

Specific Functions of Synaptically Localized Potassium Channels in Synaptic Transmission at the Neocortical GABAergic Fast-Spiking Cell Synapse

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Potassium (K^+) channel subunits of the Kv3 subfamily (Kv3.1–Kv3.4) display a positively shifted voltage dependence of activation and fast activation/deactivation kinetics when compared with other voltage-gated K^+ channels, features that confer on Kv3 channels the ability to accelerate the repolarization of the action potential (AP) efficiently and specifically. In the cortex, the Kv3.1 and Kv3.2 proteins are expressed prominently in a subset of GABAergic interneurons known as fast-spiking (FS) cells and in fact are a significant determinant of the fast-spiking discharge pattern. However, in addition to expression at FS cell somata, Kv3.1 and Kv3.2 proteins also are expressed prominently at FS cell terminals, suggesting roles for Kv3 channels in neurotransmitter release. We investigated the effect of 1.0 mM tetraethylammonium (TEA; which blocks Kv3 channels) on inhibitory synaptic currents recorded in layer II/III neocortical pyramidal cells. Spike-evoked GABA release by FS cells was enhanced nearly twofold by 1.0 mM TEA, with a decrease in the paired pulse ratio (PPR), effects not reproduced by blockade of the non-Kv3 subfamily K^+ channels also blocked by low concentrations of TEA. Moreover, in Kv3.1/Kv3.2 double knock-out (DKO) mice, the large effects of TEA were absent, spike-evoked GABA release was larger, and the PPR was lower than in wild-type mice. Together, these results suggest specific roles for Kv3 channels at FS cell terminals that are distinct from those of Kv1 and large-conductance Ca^{2+} -activated K^+ channels (also present at the FS cell synapse). We propose that at FS cell terminals synaptically localized Kv3 channels keep APs brief, limiting Ca^{2+} influx and hence release probability, thereby influencing synaptic depression at a synapse designed for sustained high-frequency synaptic transmission.

Key words: Kv3; potassium channels; interneurons; FS cells; synaptic transmission; GABA; inhibition

Introduction

GABAergic interneurons are the source of inhibitory synaptic transmission in the cortex, have essential roles in cortical function, and are believed to be involved in the pathophysiology of neuropsychiatric disorders such as epilepsy and schizophrenia (Jones, 1993; McBain and Fisahn, 2001; Noebels, 2003; Swadlow, 2003; Lewis et al., 2005).

The largest group (40–50%) of cortical GABAergic interneurons are the fast-spiking (FS) cells, which form strong inhibitory axo-axonic and axo-somatic synaptic connections and thereby exert powerful control over the output of their target neurons (Kawaguchi and Kubota, 1997). FS cells are named for their ability to fire sustained trains of brief action potentials (APs) at re-

markably high frequency (McCormick et al., 1985; Swadlow, 2003), a feature that is determined mainly by the somatic expression of unique voltage-gated K^+ channels composed of Kv3.1 and Kv3.2 pore-forming subunits (Rudy and McBain, 2001). However, in addition to Kv3.1 and Kv3.2 protein expression at FS cell somata, there is prominent expression of these proteins at FS cell synapses (Sekirnjak et al., 1997; Chow et al., 1999), yet nothing is known concerning the role of these Kv3 channels.

The impact of FS cells on cortical circuits depends not only on the anatomical localization but also on the functional properties of these synapses, including the efficacy and dynamics of neurotransmitter release. The prominent expression of Kv3 proteins at FS cell terminals suggests that Kv3 channels could have important roles in regulating these functional properties, a possibility that we investigated in the present study. Our results suggest that Kv3 channels are major contributors to repolarization of the AP at the FS cell terminal, governing spike duration, Ca^{2+} entry, and neurotransmitter release, thereby influencing the efficacy and short-term dynamics of the FS cell synapse.

Materials and Methods

Recording of extracellularly evoked IPSCs. Acute brain slices (300 μ m) were prepared from 16- to 24-d-old mice essentially as described previ-

