Long-Term Potentiation in the Dentate Gyrus Is Not Linked to Increased Extracellular Glutamate Concentration

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Jay, T. M., E. Zilkha, and T. P. Obrenovitch. Long-term potentiation in the dentate gyrus is not linked to increased extracellular glutamate concentration. J. Neurophysiol. 81: 1741–1748, 1999. Long-term potentiation (LTP) of excitatory transmission is a likely candidate for the encoding and storage of information in the mammalian brain. There is a general agreement that LTP involves an increase in synaptic strength, but the mechanisms underlying this persistent change are unclear and controversial. Synaptic efficacy may be enhanced because more transmitter glutamate is released or because postsynaptic responsiveness increases or both. The purpose of this study was to examine whether increased extracellular glutamate concentration was associated with the robust and well-characterized LTP that can be induced in the rat dentate gyrus. To favor the detection of any putative change in extracellular glutamate associated with LTP, our experimental strategy included the following features. 1) Two separate series of experiments were carried out with animals under pentobarbital or urethan anesthesia; 2) changes in extracellular concentration of glutamate were monitored continuously by microdialysis coupled to enzyme amperometry; and 3) dialysate glutamate levels and changes in the slope of excitatory postsynaptic potential evoked by activation of the perforant path were recorded precisely at the same site. Tetanic stimulation of the perforant path increased persistently test-evoked responses in the dentate gyrus (by 19 and 14% in barbiturate and urethan group, respectively), but there was no glutamate change either during or after LTP induction and no indication of increased glutamate efflux when low-frequency stimulation was applied. The results do not rule out a possible contribution of enhanced glutamate exocytosis to LTP induction and/or maintenance because such a presynaptic change may not be detectable extracellularly. However, our findings and other data supporting the notion that neurotransmitter glutamate may hardly leak out of the synaptic cleft conflict with the hypothesis that LTP could also involve a broad synaptic spillover of glutamate.

INTRODUCTION

Brief high-frequency stimulation of presynaptic fibers can induce a long-lasting enhancement of synaptic transmission, and this phenomenon [i.e., long-term potentiation (LTP)] may be one of the possible cellular mechanisms underlying learning and memory (Bliss and Collingridge 1993). Most of the excitatory synapses where LTP can be elicited are operated by glutamate, a transmitter that activates both ionotropic and metabotropic receptors. Although it is generally acknowledged that induction of LTP involves depolarization with subsequent postsynaptic Ca++ influx through N-methyl-D-aspartate (NMDA) receptor-operated ion channels, how this initial Ca++ rise is converted into sustained enhancement of the synaptic response is still unresolved. Synaptic strength may be persistently enhanced because more transmitter glutamate is released or because postsynaptic responsiveness increases or both (Kullmann and Siegelbaum 1995). Early in vivo experimental data were interpreted as indicative of LTP induction resulting in long-lasting enhancement of transmitter release (Bliss et al. 1986; Dolphin et al. 1982; Lynch et al. 1985; Skrede and Malthe-Sorensen 1981). For example, samples of extracellular perfusate collected from a push–pull cannula implanted into the dentate gyrus contained more glutamate after LTP induction (2- to 3-fold basal levels for 1.5 h after LTP induction) (Bliss et al. 1986), and the specific NMDA receptor antagonist d(-)-aminophosphonovaleter (APV) blocked both LTP induction and glutamate increase (Errington et al. 1987). However, these data could not be confirmed (Aniksztejn et al. 1987, 1989; Roisin et al. 1990), and the finding that both glutamate and aspartate increased in the extracellular fluid after LTP (Bliss et al. 1986) weakens the notion that these changes indicated enhanced exocytosis. Indeed, aspartate is not accumulated in synaptic vesicles (Burger et al. 1989; Fykse et al. 1992) and therefore may not be a neurotransmitter (Nicholls 1993; Orrego and Villameuva 1993; however, see Gundersen et al. 1998).

