Inhibition of Ca\(^{2+}\)-activated large-conductance K\(^+\) channel activity alters synaptic AMPA receptor phenotype in mouse cerebellar stellate cells.

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ABSTRACT

Many fast spiking inhibitory interneurons, including cerebellar stellate cells, fire brief action potentials, and express AMPA type glutamate receptors (AMPAR) that are permeable to Ca\(^{2+}\) and do not contain the GluR2 subunit. In a recent study we found that increasing action potential duration promotes GluR2 gene transcription in stellate cells. We have now tested the prediction that activation of potassium channels that control the duration of action potentials can suppress the expression of GluR2-containing AMPARs at stellate cell synapses. We find that large-conductance Ca\(^{2+}\)-activated potassium channels (BK) mediate a large proportion of the depolarization evoked non-inactivating potassium current in stellate cells. Pharmacological blockade of BK channels prolonged the action potential duration in postsynaptic stellate cells and altered synaptic AMPAR subtype from GluR2-lacking to GluR2-containing Ca\(^{2+}\)-impermeable AMPARs. An L-type channel blocker abolished an increase in Ca\(^{2+}\) entry that was associated with spike broadening and also prevented the BK channel blocker-induced switch in AMPAR phenotype. Thus blocking BK potassium channels prolongs the action potential duration and increases the expression of GluR2-containing receptors at the synapse by enhancing Ca\(^{2+}\) entry in cerebellar stellate cells.
AMPA receptors mediate the vast majority of excitatory synaptic transmission in the CNS. Of the four AMPAR subunits, GluR2 subunits are critical in determining a number of biophysical properties of AMPARs. Incorporation of GluR2 subunits reduces the Ca\(^{2+}\) permeability and channel conductance of AMPARs and prolongs the decay time of synaptic currents (Cull-Candy et al., 2006; Liu and Zukin, 2007). The abundance of GluR2 mRNA varies considerably between cell types. While pyramidal cells in the hippocampus and cortex, and Purkinje cells in the cerebellum have a high GluR2 mRNA content, many GABAergic interneurons express low levels of GluR2 mRNA and have synaptic AMPARs that lack GluR2 subunits. For example, a rapid synaptic current through GluR2-lacking AMPARs in hippocampal GABAergic interneurons is functionally important for the long range synchrony of gamma oscillation (Fuchs et al., 2001). We have recently shown that a prolonged decay time of synaptic currents due to enhanced GluR2 gene expression in cerebellar GABAergic interneurons markedly increases the ability of synaptic potentials to evoke an action potential (Savtchouk and Liu, 2011). Thus controlling GluR2 gene expression in GABAergic interneurons can have a profound impact on the neuronal network activity.

In a recent study we have shown that noradrenaline increases GluR2 gene transcription and promotes the incorporation of GluR2-containing receptors at synapses in cerebellar stellate cells (Liu et al., 2010). This leads to a switch in synaptic AMPAR phenotype from GluR2 lacking, Ca\(^{2+}\)-permeable to GluR2-containing receptors. Noradrenaline also prolongs the action potential (AP) duration in cerebellar stellate cells by enhancing an h current (Saitow and Konishi, 2000; Liu et al., 2010). Since a subset of K\(^+\) channels are known to reduce the duration of APs, this raises the possibility that K\(^+\) currents via these channels may also modulate the expression of postsynaptic AMPARs by controlling AP repolarization.

Several types of GABAergic interneurons are known to express high levels of potassium channels, including Kv3 and BK channels (Perney et al., 1992; Rudy and McBain, 2001; Sugino et al., 2006; Bartos et al., 2007). These channels display a characteristic high activation threshold and generate non-inactivating currents, which accelerate the repolarization of APs. These properties reduce the AP duration and allow neurons to fire APs at high frequency (Wang et al., 1998; Salkoff et al, 2006). One intriguing possibility is that the brief duration of such APs suppresses the expression of GluR2 subunits in these neurons. Here we have tested this hypothesis using cerebellar stellate cells. These inhibitory interneurons express GluR2-lacking AMPARs at the parallel fiber synapse and fire spontaneous APs that are of a brief duration.

Using pharmacological inhibitors we find that BK channels mediate a large fraction of the non-inactivating potassium current in stellate cells. Application of BK channel blockers prolongs the AP duration and promotes the incorporation of GluR2-containing AMPARs at stellate cell synapses, changing the synaptic AMPARs from GluR2-lacking, Ca\(^{2+}\)-permeable to GluR2-containing, Ca\(^{2+}\)-impermeable
receptors. This change is triggered by an enhanced Ca$^{2+}$ influx through L-type voltage-gated Ca$^{2+}$ channels during an AP. Our results suggest that reducing AP duration in cerebellar GABAergic interneurons is a novel mechanism that suppresses synaptic GluR2-containing receptor expression.
Cerebellar slices (250 µm) were obtained from 18- to 22-day-old C57BL/6J mice as described (Liu et al., 2010). Slices were maintained in ACSF (in mM: 125 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 26 NaHCO₃, 1.25 NaH₂PO₄, and 25 glucose, saturated with 95% O₂-5% CO₂, pH 7.3) for 60 minutes prior to recordings.