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ously (Lau et al., 2000). Synaptic currents were recorded via whole-cell voltage clamp of layer II/III pyramidal cells of mouse primary somatosensory ("barrel") cortex. A concentric bipolar-stimulating electrode (125 μm in diameter; FHC, Bowdoinham, ME) was placed either in the layer IV barrel hollow of the same column as the recorded cell or lateral ($\sim 100 \mu\text{m}$) to the recording pipette within layer II/III. IPSCs were evoked by 0.2 ms pulses, typically 20–100 μA , to keep IPSC magnitude low to reduce voltage-clamp errors. Recordings were performed with patch pipettes (2–2.5 M Ω) filled with intracellular solution that, in most experiments, contained the following (in mM): 130 CsMeSO₄, 8.0 NaCl, 4.0 Mg²⁺-ATP, 0.3 Na⁺-GTP, 10 HEPES, 0.5 EGTA, and 6.0 lidocaine N-ethyl bromide (QX-314), with pH adjusted to 7.40 with CsOH. Recordings were performed at 28–32°C. Reported values for membrane potential were corrected for the error attributed to liquid junction potential (–15 mV). To minimize voltage-clamp errors, we used low-resistance pipettes and series resistance compensation, and we recorded at potentials close to the chloride equilibrium potential (E_{Cl}) to reduce extracellularly evoked IPSC (eIPSC) amplitudes. A cell was rejected if series resistance (R_s) after break-in was $>18 \text{ M}\Omega$ (typically, 10–12 M Ω), if R_s could not be compensated to $<10 \text{ M}\Omega$ (typically, 4–8 M Ω), or if R_s changed by $>20\%$ during the experiment.

Currents were recorded with an Axopatch 200B amplifier (Molecular Devices, Union City, CA), low-pass filtered at 5 kHz, digitized at 16 bit resolution (Digidata 1322A; Molecular Devices), and sampled at 20 kHz. pClamp9 software (Molecular Devices) was used for data acquisition, and analysis was performed with Origin (Microcal, Northampton, MA) and the Clampfit module of pClamp.

Paired recordings. Simultaneous whole-cell recordings were performed from FS cell→pyramidal cell (FS→PC) pairs in layer II/III primary somatosensory cortex by using patch electrodes (5–7 M Ω) filled with a solution containing the following (in mM): 65 KCl, 65 K-gluconate, 10 HEPES, 0.5 EGTA, 4.0 Mg-ATP, and 0.3 Na-GTP plus 0.5% biocytin, pH 7.40, with KOH. PV-GFP (parvalbumin–green fluorescent protein) mice (Di Cristo et al., 2004) were used to aid in the identification of FS cells. Both cells were recorded under current-clamp conditions with the use of two Axoclamp 2B amplifiers (Molecular Devices). Voltage output was filtered at 10 kHz, and data were acquired at a sampling frequency of 20 kHz.

Two-photon imaging. Experiments were performed by using a 60 \times , 0.9 numerical aperture water immersion objective (IR1, Olympus, Tokyo, Japan) on an Olympus BX-62 upright confocal laser-scanning microscope modified for two-photon imaging. The pipette solution contained the Ca²⁺ indicator Fluo-4 (50–200 μM) and 25–50 μM Alexa-594 biocytin (Molecular Probes, Eugene, OR). Two-photon excitation was elicited with a dye-pumped Ti-Sapphire Mai-Tai laser (Spectra-Physics, Mountain View, CA) generating <100 fs pulses at 80 MHz. Both dyes were excited at 810 nm. Presumed terminals were imaged with a 5–10 \times digital zoom, and AP-evoked fluorescence changes were detected in line scan mode, with fluorescence at each point acquired every 2.14 ms. Imaging data were analyzed by using Fluoview, ImageJ, and Igor software (Wave-Metrics, Lake Oswego, OR).

Statistical analysis. Unless indicated otherwise, data are expressed as the mean \pm SD, with p values derived from paired t tests; * $p < 0.05$ and ** $p < 0.01$ were used to indicate statistical significance. For pooled IPSC amplitudes, which were not distributed normally, the Mann–Whitney test was used.

Results

Low concentrations of tetraethylammonium enhance inhibitory synaptic transmission in the neocortex via presynaptic mechanisms

Given that Kv3 channels are important determinants of AP repolarization at FS cell somata and are expressed at high density at FS cell synapses, we predicted that blockade of these channels would broaden the AP at the FS cell terminal. However, because of the inaccessibility of the FS cell terminal to direct electrophysiological recording with the use of currently available techniques, we made inferences as to the role of Kv3 channels in spike repolar-

ization at these terminals, based on recordings of postsynaptic events.

We recorded eIPSCs in layer II/III pyramidal neurons in the presence of 10 mM CNQX and 50 mM D-APV. In most cases, a CsMeSO₄-based internal solution was used. The predicted E_{Cl} under these conditions was approximately –70 mV, and eIPSCs were recorded as outward currents during depolarizations to membrane potentials between –45 and –15 mV (Fig. 1A). eIPSCs reversed near E_{Cl} (data not shown) and were blocked by GABA_A receptor antagonists (Fig. 1C).

