 1*A*, bottom). Rhythms returned upon washout of CNQX (data not shown). Whole cell recording confirmed the presence of spontaneous EPSCs in CA3 pyramidal neurones (Fig. 1*B*). These results accord with the conclusion that SPWs are excitatory events. As is the case for SPWs *in vivo*, the *in vitro* SPWs were associated with high frequency (~150–200 Hz) 'ripple' oscillations (Fig. 1*C–D*).

Figure 2*A* compares a spontaneous recording of SPWs in CA3 stratum radiatum of slices prepared from temporal

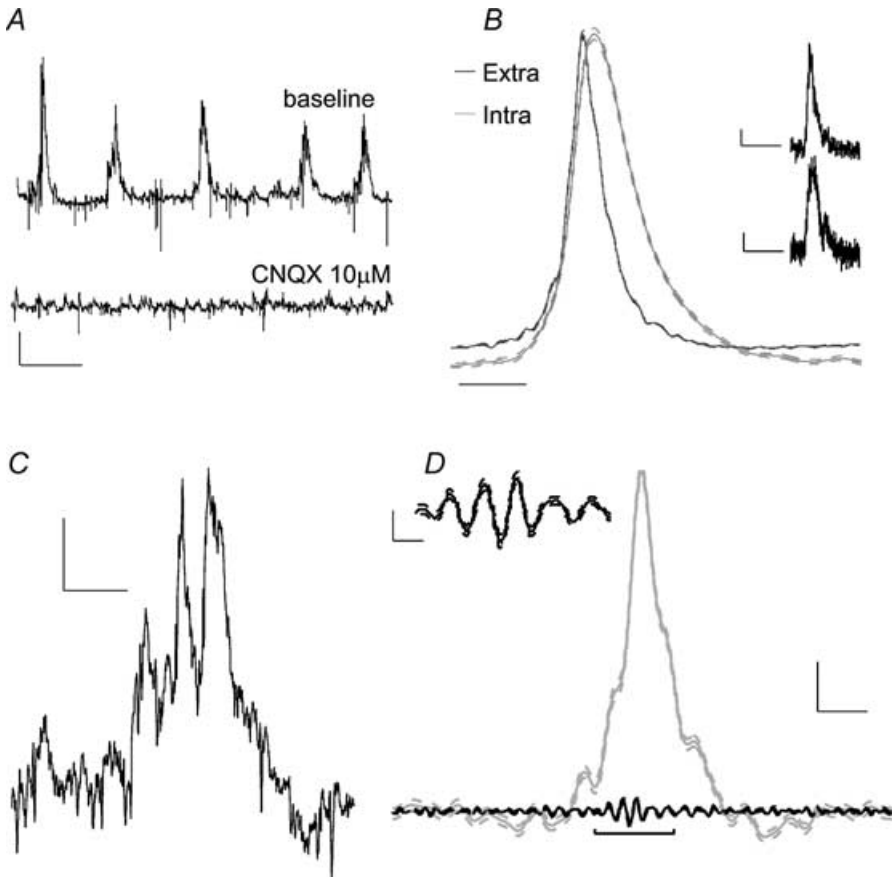

Figure 1. Properties of spontaneously occurring SPWs in temporal slices from rat hippocampus
A, SPWs are eliminated by infusion of the AMPA receptor antagonist CNQX. Representative traces from CA3 stratum pyramidale prior to (above) and at the end of a 30 min CNQX wash-in (below) are shown. Calibration: 250 ms, 200 μV. *B*, simultaneous extracellular and intracellular recordings from CA3 stratum pyramidale during SPW activity. Over 800 events were averaged to produce these records (standard error indicated by dashed line), and the traces were normalized to peak amplitude for illustration purposes. Individual records are also shown (inset: scale bars for extracellular trace (above) represent 100 ms and 200 μV; scale bars for intracellular record (below) represent 100 ms and 10 pA). *C*, representative example of a single SPW recorded in CA3b stratum pyramidale. Small amplitude, high frequency activity is apparent, particularly on the ascending phase. Calibration: 30 ms, 50 μV. *D*, SPWs were accompanied by high frequency (150–200 Hz) ripple oscillations. 235 SPW/ripple complexes recorded from CA3b stratum pyramidale were averaged and bandpass filtered from 0.3 to 100 Hz to isolate the SPW (grey) or bandpass filtered from 150 to 300 Hz to isolate the ripple (black). Errors are shown as dashed lines above and below the traces. Calibration: 20 ms, 10 μV. A segment of the ripple record (indicated by bar) is enlarged and shown (inset). Scale bars (inset) represent 5 ms and 2.5 μV.

