Long-term potentiation of GABAergic synaptic transmission in neonatal rat hippocampus

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1. The plasticity of GABAergic synapses was investigated in neonatal rat hippocampal slices obtained between postnatal days 3 and 6 using intracellular recording techniques. Ionotropic glutamate receptor antagonists were present throughout the experiments to isolate $GABA_A$ receptor-mediated postsynaptic potentials ($GABA_A$ PSPs) or currents ($GABA_A$ PSCs).

2. Repetitive depolarizing pulses (20 pulses, 0·5 s duration, at 0·1 Hz, each pulse generating 4–6 action potentials) induced a long-term potentiation in the slope and amplitude of the evoked $GABA_A$ PSPs and $GABA_A$ PSCs.

3. Long-term potentiation was prevented by intracellular injection of the calcium chelator BAPTA (50 mM), or when the voltage-dependent calcium channels blockers Ni^{2+} (50 μM) and nimodipine (10 μM) were bath applied.

4. Repetitive depolarizing pulses induced a persistent (over 1 h) increase in the frequency of spontaneous $GABA_A$ PSCs.

5. Repetitive depolarizing pulses induced a long-lasting increase in the frequency of miniature $GABA_A$ PSCs, without altering their amplitude or decay-time constant.

6. It is concluded that the postsynaptic activation of voltage-dependent calcium channels leads to a long-term potentiation of GABAergic synaptic transmission in neonatal rat hippocampus. This form of plasticity is expressed as an increase in the probability of GABA release or in the number of functional synapses, rather than as an upregulation of postsynaptic $GABA_A$ receptor numbers or conductance at functional synapses.

The activity-dependent plasticity of glutamatergic synapses has been described extensively (Nicoll & Malenka, 1995) and is believed to play a crucial role in learning and memory processes. Because activity-dependent plasticity of GABAergic synaptic transmission could also modify the input–output relationship of the neurones, study of this form of plasticity is essential.

Both long-term potentiation and long-term depression of GABAergic synaptic transmission have been reported in hippocampal (Stelzer *et al.* 1987, 1994; McLean *et al.* 1996), cortical (Komatsu, 1994) and cerebellar (Kano *et al.* 1992; Kano *et al.* 1996) neurones. While a postsynaptic rise in intracellular calcium concentration appears to be a common trigger for the induction of long-term changes in the strength of GABAergic synaptic transmission (Kano *et al.* 1992; Komatsu, 1996; Hashimoto *et al.* 1996; McLean *et al.* 1996; Morishita & Sastry, 1996), the mechanisms underlying the expression may differ.

Long-term changes in synaptic efficacy may be expressed as presynaptic alterations in neurotransmitter release or as postsynaptic modifications in the sensitivity to released neurotransmitter. One approach to address postsynaptic modifications is to measure the amplitude of responses induced by application of neurotransmitter agonist. This method does not, however, provide compelling evidence since exogenously applied agonist may activate extrasynaptic receptors that could be under different modulatory control than the synaptic ones (Boxall & Marty, 1997). A more direct approach is to measure the amplitude and frequency of spontaneous synaptic currents that occur independently of action potential firing. A change in the amplitude of these events, referred to as miniature postsynaptic currents, is usually considered to reflect a postsynaptic modification, while a change in their frequency is considered to reflect a presynaptic modification. This method, however, requires that a significant number of synapses impinging on the recorded neurone are affected to make postsynaptic modifications detectable.

In a previous study we reported that early in development GABAergic synaptic transmission expresses a calcium-dependent bidirectional plasticity in the neonatal rat hippocampus (McLean *et al.* 1996). Thus, concomitant activation of $GABA_A$ and NMDA receptors during a high-

frequency stimulation leads to long-term depression of GABAergic synaptic transmission, while activation of only $GABA_A$ receptors leads to a long-term potentiation of GABAergic synaptic transmission. The long-term potentiation of GABAergic synaptic transmission requires a membrane depolarization, provided by the activation of $GABA_A$ receptors, and a rise in intracellular calcium concentration, probably resulting from an influx of calcium through voltage-dependent calcium channels. In the present study, we show that direct activation of postsynaptic voltage-dependent calcium channels in the absence of synaptic stimulation results in a long-term potentiation (LTP) of evoked and spontaneous $GABA_A$ receptor-mediated postsynaptic potentials or currents in neonatal rat CA3 pyramidal neurones (LTP_{GABA_A}). The conditioning stimulus also leads to a long-lasting increase in the frequency, but not amplitude, of spontaneous and miniature $GABA_A$ receptor-mediated postsynaptic currents. Therefore, LTP_{GABA_A} is expressed as an increase in the probability of GABA release or in the number of functional GABAergic synapses, but not as an upregulation of postsynaptic $GABA_A$ receptors at previously functional GABAergic synapses.

METHODS

Experiments were performed on hippocampal CA3 pyramidal neurones obtained from postnatal day P3–P6 male Wistar rats. Brains were removed under ether anaesthesia and submerged in cold (0 °C) artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl, 126; KCl, 3·5; $CaCl_2$, 1·3; NaH_2PO_4, 1·2; $NaHCO_3$, 25; and glucose, 11; pH 7·4 when equilibrated with 95% O_2/5% CO_2. Hippocampal slices (600 μm thick) were cut with a McIlwain tissue chopper, incubated for at least 1 h in ACSF at room temperature (20–23 °C), and transferred individually into a submerged recording chamber superfused with ACSF (2·5–3 ml min^{-1}, 34 °C).