Kv3 channels are highly tetraethylammonium-sensitive (TEA-sensitive; IC₅₀ of 150–300 μM), with near-complete block at 1.0 mM (Coetzee et al., 1999). Bath application of 1.0 mM TEA increased the eIPSC amplitude nearly twofold ($73.7 \pm 26.9\%$ increase over control; $n = 14$; $p < 0.01$) (Fig. 1A,C) without changing the IPSC kinetics (Fig. 1B). In addition, TEA application did not affect pyramidal cell input resistance (Fig. 1D₁). Furthermore, the application of 1.0 mM TEA did not affect currents evoked by a brief local application of GABA (Fig. 1D₂). Together, these results suggest that the TEA-induced enhancement of the eIPSC is attributable to a presynaptic mechanism (i.e., an increase in evoked GABA release). Also consistent with this interpretation, TEA decreased the paired pulse ratio (PPR) from 0.86 ± 0.09 to 0.66 ± 0.08 ($n = 7$; $p < 0.005$) (Fig. 1E–G) and decreased the coefficient of variation of eIPSCs (data not shown). Results obtained with a CsCl-based internal solution (Choi et al., 2002) were not statistically different from data obtained with the CsMeSO₄-based internal solution (data not shown).

The effect of TEA on inhibitory synaptic transmission is likely attributable to blockade of presynaptic Kv3 channels

Whereas 1.0 mM TEA produces near-complete block of Kv3 channels, low concentrations of TEA also block other K⁺ channels with similar affinity. In heterologous expression systems, 1.0 mM TEA blocks homomeric channels composed of Kv1.1 and, to a lesser extent, Kv1.6 subunits (IC₅₀ values of ~ 0.5 and 1.7–7.0 mM, respectively) as well as Slo1 large-conductance (BK, or maxi-K) Ca²⁺-activated K⁺ channels (IC₅₀ of 80–330 μM) (Coetzee et al., 1999). However, although there is no blocker specific for Kv3 channels, toxins exist that are specific for the non-Kv3 channels also blocked by low TEA concentrations. For example, 100 nM dendrotoxin-I (DTx), which blocks K⁺ channels containing one or more Kv1.1, Kv1.2, or Kv1.6 subunits (IC₅₀ values of 12–21, 2.8–24, and 0.3–1.5 nM, respectively, for heterologously expressed homomultimers) (Coetzee et al., 1999), had no effect on the amplitude of eIPSCs (IPSC amplitude was $96.1 \pm 8.7\%$ of control; $n = 4$; $p = 0.60$) (Fig. 1C). This indicates that the large effect of TEA on the eIPSC is not produced by blockade of Kv1 channels and suggests that DTx-sensitive Kv1 channels do not contribute significantly to repolarization of the AP at interneuron synapses, similar to the situation at FS cell somata (Erisir et al., 1999).

Bath application of 100 nM iberiotoxin (IbTx) or charybdotoxin (ChTx), both of which block BK channels with high affinity (IC₅₀ values of 6–11 and 2–40 nM for heterologously expressed Slo1, respectively), produced a small but statistically significant enhancement of the eIPSC ($18.6 \pm 8.6\%$ increase over control; $p < 0.05$; $n = 6$) (Fig. 1C), with no effect on the shape of the eIPSC. However, blockade of IbTx/ChTx-sensitive BK channels cannot account for most of the effect of TEA, because TEA still had a large effect when it was applied after a previous application of IbTx or ChTx (58.5% increase) (Fig. 1C).