hippocampus (above) to a trace from a slice from septal hippocampus in which no SPW activity was observed (below). A protocol similar to that used in the Larson *et al.* (1993) study and described in Methods was used in an attempt to induce long-term potentiation in both groups of slices (Fig. 2*B–D*). In a group of nine slices prepared from mid-to-septal hippocampus, LTP reached an initial maximum of approximately 175% of baseline values ($175.2 \pm 12.6\%$ for slope and $171.8 \pm 9.4\%$ for amplitude immediately following induction) and stabilized at a level of approximately 145% ($144.0 \pm 10.4\%$ for slope and $148.2 \pm 10.3\%$ for amplitude at 60 min after induction). In contrast, LTP in the SPW-exhibiting slices from ventral hippocampus ($n = 9$) was initially lower in magnitude ($148.7 \pm 11.4\%$ for slope and $151.1 \pm 5.6\%$ for amplitude immediately following induction) and declined steadily over the course of 1 h to approximately 110% of initial baseline values ($105.9 \pm 2.6\%$ for slope and $113.2 \pm 3.2\%$ for amplitude at 60 min post induction). In other words, LTP was significantly lower in temporal slices with SPWs than in the group of septal slices with no SPW activity ($P < 0.0001$). On average, baseline responses in the group of temporal slices (1.64 ± 0.13 mV) were greater than their septal slice counterparts (1.33 ± 0.12 mV), indicating that the LTP deficits were not due to impaired responses in the former.

SPWs are thought to originate in the dense associational system of CA3 (Buzsaki, 1986; Suzuki & Smith, 1987; Kubota *et al.* 2003). In support of this, cross-correlation analyses showed that SPWs in CA1 closely followed SPWs in CA3 with an average delay of 4.0 ± 0.2 ms. Moreover, SPWs in CA1 were eliminated by physically cutting the connection between CA3 and CA1 (Fig. 3*A*), while SPWs in CA3 were for the most part unaffected by the procedure. Previous studies have reported that dissection of CA3 from CA1 does not affect many routinely tested measures of CA1 synaptic physiology, including induction of LTP (Lynch *et al.* 1982; Cohen &