**Electrophysiology:** Whole cell voltage and current clamp recordings were made with an Axopatch 200B and Multiclamp 700B amplifier (Axon instruments, Foster city, CA) in ACSF bubbled with 95% O₂ and 5% CO₂. Stellate cells were identified by their location in the outer two-thirds of the molecular layer in a cerebellar slice and by their ability to fire spontaneous action potentials in the cell attached configuration. Synaptic currents, Ca²⁺ currents; K⁺ currents and action potentials were filtered at 2-5 kHz and digitized at 20 kHz. The pipette resistance was 5-7 MΩ. All recordings were performed at room temperature.

K⁺ currents were recorded using a pipette solution containing (in mM) 135 KCl, 4.6 MgCl₂, 0.1 CaCl₂, 10 HEPES, 1 EGTA, 4 ATP-Na, 0.4 GTP-Na, pH 7.3 (75 - 95% compensation; series resistance =20.3 ± 1.5 MΩ, n = 11). Amphotericin B (0.6 mg/ml) was added to the potassium-based pipette solution in perforated patch recordings (typical series resistance = 25 ± 3 MΩ, n = 4). The bath solution contained 300 nM tetrodotoxin (TTX), 20 µM ZD7288, 1 mM kynurenic acid (KYNA) and 100 µM picrotoxin (PTX) to block Na⁺ channels, h currents, ionotropic glutamate receptors and inhibitory transmission, respectively. Spontaneous action potentials (APs) were recorded using a whole cell patch configuration in ACSF that contained 1 mM KYNA, 100 µM PTX. The pipette solution contained (in mM) 115 KMeSO₃, 2 MgCl₂, 0.16 CaCl₂, 0.5 EGTA, 10 HEPES, 4 ATP-Na, 0.4 GTP-Na, 14 Tris₂-creatine phosphate, 0.6 mg/ml amphotericin B for perforated patch recordings), pH 7.3. The frequency of spontaneous APs were recorded extracellularly in the presence of 100 µM PTX and 1 mM KYNA, using a cell-attached configuration with a glass electrode filled with ACSF.

Ca²⁺ currents were measured using a voltage clamp protocol that mimicked the AP waveform. The waveforms of a control AP (control-AP) and an AP in the presence of 1 mM TEA (TEA-AP) were recorded in current clamp from a stellate cell, and had an AP half-width of 1.5 ms and 2.3 ms and an after-hyperpolarization of −30 mV and −9 mV, respectively. They were therefore used as voltage commands. The pipette solution contained (in mM): 119 CsCl, 9 EGTA, 10 HEPES, 1.8 MgCl₂, 14 Tris₂-creatine phosphate, 4 ATP-Mg, 0.4 GTP-Na, 10 TEA, 1 QX314, pH 7.3. The external solution included 10 mM TEA, 300 nM TTX, 10 µM ZD7288, 1 mM KYNA, 100 µM PTX to block potassium, sodium and h-currents and synaptic currents, respectively. Cd²⁺ (100 µM) was used to block Ca²⁺ channels. The Ca²⁺ current was monitored as the difference current (I - I_Cd).

Adequacy of the space clamp during action potential waveforms is an issue. However, stellate cells are electrically compact with an average cell capacitance of 6.0 ± 0.3 pF (n =16) and input resistance of 2.0 ±
0.5 GΩ. Mean series resistance was 24.6 ± 1.1 MΩ. Action potential waveforms evoked small Ca²⁺ currents (108 ± 15 pA, n = 16) and the expected voltage error is less than 2.5 mV. As an experimental test, we determined the time delay between the peak of the action potential waveform and the notch in the rising phase of the Ca²⁺ current (that correlates with the peak of membrane depolarization), and found a short delay with a latency of 0.20 ± 0.04 ms (n = 8), which appears similar to other studies (Yang and Wang, 2006). Also, if the stellate cells were not adequately clamped during APs due to a voltage error, then reducing Ca²⁺ current would be expected to result in more rapid decay kinetics of AP-evoked calcium currents. Although the amplitude of Ca²⁺ currents decreased by half as the extracellular Ca²⁺ concentration decreased from 2 mM to 1 mM, we observed no significant difference in the decay kinetics of the AP-evoked calcium currents (1.13 ± 0.09 ms in 2 mM Ca²⁺ and 1.03 ± 0.09 ms in 1 mM Ca²⁺).