Intracellular recordings were performed with electrodes filled with 3 M KCl (current-clamp mode, 50–60 MΩ) or 2 M CsCl (voltage-clamp mode, 40–50 MΩ). In some experiments BAPTA (50 mM, dissolved in 3 M KCl, 50–60 MΩ) was iontophoretically applied to the recorded cells (−0·5 nA, 500 ms, 10–30 min). Cells were considered to be BAPTA loaded when both spike-frequency adaptation and after-hyperpolarization were blocked. For experiments performed in the presence of the voltage-dependent calcium channel blockers Ni^{2+} (50 μM) and nimodipine (10 μM), the slices were incubated at room temperature in ACSF containing the blockers for at least 30 min before the recording session. The blockers were then present throughout the experiment. Both current-clamp and voltage-clamp recordings were performed using an Axoclamp-2A amplifier (Axon Instruments). Evoked, spontaneous and miniature $GABA_A$ receptor-mediated postsynaptic currents ($GABA_A$ PSCs) were recorded in the single-electrode discontinuous voltage-clamp mode with a sampling rate of 3·5 kHz, a time constant of 20 ms and a gain of 25 nA mV^{-1}. To ensure a correct clamp, the voltage at the head stage of the amplifier was monitored on a separate oscilloscope. Recordings of miniature $GABA_A$ PSCs were performed at a holding potential of −100 mV to obtain a large signal-to-noise ratio. Currents were stored on a DAT and analysed off line on a personal computer with Acquis 1 software (G. Sadoc, Biologic, France). The detection threshold was usually set at 8 or 10 pA. This threshold was 2-fold greater than the baseline noise (estimated in the presence of ionotropic GABAergic and glutamatergic receptors antagonists). The fact that no false events would be identified was confirmed by visual inspection for each experiment. The amplitude histogram and cumulative distribution were constructed using data recorded from a fixed time epoch (3 min). The decay time constant was determined on several single miniature $GABA_A$ PSCs in which no other events were present during the decaying phase. A least-square fitting with a single exponential function was used for the analysis of the decaying phase of miniature $GABA_A$ PSCs.

Electrical stimulation (20–50 μs, 10–40 V, 0·03 Hz) was performed with a bipolar tungsten electrode positioned in the hilus.

All data are expressed as means ± S.E.M.. Student's paired t test was used to compare pooled data from different cells. The non-parametric Kolmogorov–Smirnov test (K–S test) was used to compare amplitude and frequency of spontaneous and miniature synaptic events for a given cell as the histograms deviated from the normal distribution. The level of significance was chosen to be $< 0{\cdot}05$.

Drugs used were: 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX; Tocris); D(-)2-amino-5-phosphonovaleric acid (D-AP5; Tocris); bicuculline (Tocris); tetrodotoxin (TTX; Latoxan); nimodipine (Sigma) and BAPTA (Molecular Probes). The hypertonic ACSF had the same composition as the control except that sucrose (50 mM) was added.

RESULTS

Repetitive depolarizing pulses induce a long-term potentiation of $GABA_A$ receptor-mediated synaptic potentials

To investigate the effects of depolarizing pulses on GABAergic synaptic transmission the following protocol was used. Evoked monosynaptic $GABA_A$ receptor-mediated postsynaptic potentials or currents, hereafter referred to as $GABA_A$ PSPs or $GABA_A$ PSCs, were isolated in the presence of the ionotropic glutamatergic receptor antagonists CNQX (10 μM) and D-AP5 (50 μM) (Fig. 1*A*). After stable baseline responses were obtained for at least 10 min, 20 depolarizing pulses (from −90 to −40 ± 5 mV, 0·5 s duration), each pulse generating 3–6 action potentials, were applied to CA3 pyramidal cells at a frequency of 0·1 Hz. The depolarizing pulses were applied in the absence of synaptic stimulation (Fig. 1*B*). This conditioning stimulus induced a long-term increase in both the amplitude and slope of evoked $GABA_A$ PSPs. A typical result of such an experiment is shown in Fig. 1*A–D*; the slope of the evoked $GABA_A$ PSPs slowly increases to reach a maximal value of about 150% of the control value 2 min after the conditioning stimulus and then remains increased for 60 min (Fig. 1*D*). The effect of depolarizing pulses on evoked $GABA_A$ PSPs were highly reproducible, occurring in 10 out of 13 cells tested. On average, the slope of the evoked $GABA_A$ PSPs was increased by 165 ± 5% (range, 130–180%) of the control value 60 min after the depolarizing pulses (4·1 ± 0·8 mV ms^{-1} *vs.* 6·9 ± 0·6 mV ms^{-1}, $n = 10$, $P = 0{\cdot}013$; Fig. 1*E*) without any changes in membrane input resistance (97 ± 5 *vs.*

96 ± 3 MΩ, $n = 10$, $P = 0{\cdot}88$; Fig. 1*A*), reversal potential (0·1 ± 1·3 *vs.* 0·8 ± 1·2 mV, $n = 5$, $P = 0{\cdot}34$; not shown but see Fig. 3*D* and *E*) nor latency to peak (16·4 ± 2·1 *vs.* 15·7 ± 1·8 ms, $n = 10$, $P = 0{\cdot}4$; Fig. 1*A*) of $GABA_A$ PSPs. As shown in Fig. 1*A* and *D*, the potentiated PSPs were completely blocked by bath application of bicuculline (10 μM), showing that they were entirely mediated by the activation of $GABA_A$ receptors. The long-term potentiation of $GABA_A$ PSPs induced by repetitive depolarizing pulses will be referred to hereafter as LTP_{GABA_A}.

LTP_{GABA_A} requires the activation of voltage-dependent calcium channels

Most forms of synaptic plasticity require a postsynaptic rise in intracellular calcium concentration ($[Ca^{2+}]_i$) (Kano *et al.* 1992; Nicoll & Malenka, 1995; Komatsu, 1996; McLean *et al.* 1996). To test the involvement of a postsynaptic calcium rise in the induction of LTP_{GABA_A}, the calcium chelator BAPTA (50 mM) was added to the recording solution. The effectiveness of the injection into the recorded neurone was monitored by observation of the spike frequency adaptation and the slow after-hyperpolarizing potential (Fig. 2*A*). When CA3 pyramidal neurones were loaded with BAPTA, repeated depolarizing pulses (inset in Fig. 2*C*) failed to induce an increase in the slope of evoked $GABA_A$ PSPs (3·6 ± 0·7 *vs.* 4·0 ± 1·6 mV ms^{-1} 30 min after the conditioning stimulus, $n = 5$, $P = 0{\cdot}39$) (Fig. 2*B* and *C*). Therefore a postsynaptic rise in $[Ca^{2+}]_i$ is required for the induction of LTP_{GABA_A}.