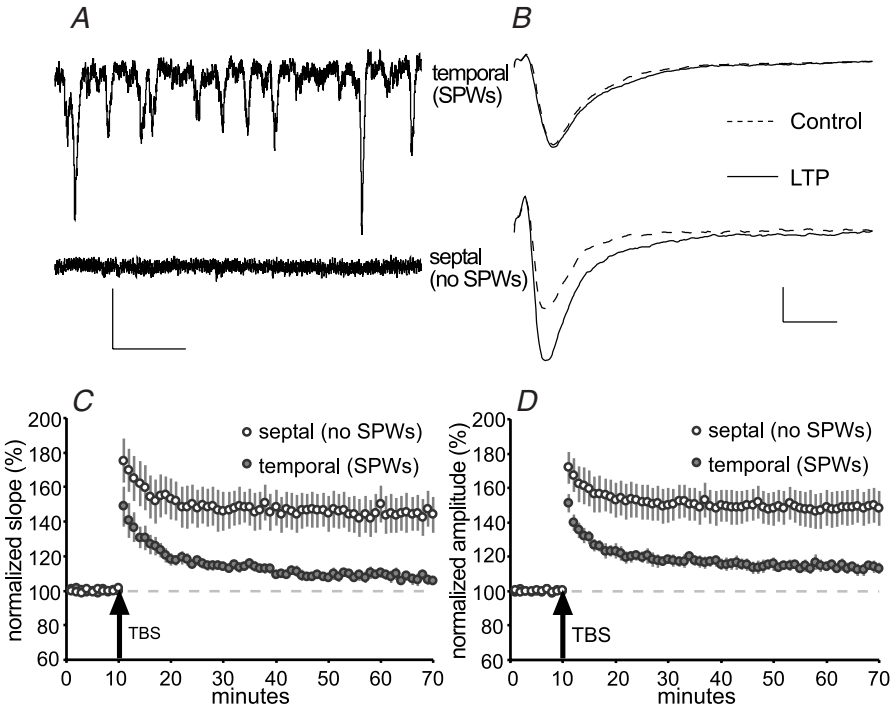

Figure 2. LTP was significantly decreased in slices from temporal hippocampus with spontaneously occurring SPWs

A, spontaneous field recording in CA3 stratum radiatum of slices prepared from temporal hippocampus (above) or septal hippocampus (below). SPW activity was only observed in the former case. Note that the phase of *in vitro* SPWs was negative-going in stratum radiatum, in contrast to the positive-going waves recorded in stratum pyramidale (see Fig. 1). Scale bars represent 500 ms and 0.2 mV. *B*, control and potentiated responses in CA3 stratum radiatum of a SPW-producing temporal slice (above) and a septal slice exhibiting no SPW activity (below). LTP decayed to near baseline in the presence of SPWs. Calibration: 5 ms, 0.4 mV. *C* and *D*, summary of all data for slope (*C*) and amplitude (*D*) measures across time. Slope and amplitude were normalized to 10 min baseline values. Theta bursts were delivered at the time indicated with an arrow.

Abraham, 1996). In accord with this, baseline responses in the present study were not significantly different in surgically isolated CA1 (1.61 ± 0.11 mV) than intact CA1 (1.53 ± 0.21 mV). Thus, this manipulation provided a means for examining LTP in temporal hippocampal slices in the absence of SPWs. Figure 3*B* illustrates the difference between LTP in intact *versus* isolated CA1 of slices from the temporal hippocampus. As is apparent in Fig. 3*B*–*D*, slices in which CA1 SPWs were suppressed by dissecting the projection from CA3 exhibited robust LTP in CA1 ($149.4 \pm 7.7\%$ for slope and $141.6 \pm 7.9\%$ for amplitude at 60 min post induction, $n = 5$). Conversely, slices in which the CA3–CA1 projection was preserved displayed SPW activity and expressed potentiation that decayed to close to baseline values by 30–60 min post induction ($99.7 \pm 11.2\%$ for slope and $103.4 \pm 9.8\%$ for amplitude at 60 min post induction, $n = 5$). These values were significantly lower than LTP in CA1 of slices with a cut between CA3 and CA1 and consequently no SPWs ($P < 0.0001$). Figure 3*E* shows LTP data for a group of four septal slices in which CA1 was physically separated from CA3 in the same manner as in the temporal slices. As is apparent, LTP stabilized at a level that was approximately 40% above baseline, suggesting that the procedure of cutting the projection from CA3 to CA1 does not enhance LTP in CA1 and thus is probably not responsible for the recovery of LTP observed in the temporal slices.

Single stimulation pulses delivered at a frequency of 5 Hz reverse LTP if delivered shortly after induction, and this effect is blocked by adenosine antagonists (Larson *et al.* 1993). The next set of experiments was conducted to determine if endogenous adenosine might also mediate the detrimental effects on LTP in slices exhibiting SPWs. The A1-selective adenosine receptor antagonist DPCPX was infused at a concentration of 250–500 nM for at least 30 min prior to the delivery of theta burst stimulation. Consistent with earlier reports (Dunwiddie & Hoffer, 1980; Arai & Lynch, 1992), the drug increased the amplitude of evoked responses by approximately 50%. In addition, DPCPX prevented the disruption of LTP in the presence of SPW activity. In a group of eight temporal hippocampal slices exhibiting SPWs and infused with DPCPX, LTP was significantly larger and more stable ($153.6 \pm 18.3\%$ for