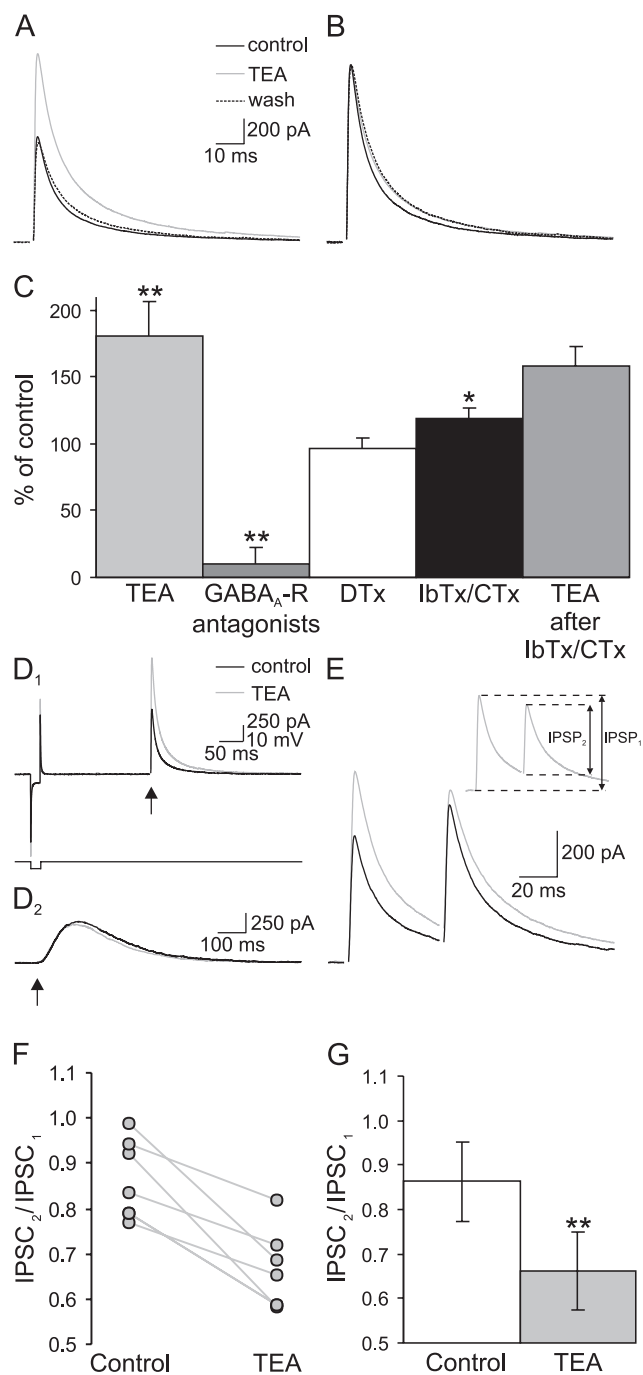


Figure 1. TEA increases the amplitude of eIPSCs recorded in neocortical pyramidal cells by blocking presynaptic Kv3 channels. **A**, Bath application of 1.0 mM TEA produced a reversible 78.7% increase in the amplitude of the eIPSC (control, solid black line; TEA, solid gray line; washout, dashed black line). Traces are averages of 10 individual sweeps. **B**, Same as **A**, with traces scaled to the peak value of the eIPSC obtained after TEA application to illustrate that the eIPSC decay kinetics did not change. **C**, Summary data; * $p < 0.05$ and ** $p < 0.01$. **D**, TEA had no effect on pyramidal cell input resistance. **D**₁, In this example, brief hyperpolarizing 10 mV steps to -40 mV from the holding potential of -30 mV elicited current transients that were identical before (black) and after (gray) the application of 1.0 mM TEA, whereas the current response to extracellular stimulation (indicated by the vertical arrow) was enhanced. **D**₂, TEA had no effect on the current response to the local application of GABA (500 μ M; dissolved in ACSF) via pressure ejection (5–20 ms; 2–10 psi) (before, 529.0 ± 90.2 pA; after, 560.1 ± 83.0). **E**, Consistent with a presynaptic mechanism, TEA produced a large decrease in the PPR (ratio of eIPSC₂ to eIPSC₁, shown in inset). Currents elicited in response to a pair of identical stimuli delivered at an interstimulus interval of 50 ms (20 Hz) before (black) and after (gray) the application of 1.0 mM TEA are shown. **F**, TEA produced a decrease in the PPR in all experiments ($n = 7$). **G**, Summary of PPR data; ** $p < 0.01$.