These results indicate that stellate cells were adequately voltage-clamped in these experiments.

Cerebellar slices were incubated with 100 nM iberiotoxin or 1 mM TEA for 3 hours, in the presence of 1 mM (or 5 mM) KYNA, 100 µM PTX at room temperature. As a control, cerebellar slices were incubated in ACSF that contained 1 mM KYNA, 100 µM PTX (“control solution”). In one experiment slices were treated with 100 nM iberiotoxin (+KYNA+PTX) for 1 hour followed by 2 h in control solution. KYNA and TEA were washed out 15 min before recordings. Spontaneous excitatory postsynaptic currents (sEPSCs) were recorded from stellate cells using a Cs-based pipette solution (in mM: 135 CsCl, 10 HEPES, 10 EGTA, 2 NaCl, 4 ATP-Mg, 5 TEA, 1 QX314, 0.1 spermine, pH 7.3) in ACSF containing 100 µM PTX. Synaptic events that did not have a smooth rise and decay phase were rejected. Average sEPSCs at each holding potential (typically average of 50-100 events over 10-15 min) were measured using N version 4.0 (written by Steve Traynelis, Emory University) as described previously (Liu and Cull-Candy, 2000). The rectification index of sEPSC I-V relationship was defined as the ratio of the current amplitude at +40 mV to the predicted linear value at +40 mV (extrapolated from linear fitting of the currents at negative potentials). The decay time constant of individual synaptic events was determined by fitting the decay phase of an EPSC with a single exponential function, since the weighted decay time constants calculated from a double exponential decay fit are similar to those obtained with a single exponential fit (Savtchouk and Liu, 2011).

All values are expressed as mean ± SEM. Statistical significance was assessed by a two-tailed Student’s t-test. TTX, iberiotoxin and ZD7288 were obtained from Tocris Bioscience. Water soluble amphotericin B was obtained from Sigma.
RESULTS

Cerebellar stellate cells express large-conductance Ca\(^{2+}\)-activated potassium channels.

Cerebellar stellate cells fire brief APs (Liu et al., 2010). To identify the K\(^{+}\) channels that control the AP duration in stellate cells, cells were voltage-clamped at -100 mV and stepped from -90 mV to +60 mV in 10 mV increments. Using whole cell recordings we found that depolarization beyond -40 mV elicited a transient outward current followed by a sustained steady state current (Fig 1A). The conductance of this current reached its maximum at -10 mV and declined as the membrane potential further depolarized (Fig 1B). To test whether cell dialysis altered the amplitude of total potassium currents, we performed perforated patch recordings. The potassium current waveform and I-V relationship of the transient current and the non-inactivating current were indistinguishable from those obtained using whole cell recordings (Fig 1A). Therefore further characterization of potassium currents were conducted using whole cell patch recordings.

Several types of potassium channels, including Kv3.1 and large conductance Ca\(^{2+}\) activated K\(^{+}\) (BK) channels, are known to conduct a high threshold non-inactivating K\(^{+}\) current with rapid activation kinetics, which are ideally suited for generating short duration APs. We therefore tested whether these channels contribute to the net K\(^{+}\) current in stellate cells. A low concentration of TEA is known to preferentially block a subset of K\(^{+}\) channels, including Kv3 and BK channels (Wang et al., 1998; Rudy and McBain, 2001). We found that bath application of 1 mM TEA reduced the outward transient current at 0 mV by 53.2 ± 1.8% and the non-inactivating current by 42.8 ± 6.2% (n = 4; P < 0.05; Fig 1B). To distinguish between Kv3 and BK channels, we used a pharmacological approach. Kv3 channels, but not BK channels, are sensitive to 1 mM 4-AP (Coetzee et al, 1999). Since 4-AP inhibited the non-inactivating current by 13.3 ± 3.6% (n = 5, P < 0.05, Fig 1D-E), Kv3 channels may marginally contribute to the depolarization-evoked outward current. In contrast, a specific BK channel blocker, 100 nM iberiotoxin, markedly inhibited the sustained outward current (70.4 ± 4.8%; n = 4; P < 0.005, Fig 1C). Iberiotoxin-sensitive currents were activated at -20 mV and exhibited little inactivation (Fig 1C). Therefore non-inactivating potassium currents in stellate cells are predominantly mediated by BK channels. In the presence of iberiotoxin, application of TEA produced a small reduction in K\(^{+}\) currents (Fig 1E), and thus BK channels mainly contribute to TEA-sensitive currents.

Inhibition of BK channels prolongs AP duration.

