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Presynaptic action potential waveform determines cortical synaptic latency

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Non-technical summary Synaptic delay at cortical synapses is determined by the presynaptic release probability. We show here that the duration and amplitude of the presynaptic action potential also determine synaptic latency at neocortical and hippocampal excitatory synapses. Broadening the presynaptic spike with blockers of potassium channels increased latency by 1–2 ms. Decreasing the amplitude of the presynaptic action potential by partly blocking sodium channels reduced synaptic latency by ∼0.5 ms. These changes may contribute to stabilization of synaptic timing during repetitive stimulation. The regulation of synaptic timing by these pharmacological agents could not be attributed to modulation of axonal conduction. Rather, the effects are compatible with modifications of the kinetics of the presynaptic calcium current. We conclude that synaptic latency at cortical neurons is not constant but dynamically regulated by presynaptic action potential waveform.

Abstract Synaptic latency at cortical synapses is determined by the presynaptic release probability (P_r). Short- and long-term presynaptic plasticity is associated with modulation of synaptic delay. We show here that the duration and amplitude of the presynaptic action potential also determine synaptic latency at neocortical and hippocampal excitatory synapses. Blockade of voltage-gated potassium (Kv) channels with 4-aminopyridine or dendrotoxin-I, but not tetraethylammonium, induced a 1–2 ms shift in latency at excitatory synaptic connections formed by pairs of neocortical pyramidal neurons. 4-Aminopyridine or dendrotoxin-I, but not tetraethylammonium, increased the duration of the action potential recorded in the axon, suggesting that presynaptic spike duration is controlled by axonal Kv1 potassium channels. Spike width-dependent changes in latency have been identified at the mossy fibre–CA3 cell synapses and contribute to stabilization of synaptic timing during repetitive stimulation. The effects of presynaptic spike amplitude on synaptic latency were also examined. Decreasing the amplitude of the presynaptic action potential with 15–30 nM TTX reduced synaptic latency by ∼0.5 ms. The regulation of synaptic timing by potassium and sodium channel blockers could not be attributed to modulation of axonal conduction. Rather, these effects are compatible with modifications of the kinetics of the presynaptic calcium current. We conclude that synaptic latency at cortical neurons is not constant but dynamically regulated by presynaptic action potential waveform.

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Abbreviations AP, action potential; 4-AP, 4-aminopyridine; DCG-IV, (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine; DTx-I, dendrotoxin-I; IR, infrared; Kv channel, voltage-gated K^+-selective channel; PPR, paired-pulse ratio; P_r, release probability

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Introduction

Nerve cells transmit information not only by their firing rate, but also by the fine temporal organization of their discharge (Rieke *et al.* 1997). In simple neuronal networks, the timing between connected neurons is usually described by the synaptic latency, which is the sum of the conduction time and the synaptic delay (Sabatini & Regehr, 1999). We have recently shown that EPSC latency at monosynaptically connected pairs of layer 5 (L5) or CA3 pyramidal neurons is not fixed, but rather determined by the presynaptic release probability (P_r; Boudkkazi *et al.* 2007). Synaptic latency was found to be inversely correlated with synaptic strength and sensitive to changes in the extracellular Ca^{2+} to Mg^{2+} ratio. Changes in synaptic latency were also observed during paired-pulse synaptic plasticity and presynaptic long-term potentiation and depression. We furthermore established that the observed covariation in latency and synaptic strength was a synergistic combination that significantly affects postsynaptic spiking (Boudkkazi *et al.* 2007), suggesting that it might be a putative code for short- and long-term synaptic dynamics in cortical networks.

Classical studies on giant synapses (Katz & Miledi, 1967; Llinas *et al.* 1981; Augustine *et al.* 1985) showed that the duration of the presynaptic waveform strongly determines synaptic latency because calcium entry essentially occurs during the repolarization of the action potential. However, the precise role of the presynaptic action potential waveform is still unclear at central synapses of the mammalian brain. This question is potentially of great importance, since the shape of the presynaptic action potential (AP) is subject to considerable variation in mammalian axons in physiological conditions. In mossy fibre terminals, the AP duration is extended following repetitive stimulation (Geiger & Jonas, 2000). Likewise, in L5 pyramidal neurons the duration of the axonal spike increases when voltage-gated K^+-selective (Kv) channels are inactivated by sustained depolarization of the presynaptic neuron (Shu *et al.* 2006, 2007; Kole *et al.* 2007). Finally, the shortening of AP duration observed during postnatal development in the calyx of Held terminal is associated with a decrease in the delay of the presynaptic calcium current and a shortening of synaptic latency (Taschenberger & von Gersdorff, 2000), suggesting that presynaptic AP waveform could be a major determinant of synaptic latency.

We show here that extension of the axonal spike duration with Kv1 channel blockers (20 μM 4-aminopyridine (4-AP) or dendrotoxin-I (DTx-I)) increased synaptic latency in the millisecond range. This spike-dependent latency is independent of a presynaptic release because release-dependent variation in latency was still observed in the presence of Kv channel blockers. In addition, it does not result from a modification of the conduction properties because the latency of antidromically evoked APs was not affected. However, AP waveform-dependent regulation of synaptic latency is compatible with modification of the kinetics of the presynaptic calcium current. Functionally, this spike width-dependent variation in latency is operant during repetitive stimulation to stabilize synaptic latency. In parallel, we also show that the reduction of AP amplitude with TTX shortens latency. Our study therefore demonstrates that synaptic latency at excitatory contacts is finely tuned by both P_r and presynaptic spike waveform.

Methods

Slices and slice cultures

Cortical slices (350–400 μm thick) were obtained from 13- to 20-day-old Wistar rats. All experiments were carried out according to the European and Institutional guidelines for the care and use of laboratory animals (Council Directive 86/609/EEC and French National Research Council). In addition, our experiments comply with *The Journal of Physiology* policy on animal experimentation described by Drummond (2009). Rats were deeply anaesthetized with chloral hydrate (intraperitoneal, 200 mg kg^{-1}) and killed by decapitation. Slices were cut in an ice-cold solution containing (mM): 280 sucrose, 26 $NaHCO_3$, 10 D-glucose, 10 $MgCl_2$, 1.3 KCl and 1 $CaCl_2$, and were bubbled with 95% O–5% CO_2, pH 7.4. Slices recovered (1 h) in a solution containing (mM): 125 NaCl, 26 $NaHCO_3$, 3 $CaCl_2$, 2.5 KCl, 2 $MgCl_2$, 0.8 NaH_2PO_4 and 10 D-glucose, and were equilibrated with 95% O_2–5% CO_2.

Interface hippocampal slice cultures were prepared as described previously (Stoppini *et al.* 1991; Debanne *et al.* 2008). In brief, hippocampal slices (250 μm) were obtained from 6- to 10-day-old Wistar rats and were grown on culture inserts. Culture medium was replaced three times per week. Slice cultures were maintained at 35°C for at least 4 days *in vitro* before experiments.

Each slice was transferred to a submerged chamber mounted on an upright microscope (Olympus, equipped with a ×40 water-immersion objective). Layer 5 pyramidal neurons were visualized using differential interference contrast infrared videomicroscopy.

Recording and data analysis

Dual whole-cell recordings were obtained as detailed previously (Boudkkazi *et al.* 2007; Debanne *et al.* 2008). The external solution contained (mM): 125 NaCl, 26 $NaHCO_3$, 3 $CaCl_2$, 2.5 KCl, 2 $MgCl_2$, 0.8 NaH_2PO_4 and 10 D-glucose, and was equilibrated with 95% O_2–5% CO_2. Patch pipettes (5–10 MΩ) were filled with a solution containing (mM): 120 potassium gluconate, 20 KCl, 0.5 EGTA, 10 Hepes, 2 Na_2ATP, 0.3 NaGTP and

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2 $MgCl_2$, pH 7.4. Some experiments were performed with another presynaptic pipette solution containing (mM): 140 $CsMeSO_4$, 10 Hepes, 0.5 EGTA, 4 MgATP and 0.3 NaATP, pH 7.3. Recordings were made at 34°C in a temperature-controlled recording chamber (Luigs & Neumann, Ratingen, Germany). Classically, the presynaptic neuron was recorded in current clamp with an Axoclamp 2B amplifier (Axon Instruments) and the postsynaptic cell in voltage clamp with an Axopatch 200B amplifier (Axon Instruments). Pre- and postsynaptic cells were held at their resting membrane potential (~ –65 mV). Presynaptic APs were generated by injecting brief (5–10 ms) depolarizing pulses of current at a frequency of 0.3 Hz. Short-term facilitation was assessed with two presynaptic stimulations delivered at a frequency of 20 Hz (Debanne *et al.* 1996; Boudkkazi *et al.* 2007). The voltage and current signals were low-pass filtered (3 kHz), and acquisition of 500 ms sequences was performed at 10–15 kHz with Acquis1 (G. Sadoc, CNRS, Gif-sur-Yvette, France) or DAAD software (N. Ankri, INSERM UMR 641, Marseille, France).