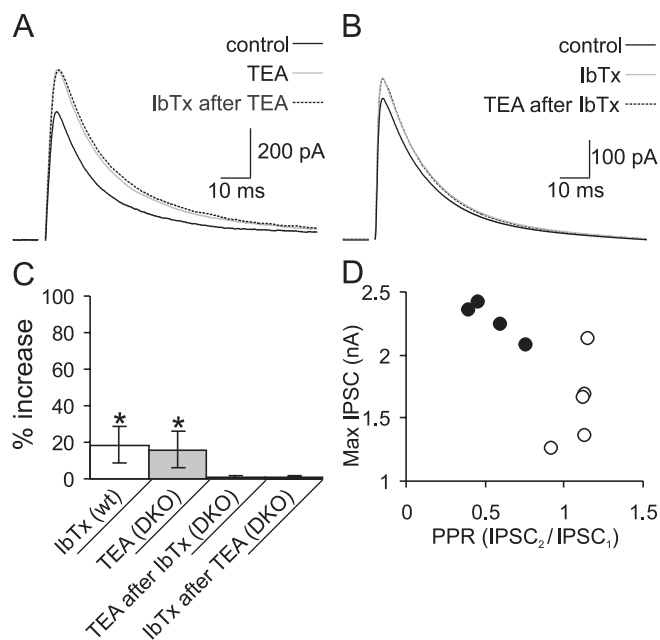


Figure 2. The large effect of TEA on the eIPSC is absent in DKO mice. **A**, Representative example illustrating the eIPSC recorded in a pyramidal cell from a slice prepared from a DKO mouse under control conditions (solid black line), after the application of 1.0 mM TEA (solid gray line), and with the subsequent application of IbTx in the presence of TEA (dashed black line). **B**, An example showing control (solid black line), 100 nM IbTx (solid gray line), and the subsequent application of TEA in the presence of IbTx (dashed black line; note that this trace overlaps with the gray trace). **C**, Summary data; * $p < 0.05$. In the DKO mice, IbTx and TEA reciprocally occlude one another. **D**, The maximal eIPSC was 52.4% larger ($n = 5$ for each genotype; $p < 0.005$) and the PPR was 48.9% smaller ($p < 0.05$) in slices prepared from DKO mice compared with wild type.

The sum of the evidence suggests that TEA enhances GABA release from neocortical interneurons (most likely FS cells) predominantly by blocking presynaptic Kv3 channels, indicating that these channels are key contributors to the repolarization of the spike at the FS cell synapse. This result is consistent with immunohistochemical data showing a high density of Kv3.1 and Kv3.2 proteins at neocortical FS cell basket synapses (Sekirnjak et al., 1997; Chow et al., 1999).

The large TEA-induced enhancement of evoked GABA release is absent in Kv3.1/Kv3.2 double knock-out mice

To obtain more direct evidence that the effect of TEA on spike-evoked GABA release is mainly attributable to blockade of presynaptic channels containing Kv3.1 and Kv3.2 proteins, we performed experiments using Kv3.1/Kv3.2 double knock-out (DKO) mice (Ozaita et al., 2004). In slices prepared from DKO mice, the large TEA-induced enhancement of eIPSCs seen in wild-type animals was absent, and TEA produced only a small increase in the eIPSC (Fig. 2A, C). However, the effect of TEA on the amplitude of the eIPSC in DKO mice ($15.7 \pm 9.8\%$ increase over control; $n = 9$; $p < 0.05$) was similar in magnitude to the effect of IbTx/ChTx in both wild-type mice (Fig. 1C) and DKO mice (Fig. 2C), suggesting that the small effect of TEA in DKO mice is attributable to a blockade of BK channels. Consistent with this interpretation, TEA occluded the effect of IbTx in DKO mice (99.1% of control; $n = 2$) (Fig. 2A, C), and IbTx occluded the effect of TEA ($0.7 \pm 1.0\%$ increase over TEA alone; $n = 4$) (Fig. 2B, C). These results demonstrate that the large effect of TEA on the eIPSC in wild-type mice is attributable to a blockade of channels containing Kv3.1 and Kv3.2 subunits in interneuron terminals, confirming the conclusions reached via pharmacological

analysis. Furthermore, we also found that the maximal eIPSC (Choi et al., 2002) was larger and the PPR smaller in DKO mice compared with wild type (Fig. 2D).

Low concentrations of TEA enhance the amplitude of unitary IPSPs in FS→PC pairs

The majority of the extracellularly eIPSC likely arises from FS cells, because FS cells constitute 40–50% of all neocortical GABAergic interneurons and form numerous proximal synapses that exhibit high release probability (Kawaguchi and Kubota, 1997). However, many non-FS interneurons also are stimulated under these recording conditions. Hence we performed dual whole-cell current-clamp recordings from identified FS→PC pairs (Fig. 3). TEA (1.0 mM) increased the amplitude of the unitary IPSPs (uIPSPs) recorded in pyramidal cells in response to AP generation in a synaptically connected FS cell ($61.6 \pm 25.6\%$ of control; $n = 6$; $p < 0.05$), an effect similar in magnitude to (and statistically similar to) that observed in extracellular stimulation experiments ($p = 0.90$ by two-tailed unpaired t test) (Fig. 3B–D). The increase in uIPSP amplitude also was accompanied by a decrease in the PPR ($36.8 \pm 11.8\%$ decrease compared with control, from 0.75 ± 0.18 to 0.58 ± 0.10 ; $n = 5$; $p < 0.05$) (Fig. 3C,D).

In addition, 100 nM IbTx produced a small but statistically significant enhancement of the uIPSP amplitude ($11.0 \pm 9.5\%$ increase over control; $n = 4$; $p < 0.05$) that was similar in magnitude to (and not statistically different from) the IbTx/ChTx-induced increase in the extracellular eIPSC ($p = 0.25$ by two-tailed unpaired t test) (Fig. 3D), confirming the presence of BK channels at FS cell terminals.