Since NMDA receptors were blocked, a likely mechanism for the rise in $[Ca^{2+}]_i$ is the activation of voltage-dependent calcium channels (VDCCs) during depolarization of the neurones. To test this hypothesis, we investigated the effect of the VDCC blockers Ni^{2+} (50 μM) and nimodipine (10 μM), on the induction of LTP_{GABA_A}. In the presence of

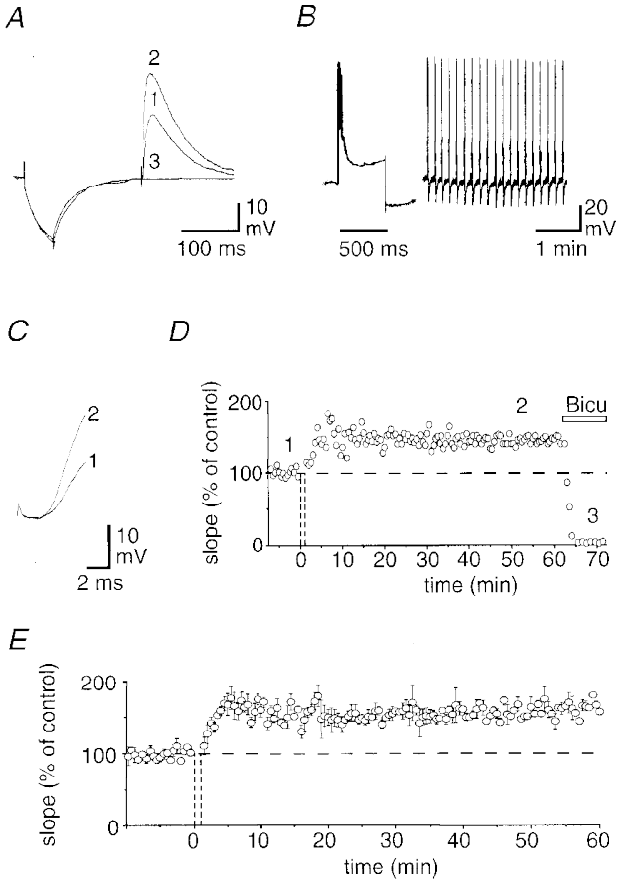

Figure 1. LTP_{GABA_A} is induced by repeated depolarizations

A, superimposed mean of 10 consecutive evoked $GABA_A$ PSPs recorded at the times marked by the numbers on the graph (*D*). In this and the following figures, CNQX (10 μM) and D-AP5 (50 μM) were present throughout the experiment. The input resistance of the cell was monitored as a negative deflection to a −0·3 nA current injection. Membrane potential, −80 mV. *B*, standard protocol used to elicit LTP. Twenty depolarizing pulses were applied at a frequency of 0·1 Hz. The stimulation was stopped during the conditioning stimulus. *C*, superimposed evoked $GABA_A$ PSPs show a clear increase in the initial slope following depolarizing pulses. *D*, time course of changes in the slope of evoked $GABA_A$ PSPs following depolarizing pulses and during the application of bicuculline (open bar, 10 μM Bicu) (same cell as in *A* and *B*). *E*, mean time course of changes in the slope of evoked $GABA_A$ PSPs following depolarizing pulses ($n = 10$).

the VDCC blockers, the slope of the evoked $GABA_A$ PSPs remained unchanged 30 min after the conditioning stimulus: $99{\cdot}3 \pm 3\%$ of the control value ($4{\cdot}1 \pm 0{\cdot}5$ *vs.* $3{\cdot}8 \pm 0{\cdot}5$ mV ms^{-1}, $n = 5$, $P = 0{\cdot}3$) (Fig. 2*D* and *E*). We observed, however, a short-lasting (around 5 min) transient depression of evoked $GABA_A$ PSP amplitude ($68 \pm 5\%$, 2 min after the conditioning stimulus). These results contrast with the usual increase in the slope of evoked $GABA_A$ PSPs observed in interleaved control slices ($154 \pm 6\%$, 30 min after depolarizing steps, $n = 3$).

LTP_{GABA_A} is associated with a long-lasting increase in the frequency of spontaneous $GABA_A$ PSCs

To study the locus of LTP_{GABA_A} expression, we investigated the effect of depolarizing pulses on spontaneous $GABA_A$ PSCs. We first verified that LTP_{GABA_A} could be induced in the voltage-clamp mode. Neurones were held at −80 mV in the presence of CNQX (10 μM) and D-AP5 (50 μM) (Fig. 3*A*) except during the conditioning stimulus, which was applied in the current-clamp mode (Fig. 3*B*). An example of such an experiment is shown in Fig. 3*A–C*. Three minutes after the application of depolarizing pulses, a stable long-lasting increase in amplitude of the evoked $GABA_A$ PSCs was observed (120 ± 7 to 160 ± 2 pA, 40 min after the depolarizing pulses; Fig. 3*C*). The long-lasting increase (over 1 h) in the amplitude of the evoked $GABA_A$ PSCs was not associated with a significant change in their reversal potential as shown in Fig. 3*D* and *E*. The group data from five neurones revealed that the amplitude of evoked $GABA_A$ PSCs increased to $139 \pm 8\%$ of control values (range, 124–166%) and that their reversal potential was $-1 \pm 1{\cdot}3$ mV before and $0{\cdot}72 \pm 1$ mV 30 min after repeated depolarizing pulses ($P = 0{\cdot}2$).