Mossy fibre EPSCs recorded in CA3 neurons were evoked by minimal stimulation in the dentate gyrus of organotypic slice cultures and were characterized by a marked frequency facilitation and by their sensitivity to group II metabotropic glutamate receptor agonist DCG-IV ((2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl) glycine; Nicoll & Schmitz, 2005). For these particular experiments, external calcium and magnesium concentrations were 1 and 3 mM, respectively, in control condition. When 4-AP was added, the calcium to magnesium ratio was decreased to 0.5/9 to avoid epileptiform activity. However, in all the other experiments (i.e. paired recordings of L5–L5 and CA3–CA3 neurons), the calcium to magnesium ratio remained constant (3/2) before and after application of 4-AP or DTX-I.

Synaptic responses were averaged following alignment of the presynaptic action potentials using automatic peak detection (Detectivent 4.0, N. Ankri, INSERM). The presence or absence of a synaptic connection between two neurons was determined on the basis of averages of 30–50 individual traces, including failures (Debanne *et al.* 2008). With this technique, even very small responses (<0.2 mV or <10 pA) could easily be detected. In practice, the smaller synaptic responses were 0.1 mV and 4 pA (Boudkkazi *et al.* 2007). The analysis was restricted to a corpus of connections with mean amplitude larger than 10 pA. The latency of individual EPSCs was measured from the peak of the presynaptic AP measured in the cell body to 5% of the EPSC amplitude (Boudkkazi *et al.* 2007).

In a few experiments, simultaneous recordings were obtained from the soma in whole-cell configuration and the axon in 'loose whole-cell' configuration. Typically, the axon was recorded 10–15 min after whole-cell access of the somatic compartment. The axon was visualized with differential interference contrast infrared video-microscopy, and negative pressure was applied to the pipette to obtain a high-resistance seal on the axon. Occasionally, a short length of the axon was pulled into the lumen of the patch pipette. In order to obtain a loose whole-cell patch recording, a brief suction of negative pressure was applied to the pipette that was occasionally maintained during the recording. In these conditions, the spike measured in the axon had a positive polarity (amplitude 0.3–10.1 mV). Its waveform was comparable to the spike waveform recorded in whole-cell configuration in the cell body (Scott *et al.* 2007; Boudkkazi *et al.* 2007), indicating that the time course of the recorded spike is minimally distorted in this recording configuration. The estimated conduction velocity between the axon and the soma (~0.1 m s^{-1}) was compatible with previous values measured in neocortical and pyramidal cell axons (Meeks *et al.* 2005; Palmer & Stuart, 2006). Axonal APs were averaged following alignment of the somatic APs using automatic peak detection (Detectivent 4.0). Axonal APs were analysed on the basis of acquisition of 300–2000 individual traces. All values shown in the figures correspond to averages over at least 2 min (i.e. >20 trials).

High-frequency components in the signals were filtered with a median filter (rank 1 or 2) and in some cases de-noising filtering (wavelet methods) was used. Special care was taken to verify that filtering of signals did not affect the original signals by superposition of the raw and filtered signals.

Drugs and statistical analysis

Tetraethylammonium (TEA) and 4-AP were obtained from Sigma, DCG-IV from Tocris and DTx-I from Latoxan. Data are presented as means ± S.E.M.

Results

Prolonged latency by extended spike duration with potassium channel blockers

To explore the consequences of AP broadening on EPSC latency, the latency of L5–L5 EPSCs was compared when the presynaptic cell was recorded with a control pipette solution (potassium gluconate) or Cs^+ as the main cation. As Cs^+ applied intracellularly is poorly permeable, this experimental approach allows inhibition of voltage-gated K^+ channels selectively in the presynaptic compartment. In this case, the presynaptic cell was voltage clamped to avoid uncontrolled presynaptic spikes. Interestingly, EPSP latency was significantly longer in caesium gluconate (4.0 ± 0.2 ms, $n = 6$) compared with potassium

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gluconate (1.4 ± 0.1 ms, $n = 9$, Student's unpaired t test $P < 0.001$; Fig. 1). To further confirm that the prolonged latency was the result of the broadening of the presynaptic spike, we studied the effects of extracellular Kv channel blockers on synaptic latency in the same synaptic connections.

Bath application of TEA (2 mM) increased somatic AP amplitude (from 85.4 ± 4.5 to 89.3 ± 4.2 mV, $n = 6$, Student's paired t test $P < 0.05$) as well as somatic AP duration (from 1.6 ± 0.1 to 3.0 ± 0.3 ms, $n = 6$, Student's paired t test $P < 0.05$; Fig. 2*Aa* and *b*). Compared with

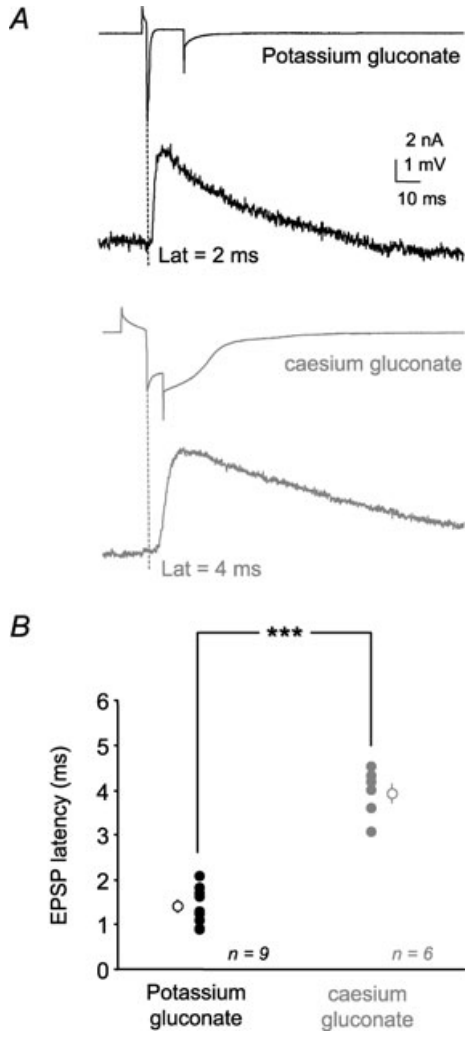


Figure 1. Delayed synaptic latency with Cs^+ in the presynaptic pipette
A, comparison of synaptic latency (Lat) at L5–L5 connections when the presynaptic neuron is recorded with potassium gluconate (black traces) or caesium gluconate (grey traces) in the intracellular solution. Note the longer EPSP latency in the presence of Cs^+. *B*, analysis of EPSP latency in potassium gluconate and caesium gluconate. Note that EPSP latency was on average significantly longer with Cs^+ as the main intracellular cation.

experiments using presynaptic Cs^+, TEA slightly increased EPSC latency (from 1.3 ± 0.2 to 1.5 ± 0.2 ms, $n = 6$, Student's paired t test $P < 0.05$; Fig. 2*Ac* and *d*). Moreover, no significant change in paired-pulse ratio (PPR) was observed (from 50 ± 8 to $50 \pm 8\%$ in TEA, $n = 6$, Student's paired t test $P > 0.10$). To further explore the effect of TEA on the presynaptic AP, simultaneous soma and axon recordings were performed (Fig. 2*Ba*). While TEA increased the somatic AP width by almost a factor of 2 (from 1.7 ± 0.1 to 2.9 ± 0.4 ms, i.e. $175 \pm 14\%$, $n = 7$, Student's paired t test $P < 0.01$), its effect on axonal AP duration diminished with the axonal distance (Fig. 2*Bc*). In fact, in the proximal part of the axon ($<45\ \mu$m), the spike increased (from 1.4 ± 0.2 to 2.5 ± 0.4 ms, $n = 4$, Student's paired t test $P < 0.01$) but not in its more distal part ($>45\ \mu$m, from 1.0 ± 0.1 to 1.0 ± 0.1 ms, $n = 3$, Student's paired t test $P > 0.10$; Fig. 2*Bc*). These results indicate that TEA-sensitive Kv channels are expressed in the soma and the proximal part of L5 pyramidal axons, but not in more distal axonal membranes.