TEA enhances AP-evoked Ca^{2+} transients imaged in FS cell terminals

The results are consistent with a model in which Kv3 channel blockade reduces the rate of spike repolarization at the FS terminal, leading to spike broadening, enhanced Ca^{2+} influx, and hence to increased neurotransmitter release. Because whole-cell recording from the FS cell terminal is probably physically impossible, the AP itself or AP-evoked Ca^{2+} currents cannot be recorded directly. Thus we used two-photon laser-scanning microscopy combined with whole-cell recording and Ca^{2+} imaging to visualize AP-evoked Ca^{2+} transients in FS cell terminals (Fig. 4). Consistent with the proposed mechanism for TEA-induced enhancement of GABA release from FS cells, the application of 1.0 mM TEA enhanced the AP-evoked Ca^{2+} transient in FS cell terminals ($213.2 \pm 24\%$ of control; $p < 0.02$; range, 136–297%; $n = 6$ terminals from three FS cells) (Fig. 4D–F). After recovery of the somatic AP waveform after washout of the TEA, the Ca^{2+} transients also recovered to baseline levels ($105.6 \pm 10.9\%$ of control; $p > 0.05$).

Low TEA concentrations broaden the AP at the FS cell soma (Erisir et al., 1999; Lau et al., 2000) (Fig. 4D). It is extremely unlikely that somatic AP broadening alone will increase Ca^{2+} influx at the FS cell terminal; purely passive conduction of somatic depolarizations to the terminal will be very inefficient because of, among other factors, the low-pass cable-filtering properties along with the extent ($>100 \mu\text{m}$) and highly branched nature (Fig. 4A) of the axon. Furthermore, during active propagation, the AP will be shaped by the conductances present along the axon. Nevertheless, to confirm our expectations, we demonstrate (supplemental Fig. S1, available at www.jneurosci.org as supplemental material) that TEA applied near the recorded cell and its surrounding perisomatic inhibitory synapses produced an enhancement of IPSC amplitude similar in magnitude to that

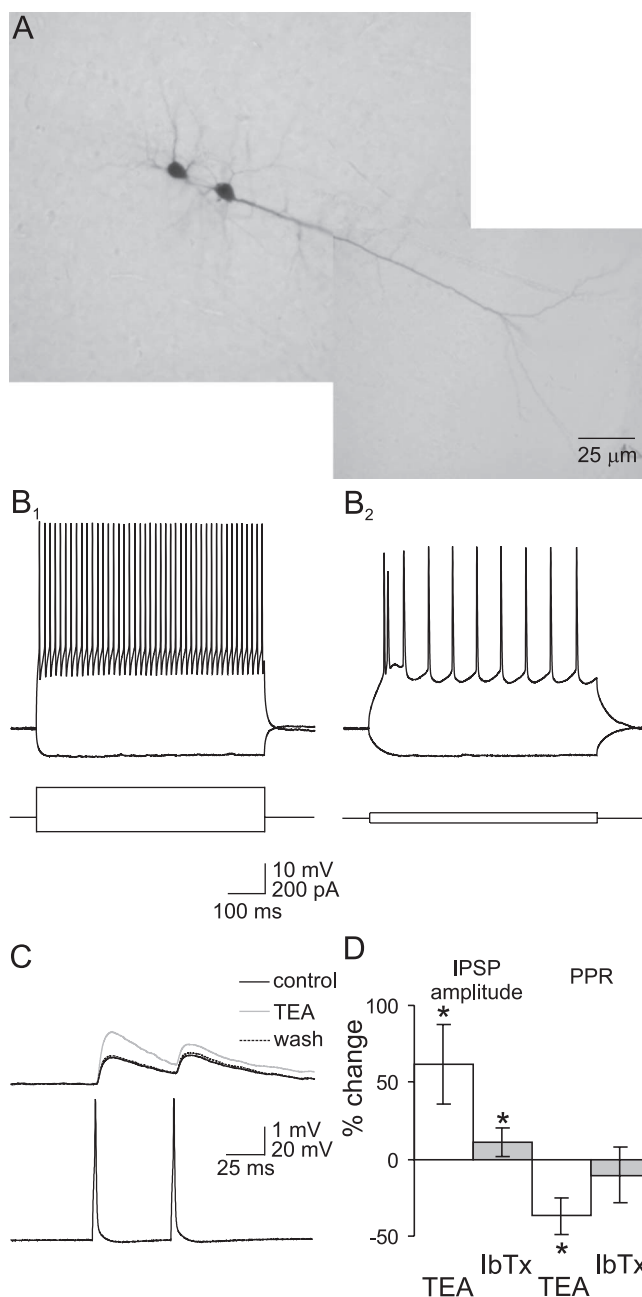


Figure 3. TEA enhances the amplitude of uIPSPs in dual whole-cell recordings of FS cell→pyramidal cell pairs in layer II/III of the primary somatosensory cortex. **A**, Immunoperoxidase labeling of the FS cell→pyramidal cell pair studied in **B–D**. **B**, Fast-spiking and regular-spiking firing patterns of the presynaptic FS cell (**B**₁) and the postsynaptic pyramidal cell (**B**₂), respectively, in response to depolarizing current injection. **C**, An example of an FS cell→pyramidal cell connection. The presynaptic FS cell was stimulated with pairs of brief, identical depolarizing pulses (1.0 nA, 1.5 ms) with a 50 ms interspike interval (20 Hz) and with 10 s intervals between each paired pulse. The IPSPs are averages of 25 sweeps. The wash (dashed black line) overlaps with the control trace (solid black line). **D**, Summary data; $*p < 0.05$.