Preliminary data obtained by monitoring extracellular glutamate with an implantable biosensor suggested a sustained increase in basal glutamate after LTP induction and a twice larger peak of stimulus-dependent (2 Hz) glutamate release measured 60 min after LTP induction (Galley et al. 1993), but a full report of this study was not published.

Although the “positive” results outlined previously are often taken as strong evidence for a presynaptic component of LTP (Richter-Levin et al. 1995), whether increased extracellular glutamate is associated with LTP induction and maintenance is still unclear. This is an important issue because persistently increased extracellular levels of glutamate may suggest that spillover of transmitter from one synapse to the adjacent ones contributes to LTP (Asztely et al. 1997; Kullmann et al. 1996) and/or interneuronal spread of LTP (Harris 1995). The main purpose of this study was to examine this question in vivo by application of effective and sensitive methods to the robust and well-characterized dentate gyrus LTP. Glutamate concentration in the dialysate was measured by enzyme-amperometry (biosensor) as it emerged from a microdialysis probe implanted
into the hippocampus of anesthetized rats. As general anesthesia may interfere with neurotransmitter release (Miao et al. 1993; Schlamn and Hemmings 1995), rats were anesthetized with either sodium pentobarbitone or urethan, two anesthetics agents with which dentate gyrus LTP was demonstrated in vivo (Douglas and Goddard 1975; Winson and Dahl 1986). Field potentials evoked in the dentate gyrus by electrical stimulation of the perforant path were recorded precisely at the microdialysis sampling site. The effects of repetitive low-frequency (2 Hz) stimulation were also examined before and after LTP induction, as previously performed by Galley and co-workers (1993).

**METHODS**

**Animal preparation**

Adult male Sprague-Dawley rats (300–400 g; IFFA-Credo, L’Arbresle, France) were anesthetized throughout with either sodium pentobarbitone (65 mg/kg ip, subsequently supplemented as necessary; n = 10) or urethan (1.5 g/kg ip; n = 8) and positioned in a stereotaxic frame. Body temperature was maintained at 36.5–37.5°C by using a heating blanket.

A concentric microdialysis probe with an external 62-μm Ni-chrome electrode (Fig. 1A) (2-mm dialysis fiber length; Applied Neuroscience, London, U. K.) was slowly lowered into the hilus of the dentate gyrus (coordinates: 4.2 mm posterior to bregma, 2.5 mm lateral to midline) (Paxinos and Watson 1986). A concentric bipolar stainless steel stimulating electrode (300 μm OD) was placed ipsilaterally into the angular bundle of the medial perforant path (coordinates: 8.0 mm caudal to bregma, 4.3 mm lateral to midline) (Paxinos and Watson 1986). Recording and stimulating electrodes were lowered under electrophysiological control, and the depth, adjusted to optimize the slope of the positive-going population excitatory postsynaptic potential (EPSP), was generally 3 mm below the cortical surface for both electrodes. The tip of the recording dialysis electrode was in or just below the granule cell layer so that the dialysis membrane would straddle CA1 and the dentate gyrus. The recording electrode was consistently oriented toward the stimulating electrode relative to the dialysis fiber to preserve the integrity of the perforant path between stimulating and recording electrodes (Fig. 1B).

**Recording of extracellular field potentials**

Test pulses (100 μs, 0.033 Hz) were delivered via an isolated constant current unit at an intensity that evoked a population spike of 20–40% of its maximum (200–500 μA). Field potentials were amplified, filtered (band-pass 0.1 Hz to 3 kHz), digitized at 20 kHz, and stored to disk as averages of four consecutive responses for off-line analysis. LTP was induced as follows: six series at 2-min intervals of six high-frequency trains (400 Hz for 20 ms) at 0.1 Hz. To increase the number of potentiated synapses the stimulus intensity was increased during the tetanus by 200 μA. Low-frequency stimulation (LFS) protocols (1,200 stimuli at 2 Hz) were conducted at baseline intensity in naive (nonpotentiated) and potentiated synapses.