Next, the effect of blocking Kv channels with 4-AP was tested on synaptic latency. Bath application of 4-AP (2 mM) increased both somatic AP amplitude (from 79 ± 4 to 85 ± 3 mV, $n = 6$, Student's paired t test $P < 0.10$; data not shown) and somatic AP duration (from 1.3 ± 0.1 to 1.6 ± 0.1 ms, $n = 6$, Student's paired t test $P < 0.05$). In contrast with TEA, 4-AP produced a large increase in both EPSC amplitude (from 27 ± 5 to 34 ± 5 pA, $n = 6$, Student's paired t test $P < 0.01$) and EPSC latency (1.4 ± 0.3 to 2.6 ± 0.3 ms, $n = 6$, Student's paired t test $P < 0.01$; Fig. 3*Aa–c*). Interestingly, the enhanced transmission was associated with a reduced PPR (from 68 ± 7 to $37 \pm 2\%$, $n = 6$, Student's paired t test $P < 0.01$), indicating that it was mediated by an increase in P_r. As a consequence of P_r-dependent latency (Boudkkazi *et al.* 2007), the 4-AP-induced effect could be underestimated, because increasing P_r leads to reduced latency, which tends to counterbalance the effect of 4-AP. For this reason, latencies were measured on EPSCs of similar amplitudes (average from 37 ± 3 to 37 ± 3 pA, $n = 6$, Student's paired t test $P > 0.10$; Fig. 3*Ac*, inset). In these conditions, the latency increased from 1.3 ± 0.3 to 3.1 ± 0.4 ms in the presence of 4-AP ($n = 6$, Student's paired t test $P < 0.01$; Fig. 3*Ad*). To examine the effects of 4-AP on spike duration along the axo-somatic axis, simultaneous somatic and axonal recordings were obtained from L5 neurons (Fig. 3*Ba*). 4-Aminopyridine produced a larger prolongation of the spike duration in the axon (from 1.2 ± 0.1 to 2.8 ± 0.4 ms $n = 10$, Student's paired t test $P < 0.01$) than in the soma (from 1.6 ± 0.1 to 1.9 ± 0.1 ms, $n = 10$, Student's paired t test $P < 0.01$; Fig. 3*Bb–c*). In conclusion, these data demonstrate that 4-AP-sensitive Kv channels control latency at excitatory cortical synapses.

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Axonal Kv1 channels control synaptic latency

We next identified the type of axonal Kv channel involved in the spike width-dependent shift in latency. Prolonged latency was observed with 2 mM 4-AP but not with 2 mM TEA, suggesting the involvement of A- or D-type Kv channels (Storm, 1990). To distinguish between these two possibilities, additional experiments were performed with a low concentration of 4-AP (20 μM) that preferentially blocks D-type Kv current. In these conditions, EPSC amplitude increased (from 17 ± 3 to 20 ± 4 pA, $n = 6$, Student's paired t test $P < 0.01$), the PPR decreased (from 62 ± 7 to 41 ± 4%, $n = 6$, Student's paired t test $P < 0.01$), the P_r-independent shift in latency (i.e. measured on similar EPSC amplitudes) increased from 1.0 ± 0.2 to 1.7 ± 0.2 ms ($n = 6$, Student's paired t test, $P < 0.01$) and axonal spike duration increased (from 0.8 ± 0.1 to 1.6 ± 0.1 ms, Student's paired t test, $P < 0.01$, $n = 9$; Fig. 4*A*). D-type potassium channels (Kv1) located in the axon control spike width and are highly sensitive to dendrotoxin (Shu *et al.* 2006, 2007; Kole *et al.* 2007). We therefore tested whether DTx-I, which specifically blocks Kv1.1, Kv1.2 and Kv1.6 subunits, also prolonged the synaptic latency at L5–L5 synapses. As previously shown (Kole *et al.* 2007), DTx-I (200 nM) was found to increase the amplitude of EPSCs (from 28 ± 5 to 33 ± 6 pA, $n = 7$), prolong the axonal spike (from 0.9 ± 0.1 to 1.5 ± 0.2 ms, $n = 3$, distance >45 μm), and reduce PPR (from 67 ± 6 to 44 ± 5%, $n = 7$, Student's paired t test $P < 0.01$). In addition, DTx-I was found to prolong the latency of EPSCs of comparable amplitude (23 pA; from 0.9 ± 0.2 to 1.4 ± 0.3 ms, $n = 7$; Student's paired t test, $P < 0.01$; Fig. 4*B*). Thus, axonal Kv1 channels that mediate the D-type current determine synaptic latency.

Axonal spike duration determines synaptic latency

The latency between the presynaptic AP in the somatic compartment and the EPSC onset depends, in theory, on the following factors: (1) the conduction velocity along the axon; (2) the back-propagating time from the spike initiation zone to the soma; (3) the coupling between the spike in the presynaptic terminal and the calcium influx; and (4) the kinetics of Ca^{2+} binding to the Ca^{2+} sensor. We first tested the effect of 4-AP on axonal propagation. Antidromic APs were evoked in L5 neurons with an extracellular stimulating electrode located in the white matter (at approximately 200 μm from the soma). The prolongation of the spike produced by 4-AP (2 mM) was associated with a slight reduction in latency (from 1.2 ± 0.4 to 1.1 ± 0.3 ms, $n = 4$, Student's paired t test $P < 0.01$; Fig. S1*A* and *B*). Therefore, the prolonged latency is not due to modification of the conduction velocity, and other mechanisms should be envisaged.

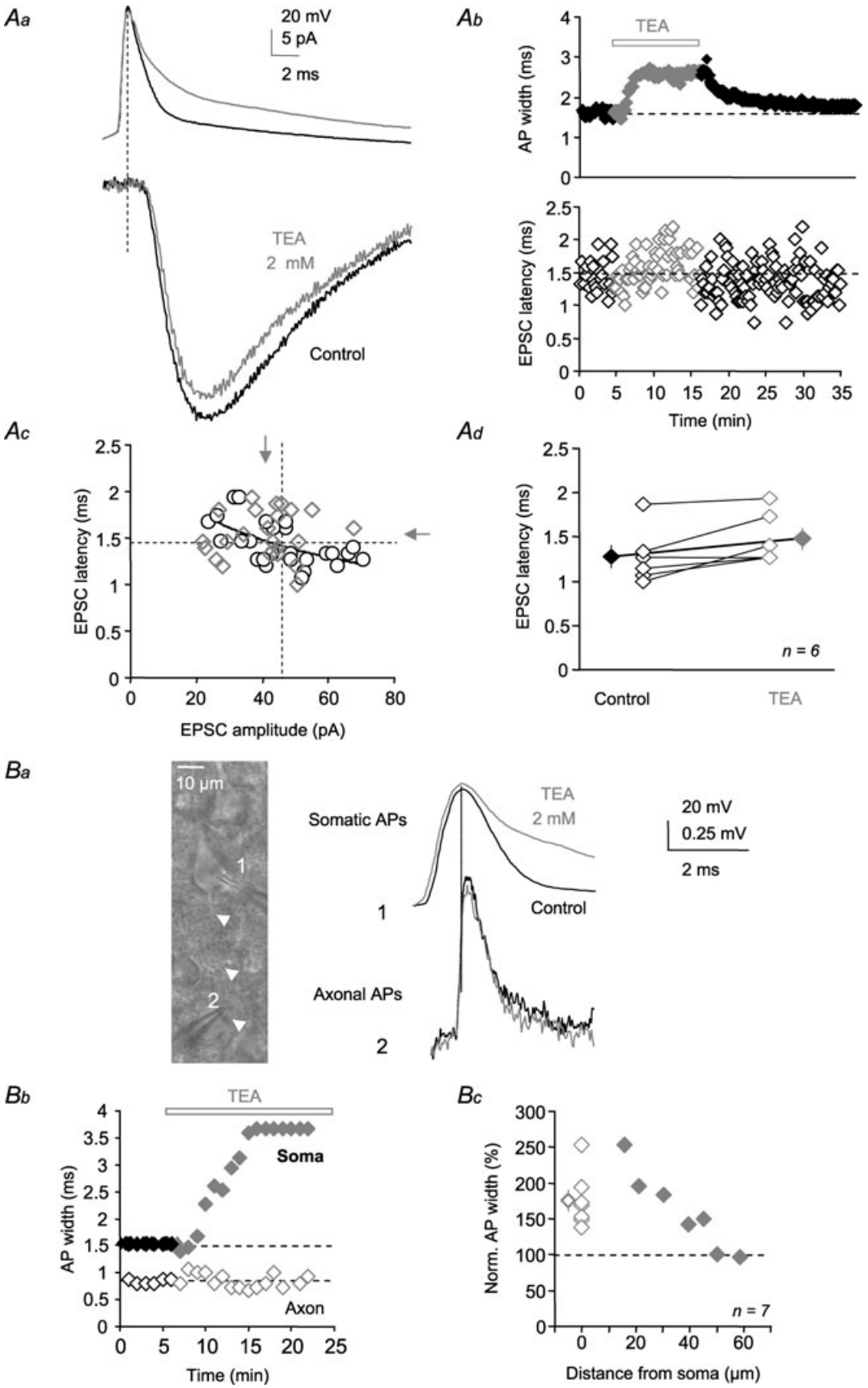
At invertebrate synapses, latency is largely determined by the duration of the presynaptic depolarization (Katz & Miledi, 1967; Augustine *et al.* 1985). To further determine the role of axonal spike duration in the synaptic latency at L5–L5 synapses, the variation in latency (ΔLat) produced by the Kv channel blockers was plotted as a function of the induced variation in AP duration measured in the axon. Overall, the increase in synaptic latency produced by the blockers was well predicted by the extension of the AP width in the axon (linear regression: $y = 0.72x + 0.11$, $r^2 = 0.95$; Fig. 5), indicating that AP extension induced by Kv channel blockers is a major determinant of synaptic latency at L5–L5 synapses.

