Pyramidal neurons scale the strength of all of their excitatory synapses up or down in response to long-term changes in activity, and in the direction needed to stabilize firing rates. This form of homeostatic plasticity is likely to play an important role in stabilizing firing rates during learning and developmental plasticity, but the signals that translate a change in activity into global changes in synaptic strength are poorly understood. Some but not all of the effects of long-lasting changes in activity on synaptic strengths can be accounted for by activity-dependent release of the neurotrophin brain-derived neurotrophic factor (BDNF). Other candidate activity signals include changes in glutamate receptor (GluR) activation, changes in firing rate, or changes in the average level of postsynaptic depolarization. Here we combined elevated KCl (3–12 mM) with ionotropic receptor blockade to dissociate postsynaptic depolarization from receptor activation. Chronic (48 hr) depolarization, ranging between −62 and −36 mV, parametrically reduced the quantal amplitude of excitatory synapses in a BDNF-independent manner. This effect of depolarization did not depend on AMPA, NMDA, or GABA, receptor signaling, action-potential generation, or metabotropic GluR activation. Together with previous work, these data suggest that there are two independent signals that regulate activity-dependent synaptic scaling in pyramidal neurons: low levels of BDNF cause excitatory synapses to scale up in strength, whereas depolarization causes excitatory synapses to scale down in strength.

Key words: BDNF; synaptic scaling; activity-dependent; synaptic plasticity; KCl; depolarization; mEPSC

Neurons use a variety of homeostatic mechanisms to counter the destabilizing effects of plasticity and keep firing rates within functional boundaries (Turrigiano, 1999). One of these mechanisms is synaptic scaling: pyramidal neurons are able to upregulate or downregulate the quantal amplitude of all of their excitatory synapses in an activity-dependent manner, and in the direction needed to maintain stability (Turrigiano et al., 1998). Because this process scales synapses proportionally, it preserves the relative differences in strength between different synaptic inputs. Synaptic scaling has a slow time course (24–48 hr) and is at least partially mediated by changes in the number of postsynaptic glutamate receptors (GluRs) (O’Brien et al., 1998; Turrigiano et al., 1998; Watt et al., 2000).

For synaptic scaling to work, neurons need to integrate some measure of activity over a long time scale and transduce this measurement into a signal that modifies quantal amplitude. Brain-derived neurotrophic factor (BDNF) appears to mediate some of the effects of activity on quantal amplitude (Rutherford et al., 1998). BDNF is released by pyramidal neurons in an activity-dependent manner (Castren et al., 1992; Bałkowiec and Katz, 2000) and binds to TrkB receptors on both pyramidal and inhibitory interneurons (Kokaia et al., 1993; Miranda et al., 1993). BDNF is especially important for the activity-dependent regulation of inhibitory circuits: high concentrations of BDNF lead to increased inhibition in a variety of preparations (Isackson et al., 1991; Zafra et al., 1991; Castren et al., 1992; Ghosh et al., 1994; Rutherford et al., 1997; Vicario-Abejon et al., 1998; Huang et al., 1999; Marty et al., 2000; Seil and Drake-Baumann, 2000) and also increase the amplitude of miniature EPSCs (mEPSCs) onto inhibitory interneurons (Rutherford et al., 1998).

Although BDNF has important effects on quantal amplitude and neuronal excitability (Rutherford et al., 1998; Desai et al., 1999), changes in BDNF alone cannot account for the bidirectional regulation of quantal amplitude by long-lasting changes in activity. Lowering activity increases mEPSC amplitude by reducing the release of endogenous BDNF (Rutherford et al., 1998). However, adding exogenous BDNF does not reduce mEPSC amplitudes below control values, suggesting that increased activity does not scale down mEPSC amplitude by increasing the release of BDNF. Thus, although activity can bidirectionally scale mEPSC amplitude, BDNF can only account for part of this regulation. This raises the question of what signal transduces high levels of activity into a reduction in mEPSC amplitude. Possible signals include changes in firing rate, changes in glutamate receptor activation (Lissin et al., 1998; O’Brien et al., 1998), and...
changes in the average level of postsynaptic depolarization. We used elevated external KCl (3–12 mM) in ionotropic receptor blockade to dissociate the effects of postsynaptic depolarization from glutamate receptor activation. Chronic depolarization parametrically reduced mEPSC amplitudes in the absence of AMPA-kainate, NMDA, and GABA_A receptor activation. The effects of depolarization were independent of action-potential generation and BDNF signaling. Together with previous results, these findings suggest that BDNF and postsynaptic membrane potential changes in neuronal health, whole-cell properties, mEPSC amplitude, rise time, and frequency. The NaOH controls were combined with other treated controls for the LY341495 experiment. Nifedipine (Sigma) (5 μM) was used to block L-type voltage-gated calcium channels (VGCCs). Nifedipine was made up as a 5 mM stock in DMSO (Sigma). Control cultures were treated with DMSO alone (0.1%). DMSO did not adversely affect neuronal properties or block scaling. The nifedipine was not refreshed.