**On-line enzyme-amperometric analysis of dialysate glutamate**

Microdialysis probes were perfused with artificial cerebrospinal fluid (composition in mM: 125 NaCl, 2.5 KCl, 1.18 MgCl₂, 1.26 CaCl₂, and 0.2 NaH₂PO₄, pH 7.3 adjusted with 1 M NaOH) at 1 μl/min with a syringe pump (CMA/100; CMA/Microdialysis, Stockholm, Sweden).

The outflow tube of the microdialysis probe was directly connected to a glutamate biosensor (i.e., enzyme-amperometric flow cell). This method was selected for monitoring changes in dialysate glutamate because it is so far the most effective in terms of sensitivity, specificity, and time resolution, and the delay between neurochemical changes and their detection is reduced to 2–3 min (Zilkha et al. 1995). Biosensors (Applied Neuroscience) consisted of two blocks of perspex. One contained the reference (Ag/AgCl) electrode, the auxiliary electrode (platinum), and the inlet and outlet ports to the cells; the other incorporated the platinum working electrode. A thin polytetrafluoroethylene (PTFE) gasket with a 0.5 × 19 mm slot provided a narrow channel between the working electrode and all the components of the opposite block listed previously. To eliminate interference resulting from direct oxidation of ascorbic acid and other electroactive compounds, a film of 1.2-diaminobenzene (Sigma Chemical, Poole, U. K.) was deposited on the working electrode by electropolymerisation. L-Glutamate oxidase (200 U/ml) was immobilized with 2.5% glutaraldehyde in phosphate buffer. For a detailed description of the method see Zilkha et al. (1995).

The glutamate signal was continuously digitized and stored on disk by using a computer (IBM PC equivalent) equipped with an A/D converter card (Metabyte DAS-20, Keithley, Reading, U. K.) and running a dedicated program (ASYST laboratory software, Keithley, Reading, U. K.). Glutamate detection was calibrated at the end of each experiment with a 20 μM standard solution of L-glutamate.

**Experimental protocol**

Test pulses were delivered every 30 s unless otherwise stated. Two-hour postimplantation, animals were subjected to the following procedure: 20-min baseline; 10-min low-frequency (2 Hz) stimulation with test pulse intensity (i.e., frequency of electrical stimulation of the dentate gyrus was increased from 0.033 to 2 Hz for 10 min); 60-min baseline, LTP induction with six series, 2 min apart, of six trains (400 Hz for 20 ms) at 0.1 Hz, delivered at test intensity plus 200 μA; 60-min recording after the end of the tetanic stimulation; 10-min LFS (described previously); and 30-min baseline; death by intraperitoneal administration of anesthetic overdose.

**Presentation and analysis of data**

The synaptic component of the evoked response was measured by calculating the maximum slope of the early rising phase of the population EPSP. For each individual rat, the data were expressed as a percent change of the mean baseline value obtained before high-frequency stimulation or LFS.

Continuous storage of glutamate data in a digitized form together with strict adherence to the previous experimental procedure allowed us to average relevant corresponding sequences from all experiments.


Results

Figure 2 illustrates the potential of microdialysis coupled to enzyme-amperometric analysis for the detection of transient changes in extracellular glutamate concentration. It shows glutamate efflux as the probe was inserted into the brain (i.e., penetration injury) (Obrenovitch and Urenjak 1997b) and the characteristic biphasic pattern produced by terminal ischemia (i.e., synchronous exocytosis followed by cytosolic efflux) (Obrenovitch and Urenjak 1997a).

Changes in EPSP slope and extracellular glutamate associated with LTP induction

Barbiturate anesthesia. Two experiments were omitted in this series because suitable LTP could not be induced (technical difficulties with implantation). With pentobarbital-anesthetized rats, high-frequency stimulation of the medial perforant pathway consistently resulted in a robust potentiation of the EPSP recorded in the hilus of the dentate gyrus. As shown in Fig. 3A (top graph), the EPSP slope expressed as a percentage of its pretetanus value increased, reached a plateau a few minutes after the last tetanus, and stayed at the same level for up to 60 min (19.4 ± 0.5%, n = 8). The population spike component was also increased after tetanus (226 ± 7% of baseline; data not shown).