Does AP amplitude determine latency?

In parallel with the prolongation of the axonal spike duration, 4-AP (2 mM) or DTx-I also slightly increased its amplitude in the axon (respectively 9 ± 2%, $n = 10$ and 2 ± 2%, $n = 8$). To explore the consequences of AP reduction on EPSC latency, low concentrations of TTX (15–30 nM) were bath applied. In these conditions, action potentials and synaptic transmission were not totally abolished. In fact, the somatic AP amplitude decreased from 79 ± 3 to 63 ± 4 mV (i.e. 21% reduction, $n = 12$, Student's paired t test $P < 0.01$) and EPSC amplitude was decreased (from 32 ± 4 to 26 ± 4 pA, $n = 12$, Student's paired t test $P < 0.01$). This reduction in synaptic transmission was followed by a significant increase in PPR (from 61 ± 8% in control conditions to 80 ± 9% in TTX, $n = 12$, Student's paired t test $P < 0.01$; Fig. 6*A*), suggesting that the TTX-induced reduction in EPSC is due to an apparent decrease in P_r. The reduction in EPSC amplitude was surprisingly associated with a shortening of EPSC latency (Fig. 6*B*). Postsynaptic current latency decreased on average from 1.2 ± 0.1 to 0.9 ± 0.1 ms ($n = 12$, Student's paired t test $P < 0.01$). Since P_r was apparently reduced in the presence of TTX, the change in EPSC latency was probably underestimated. Therefore, for each synaptic connection, a restricted range of EPSC amplitude (width 10 pA) was defined, and EPSC latencies were compared before and after TTX application (inset in Fig. 6*Bb*). In these conditions, EPSC latency decreased from 1.3 ± 0.1 to 0.8 ± 0.1 ms in TTX ($n = 12$, Student's paired t test $P < 0.01$; Fig. 6*Bc*). This effect could not be attributed to an increase in conduction along the axon, because the latency of antidromic AP was slightly delayed (from 1.1 ± 0.3 ms in control conditions to 1.4 ± 0.3 ms in TTX, $n = 4$, Student's paired t test $P < 0.01$; Fig. S1*C* and *D*). To estimate to what extent the axonal spike was decreased, we also measured the effect of TTX in the axon. On average, the amplitude decreased by 37 ± 3% in the axon ($n = 9$). We conclude that presynaptic AP amplitude also determines synaptic latency. The moderate increase in the axonal spike amplitude induced by Kv channel blockers

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may also account for 0.13 ms of the observed effect if the amplitude-dependent change in latency is linear.

Generalization to other excitatory synapses

To determine whether Kv-dependent latency was a general feature of central synapses, we examined whether 4-AP also produced a shift in the latency at other neocortical or hippocampal synapses. Pairs of connected L5 pyramidal neurons and putative GABAergic interneurons were recorded. In contrast with L5 pyramidal neurons, these neurons displayed a bipolar shape, a brief AP (<1 ms) and deep fast after-hyperpolarization (usually >15 mV). 4-Aminopyridine enhanced synaptic transmission (237 ± 78% of the control amplitude, $n = 3$). After compensation for the increased release probability, the latency was found to be prolonged in the presence of 2 mM 4-AP (from 1.0 ± 0.1 to 2.4 ± 0.1 ms, $n = 3$, Student's paired t test $P < 0.01$; Fig. 7*A*). Likewise, 4-AP also produced a shift in the synaptic latency at connected CA3 pyramidal neurons recorded in hippocampal slice cultures (from 3.4 ± 1.0 to 5.9 ± 1.4 ms, $n = 4$, Student's paired t test $P < 0.01$; Fig. 7*B*). We conclude that spike width-dependent latency is a general principal at central excitatory synapses.

Evidence for P_r-independent prolongation of synaptic latency

Action potentials in presynaptic mossy fibre boutons from granule cell axons are broadened following repetitive high-frequency (50 Hz) stimulation as a result of inactivation of A-type K^+ current (Geiger & Jonas, 2000). If the presynaptic spike width determines the latency, repetitive stimulation of granule cell axons should reveal an activity-dependent increase in synaptic latency. CA3 pyramidal neurons were recorded in hippocampal slice cultures (day in vitro 8–15), and trains of 25 EPSCs were evoked at 20 Hz with minimal stimulation in the dentate gyrus. A large facilitation was observed (ratio of the 25th EPSC to the 1st EPSC: 322 ± 30%, $n = 12$; Fig. 8*A*). The evoked response was fully antagonized by the type 2/3 metabotropic glutamate receptor agonist DCG-IV (10 μM), confirming that it was mediated by mossy fibre inputs (data not shown). As expected from our previous work (Boudkkazi *et al.* 2007), the synaptic latency decreased during paired-pulse stimulation, but interestingly, synaptic latency reached a plateau and increased during subsequent stimulations (Fig. 8*B*). This shift out of the P_r-law suggests that a second mechanism may determine synaptic latency during repetitive activity (Fig. 8*C*). This change probably results from a broadening of the presynaptic spike. In fact, the width of APs recorded in the soma of granule cells was increased by 77 ± 6% (from 0.9 ± 0.1 to 1.9 ± 0.2 ms; $n = 26$, data not shown). Although the presynaptic spike width has not been directly measured in the terminal, additional broadening would be observed in the mossy fibre bouton (see Geiger & Jonas, 2000). It is noteworthy that the data points followed the P_r law in the presence of 40 μM 4-AP (Fig. 8*B*, inset), indicating that increased latency during repetitive stimulation depends on Kv channels. We conclude that presynaptic Kv channels stabilize synaptic latency during repetitive stimulation of mossy fibres.