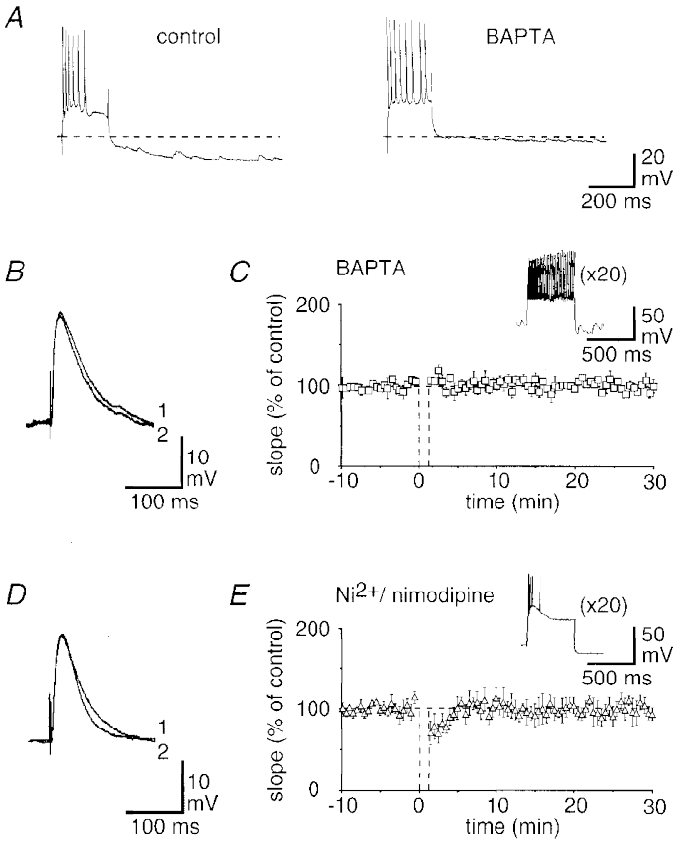

Figure 2. LTP_{GABA_A} requires the activation of postsynaptic voltage-dependent calcium channels
A, soon after impalement (left panel, control) a depolarizing current pulse evoked a burst of action potentials followed by an after-hyperpolarizing potential (AHP). Fifteen minutes after 50 mM intracellular BAPTA iontophoresis (right panel, BAPTA), the same current injection induced a sustained spike discharge with an almost undetectable AHP. Membrane potential, −65 mV. *B*, superimposed mean of 10 consecutive evoked $GABA_A$ PSPs before (1) and 30 min after (2) the application of depolarizing pulses in a BAPTA-loaded cell. Membrane potential, −80 mV. *C*, mean time course of changes in the slope of evoked $GABA_A$ PSPs following depolarizing pulses in BAPTA-loaded cells ($n = 5$). The inset illustrates the response of a cell to one of the 20 depolarizing pulses applied to elicit change in the slope of the $GABA_A$ PSPs. *D*, superimposed mean of 10 consecutive evoked $GABA_A$ PSPs before (1) and 30 min after (2) the application of repeated depolarizing pulses in the presence of Ni^{2+} (50 μM) and nimodipine (10 μM). Membrane potential, −75 mV. *E*, mean time course of changes in the slope of evoked $GABA_A$ PSPs after depolarizing pulses in the presence of Ni^{2+} (50 μM) and nimodipine (10 μM) ($n = 5$). The inset illustrates the response of a cell to one of the 20 depolarizing pulses applied to elicit change in the slope of $GABA_A$ PSPs .

Concurrent with the LTP of evoked $GABA_A$ PSC amplitude, depolarizing pulses also resulted in a persistent increase in the frequency of spontaneous $GABA_A$ PSCs (see Fig. 4*A*; $n = 6$). On average, the inter-events interval decreased from $0{\cdot}12 \pm 0{\cdot}03$ to $0{\cdot}07 \pm 0{\cdot}01$ s 60 min after the depolarizing pulses ($n = 6$, $P = 0{\cdot}03$). Due to the low incidence of single events it was possible to quantify the effect of depolarizing pulses on the amplitude of spontaneous $GABA_A$ PSCs in only three cells. Results from one of these cells are illustrated in Fig. 4. Following depolarizing steps, the inter-events interval decreased from $0{\cdot}177 \pm 0{\cdot}008$ s ($n = 332$) to $0{\cdot}097 \pm 0{\cdot}003$ s ($n = 662$) ($P = 0{\cdot}0001$) (Fig. 4*A*) as illustrated by the shift to the left of the cumulative inter-events interval distribution (Fig. 4*B*). In the same cell, the mean amplitude of spontaneous $GABA_A$ PSCs was not statistically increased: from $44{\cdot}3 \pm 1{\cdot}6$ pA ($n = 332$) to $46{\cdot}8 \pm 0{\cdot}9$ pA ($n = 662$) ($P = 0{\cdot}53$) (Fig. 4*C*). Pooled data from the three cells in which analysis of amplitude was possible gave a mean decrease in the inter-events interval of $69 \pm 5\%$ of the control value (range, 55–81 %), whereas the amplitude remained constant ($103 \pm 4\%$ of control value). The observation that depolarizing pulses increased the frequency, but not the amplitude, of spontaneous $GABA_A$ PSCs suggests that a postsynaptic upregulation of $GABA_A$ receptors is not involved in the expression of LTP_{GABA_A}.

LTP_{GABA_A} is associated with a long-lasting increase in the frequency, but not the amplitude, of miniature $GABA_A$ PSCs

The fact that depolarizing pulses affect spontaneous $GABA_A$ PSCs strongly suggests that the majority, if not all of the GABAergic synapses impinging on the recorded neurones were potentiated. Therefore, going a step further in elucidating the mechanisms of LTP_{GABA_A} expression, we investigated the effect of depolarizing pulses on the amplitude and frequency of miniature $GABA_A$ PSCs ($mGABA_A$ PSCs). $mGABA_A$ PSCs were isolated in the