In this group, the average level of glutamate in the dialysate was 1.19 ± 0.08 μM (n = 8) during the 30 min preceding LTP induction. There was no detectable change in the dialysate concentration of glutamate during LTP induction and the subsequent 30 min (Fig. 3A, bottom graph).

Urethan anesthesia. Two experiments were omitted in this series, one because LTP could not be induced and the other because of excessive oscillations of the glutamate signal because of microdialysis perfusion irregularities. The magnitude of LTP elicited in the medial perforant pathway of urethan-anesthetized rats was comparable with that measured in pentobarbital-anesthetized rats. After tetanus, EPSP remained significantly potentiated for >60 min (increase by 13.8 ± 0.5%; n = 6; Fig. 3B, top graph), and the population spike component increased to 197 ± 13% of the baseline (data not shown).

In this group the average level of glutamate in the dialysate was 1.14 ± 0.11 μM (n = 6) during the 30 min preceding LTP induction, not significantly different from that measured under barbiturate anesthesia. No consistent change in extracellular glutamate was detected, either during or after LTP induction (Fig. 3B, bottom graph). In one experiment, a small positive shift (0.05–1.0 μM) of dialysate glutamate appeared to develop with LTP induction, but similar changes were observed in another experiment in absence of any electrical stimulation.

Effect of LFS (2 Hz) on naive and potentiated synapses and extracellular glutamate

Barbiturate anesthesia. LFS (2 Hz) of the perforant pathway applied either 1 h before or after tetanus for 10 min produced a similar transient depression in the dentate gyrus synaptic transmission (Fig. 4, A and B). The EPSP slope decreased immediately after the beginning of LFS application, reached a plateau (reduction by 23.2 ± 0.4 and 20.7 ± 0.5% in naive and potentiated synapses, respectively; n = 5), and recovered to near-baseline values after resumption of test stimulation.

Examination of individual sequences of the glutamate signal suggested that a small transient decrease might occur during LFS. However, statistical analysis of this change was difficult because the effects of the first 2-Hz stimulus was superimposed...
showed a small, but significant decrease during stimulation (Student’s paired comparison of LFS and post-LFS glutamate values with pre-LFS values throughout LFS, and follows:

3) of values obtained for each animal by averaging the glutamate signal as follows: 1) over the 10 min immediately preceding the start of LFS, 2) throughout LFS, and 3) over the first 10 min that immediately followed LFS. Comparison of LFS and post-LFS glutamate values with pre-LFS values showed a small, but significant decrease during stimulation (Student’s paired t-test; *P < 0.01).

A small, significant reduction during stimulation (by 0.06 μM; P < 0.001, n = 6) compared with the level averaged over the preceding 10 min, but the level during LFS was not different from that averaged during the first 10 min poststimulation (P = 0.39). In contrast, with the second 2-Hz stimuli, which was not superimposed on a declining baseline, the mean level during stimulation was not different from that measured before stimulation (P = 0.68) but was slightly lower (by 0.05 μM) than during the subsequent 10-min period (P < 0.01). Figure 4C shows the changes in the dialysate concentration of glutamate when recordings obtained with LFS either applied 1 h before or after LTP induction were pooled together (n = 2 × 6). With this latter analysis, the overall mean glutamate concentration during LFS was slightly lower than the preceding one (P < 0.01).

URETHAN ANESTHESIA. The 2-Hz protocol also produced a temporary depression of EPSP by 25.4 ± 0.7 and 29.5 ± 0.4% in naïve and potentiated synapses, respectively; n = 7 and n = 5), and the recovery from this change appeared more progressive than with barbiturate. In all cases, LFS produced a transient decrease in the EPSP slope, with complete disappearance of the population spike (data not shown).