Cerebellar stellate cells fire APs spontaneously in the absence of synaptic input. We next determined the impact of BK channel blockade on the AP waveform in stellate cells. Using the whole cell current clamp technique, we recorded spontaneous APs in stellate cells in the presence of 1 mM KYNA and 100 µM PTX. Spontaneous APs were of a brief duration (Fig 2A and 2C). Iberiotoxin (100 nM) prolonged AP duration (from 1.2 ± 0.1 to 2.3 ± 0.2 ms, n = 5, P < 0.001) and reduced the after-hyperpolarization.
One mM TEA also significantly increased the AP duration and reduced the after-hyperpolarization without altering the amplitude of APs (Figure 2B-D). Changes in AP waveform elicited by iberiotoxin were indistinguishable from those elicited by TEA. Therefore BK channels exert a strong control over the AP waveform by accelerating repolarization.

**Blocking BK channels increases the expression of synaptic GluR2-containing AMPARs.**

Under basal conditions, cerebellar stellate cells express primarily GluR2-lacking, Ca\(^{2+}\)-permeable AMPARs (Liu and Cull-Candy, 2000). Does the brief AP waveform in stellate cells suppress the expression of GluR2 subunits and thus promote GluR2-lacking AMPARs at stellate cell synapse? To examine the modulatory effects of postsynaptic APs, cerebellar slices were incubated for 1h with iberiotoxin (100 nM) followed by further incubation (2h) in control solution. We have previously shown that repetitive activation of Ca\(^{2+}\)-permeable AMPARs enhances synaptic incorporation of GluR2-containing receptors (Liu and Cull-Candy, 2000). To avoid any effect arising from altered presynaptic release, KYNA and PTX were present during all control and BK channel inhibitor treatments. Incubation with KYNA and PTX for 3 hours did not alter synaptic currents (rectification index: 0.29 ± 0.03, \(n = 5\); vs without treatment, 0.34 ± 0.03 (Liu et al, 2010); \(P > 0.05\)) and therefore was used as control. KYNA was washed out for 15 min before recording.

Two pharmacological approaches were used to monitor the synaptic AMPAR subunit composition in these cells. First, inclusion of spermine in the pipette solution is known to confer a voltage-dependent block of AMPARs that lack GluR2 subunits and to produce a characteristic inwardly rectifying I-V relationship (Cull-Candy et al, 2006). sEPSCs were measured at various holding potentials. In control cells, the amplitude of sEPSCs was reduced at positive membrane potentials and displayed an I-V relationship with pronounced inward rectification, indicating that the EPSCs were mediated mainly by GluR2-lacking, Ca\(^{2+}\)-permeable AMPARs (Fig 3A, *control*). In contrast, in iberiotoxin treated cells, the I-V relationship of the sEPSC was nearly linear (Fig 3B), indicating that GluR2-containing AMPARs mediated the synaptic currents. Iberiotoxin treatment altered the rectification index from control, 0.29 ± 0.02 (\(n = 8\)) to iberiotoxin, 0.80 ± 0.11 (\(n = 5\); \(P < 0.005\); Fig 3D). Iberiotoxin increased the sEPSC amplitude at +40 mV from 7.7 ± 0.4 pA under control conditions to 18.5 ± 1.4 pA following iberiotoxin treatment (\(n = 5\), \(P < 0.001\)). At +40 mV the synaptic current is mediated mainly by AMPARs containing GluR2 subunits and the decay time constant of sEPSCs at -60 mV was prolonged (Kolmogorov-Smirnov test, \(P < 0.000005\); Fig 3E). Incubation with iberiotoxin (100 nM) for 3 hrs produced similar effects on the rectification index and decay time of sEPSCs (Fig 3B, 3D and 3E). Thus this result is consistent with the synaptic incorporation of GluR2-containing receptors. Iberiotoxin application did not alter the amplitude and frequency of sEPSCs at -60 mV (amplitude: control, -41.8 ± 3.0 pA; iberiotoxin, -44.3 ± 4.1 pA, NS;
frequency: control, 0.12 ± 0.02 Hz; iberiotoxin, 0.15 ± 0.02 Hz, n = 5, NS) and therefore has minimal
presynaptic effects.

To confirm that this change in rectification reflects an increase in synaptic GluR2-containing
AMPARs, we used a selective Ca^{2+}-permeable AMPAR blocker, 1-naphthyl acetyl spermine (Naspm).
Naspm (500 nM) reduced the EPSC amplitude of control cells by ~80% (n = 6) at –60 mV, a larger
reduction than that observed for iberiotoxin treated cells (3 hrs, 47 ± 3%; n = 4; P < 0.0002; Fig 3F). Thus
BK channel blockade enhances synaptic incorporation of GluR2-containing AMPARs.