Discussion

We show here that besides the P_r-dependent latency reported previously (Boudkkazi *et al.* 2007; Saez & Friedlander, 2009), the axonal spike duration also determines synaptic latency at cortical synapses. In fact, pharmacological inactivation of Kv channels with 4-AP (2 mM or 20 μM) or DTx-I (200 nM), but not TEA (2 mM), prolonged synaptic latency by ∼2 ms. These two mechanisms are independent, because P_r-dependent latency was still observed before and after blocking Kv channels with 4-AP. The fact that the shift in latency was linearly related to the induced change in axonal spike duration strongly suggests that the underlying mechanism

Figure 2. Tetraethylammonium-sensitive Kv channels do not control synaptic latency and axonal spike duration
Aa, effectof 2 mM TEA (grey traces) on synaptic transmission at a connected pair of L5 neurons. *Ab*, time course of AP duration (filled diamonds; top panel) and EPSC latency (open diamonds; bottom) in control conditions (black symbols) and in the presence of TEA (grey symbols). Averages in control conditions are indicated by dotted lines. *Ac*, plot of EPSC latencies *versus* amplitudes measured on individual currents in control conditions (open circles) and in TEA (grey open diamonds). Note that control and TEA distributions are almost superimposed. Black curve, logarithmic fit ($y = -0.46\ln(x) + 3.19$; $r^2 = 0.36$) over the control distribution. Mean values of EPSCs and latencies are symbolized by dotted lines in control conditions and by grey arrows in TEA. *Ad*, group data corresponding to 6 pairs tested. *Ba*, left panel, differential interference contrast infrared videomicrograph of an L5 neuron recorded simultaneously at the soma in whole-cell configuration and at the axon with the loose whole-cell patch recording technique. Right panel, effect of TEA on somatic and axonal APs. Note the prolongation in the soma but not in the axon. *Bb*, time course of spike duration in the soma (filled diamonds) and in the axon (open diamonds) following bath application of TEA. Mean values in control conditions are indicated by dotted lines. *Bc*, normalized AP width as a function of distance from the soma after application of TEA (7 experiments). Note the large effect in the soma and the proximal part of the axon. The effect of TEA disappears in the distal part of the axon.

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principally involves the shift of the presynaptic calcium current resulting from the prolonged axonal spike. The Kv-dependent shift in latency was not only observed at L5–L5 synapses but also at L5–interneuron synapses and at unitary CA3–CA3 synapses, indicating that it may be a general feature of many central excitatory synapses. The Kv-dependent latency shift is functionally relevant in at least one way. It may stabilize synaptic latency during repetitive stimulation at mossy fibre–CA3 cell synapses (Fig. 8). Importantly, we also demonstrated that reducing the spike amplitude by ∼15 mV with a low concentration of TTX shortened synaptic delay (Fig. 6). In agreement with previous findings (Augustine *et al.* 1985), the reduction in presynaptic spike amplitude may produce

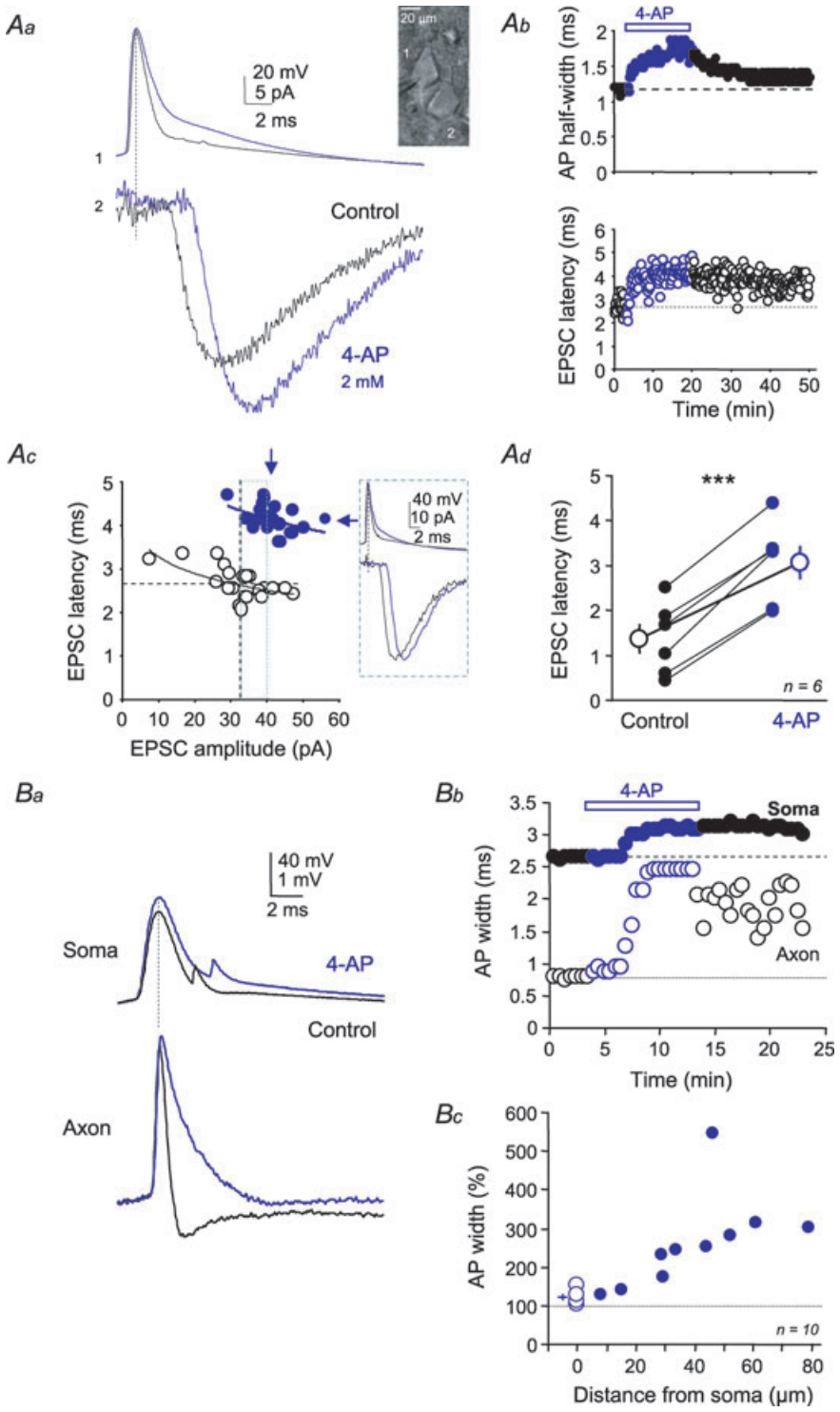


Figure 3. 4-Aminopyridine-sensitive Kv channels control synaptic latency and axonal spike duration
Aa, effect of 2 mM 4-AP (blue traces) on spike duration and synaptic transmission at a connected pair of L5 neurons. Note the large increase in EPSC latency. *Ab*, time course of presynaptic AP duration recorded in the cell body (filled circles; top panel) and EPSC latency (open circles; bottom panels) in control conditions (black symbols) and in the presence of 4-AP (blue symbols). *Ac*, plot of EPSC latencies *versus* amplitudes measured on individual currents in control conditions (black open circles) and in 4-AP (blue filled circles). Note a rightward shift of the 4-AP distribution. Each distribution is fitted by a logarithmic curve ($y = -0.56\ln(x) + 4.56$, $r^2 = 0.37$ in control conditions; $y = -0.92\ln(x) + 7.55$, $r^2 = 0.18$ in 4-AP). The P_r-independent shift in EPSC latency induced by 4-AP was determined for EPSCs of the same amplitude (dotted box). *Ad*, group data corresponding to 6 pairs tested. *Ba*, effect of 4-AP on somatic and axonal APs during simultaneous soma and axon recordings. Note the prolongation of the axonal spike. *Bb*, time course of spike duration in the soma (filled circles) and in the axon (open circles) following bath application of 4-AP. *Bc*, normalized AP width as a function of recording distance after application of 2 mM 4-AP (10 experiments). Note the dramatic extension in the distal part of the axon.

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an advance of the presynaptic calcium current, and subsequently a reduction in synaptic delay.