Under urethan anesthesia, the first LFS at 2 Hz produced a small increase in the dialysate concentration of glutamate (Fig. 5, A and B), but significance was reached only with the values averaged during 0–5 min and 5–10 min of LFS (1.15 ± 0.12 μM vs. 1.22 ± 0.13 μM; n = 7, P < 0.05). Paradoxically,
the same stimuli applied 60 min after LTP induction had no effect on dialysate glutamate (Fig. 5B, bottom graph).

**DISCUSSION**

**Methodology**

Four circumstances were combined in this study to assist the detection of any putative change in extracellular glutamate associated with LTP: 1) investigation of dentate gyrus LTP induced by high-frequency stimulation of the medial perforant pathway because it is robust and well characterized; 2) careful positioning of the recording electrode relative to the microdialysis fiber and stimulating electrode (Fig. 1) to record field potentials at the sampling site and minimize damage to the afferent fibers; 3) on-line enzyme-amperometric detection of glutamate, which provides maximal sensitivity, selectivity, and time resolution (Zilkha et al. 1995) (Fig. 2); and 4) two series of experiments with different anesthetics.

Despite our precautions to preserve the integrity of the afferent fibers between stimulating and recording electrodes, the potentiation achieved when the recording electrode was adjacent to a dialysis fiber was approximately one-half to one-third that obtained in previous experiments with a recording electrode alone (Doyère et al. 1996), suggesting that microdialysis adjacent to the recording electrode may be deleterious to LTP induction and/or field potential recording. Possible detrimental effects of microdialysis include more pronounced tissue damage and buffering of potential extracellular changes contributing to interneuronal spread of LTP (Harris 1995). However, a consistent and marked LTP was still recorded precisely at the sampling site of the microdialysis probe (Fig. 3).

The representative changes in dialysate glutamate produced by penetration injury and death (Fig. 2) and the fact that we were capable of detecting changes as small as 0.05 μM associated with LFS (Figs. 4 and 5) illustrate the suitability and performance of microdialysis coupled to enzyme-amperometric detection. In previous studies, microdialysis coupled to enzyme-amperometric detection allowed us to detect small increases in extracellular glutamate levels produced by rising the concentration of K⁺ in the perfusion medium to only 15 mM and to resolve very brief changes in glutamate associated with spreading depression (Obrenovitch and Zilkha 1995a; Obrenovitch et al. 1996). As opposed to implantable biosensors (Walker et al. 1995), flow enzyme-amperometric analysis allowed us to obtain data even during electrical stimulation.

The exposed length of the probe extended above and below the tip of the recording electrode (Fig. 1) and therefore a substantial proportion of the collecting area were not in the middle of the molecular layer where the medial perforant path fibers terminates. Despite this inevitable “dilution” of the glutamate signal arising from the molecular layer, the high sensitivity of our detection system should have been capable of recording any relevant changes in extracellular glutamate associated with induction of LTP in this layer.

No detectable change in extracellular glutamate was associated with LTP induction: implications for the presynaptic hypothesis of LTP

Our data (Fig. 3) clearly contradict the notion that LTP induction in the hippocampal dentate gyrus may be associated with an increase in extracellular concentration of glutamate. However, these data do not rule out a possible contribution of enhanced presynaptic glutamate release to LTP induction and/or maintenance because such a change may remain undetected extracellularly. This point can be exemplified with data obtained with 4-aminopyridine (4-AP), a suitable agent for selective induction of exocytotic glutamate release from synaptosomes (Tibbs et al. 1996). In contrast to in vitro studies Segovia et al. (1997) did not find any increase in dialysate levels of glutamate when 4-AP (1, 5, and 10 mM) was perfused through the probe despite a decrease in extracellular glutamine that could reflect recycling of transmitter glutamate and GABA via the glutamine synthesis pathway (Hertz 1979; Westergaard et al. 1995). In the course of the study reported herein 4-AP (1 and 10 mM) was applied through the probe in three separate experiments. This agent did not increase the dialysate levels of glutamate despite a marked enhancement of local EPSPs (Obrenovitch 1998b). Similarly, we did not detect any change in extracellular glutamate levels in the dentate gyrus during tetanic stimulation of the perforant path (Fig. 3).