Consistent with the idea that spike broadening promotes the insertion of synaptic GluR2
receptors, the I-V relationship of sEPSCs' became more linear (Fig 3C) and Naspm inhibition decreased
(Fig 3F) following TEA (1 mM) treatment for 3 hrs (Liu et al., 2010). These findings indicate that the
synaptic AMPAR phenotype in cerebellar stellate cells can be altered by the activity of BK channels.

**Blockade of BK channels enhances Ca^{2+} entry during an AP and alters synaptic AMPAR
phenotype.**

We hypothesized that prolonged APs may enhance Ca^{2+} entry that triggers an alteration in
synaptic AMPAR composition. To examine the effect of AP waveform on Ca^{2+} influx, we measured the
Ca^{2+} currents in stellate cells under voltage-clamp using AP waveforms that mimicked the control AP
(control-AP) or following TEA application (TEA-AP). The TEA-AP waveform with a half-width (2.3 ms) and
after-hyperpolarization (-9 mV) is comparable to the average iberiotoxin-AP (Fig 2C and 2D), and
therefore would also represent the AP waveform when BK channels were blocked by iberiotoxin. The
control-AP evoked an inward current and TEA-AP increased the duration of the Ca^{2+} currents. The Ca^{2+}
entry occurring during a TEA-AP (defined as the current integrated over time) was ~40% higher than that
entering during the control-AP waveform (Fig 4A-C, n = 15; P < 0.001 vs control, by a paired t-test).
Lowering the extracellular Ca^{2+} concentration from 2 to 1 mM reduced the Ca^{2+} entry during a TEA-AP by
~40% (n = 8; P < 0.05; Fig 4), primarily due to a decrease in the peak amplitude of the Ca^{2+} current. Thus
increasing the AP duration by inhibiting BK channels significantly enhanced Ca^{2+} entry.

Nifedipine (20 µM), an L-type voltage-gated Ca^{2+} channel blocker, blocked 72.2 ± 3.0% (n = 4) of
the Ca^{2+} current evoked by a TEA-AP, indicating that L-type channels were activated during an AP (Fig
4B and 4C). To determine whether enhanced Ca^{2+} entry via voltage-gated Ca^{2+} channels is required for
synaptic incorporation of Ca^{2+}-impermeable AMPARs, we included 20 µM nifedipine during the iberiotoxin
treatment (3 hrs). Following this treatment, sEPSCs displayed an inwardly rectifying I-V relationship (Fig
4D). Thus nifedipine blocked the iberiotoxin-induced increase in rectification index (Nifedipine+iberiotoxin,
RI = 0.52 ± 0.02, n = 4; P < 0.05, vs iberiotoxin; Fig 4), indicating that nifedipine prevented insertion of
new GluR2-containing AMPARs. As a control, cerebellar slices were incubated in ACSF containing 20 µM
nifedipine (without iberiotoxin) for 3 hours. Under these conditions, the rectification index of sEPSCs was
greater than that without nifedipine (RI = 0.42 ± 0.02, n = 4), but remained inwardly rectifying (P < 0.05, vs iberiotoxin; Fig 4F). Nifedipine alone prolonged the AP duration (Supplementary Fig 1), presumably by reducing the activity of Ca\(^{2+}\)-activated potassium channels. Although inclusion of nifedipine together with TEA did not further prolong AP duration, nifedipine also blocked the TEA-induced switch in sEPSC I-V relationship from inwardly rectifying to near linearity (P < 0.05, vs TEA; Fig 4E). While nifedipine can prolong cardiac action potentials by blocking Kv1.5, this channel does not appear to be expressed in cerebellar stellate cells (Lin et al 2001; Chung et al, 2001). Thus, enhanced Ca\(^{2+}\) entry via L-type Ca\(^{2+}\) channels is most likely required for synaptic incorporation of GluR2-containing receptors. To test whether activation of N-type and P-type Ca\(^{2+}\) channels also contributes to the TEA-induced change in synaptic receptors, we included \(\omega\)-conotoxin GVIA (500 nM) and \(\omega\)-Agatoxin IVA (500 nM) together with nifedipine (20 \(\mu\)M) during the TEA treatment. This combination did not further reduce the rectification index of sEPSCs, compared with nifedipine + TEA treatment (Fig 4E).
It is well established that the number and properties of postsynaptic AMPARs can be regulated by presynaptic activity. However whether AP firing in postsynaptic neurons can also alter the synaptic AMPARs remains unclear. In the present study, we tested the hypothesis that activation of potassium channels (that control the duration of APs) reduces the level of synaptic GluR2-containing receptors. BK channels mediate substantial fraction of the potassium currents in cerebellar stellate cells and activation of these channels gives rise to brief APs. We found that pharmacological blockade of BK channels enhances Ca\(^{2+}\) entry during an AP and promotes the incorporation of GluR2 receptors at cerebellar synapses. This switch in AMPAR subtype requires Ca\(^{2+}\) influx through L type voltage-gated Ca\(^{2+}\) channels. These results indicate that activation of BK channels shortens AP duration and thereby reduces in the expression of synaptic GluR2-containing AMPARs. Therefore K\(^+\) channels in postsynaptic neurons can influence synaptic AMPAR subtype by controlling membrane excitability and AP waveform.