Axonal Kv1 channels determine the synaptic latency at L5–L5 connections

We report here that synaptic latency at L5–L5 connections is increased by Cs^+ in the presynaptic neuron, 4-AP (20 μM or 2 mM) or DTx-I (200 nM), but not by TEA (2 mM). In fact, TEA markedly prolonged the spike in the cell body but had no effect on either synaptic latency or the duration of the AP measured in the axon. In contrast, 4-AP or DTx-I significantly extended the axonal AP and delayed synaptic latency, but had little effect on the somatic spike waveform. These data indicate that 4-AP-sensitive Kv channels are selectively expressed in the axon, whereas TEA-sensitive Kv channels are mainly located in the cell body compartment

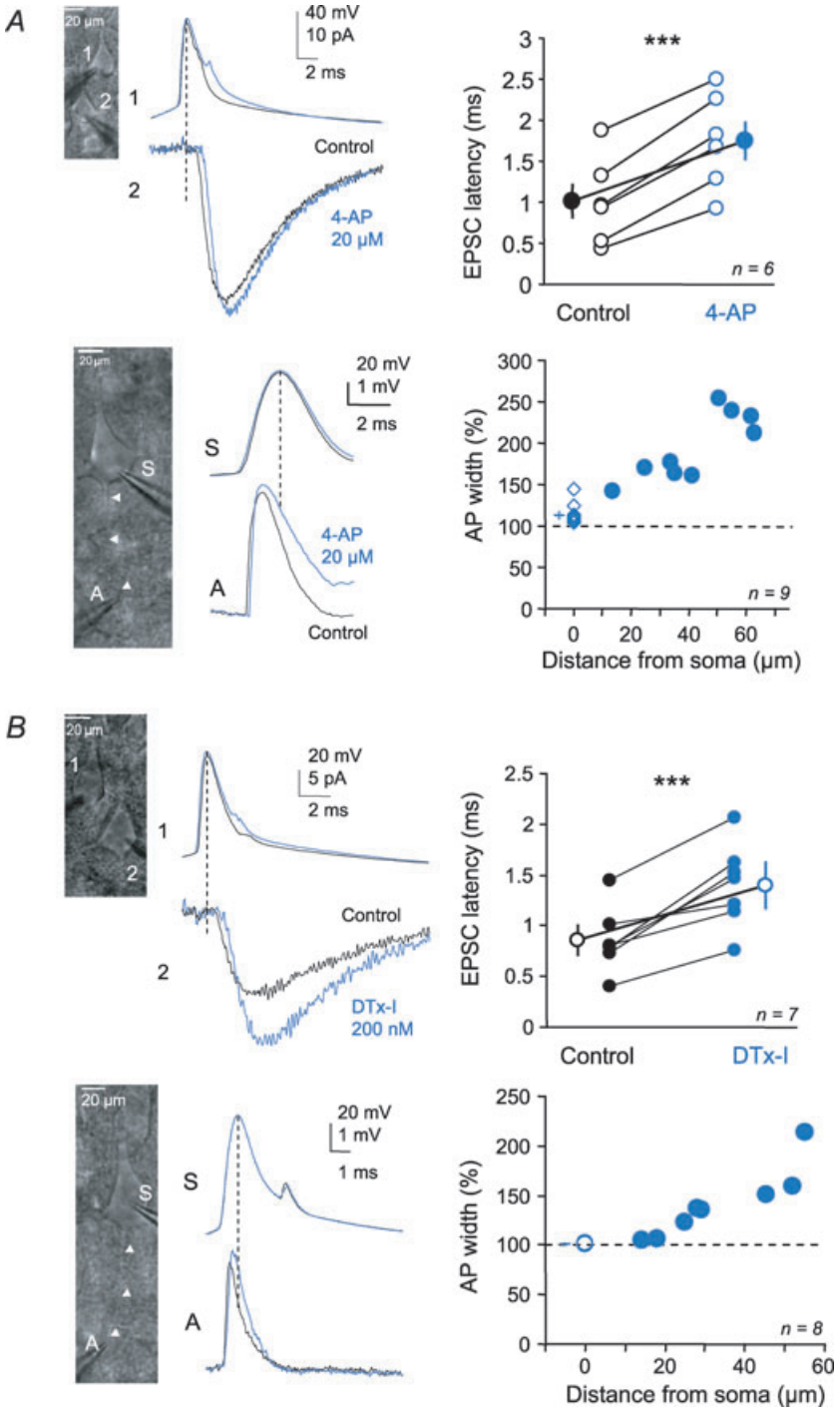


Figure 4. D-type Kv channels control synaptic latency and axonal spike duration
A, top left, effect of 20 μM 4-AP (blue traces) on EPSC latency at an L5–L5 connection. Top right, group data of EPSC latency changes measured at constant amplitude (compensated for P_r-dependent latency changes). Bottom left, 20 μM 4-AP extends axonal but not somatic AP. Bottom right, normalized effects of 20 μM 4-AP on AP width as a function of recording distance. *B*, top left, effect of DTx-I on EPSC latency. Top right, group data of EPSC latency changes measured at constant amplitude. Bottom left, DTx-I extends axonal but not somatic AP. Bottom right, normalized effects of DTx-I on AP width as a function of recording distance.

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and/or in the proximal part of the axon. In fact, cell-attached recordings from axons of rat L5 pyramidal neurons revealed that the axon initial segment and the axon proper express DTx-sensitive, slowly inactivating D-type potassium currents that are respectively 10 and seven times larger in these compartments than in the cell body (Kole *et al.* 2007). Supporting this view, another study has indicated that the DTx-sensitive K^+ currents are three to four times larger in the axon of L5 cortical neurons of the ferret (Shu *et al.* 2007). In hippocampal and cortical pyramidal neurons, the slowly inactivating D-type K^+ current is principally mediated by Kv1 channels (Stühmer *et al.* 1989; Storm, 1990; Kole *et al.* 2007). In fact, immunohistochemistry of Kv1.1 and Kv1.2 proteins has indicated that Kv1 channels are highly expressed in the axon initial segment and axon terminals (Sheng *et al.* 1993; Veh *et al.* 1995; Inda *et al.* 2006; Goldberg *et al.* 2008; Lorincz & Nusser, 2008).

Tetraethylammonium broadens action potentials in the soma, but not in the axon. Several TEA-sensitive channels controlling spike width are specifically located in the soma of cortical neurons. Spike broadening might be caused by the blockade of the large conductance, calcium-activated (I_C) K^+ current (Storm, 1987; Lancaster & Nicoll, 1987; Marrion & Tavalin, 1998) or by the blockade of Kv2.1 channels carrying I_K (Murakoshi & Trimmer, 1999; Du *et al.* 2000; Mitterdorfer & Bean, 2002). In addition, TEA blocks Kv7 channels located in the axon initial segment that mediate the M-type current (Devaux *et al.* 2004). However, these channels do not strongly influence the action potential waveform (Vervaeke *et al.* 2006; Shah *et al.* 2008).

The effects of DTx-I on EPSC amplitude were found to be smaller (26%) than those initially reported by Kole *et al.* (2007; ∼200% of the control amplitude). This discrepancy may result from the high release probability in our experiments (PPR close to 100% in Kole's paper *vs.* 60% in the present study), due to both the experimental conditions (2 mM external Ca^{2+} *vs.* 3 mM in our case) and the age of the preparation (2- to 5-week-old rats *vs.* 13- to 20-day-old rats in our case; see Angulo *et al.* 1999; Reyes & Sakmann, 1999). Thus, spike prolongation caused by DTx-I only slightly enhances synaptic strength.

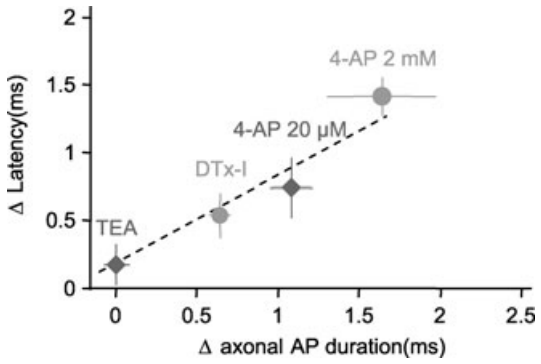


Figure 5. Axonal AP duration controls synaptic latency
Summary of the changes in EPSC latency and the corresponding effects on axonal AP duration induced by TEA, DTx-I and 4-AP (20 μM and 2 mM). Note the linear relation between latency and AP duration in the axon.

Prolonged synaptic latency has been observed at CA3–CA3 synaptic connections when the D-type potassium current was inactivated with 4-AP (Saviane *et al.* 2003). However, our study is the first that clearly establishes a relation between the change in synaptic latency and the modification in AP waveform measured in the axon of mammalian central neurons. Increased synaptic latency in the millisecond range has been reported at connected pairs of hippocampal neurons when the presynaptic A-type current was partly inactivated (Debanne *et al.* 1997). In these experiments, the prolonged latency cannot be attributed to the mechanism described here. Rather, it may result from delayed axonal conduction caused by the combination of a change in local geometry and an increase in conductance (Segev, 1990; Manor *et al.* 1991).

Delayed presynaptic calcium current

Modification of axonal spike duration strongly determined the magnitude of the shift in latency. For instance, TEA (2 mM) had no effect on both axonal spike duration and synaptic latency, whereas 4-AP (2 mM or 20 μM) or DTx-I produced a major effect on both parameters (Fig. 5). Thus, axonal spike prolongation represents a key parameter in the 4-AP-dependent prolongation of synaptic latency. However, conduction velocity does not play a critical role in this mechanism. In fact, 4-AP slightly decreased the latency of antidromically evoked AP, indicating that conduction velocity was not reduced.