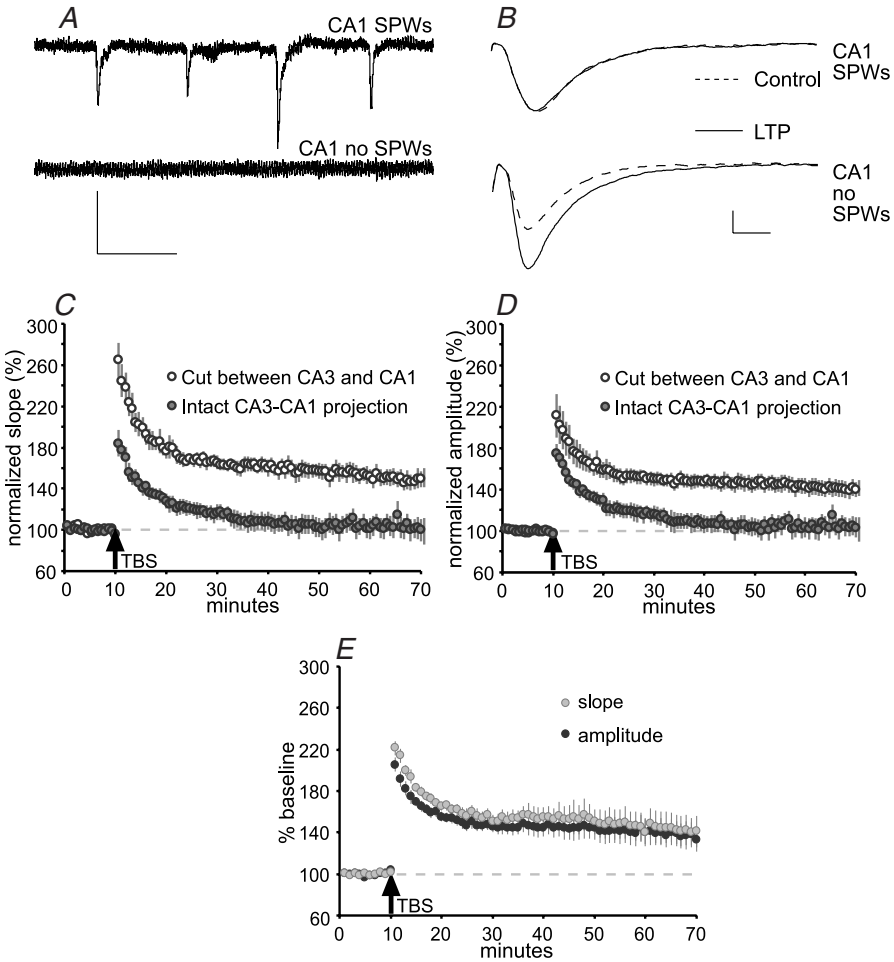

Figure 3. Elimination of SPWs in CA1 of temporal hippocampal slices by cutting the projection from CA3 allowed for induction of lasting LTP
A, representative field recordings from CA1 stratum radiatum of temporal hippocampal slices. The top trace depicts an example of SPW activity in a slice with an intact connection between CA3 and CA1, while the bottom trace shows a typical case with no SPW activity following surgical isolation of CA1 from CA3. Scale bars represent 500 ms and 0.2 mV. *B*, representative EPSPs in CA1 stratum radiatum during baseline and after the delivery of theta burst triplets in a slice with SPWs (above) and another slice in which SPWs were eliminated by removing afferent inputs from CA3 (below). Calibration: 5 ms, 0.5 mV, *C* and *D*, group data for normalized slope (*C*) and amplitude (*D*) measures in CA1. *E*, summary of data for SPW-deficient septal slices in which a cut was placed between CA3 and CA1 prior to induction of LTP. Note that LTP does not appear to be affected by the procedure.

slope and 139.8 ± 10.8% for amplitude at 60 min after theta burst delivery) than LTP in similar slices that were not treated with the antagonist ($P < 0.0001$; Figs 4*A* and *B*). This effect could not be attributed to the DPCPX-induced increase of the evoked response because DPCPX treatment did not significantly enhance LTP in a group of five septal slices exhibiting no SPW activity (153.5 ± 6.2% for slope and 155.9 ± 7.2% for amplitude at 60 min post LTP-induction). Moreover, as shown in Fig. 4*C*, stable LTP was obtained in a group of three temporal slices in which stimulation current was lowered after DPCPX infusion such that the amplitudes of baseline responses (1.53 ± 0.19) were not significantly different than those in the SPW-producing temporal slices that were not treated with DPCPX (1.64 ± 0.13 mV). Although both frequency and power of spontaneous activity appeared to be slightly increased by DPCPX treatment (Fig. 4*D–E*), this trend did not achieve statistical significance. It is possible that the large within- and across-slice variability of the SPWs obscured small effects of the drug.