Kv3 potassium channels are expressed at a high level in a subset of GABAergic neurons and in auditory neurons (Perney et al., 1992). Like BK channels, activation of Kv3 channels can shorten AP duration. Intriguingly a study that examined the gene expression profile of populations of CNS neurons revealed an inverse relationship between the levels of GluR2 mRNA and that of Kv3.1 with many GABAergic interneurons expressing high levels of Kv3 and low levels of GluR2 (Sugino et al., 2006). In this study we show that blockade of BK channels can increase the expression of GluR2-containing receptors in GABAergic interneurons in the cerebellum. Our results indicate that a brief AP duration limits the amount of Ca\(^{2+}\) that enters the cell through voltage-gated Ca\(^{2+}\) channels, which in turn reduces the expression of synaptic GluR2-containing receptors. We have recently shown that increasing AP duration by TEA promotes GluR2 gene expression in stellate cells (Liu et al., 2010). Therefore the presence of these K\(^+\) channels may be one of the determinants that control GluR2 gene expression. However other factors that affect Ca\(^{2+}\) entry are also likely to influence GluR2 gene transcription and the postsynaptic AMPA receptor phenotype. Thus the expression of BK channels by itself does not necessarily lead to a low level of GluR2 gene expression as seen in Purkinje cells and hippocampal pyramidal neurons (Womack and Khodakhah, 2002; Sailer et al, 2006).

Potassium currents in cerebellar basket and stellate cells have been previously characterized (Southan and Robertson, 1998; Molineux et al, 2005). Basket cells are located in the lower one third of the molecular layer whereas stellate cells are found in the upper two thirds of the molecular layer. 4-AP at 1 mM inhibits 15% of the net K\(^+\) current in stellate cells (present study) but does not have any inhibitory effects in basket cells (Southan and Robertson, 1998). Thus 4-AP sensitive channels (Kv3 and Kv1) are unlikely to mediate a large portion of the potassium currents in either stellate or basket cells. While our results show thatiberiotoxin produced a marked inhibition of potassium currents in stellate cells, charybdotoxin, a BK channel blocker, failed to reduce the potassium current in basket cells (Southan and
Robertson, 1998). This could be due to the presence of beta subunits that reduce the inhibitory potency of charybdotoxin (Behrens et al., 2000) or the higher EGTA (10 mM) concentration of the pipette solution used in their study that suppresses a Ca$^{2+}$ rise and thus the activity of BK channels. The transient and sustained K$^+$ currents play a distinct role in controlling AP firing in stellate cells. Low-threshold and inactivating K$^+$ currents in stellate cells prolong spike latency (Molineux et al, 2005), whereas the high threshold non-inactivating currents controls the duration of APs (present study).

We and others have previously shown that synaptic activity increases GluR2-containing AMPARs at the parallel fibre to stellate cell synapse. This requires activation of either synaptic Ca$^{2+}$-permeable AMPARs or extrasynaptic NMDARs, (Liu and Cull-Candy, 2000 & 2005; Gardner et al., 2005; Sun and Liu, 2007). Activation of metabotropic receptors can also alter synaptic AMPAR subtype in a protein synthesis-dependent manner (Kelly et al., 2009). In a recent study we have shown that stress upregulates GluR2 mRNA abundance in stellate cells and induces a lasting increase in synaptic GluR2-containing AMPARs (Liu et al., 2010). The stress-induced increase in GluR2 mRNA expression is mediated by beta adrenergic receptors. Noradrenaline increases h-currents, giving rise to membrane depolarization (Saitow and Konishi, 2000) and prolongation of AP duration in stellate cells (Liu et al., 2010). However in the present study we show that increasing AP duration via a different mechanism, blockade of BK channels, also promotes incorporation of GluR2-containing receptors to the synapse. While adrenergic receptors can be coupled to a number of signaling pathways, both noradrenaline application and the BK channel blockade–induced switch in AMPAR phenotype requires Ca$^{2+}$ influx via L-type channels. Furthermore activation of MAPK and gene transcription are necessary for the incorporation of GluR2-containing receptors triggered by noradrenaline and by TEA (Liu et al., 2010). Together these results support the idea that noradrenaline increases synaptic GluR2-containing AMPARs, at least in part by increasing AP duration in stellate cells.