seen with the bath application of TEA (supplemental Fig. S1C–E, available at www.jneurosci.org as supplemental material), whereas TEA applied close to the stimulating electrode had no effect (supplemental Fig. S1A, B, E, available at www.jneurosci.org as supplemental material). Furthermore, in FS→PC pairs, spike-like depolarizations (1.5–2.0 nA) of varying duration (from 2 ms to 1 s) delivered to presynaptic FS cells in the presence of TTX failed to evoke IPSPs in connected PCs (supplemental Fig. S2, available at www.jneurosci.org as supplemental material).

Discussion

Understanding the mechanisms of GABA release in the cortex critically depends on knowledge of the ion channels that determine the electrophysiological properties of FS cell synapses. Our results show that voltage-gated K^+ channels containing Kv3.1 and Kv3.2 subunits are major contributors to AP repolarization at the FS cell synapse, thereby governing spike-evoked Ca^{2+} entry and neurotransmitter release. BK-type Ca^{2+} -activated K^+ channels are also present at this synapse but make a smaller contribution to spike repolarization. In contrast, blockade of Kv1 channels (known to be present at FS cell terminals by immunohistochemistry) (E. M. Goldberg, S. Chang, and B. Rudy, unpublished observations) had no effect on the amplitude of eIPSCs, suggesting that these channels do not contribute to spike repolarization.

These results support the idea that the role of a particular K^+ channel in the regulation of neurotransmitter release cannot be predicted easily from its mere presence at a particular synapse. First of all, blocking a given K^+ channel may or may not broaden the spike, depending on the channels that shape the AP waveform of that particular terminal. For example, blockade of Kv1 or Kv3 subunit-containing K^+ channels with DTx or low concentrations of TEA, respectively, has no effect on spike-evoked Ca^{2+} transients in cerebellar basket cell terminals (Tan and Llano, 1999), despite the fact that Kv1 and Kv3 currents have been recorded directly from this synapse (Southan and Robertson, 2000).

Furthermore, the effects of AP broadening on presynaptic Ca^{2+} influx and hence transmitter release are difficult to predict and can depend on several factors, such as the density and gating kinetics of the voltage-gated Ca^{2+} channels present at that particular synapse (Llinàs et al., 1981; Spencer et al., 1989; Bischofberger et al., 2002). Our data indicate that at the FS cell synapse AP-evoked Ca^{2+} influx is submaximal and can be increased by spike broadening.

At the calyx of Held, the blockade of K^+ channels (likely composed of Kv3 subunits) broadens the presynaptic AP and augments neurotransmitter release (Ishikawa et al., 2003), similar to our results at the FS cell synapse. However, although IbTx-sensitive currents can be recorded at the calyx, BK channel blockade has no effect on presynaptic Ca^{2+} influx (Ishikawa et al., 2003). This is in contrast to our findings at the FS cell synapse and observations at the frog neuromuscular junction (Robitaille et al., 1993). However, at the hippocampal Schaffer collateral synapse, the block of BK channels enhances synaptic transmission only after previous AP broadening with 4-aminopyridine (Hu et al., 2001). It is possible that the APs at the calyx of Held and at CA3→CA1 synapses are too brief to allow for significant opening of the particular BK channel molecular complexes present at these synapses. Alternatively, the geometry of the presynaptic terminal (i.e., the relationship between voltage-gated Ca^{2+} chan-