In addition, raised extracellular glutamate levels may not necessarily imply enhancement of its presynaptic release (Miele et al. 1996; Obrenovitch 1998b; Timmerman and Westerink 1997). For example, neuronal activation subsequent to induced grooming in rats was accompanied by enhanced glutamate efflux, but this change was not reduced when TTX was present in the microdialysis perfusion medium (Miele et al. 1996).

Increased glutamate exocytosis may not lead to glutamate accumulation in the extracellular space because of one or several of the following elements: synapse ultrastructure (Edwards 1995), relatively small contribution of synapses to the overall cell membrane area (Rusakov et al. 1998), efficient glutamate uptake mechanisms (Elisof and Werblin 1993), and changes in extracellular glutamate of nonneuronal sources masking those much smaller of synaptic origin.

Whether LTP in the dentate gyrus is associated with increased glutamate exocytosis might be settled with in vitro studies where glutamate release is evaluated by whole cell recording of glial glutamate transport currents, i.e., by taking advantage of the electrogenerity of glutamate uptake (Barbour et al. 1991). So far application of this strategy to hippocampal slices suggested that LTP does not alter the amount of glutamate released on synaptic stimulation (Diamond et al. 1998; Eger et al. 1998). Other promising experimental strategies may be those relying on specific pharmacological agents (e.g., selective and noncompetitive inhibitors of glutamate uptake) and measurements of changes in exocytotic–endocytotic cycling (Malgaroli et al. 1995).

No detectable change in extracellular glutamate was associated with LTP induction: implications for the spillover hypothesis of LTP

It was suggested that spillover of transmitter from one synapse to the adjacent ones, first discovered at lower vertebrate inhibitory synapse (Faber and Korn 1988), may also contribute to enhance the size of quantal events in hippocampal cells during LTP, provided there is an increase of glutamate release that raises its concentration in the synaptic cleft (Kullmann et al. 1996). Despite obvious differences in the geometry and
processes governing microdialysis probe/surrounding tissue exchanges on one hand and putative transmitter spillover from one synapse to the adjacent ones on the other hand, we consider that our data and other findings conflict with the spillover hypothesis of LTP.

1) Two different groups presented evidence that LTP spreads to neighboring cells located within \( \leq 150 \mu m \) of the potentiated cell (Bonhoeffer et al. 1989; Schuman and Madison 1994). As microdialysis should be capable of revealing any glutamate efflux diffusing over such a distance (Benveniste and Hüttemeier 1990), LTP is unlikely to be associated with a broad spillover of glutamate.

2) One would anticipate that during tetanic stimulation synchronized exocytosis should result in a generalized spillover from glutamatergic synapses, but this did not occur in these experiments (Fig. 3). Similar, apparently paradoxical observations were made with drug-induced seizures (Obrenovitch et al. 1996).

3) If synaptic spillover of transmitter glutamate were to occur and to be functionally relevant, one would expect markedly increased extracellular levels of glutamate, subsequent to local application of exogenous glutamate and/or pharmacological inhibition of its uptake, to produce obvious abnormalities. This was not the case in our studies (Obrenovitch et al. 1996, 1997), even when high extracellular glutamate was superimposed on ischemia or spreading depression (Obrenovitch and Zilkha 1995b; Obrenovitch et al. 1998).

**LTP induction and increased glutamate efflux in previous studies: critical re-appraisal**

We already mentioned that earlier findings, obtained with the push–pull method and interpreted as indicative of increased glutamate exocytosis, should not be taken as strong evidence of presynaptic mechanisms in LTP (Bliss et al. 1986; Errington et al. 1987).