Our results suggest that intrinsic membrane excitability in postsynaptic cells can regulate the AMPAR phenotype. An alteration in membrane excitability, such as silencing APs or membrane depolarization, is known to modulate excitatory synaptic transmission. Prolonged blockade of AP firing has been shown to alter the amplitude of synaptic currents and AMPAR phenotype (Turrigiano and Nelson, 2000; Thiagarajan et al., 2005; Sutton et al., 2006), a homeostatic plasticity mechanism that stabilizes the activity of neuronal networks. In contrast, membrane depolarization induces a rapid change in synaptic AMPAR from GluR2-lacking to GluR2-containing receptors in immature hippocampal CA3 pyramidal cells (Ho et al., 2007). Recent studies show that activation of Kv4 voltage-gated potassium channels in the postsynaptic hippocampal pyramidal neuron elevates the threshold for long-term potentiation and modifies the subunit composition of synaptic NMDARs (Chen et al., 2006; Kim et al., 2007; Jung et al., 2008). Our results reveal that BK/Kv3 channels modulate synaptic AMPAR phenotype by controlling AP repolarization in the postsynaptic neuron. While one hour TEA treatment is sufficient to trigger the effect, a change in the expression of synaptic AMPARs requires >1 hour (Liu et al, 2010). Thus
the activity of K⁺ channels could potentially shape the synaptic inputs by influencing the expression of synaptic receptors.

Enhanced Ca²⁺ entry via L-type Ca²⁺ channels during an AP is required for the TEA/iberiotoxin-induced increase in synaptic GluR2 expression. Inhibition of N- and P-type Ca²⁺ channels in addition to nifedipine did not further attenuate the TEA-induced enhancement of GluR2 expression, indicating that these Ca²⁺ channels are unlikely to make an additional contribution. Action potentials can also evoke Ca²⁺ release from intracellular stores in cerebellar interneurons. While 10 µM ryanodine causes Ca²⁺ release from intracellular stores and increases spontaneous calcium transients at basket cell terminals, ryanodine at a higher concentration (100 µM) reduces the action potential-evoked rise in intracellular Ca²⁺ (Llano et al 2000; Conti et al, 2004). We incubated cerebellar slices with 100 µM ryanodine for 3 hours in the presence of kynurenic acid and picrotoxin. Following this treatment the rectification index of sEPSCs increased from 0.29 ± 0.02 (control, n = 8) to 0.72 ± 0.07 (n = 4; P < 0.005), which is not significantly different from that after IBTX and TEA treatment (supplementary figure 2). This could result from an increase in spontaneous calcium transients as the intracellular ryanodine concentration increases during the incubation and the rise in intracellular Ca²⁺ then facilitates the insertion of GluR2 receptors at stellate cell synapses (Liu and Cull-Candy, 2000). Therefore we cannot rule out the possibility that a Ca²⁺-induced Ca²⁺ release may contribute to the iberiotoxin/TEA-induced increase in GluR2 expression.

Our results show that blocking BK channels prolongs AP duration and increases Ca²⁺ influx through voltage-gated Ca²⁺ channels, promoting the expression of GluR2-containing AMPARs at synapses. This reduces the Ca²⁺-permeability of synaptic AMPARs and may provide a feedback mechanism for Ca²⁺ homeostasis in cerebellar stellate cells. Incorporation of GluR2-containing AMPARs also slows the decay time of synaptic currents without any alteration in the current amplitude (Fig 3). This can lead to a marked increase in AP firing probability in response to synaptic activation (Savtchouk and Liu, 2011). Thus the expression of BK channels in cerebelar stellate cells can control the kinetics of APs and synaptic AMPAR phenotype, and thereby the waveform of synaptic currents in stellate cells.
FIGURE LEGENDS

Figure 1. BK channels mediate a large portion of voltage-gated potassium currents in stellate cells. 
A. K+ current traces recorded from a stellate cell using perforated and whole cell patch clamp recordings. 
Cells were voltage-clamped at -100 mV and stepped from -90 mV to +60 mV in 10 mV increments. 
Current-voltage relationship of the peak and non-inactivating currents (perforated patch, n = 4; whole cell 
patch, n = 10). B and C. Depolarization from -100 mV to 0 mV elicited an outward current. TEA (1 mM, n 
= 4) and iberiotoxin (100 nM, n = 4) inhibited a large portion of the potassium current. Non-inactivating 
conductance-voltage relation shows that TEA and iberiotoxin (IBTX) sensitive current (= Icontrol – ITEA or 
IIBTX) are activated at -30 and -20mV, respectively. D. 4-AP (1 mM) moderately reduced potassium 
currents. E. Summary of K+ current inhibition at 0 mV by TEA, iberiotoxin and 4-AP. (*, P < 0.05; current 
amplitude, control vs inhibitor; #, P < 0.05, IBTX vs IBTX+TEA).