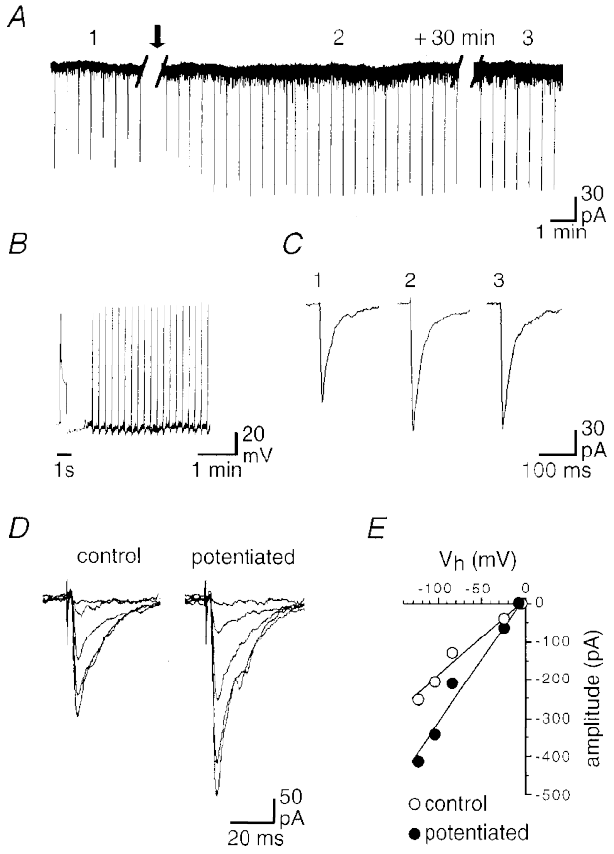

Figure 3. LTP_{GABA_A} is not associated with a change in the reversal potential of $GABA_A$ PSCs
A, low time scale recording of the $GABA_A$ PSCs. The arrow marks the time during which the conditioning protocol was applied. *B*, same cell as in *A*. Standard protocol was used to elicit LTP. Twenty depolarizing pulses (marked by the arrow in *A*) were applied in current-clamp mode at a membrane potential of −90 mV and a frequency of 0·1 Hz. Stimulation was stopped during depolarization. *C*, same cell as in *A* and *B* showing the mean of 5 consecutive evoked $GABA_A$ PSCs recorded at the times marked by the numbers in *A*. *D*, superimposed evoked $GABA_A$ PSCs recorded at different holding potentials (0 mV, −20 mV, −80 mV, −100 mV and −120 mV) before (control) and 40 min after (potentiated) the application of depolarizing pulses. *E*, current–voltage curve of the traces depicted in *D*. The control and potentiated $GABA_A$ PSCs reversed polarity at −1 and −2 mV, respectively.

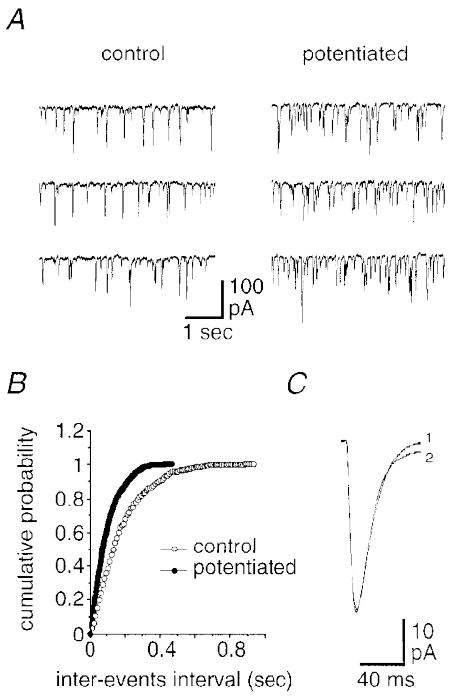

Figure 4. LTP_{GABA_A} is associated with an increase in spontaneous $GABA_A$ PSC frequency

A, spontaneous $GABA_A$ PSCs recorded before (left, control) and 30 min after (right, potentiated) repeated depolarizations. Holding potential, −100 mV. *B*, cumulative probability plots of inter-events interval of control (○) and potentiated (●) spontaneous $GABA_A$ PSCs (same cell as in *A*). *C*, mean of 30 consecutive spontaneous $GABA_A$ PSCs before (1) and 30 min after (2) repeated depolarizations (same cell as in *A*).

presence of TTX (1 μM) and the ionotropic glutamatergic receptor antagonists CNQX (10 μM) and D-AP5 (50 μM). In order to activate VDCCs in the presence of TTX, the experiments were performed with CsCl-filled electrodes and potassium channel blockers (2 mM Cs^+ and 10 mM TEA) were added to the ACSF. $mGABA_A$ PSCs ranged in amplitude from 15 to 115 pA at a holding potential of −100 mV, occurred at a frequency of approximately 0·5 Hz, and were completely abolished by bicuculline (10 μM; not shown). Repeated depolarizing voltage pulses (20 pulses,

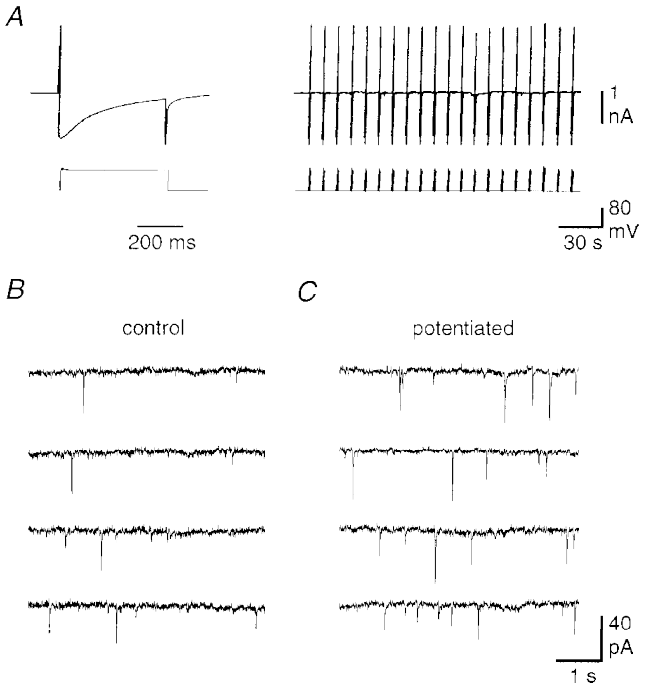

Figure 5. LTP_{GABA_A} is associated with an increase in the frequency of $mGABA_A$ PSCs

A, standard protocol used to investigate the effect of repeated depolarizing pulse on $mGABA_A$ PSCs. The recording was performed at a holding potential of −100 mV in the presence of TTX (1 μM), CNQX (10 μM), D-AP5 (50 μM), Cs^+ (2 mM) and TEA (10 mM). Twenty depolarizing voltage pulses were applied from −90 to −10 mV (500 ms duration) at a frequency of 0·1 Hz. *B* and *C*, current sweeps collected from the same cell before (*B*) and 30 min after (*C*) application of depolarizing pulses.