The above results with DPCPX suggest the possibility that SPWs and low frequency stimulation pulses interfere with LTP via a common mechanism (i.e. endogenous adenosine). The next set of experiments was performed to test if irregularly timed stimulation pulses, modelled after spontaneous SPWs, can reverse LTP in septal slices that lack endogenous SPW activity. Figure 5 shows grouped data from seven septal slices in which 1 min trains of 'SPW pulse stimulation' (SPWPS) were applied to the CA3 associational axons during the 1 min periods separating theta burst triplets and immediately after the final theta burst triplet. In some slices, one to three additional episodes of SPWPS, separated by at least 1 min, were given during the subsequent 5 min period.

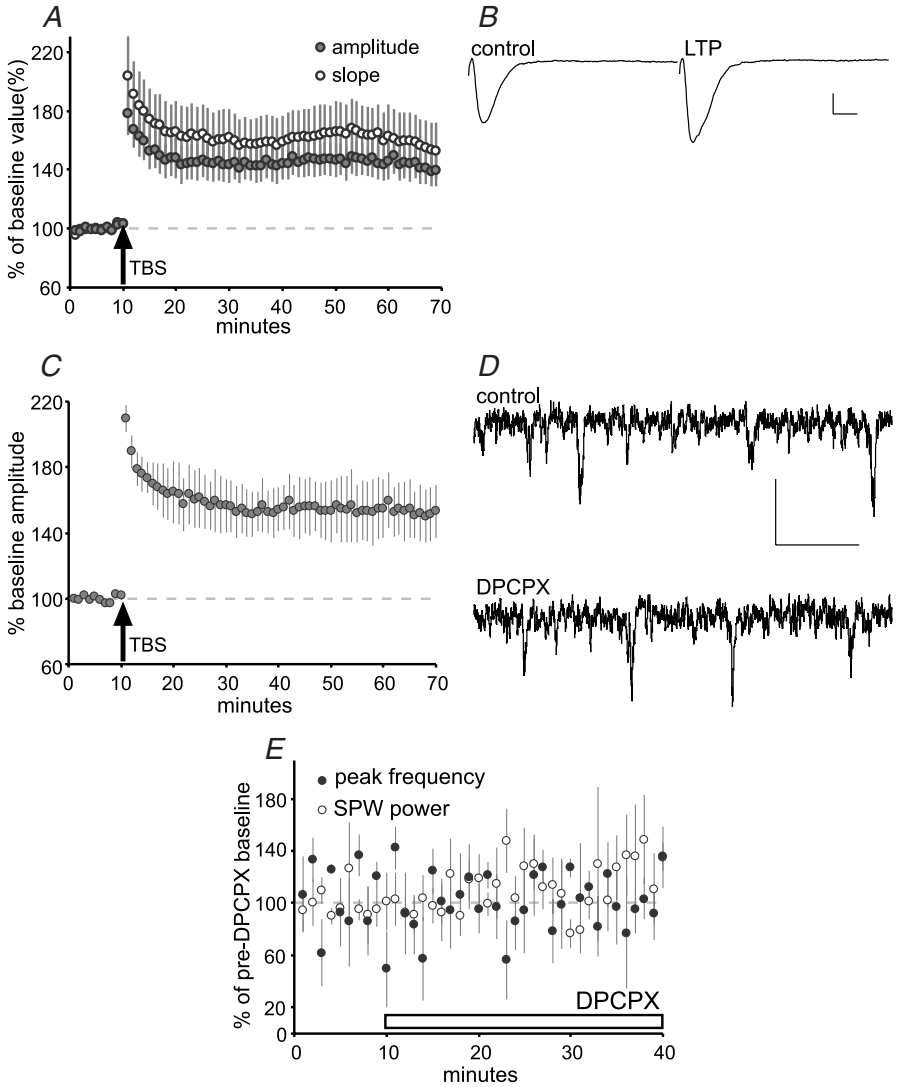

Figure 4. DPCPX, an antagonist of the A1-type adenosine receptor, blocked the impairment of LTP in SPW-producing slices
A, summary of LTP data for slices from temporal hippocampus pre-treated with DPCPX. *B*, example control and potentiated EPSPs recorded from CA3 stratum radiatum of slices infused with the adenosine antagonist DPCPX. Calibration bars represent 5 ms and 0.5 mV. *C*, LTP was not impaired in DPCPX-treated slices when stimulation current was lowered to counteract DPCPX-induced increases in baseline response amplitudes. *D*, spontaneous field recordings from CA3 stratum radiatum show that DPCPX had little effect on ongoing SPW activity. Calibration: 500 ms, 0.2 mV. *E*, peak frequency and power within the 0.1–7 Hz frequency band may have been slightly increased by DPCPX infusion, but the effects were highly variable and not significant.

In three out of seven slices, SPWPS completely prevented LTP. In the remaining four cases, LTP was lower than normal (134.4 ± 13.1% above baseline values at 60 min post induction). SPWPS was ineffective at reducing LTP when delivered tens of minutes after induction (data not shown). SPW-like activation of the CA3 associational projections appears to impair LTP to a similar degree as was observed in slices with spontaneous SPWs, but it should be noted that spontaneous SPWs arise during the 1 min intervals between theta burst triplets and that SPWPS was also applied between theta burst triplets. Thus, these activation patterns may have interfered with LTP induction, as opposed to simply erasing recently induced LTP.

Discussion