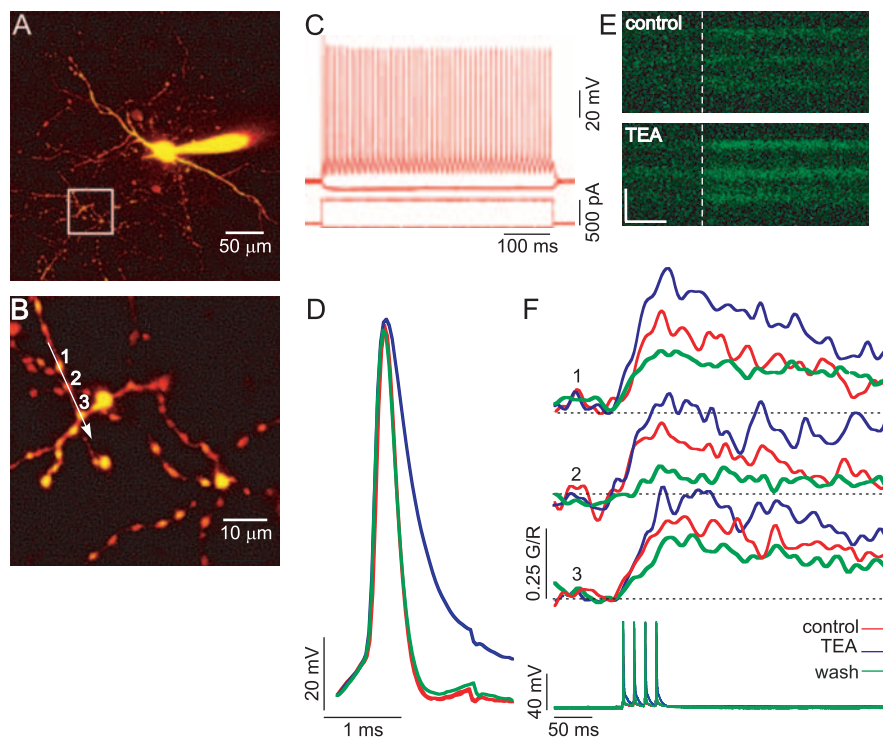


Figure 4. TEA enhances AP-evoked Ca^{2+} transients imaged in identified FS cell synaptic terminals. **A**, Recorded FS neuron imaged by two-photon excitation of Alexa 594. A pseudocolored projection of 117 images having a z separation of $0.46 \mu\text{m}$ is shown. **B**, Terminal boutons from the boxed region in **A** (projection of 136 images; z separation, $0.46 \mu\text{m}$). Subsequent high-speed line scans along the arrow were obtained from the indicated boutons (1–3) in a single z plane. **C**, Firing pattern of the recorded FS cell. **D**, As expected, 1.0 mM TEA produced a large and reversible broadening of the somatic AP. **E**, Raw line scan data of Fluo-4 emission obtained before and after four somatic APs (15 ms interstimulus interval) from boutons 1–3 (bottom to top) before (control) and 10 min after (TEA) the bath application of 1.0 mM TEA. The dashed line indicates stimulus onset. Calibration: $5 \mu\text{m}$ (vertical), 100 ms (horizontal). **F**, Ca^{2+} transients obtained from the ratio of Ca^{2+} -dependent (G; Fluo-4) and Ca^{2+} -independent (R; Alexa 594) emissions. TEA reversibly increased the spike-evoked Ca^{2+} signals in these terminals.

nels and BK channels) or differences in Ca^{2+} buffering capacity also could govern the degree of BK channel activation and hence the contribution of these channels to AP repolarization at a given terminal.

FS cells are known to fire at high frequencies both *in vitro* and *in vivo* (McCormick et al., 1985; Swadlow, 2003). Hence the dynamic properties of the FS cell synapse must be adapted to high-frequency activity. The finding that blocking Kv3 channels produced a decrease in the paired pulse ratio of FS cell→pyramidal cell inhibitory connections suggests a role for Kv3 channels in the dynamics of neurotransmitter release. We propose that, by keeping the AP at the FS cell terminal brief, Kv3 channels limit Ca^{2+} influx and hence release probability and thereby control synaptic depression during high-frequency repetitive activity. Neurotransmitter release from FS cells appears to depress less than intracortical excitatory neurotransmission during sustained epochs of activity (Galarreta and Hestrin, 1998), a difference that could be attributable to the presence of Kv3 channels at the FS cell synapse. This could explain the augmented seizure susceptibility exhibited by Kv3 knock-out mice (Lau et al., 2000; Noebels, 2003), which at first appears at odds with the increase in eIPSC amplitude observed after channel blockade. However, we hypothesize that the enhanced short-term synaptic depression that occurs with genetic elimination of Kv3 channels renders it such that the steady-state level of cortical inhibition is impaired in these mice.

There previously has been an apparent incompatibility be-

tween two well known features of FS cells, namely, high-frequency firing and short-term synaptic depression. What is the purpose of high-frequency firing if neurotransmitter release depresses with repeated activity? The present study suggests that the very same proteins that generate high-frequency firing (i.e., K⁺ channel subunits of the Kv3 subfamily) also act to limit synaptic depression and thus maintain the efficacy of neurotransmitter release at the FS cell synapse during repetitive activity.

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