from −90 to 0 mV, 0·5 s duration, at 0·1 Hz) induced a long lasting (at least 30 min) increase in the frequency, but not amplitude, of mGABA$_A$ PSCs. This effect is illustrated for a representative neurone in Fig. 5. The standard protocol used is shown in Fig. 5*A*, and the current sweeps in control conditions and 30 min after depolarizing pulses are shown in Fig. 5*B* and *C*, respectively. We could not detect significant changes in the amplitude of mGABA$_A$ PSCs following depolarizing pulses, as determined by analysis of amplitude histograms (Fig. 6*A*) and cumulative amplitude distribution (Fig. 6*C*) obtained from the same cell. The mean amplitude of mGABA$_A$ PSCs was 35·5 ± 0·9 pA ($n = 167$) in control and 37·0 ± 0·6 pA ($n = 350$) 30 min after depolarizing pulses ($P = 0·47$) (Fig. 6*B*). Neither could we detect changes in the decay time constant of the mGABA$_A$ PSCs (6·67 ± 0·3 ms in control ($n = 100$) *vs.* 6·63 ± 0·4 ms 30 min after depolarizing pulses ($n = 100$; $P = 0·3$) (Fig. 6*B*). The mGABA$_A$ PSC frequency was, however, increased, as is evident from the cumulative inter-events interval distribution (Fig. 6*D*). The inter-events interval of mGABA$_A$ PSCs recorded in this cell decreased from 1·56 ± 0·24 to 0·64 ± 0·05 s ($P = 0·037$). Similar effects were observed in four out of six neurones. On average, the inter-events interval decreased from 1·7 ± 0·18 to 0·92 ± 0·19 s ($P = 0·0013$) (mean of 55 ± 5 % of control value, range from 42 to 67 %), whereas the amplitude and decay time constant of mGABA$_A$ PSCs remained unchanged (27·4 ± 4 *vs.* 29·5 ± 5 pA ($P = 0·15$) and 8·77 ± 2·35 *vs.* 8·66 ± 2·49 ms ($P = 0·47$)). In one neurone, depolarizing pulses led to a decrease in the inter-events interval from 1·9 ± 0·2 ($n = 139$) to 1·1 ± 0·1 ($n = 229$, $P = 0·035$), associated with a significant increase in the mean amplitude of mGABA$_A$ PSCs from 33·5 ± 1·5 to 42·5 ± 1·8 pA ($P = 0·046$), but a change in their decay time constant was observed (from 6·17 ± 0·3 to 6·39 ± 0·2 ms, $P = 0·31$). This increase in the mean amplitude appeared to be due to an increase in the number of larger amplitude mGABA$_A$ PSCs (data not shown). In the remaining cell neither the amplitude nor the frequency of mGABA$_A$ PSCs was changed.

The increase in the frequency of mGABA$_A$ PSCs was not due to the external application of potassium channel blockers. Thus in control experiments, long-term recordings (1 h, $n = 3$) in ACSF containing TTX , Cs^+ (2 mM) and TEA (10 mM) did not alter the frequency of mGABA$_A$ PSCs if the postsynaptic neurone was not repetitively stimulated with depolarizing pulses (data not shown).

An increase in the frequency of mGABA$_A$ PSCs might have arisen from an increase in their amplitude, enabling

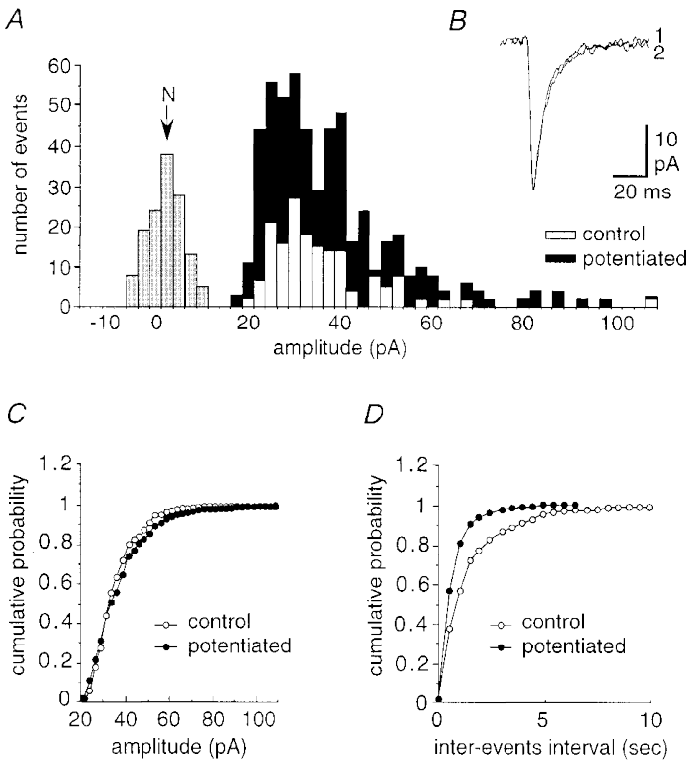

Figure 6. The frequency, but not the amplitude of mGABA$_A$ PSCs is increased during LTP$_{GABA_A}$
A, superimposed control (□) and potentiated (■) amplitude histograms obtained from the cell depicted in Fig. 5. The control and potentiated histograms are obtained from 167 and 350 events recorded during a 3 min period before and 30 min after depolarizing pulses, respectively. N indicates the noise histogram. *B*, superimposed mean of 20 consecutive mGABA$_A$ PSCs before (1) and 30 min after (2) application of depolarizing pulses. *C* and *D*, cumulative probability plots of amplitude (*C*) and inter-events interval (*D*) before (○) and 30 min after (●) depolarizing pulses.

previously undetectable events to cross the detection threshold. To address this point, $mGABA_A$ PSCs were recorded at two different holding potentials. As illustrated in Fig. 7, changing the holding potential from −50 to −100 mV resulted in an increase in the amplitude of $mGABA_A$ PSCs (Fig. 7*A*) from 16·7 ± 0·57 ($n = 153$) to 22·3 ± 0·9 pA ($n = 142$, $P = 0·0069$). The increase in the amplitude of $mGABA_A$ PSCs is further illustrated by the shift to the right of the amplitude histograms (Fig. 7*B*) and cumulative amplitude distribution (Fig. 7*D*). This increase in amplitude of the $mGABA_A$ PSCs was not associated with a significant change in their frequency. The mean inter-events interval of $mGABA_A$ PSCs was 0·75 ± 0·06 s at −50 mV and 0·83 ± 0·08 s at −100 mV ($P = 0·4$). Similar experiments were repeated on five neurones. On average the amplitude of $mGABA_A$ PSCs increased from 17·8 ± 1·6 to 27·0 ± 2·9 pA ($P = 0·006$) without any significant change in the mean inter-events interval (0·61 ± 0·1 s *vs.* 0·65 ± 0·1 s, $n = 5$, $P = 0·3$). In contrast, application of hypertonic ACSF (50 mM sucrose) significantly decreased the inter-events interval of $mGABA_A$ PSCs (0·56 ± 0·08 s *vs.* 0·35 ± 0·05 s, $n = 5$, $P = 0·034$), without any effect on their amplitude