The present study used an *in vitro* model to investigate the relationship between SPWs and LTP. Spontaneously occurring SPW activity in rodent hippocampal slices has been previously described by the authors and others (Papatheodoropoulos & Kostopoulos, 2002; Wu *et al.* 2002; Kubota *et al.* 2003; Maier *et al.* 2003). Results presented here regarding the mechanisms of *in vitro* SPW generation are consistent with reports by Maier and colleagues describing SPWs in mouse hippocampus *in vitro* (Maier *et al.* 2003). In both cases, the oscillations were blocked by the AMPA receptor antagonist CNQX, associated with high-frequency ripples, and propagated from CA3 to CA1.

The novel finding of this study is that LTP is impaired in conventional slices from temporal hippocampus that show spontaneous SPW activity. Papatheodoropoulos & Kostopoulos (2000) reported previously that LTP was smaller in temporal than in septal hippocampal slices and attributed the difference to biochemical and/or anatomical variations between the two areas. The effect reported here cannot be accounted for in this manner because robust LTP was obtained in field CA1 of temporal slices by cutting the projection from CA3 to CA1 and thereby eliminating SPWs. The possibility that the microdissection procedure itself restored LTP seems unlikely. LTP in previous studies involving surgically isolated CA1 was similar to LTP in non-dissected hippocampal slices (Lynch *et al.* 1982; Cohen & Abraham, 1996). Moreover, in the present study, LTP was not enhanced by cuts made between CA3 and CA1 in septal slices with no SPWs.

The deficits in LTP in the presence of SPWs were not observed when slices were pre-treated with an adenosine receptor antagonist, suggesting that the effect may be mediated by extracellular adenosine. Adenosine antagonists also block reversal of LTP by low frequency stimulation pulses (Larson *et al.* 1993; Staubli & Chun, 1996). The point that SPWs and low frequency stimulation pulses affect LTP via the same mechanism is important because it raises the possibility that a naturally occurring form of the reversal phenomenon may exist. If this were the case, it would lead to a novel hypothesis regarding SPW function given that LTP reversal has previously been proposed to represent a neural mechanism for forgetting (Staubli & Lynch, 1990; Huang & Hsu, 2001). Intermittent, low frequency stimulation pulses patterned after SPWs disrupted long-term potentiation when given immediately following LTP induction; this is also the most effective time period for reversing LTP with 5 Hz stimulation.