Figure 2. Inhibition of BK channels increases the AP duration in stellate cells. A, B. Application of 
IBTX (100 nM) and TEA (1 mM) altered the AP waveform. C, D. IBTX (n = 5) and TEA (n = 10) prolonged 
the duration and reduced the after-hyperpolarization of spontaneous APs. E, F. IBTX did not alter the 
spontaneous AP frequency in stellate cells. Spontaneous APs were recorded in a cell-attached 
configuration. (*, P < 0.05; **, P < 0.005; ***, P < 0.0005).

Figure 3. Blocking BK channels in stellate cells induces a change in rectification of the I-V 
relationship and the Naspm-dependent inhibition of sEPSCs. Cerebellar slices were incubated with 
KYNA (1 mM) and PTX (100 µM) in the absence (control) or presence of IBTX (100 nM, 1h and then 2h in 
ACSF that contained KYNA and PTX, or IBTX for 3 hrs) or TEA (1 mM, 3 hrs) in the presence of KYNA 
and PTX. sEPSCs were recorded when spermine was included in the pipette. A. Average sEPSCs 
displayed an inwardly rectifying I-V relationship in control, indicating the presence of Ca2+-permeable, 
GluR2-lacking AMPARs (n = 8). B, C. Following IBTX (1 hr + 2hrs in control solution, n = 5; 3 hrs, n = 3) 
and TEA (n = 14) treatment the synaptic current in stellate cells showed a near linear I-V relationship, 
indicating that it was mediated mainly by GluR2-containing AMPARs. D. Summary of rectification index of 
EPSCs. E. Cumulative distribution of decay time constant of individual EPSCs at -60 mV from 3 -14 cells 
under each condition (Kolmogorov-Smirnov test, P < 0.0001). F. Application of Naspm (500 nM) 
produced a greater inhibition of sEPSCs in control cells (n = 4) than in IBTX (3 hr, n = 4) and TEA-treated 
cells (3 hr, n = 5). (**, P < 0.005; ***, P < 0.0005).

Figure 4. Ca2+ influx through L-type Ca2+ channels is required to trigger K+ channel blockade-
induced change in sEPSC rectification. A. Ca2+ currents during a control-AP and a TEA-AP. B. 
Nifedipine (NF, 20 µM) blocked most of the Ca2+ current using step depolarization and TEA-AP as the 
voltage commands. C. Summary of Ca2+ charge. D, E. Nifedipine blocked the IBTX (n = 4) and TEA (n = 
7)-induced change in I-V relationship. Inclusion of ω-conotoxin GVIA (500 nM) and ω-Agatoxin IVA (500
nM) together with nifedipine (20 µM) during TEA treatment did not further reduce the rectification index of sEPSCs (n = 4). F. Summary of rectification index of EPSCs. (*, P < 0.05; **, P < 0.005; ***, P < 0.0005).
REFERENCES


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Figure 2. Liu et al.

A. sAPs

B. TEA

C. Half width of sAPs (ms)

D. AHP of sAPs (mV)

E. Frequency of sAPs (Hz)

F. Frequency of sAPs (Hz)
Figure 3. Liu et al.
Figure 4. Liu et al.

A. 
Control  TEA  TEA

\[ \text{Ca}^{2+} \text{current} \]

\[ 2 \text{ mM Ca}^{2+} \quad 2 \text{ mM Ca}^{2+} \quad 1 \text{ mM Ca}^{2+} \]

B. 

\[ \text{AP waveform} \]

\[ \text{Control} \quad \text{TEA} \quad \text{TEA} \]

\[ 2 \text{ mM Ca}^{2+} \quad 2 \text{ mM Ca}^{2+} \quad 2 \text{ mM Ca}^{2+} \]

C. 

\[ \text{Rectification index of sEPSCs} \]

\[ \text{IBTX treatment} \quad \text{TEA treatment} \]

\[ 2 \text{ mM} \quad 2 \text{ mM} \quad 1 \text{ mM} \quad 2 \text{ mM} \quad 2 \text{ mM} \]

D. 

\[ \text{sEPSCs} \]

\[ \text{IBTX + NF} \]

\[ -60 \text{ mV} \quad +40 \text{ mV} \]

\[ 20 \text{ pA} \]

\[ 10 \text{ ms} \]

E. 

\[ \text{TEA + NF} \quad + \omega-\text{CTX} + \omega-\text{AGA} \]

\[ -60 \text{ mV} \quad +40 \text{ mV} \]

\[ 20 \text{ pA} \]

\[ 10 \text{ ms} \]

F. 

\[ \text{Reduction index of sEPSCs} \]

\[ \text{Control} \quad \text{IBTX treatment} \quad \text{TEA treatment} \]

\[ -\text{NF} \quad +\text{NF} \]