What is the mechanism underlying axonal spike width-dependent prolongation of synaptic latency? Although direct recording from presynaptic terminals of L5 neurons is almost impossible because of their small size, our data are consistent with the fact that prolonged axonal duration delays the presynaptic calcium current. As the presynaptic spike overshoots at approximately +50 mV, the peak of the calcium current develops essentially during the repolarization phase of the presynaptic spike (Katz & Miledi, 1967; Llinas *et al.* 1981; Augustine *et al.* 1985; Sabatini & Regehr, 1996, 1997; Bischofberger *et al.* 2002; Lin & Faber, 2002). Thus, any prolongation in the duration of the presynaptic spike, such as that produced by 4-AP, will delay the calcium current (see Bischofberger *et al.* 2002) and will subsequently shift release towards longer latencies. The precise characterization of the underlying mechanisms is the matter of a separate study.

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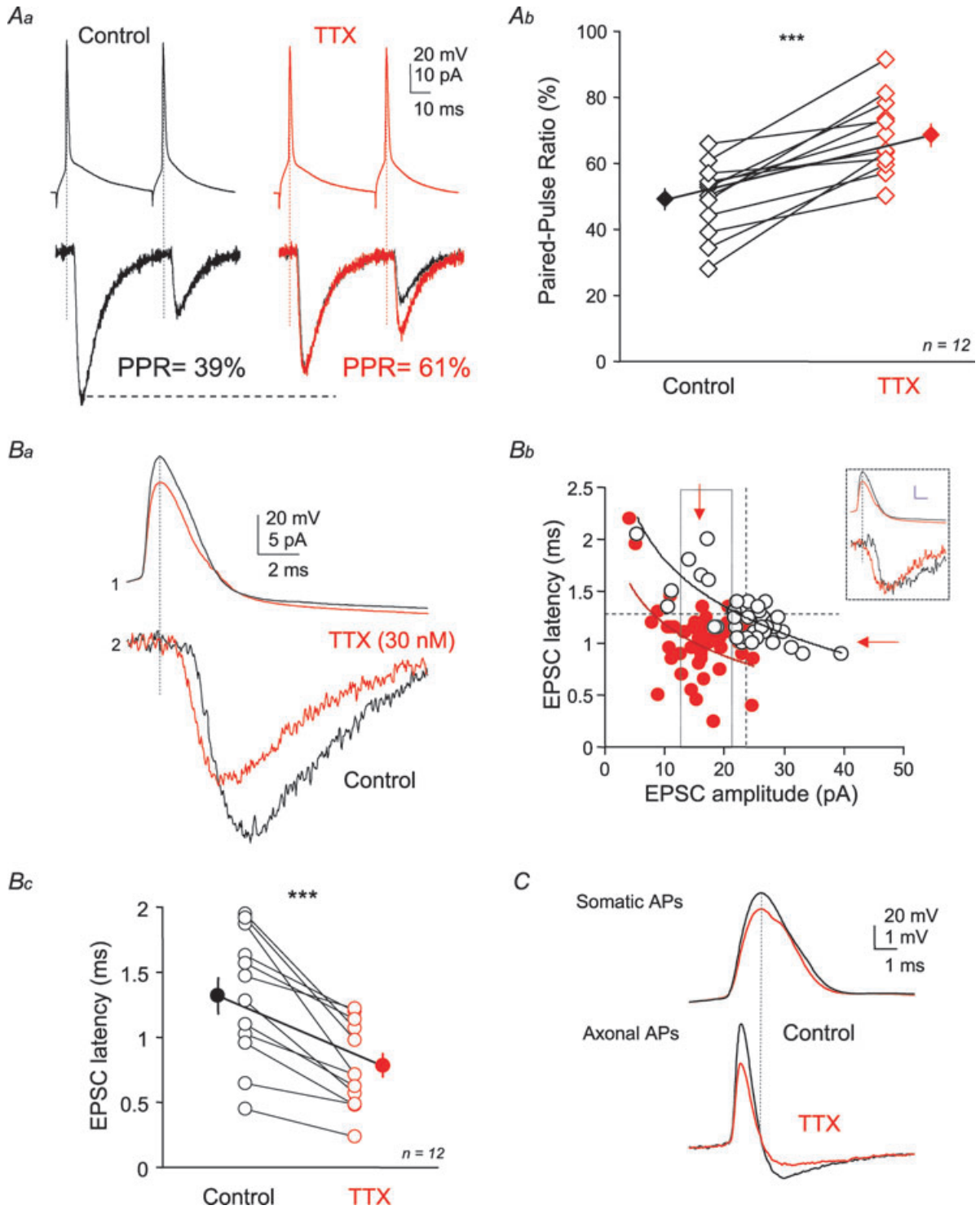


Figure 6. Reduced latency induced by low concentration of TTX
A, TTX (15–30 nM) reduces synaptic transmission and increases paired-pulse ratio at L5–L5 connections. A low concentration of TTX reduces the presynaptic spikes but does not block synaptic transmission. Representative example (*Aa*) and group data on 12 connections (*Ab*). Note the significant increase in paired-pulse ratio (***$P < 0.01$, Student's paired *t* test). *B*, a low concentration of TTX shortens synaptic latency evoked by the first AP. Example (*Ba*) and analysis of the EPSC latency *vs.* amplitude relation in the same pair (*Bb*). The P_r-independent shift in latency induced by TTX was determined with EPSCs of the same amplitude (dotted box and right inset). *Bc*, group data of the change in latency after compensation for P_r. *C*, effect of TTX on somatic and axonal APs during simultaneous soma and axon recordings.

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Amplitude of the presynaptic AP

An increase in AP amplitude in the axon constitutes the third mechanism that may account for the prolongation of EPSC latency observed when Kv1 channels are inactivated. In fact, the axonal spike was increased by 9% in the presence of 4-AP. A similar increase has been previously reported in the cell body (Storm, 1987) and results from the blockade of Kv channels that normally interrupt the rising phase of the AP. To study the effect of the amplitude of the presynaptic spike we applied a low concentration of TTX that efficiently reduced the amplitude of the AP but did not block synaptic transmission. We showed that reducing presynaptic AP amplitude with 15–30 nM TTX shortened EPSC latency by 0.5 ms. This effect is also compatible with the modification of coupling between the presynaptic waveform amplitude and the activation of calcium current reported at giant synapses (Katz & Miledi, 1967; Augustine *et al.* 1985; Bischofberger *et al.* 2002). Here, the reduction in amplitude of the presynaptic spike probably shortens the latency of the presynaptic calcium tail current. Our data indicate that the modification in amplitude accounts only for a relatively small portion of the induced change in latency. The 37% reduction of the axonal spike amplitude induced by TTX prolongs the latency by 0.5 ms, suggesting that the 9% increase in axonal spike amplitude would produce a delay in the range of 0.1–0.2 ms if this relation is linear. The amplitude-dependent reduction in synaptic delay might be functionally important. The prolongation of the axonal spike observed during repetitive stimulation (Geiger & Jonas, 2000) or somatic depolarization (Shu *et al.* 2006) is associated with a reduction in amplitude. Thus, it may partly counterbalance the increase in latency produced by the prolongation of the spike.