It should be noted that the hypothesis introduced above is in disagreement with earlier reports that concluded that SPWs act as a natural tetanizer to induce LTP (Buzsaki *et al.* 1987; King *et al.* 1999). However, the methods used in the previous experiments to assess LTP differed greatly from those employed here. In the former study, low frequency stimulation (0.1 Hz) was applied in the presence of the $GABA_A$ receptor antagonist bicuculline, resulting in a long-term enhancement of the orthodromic population spike in CA1 of rat hippocampal slices (Buzsaki *et al.* 1987). Interpretation of this result with regard to SPW function is based on the assumption that the evoked population burst in the presence of bicuculline is analogous to SPWs *in vivo*. It can be argued that the waves recorded in the present study are more likely to correspond to SPWs *in vivo*, given that they occur spontaneously

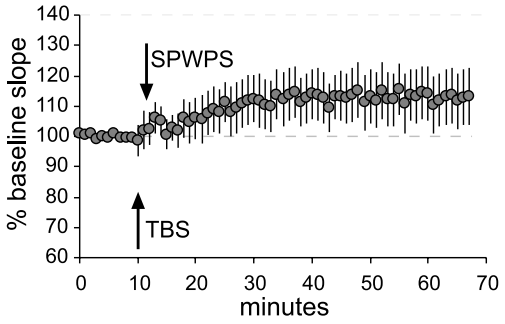

Figure 5. Stimulation pulses modelled after spontaneous SPW activity had a detrimental effect on LTP
In these experiments, the same theta burst triplet protocol was employed to induce LTP except that irregularly timed stimulation pulses (i.e. SPW pulse stimulation; SPWPS) with a mean interpulse interval of 385 ms were delivered throughout the 60 second intervals that separated the four theta burst triplets. Up to 3 additional SPWPS episodes were delivered in the 5 min following theta burst stimulation. LTP was completely reversed in 3 out of 7 cases; the data shown here represent the average values across all 7 slices.

and are accompanied by high frequency ripples. In the study by King *et al.* (1999), excitatory stimulation was directly paired with SPW events for a brief 'training' period lasting several minutes. Following training, SPW-associated depolarizations, measured either directly via intracellular recording or indirectly by extracellular spike detection, were increased for at least 15 min (King *et al.* 1999). In the present study, stimulation pulses were not correlated with ongoing SPW activity, and LTP was measured as an increase in the size of synaptic responses rather than as an increase in SPWs themselves. Thus, the design and endpoint measurements of the present experiments were fundamentally different from those in the protocol used by King and colleagues, and this may explain the discrepancy in results.

In summary, the present results show that LTP induced by theta burst stimulation is disrupted in slices exhibiting spontaneous SPWs and indicate that this effect is mediated by adenosine receptors. The latter point links the present data to previously described effects involving reversal of LTP. In support of this, irregular low frequency stimulation patterns modelled after spontaneous SPWs impaired recently induced LTP in septal slices. During active exploratory behaviours, cholinergic neurones of the basal forebrain are activated and sharp waves are replaced by theta rhythm in hippocampus; under these conditions, induction of LTP is reportedly facilitated (Leung *et al.* 2003). The present results raise the possibility that sharp waves may constitute a processing state of the hippocampal network that, in contrast to theta, interferes with the induction and/or stabilization of LTP.

References

Arai A & Lynch G (1992). Factors regulating the magnitude of long-term potentiation induced by theta pattern stimulation. *Brain Res* **598**, 173–184.

Buzsaki G (1986). Hippocampal sharp waves: their origin and significance. *Brain Res* **398**, 242–252.

Buzsaki G, Haas HL & Anderson EG (1987). Long-term potentiation induced by physiologically relevant stimulus patterns. *Brain Res* **435**, 331–333.

Buzsaki G, Leung LW & Vanderwolf CH (1983). Cellular bases of hippocampal EEG in the behaving rat. *Brain Res* **287**, 139–171.

Cohen AS & Abraham WC (1996). Facilitation of long-term potentiation by prior activation of metabotropic glutamate receptors. *J Neurophysiol* **76**, 953–962.

Dunwiddie TV & Hoffer BJ (1980). Adenine nucleotides and synaptic transmission in the in vitro rat hippocampus. *Br J Pharmacol* **69**, 59–68.