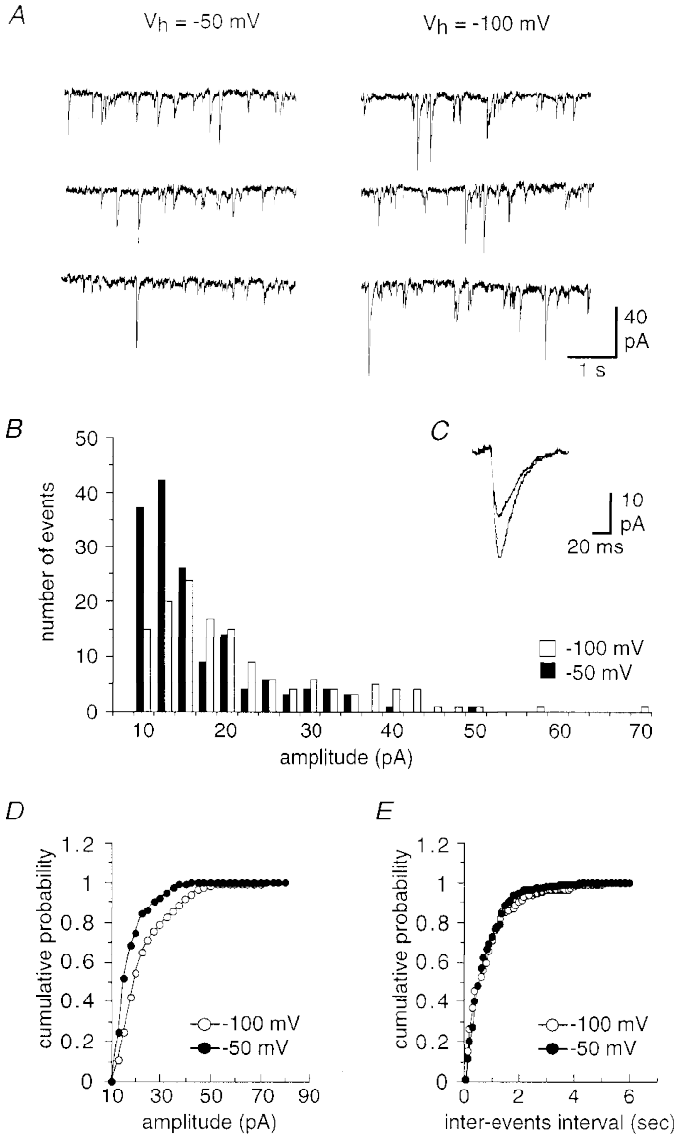

Figure 7. Genuine increase in the number of $mGABA_A$ PSCs during LTP_{GABA_A}

A, to determine whether the increase in the frequency of $mGABA_A$ PSCs is due to the detection of previously undetected events, $mGABA_A$ PSCs were recorded at two different holding potentials, −50 and −100 mV in the presence of TTX (1 μM), CNQX (10 μM), D-AP5 (50 μM), Cs^+ (2 mM) and TEA (10 mM). *B*, superimposed amplitude histograms of $mGABA_A$ PSCs recorded at −50 mV (■) and −100 mV (□). The histograms were obtained from, respectively, 153 and 142 events recorded during a 3 min period. *C*, superimposed mean of 20 consecutive $mGABA_A$ PSCs recorded at −50 mV and −100 mV. *D* and *E*, cumulative probability plots of amplitude (*D*) and inter-events interval (*E*) of $mGABA_A$ PSCs recorded at −100 mV (○) and −50 mV (●).

(26·8 ± 3·5 *vs.* 28·2 ± 3·4 pA, $n = 5$, $P = 0·3$) (data not shown). Thus, under our recording conditions, changes in the amplitude of $mGABA_A$ PSCs would not significantly influence their apparent frequency. These data therefore suggest that the depolarization-induced increase in $mGABA_A$ PSC frequency is likely to be due to a real increase in the number of events rather than an increase in amplitude of previously undetectable events.

DISCUSSION

The principal conclusions of the present study are: (i) repeated depolarizations lead to a long-term potentiation of $GABA_A$ receptor-mediated synaptic transmission (LTP_{GABA_A}) in neonatal rat hippocampus; (ii) the induction of LTP_{GABA_A} involves postsynaptic calcium-dependent mechanisms; and (iii) LTP_{GABA_A} is expressed as an increase in the probability of GABA release or as the functional expression of previously 'silent' GABAergic synapses.

Induction of LTP_{GABA_A}

The observation that LTP_{GABA_A} does not develop when buffering postsynaptic calcium with BAPTA indicates that the induction involves the activation of postsynaptic calcium-dependent mechanisms. An increase in intracellular calcium concentration ($[Ca^{2+}]_i$) could be produced by an influx of calcium through voltage-dependent calcium channels (VDCCs) or NMDA channels, or by a release of calcium from internal stores. In the present study, D-AP5 was present throughout the experiment, thus ruling out the involvement of NMDA channels. In fact, in a previous study we have shown that activation of NMDA receptors following a high-frequency stimulation leads to a long-term depression of $GABA_A$ PSPs in neonates (McLean *et al.* 1996). In contrast, LTP_{GABA_A} was prevented in the presence of the VDCC blockers Ni^{2+} and nimodipine. In the presence of these blockers, we observed a short-lasting depression of evoked $GABA_A$ PSPs following the conditioning stimulus. A likely mechanism for this transient decrease could be the phenomenon described in the cerebellum (Llano *et al.* 1991; Vincent & Marty, 1993) and the hippocampus (Pitler & Alger, 1994) termed depolarization-induced suppression of inhibition (DSI). In a recent study, Lenz and collaborators (1998) have shown that DSI is induced by the activation of N- and L-type channels. Thus, the transient depression observed in the presence of Ni^{2+} and nimodipine may be due to the activation of N-type calcium channels that are not blocked by these VDCC blockers. Alternatively, the transient depression may be due to a calcium-dependent downregulation of postsynaptic $GABA_A$ receptors (Chen & Wong, 1995).