Despite our efforts to reproduce as much as possible the experimental procedure of Galley et al. (1993), we could not confirm their findings (sustained increase in basal and stimulus-dependent glutamate efflux during LTP). From our experience with glutamate biosensors, we propose that the changes in amperometric current obtained with their implanted biosensors might not have reflected actual changes in glutamate. Implantable devices are prone to interferences (i.e., changes taking place in the tissue may alter the device sensitivity and/or selectivity), and a thorough validation of implantable devices is necessary, especially when only small changes in extracellular glutamate are expected (Obrenovitch 1998a; Obrenovitch and Zilkha 1998).

In two studies, LTP was induced in vivo, and depolarization-induced release of endogenous glutamate subsequently tested ex vivo in potentiated and control hippocampal preparations. Ghijsen et al. (1992) found that the \( K^+ \)-induced \( Ca^{2+} \)-dependent release of glutamate from CA1 subslices was increased 150% in rats killed 30 min after LTP induction. Canevari et al. (1994) reported that \( K^+ \) and veratridine release of glutamate was enhanced in synaptosomes prepared from potentiated dentate gyrus. Both groups concluded that these results suggest a persistent increase in the presynaptic vesicular pool of glutamate during LTP, but the following alternative interpretation was overlooked: enhanced glutamate efflux from tissue previously subjected to tetanic stimulation may actually reflect its increased sensitivity to the depolarizing agents used for glutamate release induction. Two intriguing findings are in line with the latter hypothesis. 1) GABA release from CA1 subslices was also enhanced 30 min after LTP induction (Ghijsen et al. 1992); 2) with 4-AP, a depolarizing agent that triggers exocytosis more specifically than \( K^+ \) and veratridine (Tibbs et al. 1996), glutamate release was not significantly enhanced in synaptosomes prepared from potentiated dentate gyrus (Canevari et al. 1994).

**Effects of low-frequency electrical stimulation**

As previously reported, repetitive LFS of the medial perforant path results in transient but not sustained depression in both the EPSP and population spike in the dentate gyrus (Errington et al. 1995; Harris et al. 1979). Such decrements of the synaptic response under repetitive stimulation could result from presynaptic or postsynaptic mechanisms. Along with the feedforward inhibitory influence on granule cells (defined as inhibition driven by excitatory afferents of inhibitory interneurons) involving GABA-mediated mechanisms recruited at frequency above 1 Hz (Sloviter 1991), a difference in the strength of the excitatory drive could also contribute to the depression of the synaptic response. It is also clear that under repetitive stimulation the releasable transmitter in a limited store is depleted, and the presynaptic terminals are not instantaneously replenished. Given the brief time window in which the depression of the signal occurs, one would have predicted an immediate change in the concentration of glutamate.

We are confident that the slight shifts in amperometric current associated with 10 min of 2-Hz electrical stimulation reflected changes in extracellular glutamate (Figs. 4 and 5). A small, transient decrease was observed in barbiturate-anesthetized rats, whereas a small, apparently more persistent increase occurred in the urethan group. The biological significance of these changes and their possible origins are difficult to assess. One interesting feature, however, is the difference in pattern of changes obtained with barbiturate and urethan anesthesia, suggesting that anesthetics may mask or favor different mechanisms (exocytosis, uptake, or metabolism) influencing glutamate homeostasis. Although the precise mechanism of the anesthetics, urethan and pentobarbital are unknown, these results could be explained by a differential contribution of alteration in the inhibitory response. Barbiturates like pentobarbital are known to enhance the \( GABA_A \) receptor-mediated, chloride-dependent inhibition, whereas urethan appears to have negligible effects on GABAergic transmission (Engström et al. 1990).

In conclusion, our data demonstrate that LTP is not associated with increased extracellular concentration of glutamate. This finding does not rule out a possible contribution of enhanced presynaptic glutamate release to LTP induction and/or maintenance but conflicts with the notion that LTP may involve a broad synaptic spillover of glutamate.

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