Holscher C, Anwyl R & Rowan MJ (1997). Stimulation on the positive phase of hippocampal theta rhythm induces long-term potentiation that can be depotentiated by stimulation on the negative phase in area ca1 in vivo. *J Neuroscience* **17**, 6470–6477.

Huang CC & Hsu KS (2001). Progress in understanding the factors regulating reversibility of long-term potentiation. *Rev Neurosci* **12**, 51–68.

Hyman JM, Wyble BP, Goyal V, Rossi CA & Hasselmo ME (2003). Stimulation in hippocampal region CA1 in behaving rats yields long-term potentiation when delivered to the peak of theta and long-term depression when delivered to the trough. *J Neuroscience* **23**, 11725–11731.

King C, Henze DA, Leinekugel X & Buzsaki G (1999). Hebbian modification of a hippocampal population pattern in the rat. *J Physiol* **521**, 159–167.

Kubota D, Colgin LL, Casale M, Brucher FA & Lynch G (2003). Endogenous waves in hippocampal slices. *J Neurophysiol* **89**, 81–89.

Larson J, Wong D & Lynch G (1986). Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. *Brain Res* **368**, 347–350.

Larson J, Xiao P & Lynch G (1993). Reversal of LTP by theta frequency stimulation. *Brain Res* **600**, 97–102.

Leung LS, Shen BX, Rajakumar N & Ma JY (2003). Cholinergic activity enhances hippocampal long-term potentiation in CA1 during walking in rats. *J Neuroscience* **23**, 9297–9304.

Lynch G, Halpain S & Baudry M (1982). Effects of high-frequency synaptic stimulation on glutamate receptor binding studied with a modified in vitro hippocampal slice preparation. *Brain Res* **244**, 101–111.

Maier N, Nimmrich V & Draguhn A (2003). Cellular and network mechanisms underlying spontaneous sharp wave-ripple complexes in mouse hippocampal slices. *J Physiol* **550**, 873–887.

Papatheodoropoulos C & Kostopoulos G (2000). Decreased ability of rat temporal hippocampal CA1 region to produce long-term potentiation. *Neurosci Lett* **279**, 177–180.

Papatheodoropoulos C & Kostopoulos G (2002). Spontaneous, low frequency (approximately 2–3 Hz) field activity generated in rat ventral hippocampal slices perfused with normal medium. *Brain Res Bull* **57**, 187–193.

Pavlides C, Greenstein YJ, Grudman M & Winson J (1988). Long-term potentiation in the dentate gyrus is induced preferentially on the positive phase of theta-rhythm. *Brain Res* **439**, 383–387.

Staubli U & Chun D (1996). Proactive and retrograde effects on LTP produced by theta pulse stimulation: mechanisms and characteristics of LTP reversal in vitro. *Learn Mem* **3**, 96–105.

Staubli U & Lynch G (1987). Stable hippocampal long-term potentiation elicited by 'theta' pattern stimulation. *Brain Res* **435**, 227–234.

Staubli U & Lynch G (1990). Stable depression of potentiated synaptic responses in the hippocampus with 1–5 Hz stimulation. *Brain Res* **513**, 113–118.

Staubli U & Scafidi J (1999). Time-dependent reversal of long-term potentiation in area CA1 of the freely moving rat induced by theta pulse stimulation. *J Neurosci* **19**, 8712–8719.

Suzuki SS & Smith GK (1987). Spontaneous EEG spikes in the normal hippocampus. I. Behavioral correlates, laminar profiles and bilateral synchrony. *Electroencephalogr Clin Neurophysiol* **67**, 348–359.

Vertes RP & Kocsis B (1997). Brainstem-diencephalo-septohippocampal systems controlling the theta rhythm of the hippocampus. *Neuroscience* **81**, 893–926.

Wu C, Shen H, Luk WP & Zhang L (2002). A fundamental oscillatory state of isolated rodent hippocampus. *J Physiol* **540**, 509–527.

Acknowledgements

This research was funded by Matsushita Electric Industrial Co., Ltd Grant MEI-27599. L.L.C. was funded by NIA Training Grant 5T32 AG00096-20. The authors thank Fernando A. Brucher for helpful discussions.