The types of VDCCs that are involved in or play the greater role in the induction of LTP_{GABA_A} remain to be investigated. This question may be of particular interest if a differential distribution (soma *vs.* dendrites) of calcium-type channels exists in neonatal pyramidal neurones, as has been described in adult neurones (Christie *et al.* 1995; Magee & Johnston, 1995). In the present study, the protocol applied to induce LTP_{GABA_A} is likely to activate both high-voltage- and low-voltage-activated calcium channels. At the concentration used, Ni^{2+} blocks T- and R-type channels, and nimodipine blocks L-type channels, while not significantly affecting other calcium channels (Mogul & Fox, 1991; Christie *et al.* 1995; Randall & Tsien, 1995). These calcium channels are present in the neonatal hippocampal pyramidal neurone, with kinetic and pharmacological properties comparable to those described in adult neurones (Thompson & Wong, 1991). The role of internal calcium stores has not been investigated in the present study, but their possible contribution in the induction of LTP_{GABA_A}, as reported in cortical (Komatsu, 1996) and cerebellar neurones (Hashimoto *et al.* 1996), cannot be excluded.

Even if the depolarizing pulses were applied in the absence of electrical stimulation of GABAergic fibres, it could be argued that a pairing-like protocol occurs because of the high-frequency barrage of spontaneous $GABA_A$ PSPs present in neonatal CA3 pyramidal neurones. The potentiation of miniature $GABA_A$ PSCs, which occurred at a 10 times lower frequency, strongly argues against such a mechanism. This finding stands in clear contrast with the data described for glutamatergic synaptic transmission. In adult CA1 pyramidal neurones and dentate gyrus granule cells, direct activation of VDCCs only leads to a short-term potentiation of evoked (Kullmann *et al.* 1992; Wang *et al.* 1997) and miniature excitatory postsynaptic currents (Wyllie *et al.* 1994). Thus, while the induction of LTP at hippocampal glutamatergic synapses requires both a postsynaptic influx of calcium and the activation of a presynaptic element (Isaac *et al.* 1995; Durand *et al.* 1996; Wang *et al.* 1997), a postsynaptic increase in $[Ca^{2+}]_i$ appears to be a minimal and sufficient requirement to trigger LTP at GABAergic synapses.

Locus of LTP_{GABA_A} expression

Locus of LTP expression has been subject to numerous investigations for both excitatory and inhibitory synaptic transmission. LTP can be expressed presynaptically, manifested as an increased transmitter release, or postsynaptically, manifested as an increased sensitivity to released transmitter. In cerebellar Purkinje cells, activation of VDCCs leads to an increase in spontaneous IPSC amplitude (Kano *et al.* 1992) due to an upregulation of postsynaptic $GABA_A$ receptors (Kano *et al.* 1992, 1996). Similarly, in the kindling-induced epilepsy, the long-lasting potentiation of inhibitory synapses in the dentate gyrus involves an increase in the number of postsynaptic $GABA_A$ channels at functional synapses (Nusser *et al.* 1998). Conversely, a retrograde inhibitory control of GABA release following a postsynaptic rise in $[Ca^{2+}]_i$ has been clearly observed in both cerebellum (Vincent & Marty, 1993) and hippocampus (Pitler & Alger, 1994).

In the present study we observed a clear increase in the spontaneous GABAergic synaptic activity. This observation

strongly supports the idea that a significant number of the GABAergic synapses impinging on the recorded neurone were potentiated, probably by the calcium influx induced by back-propagating action potentials (Christie *et al.* 1995). We therefore investigated the effect of depolarizing pulses on $mGABA_A$ PSCs to determine the mechanism of LTP_{GABA_A} expression. We observed a nearly 2-fold increase in the frequency of $mGABA_A$ PSCs without any significant effect on their amplitude or decay-time constant. We were concerned that our measured depolarization-induced increase in $mGABA_A$ PSCs might be related to the detection of events that were below the detection threshold before voltage pulses. We showed, however, that our analysis routine could reliably detect changes in $mGABA_A$ PSC amplitude, if they have to occur, without altering their apparent frequency. Thus the increase in $mGABA_A$ PSC frequency observed after depolarizing pulses results from a real increase in the number of events, without any change in their amplitude.

An increase in the frequency of $mGABA_A$ PSCs is consistent with a presynaptic increase in the probability of GABA release or with an increase in the number of functional GABAergic synapses. Such an increase in the number of functional GABAergic synapses could again be explained by either (i) an all-or-none upregulation of postsynaptic $GABA_A$ receptors at previously non-functional synapses, as proposed for glutamatergic synapses on CA1 pyramidal neurones (Isaac *et al.* 1995; Liao *et al.* 1995) or (ii) alternatively, by the presynaptic switching on of previously silent synaptic connections, as proposed for glycinergic synapses on Mauthner cells (Charpier *et al.* 1995; Oda *et al.* 1995) and mossy fibre synapses on CA3 pyramidal neurones (Tong *et al.* 1996).

Although the mechanisms of LTP_{GABA_A} expression have not been fully elucidated, the lack of effect on the amplitude of $mGABA_A$ PSCs suggests that it cannot be accounted for by an upregulation in the number of postsynaptic $GABA_A$ receptors at previously functional GABAergic synapses as recently shown in the adult hippocampus (Nusser *et al.* 1998). In addition, in adult dentate granule cells, a transient postsynaptic rise in $[Ca^{2+}]_i$ induced a long-lasting prolongation in the decay of miniature IPSCs (Soltesz & Mody, 1995). Such an effect on the $mGABA_A$ PSC decay was also not observed in the present study. This difference raises the interesting possibility that different mechanisms can generate persistent alterations in the efficacy of GABAergic synaptic transmission depending on the developmental stage, the neuronal type or the protocol used.

An important issue concerns the possible function that LTP_{GABA_A} might serve in the developing hippocampus. A growing body of evidence points to the link between activity-dependent plasticity and functional maturation of the neuronal network (Crair & Malenka, 1995; Kirkwood *et al.* 1995; Durand *et al.* 1996; Fitzsimonds & Poo, 1998). In neonatal rat hippocampus, activation of $GABA_A$ receptors by endogenous GABA induces a membrane depolarization leading to the generation of action potential associated with a subsequent rise in $[Ca^{2+}]_i$ (Ben-Ari *et al.* 1997). In addition, in a previous study we have reported that, early in development, high-frequency stimulation of GABAergic interneurones leads to a calcium-dependent LTP_{GABA_A} only when GABA depolarizes the pyramidal cells (McLean *et al.* 1996). Thus, as development progresses, spontaneously released GABA may lead to a functional maturation of GABAergic synaptic transmission in the form of long-term changes in synaptic efficacy.

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