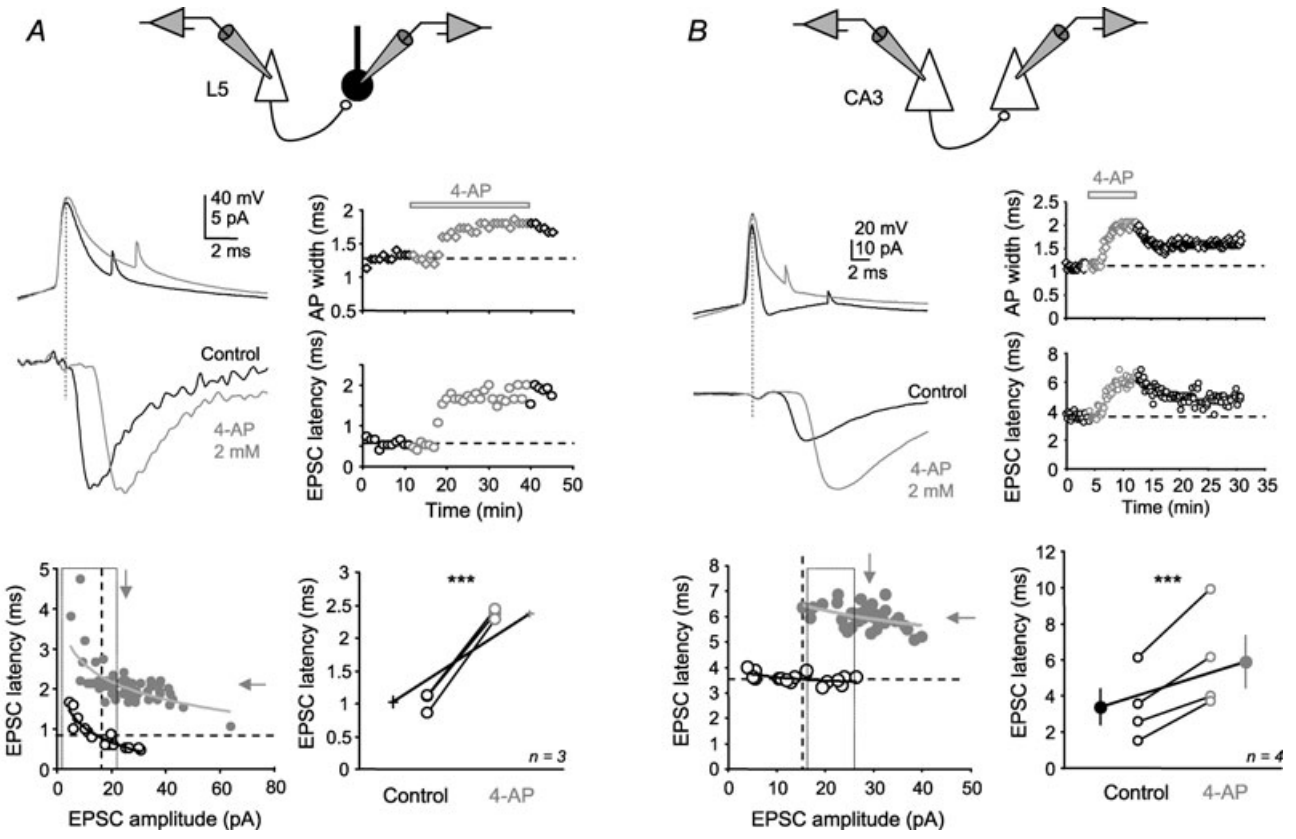


Figure 7. Generalization of Kv-dependent latency to other excitatory synapses
A, effect of 2 mM 4-AP on EPSC latency at connections between L5 pyramidal neuron and inhibitory interneuron. Scheme shows experimental configuration. Top left, 4-AP (2 mM) increased EPSC latency. Top right, time courses of the EPSC latency and AP width. Bottom left, latency *vs.* amplitude distributions in control conditions (black open circles) and in the presence of 4-AP (grey filled circles). Bottom right, group data of EPSC latency changes measured at constant amplitude. *B*, effect of 2 mM 4-AP on EPSC latency at a CA3–CA3 connection. Scheme shows experimental configuration. Top left, raw data. Top right, time courses of the EPSC latency and AP width. Bottom left, latency *vs.* amplitude distributions in each set of experimental conditions. Note the large increase in latency in the presence of 4-AP.

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Functional implications

The presynaptic spike width-dependent latency is potentially implicated in the stabilization of synaptic timing during repetitive stimulation. We show that at the mossy fibre–CA3 cell synapse the change in synaptic latency during facilitation initially follows the P_r-dependent rule (i.e. facilitation with a reduction in latency; Boudkkazi *et al.* 2007), but progressively diverges from this law after the fifth stimulation and finally returns to high values despite a very large EPSC facilitation. This observation is consistent with the extension of the AP at the mossy fibre terminal due to the inactivation of A-type Kv channels during repetitive stimulation (Geiger & Jonas, 2000). Although we did not measure directly the spike extension in the mossy fibre terminal, our results indicate that repetitive firing in granule cells increased the duration of the spike width in the soma. Interestingly, this behaviour was absent in the presence of 4-AP.

At the calyx of Held, a parallel reduction in the AP duration and the synaptic delay is observed during development (Taschenberger & von Gersdorff, 2000). This presynaptic acceleration is thought to result from the maturation of presynaptic Nav and Kv channels. Thus, our study provides a causal link between the speeding up of synaptic transmission and the changes in presynaptic AP waveform during development.

Activity-dependent changes in the presynaptic AP waveform represent a powerful means for precise control of the timing of synaptic transmission. We show here that inactivation of presynaptic Kv1 channels is a major mechanism for stabilizing synaptic latency. We may also speculate that the timing of synaptic transmission may be controlled through the activity-dependent regulation of Kv1 (Grosse *et al.* 2000; Raab-Graham *et al.* 2006; Cudmore *et al.* 2010) or Nav channel activity (Cantrell & Catterall, 2001; Carlier *et al.* 2006).

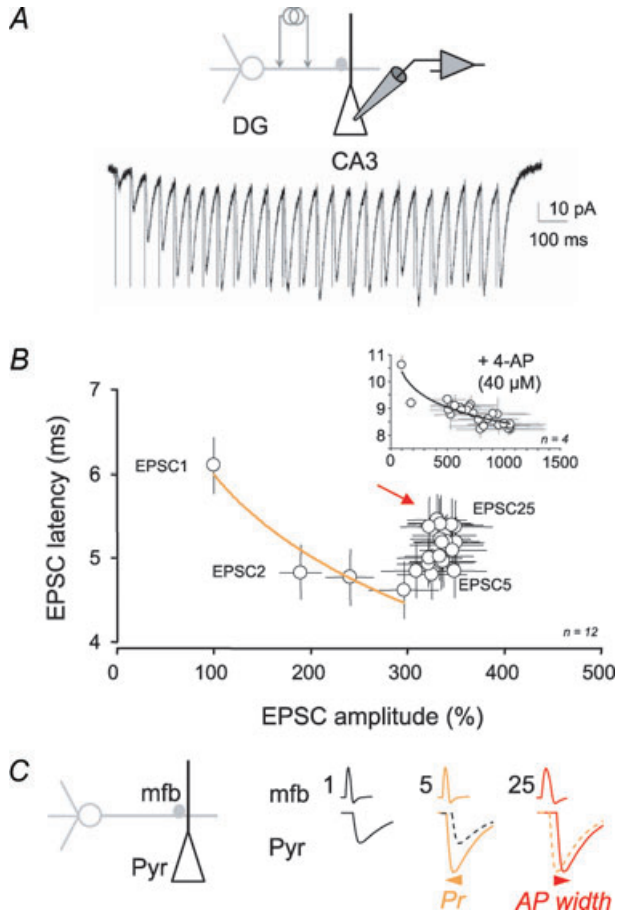


Figure 8. P_r-independent modulation of latency at DG–CA3 synapse
A, scheme shows experimental configuration. A CA3 pyramidal cell was recorded in voltage clamp in the whole-cell configuration of the patch-clamp technique, and a train of 25 mossy fibre EPSCs was evoked by stimulating a granular cell of the dentate gyrus in organotypic hippocampal slices. Bottom, example of short-term facilitation of the mossy fibre synapse stimulated at 20 Hz. *B*, plot of EPSC latencies *vs.* normalized amplitude for pooled data ($n = 12$). Note that amplitude-dependent latency variation (fitted by a logarithmic curve in orange; $y = -1.4\ln(x) + 12.5$, $r^2 = 0.92$) was only present for the first EPSCs, whereas the last EPSCs diverge from this law (red arrow). Inset, amplitude-dependent latency distribution in the presence of 4-AP (40 μM) is fitted by a logarithmic curve ($y = -0.8\ln(x) + 14.1$, $r^2 = 0.75$). *C*, summary of the latency stabilization during repetitive stimulation at the mossy fibre–CA3 cell synapse. From stimulation 1 to 5, latency decreases because of enhanced P_r (orange traces and arrowhead), whereas latency increases from stimulation 5 to 25 because of AP broadening (red traces and arrowhead). Abbreviations: Mfb, mossy fibre bouton; and Pyr, CA3 pyramidal cell.

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Author contributions

S.B., L.F.M. and D.D. conceived and designed the experiments. S.B., L.F.M. and D.D. collected, analysed and interpreted the data. S.B. and D.D. drafted the manuscript. The experiments were performed in the laboratory of D.D. at INSERM U641, Marseille, France. All authors approved the final version of the manuscript.

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