RESULTS

Chronic depolarization scales mEPSC amplitudes

Previous reports have emphasized the importance of glutamate receptor activation for activity-dependent synaptic scaling (Lissin et al., 1998; O’Brien et al., 1998). To test this hypothesis while controlling for activity, we chronically depolarized cells with a range of KCl concentrations (3–12 mM) while blocking ionotropic glutamate and GABA_A receptors with CNQX (40 μM), APV (50 μM), and bicuculline (10 μM) (henceforth referred to as ionotropic receptor blockade). This allowed us to dissociate receptor signaling from postsynaptic depolarization. Acute whole-cell current-clamp recordings showed that varying the potassium concentration in ionotropic receptor blockade depolarized pyramidal neurons from −62 ± 2.6 mV (in 3 mM KCl) to −51 ± 1.7 mV (6 mM), −45 ± 2.5 mV (9 mM), and −36 ± 0.8 mV (12 mM) (Fig. 1B). Note that some neurons at the higher potassium concentrations stopped firing, presumably because of sodium channel inactivation (Fig. 1A). Under ionotropic receptor blockade, the KCl concentration normally found in the medium (5.4 mM) yields cells that are still quite depolarized (i.e., interpolating from Figure 1 yields a value of approximately −55 mV). To get a larger range of membrane potentials, neurons were grown in 3 mM KCl medium. Control cultures raised in low potassium (3 mM) had larger mEPSCs (−19.7 ± 2.5 pA) (Fig. 2) than control cultures raised in regular medium (−13.6 ± 0.7 pA; data not shown), indicating that reduced depolarization, which is likely to reduce ongoing activity, produced an increase in quantal amplitude.

After 48 hr of treatment with different concentrations of KCl in the presence of ionotropic receptor blockers, cultures were perfused with regular ACSF in the presence of TTX (0.1 μM), APV...
and bicuculline (20 μM). AMPA-mediated mEPSCs were then recorded from visually identified pyramidal neurons.

Quantal amplitude was significantly decreased by elevated KCl (p < 0.015; single-factor ANOVA), from 23.5 ± 2.6 pA (3 mM), to 19.1 ± 2.7 pA (6 mM), −15.1 ± 1.5 pA (9 mM), and −13.1 ± 0.8 pA (12 mM) (Fig. 2). This demonstrates that chronic (48 hr) depolarization can reduce mEPSC amplitude in the absence of ionotropic excitatory or inhibitory receptor activation.

Neurons treated with a high level of potassium and with ionotropic receptor blockade appeared healthy and showed no significant changes in resting membrane potential, input resistance, series resistance, or whole-cell capacitance when compared with control (3 mM) cells. Also, there was no consistent change in mEPSC kinetics (Fig. 2B) or in frequency between conditions (one-way ANOVA, p = 0.88, data not shown). This is in agreement with other studies that show that altering activity for 2 d does not significantly influence the placement or number of excitatory synapses onto pyramidal neurons (Micheva and Beaulieu, 1995) (V. Kilman and G. G. Turrigiano, unpublished observations).

**APV reduces firing rates in bicuculline**

We have shown previously that blocking NMDA receptor activation does not lead to an increase in quantal amplitude (Turrigiano et al., 1998). However, others have suggested that NMDA receptor activation is needed for the reduction in quantal amplitude produced by elevated activity, based on experiments showing that APV partially blocks the reduction in quantal amplitude induced by treatment with GABA<sub>A</sub> receptor antagonists (Lissin et al., 1998). An alternative explanation for this result is that blockade of NMDA receptors in the presence of GABA<sub>A</sub> receptor antagonists such as bicuculline results in lower firing rates and less depolarization overall than bicuculline alone. To test this hypothesis, we measured the firing rates of pyramidal neurons treated acutely with bicuculline (20 μM) or bicuculline plus D-APV (100 μM).

Acute application of bicuculline increased the firing rate of pyramidal neurons to 2.8 ± 0.5 Hz compared with a firing rate of 0.7 ± 0.6 Hz in controls (p ≤ 0.03). In addition neurons tended to fire in bursts of action potentials riding on top of a significant
level of depolarization (Fig. 3A). Neurons recorded in ACSF containing bicuculline and APV had an average firing rate of 1.4 ± 0.3 Hz, which was significantly lower than firing rates in bicuculline alone; in addition, the depolarizing envelope during the burst was significantly reduced (p = 0.04) (Fig. 3). Most importantly, the firing rate in bicuculline and APV was not significantly higher than that in regular ACSF (p = 0.37, NS)

**NMDA receptor activation does not promote scaling**

To assay directly for any role of NMDA receptor signaling in reducing mEPSC amplitudes, we treated cultures for 2 d with 12 mM KCl in the presence of CNQX and bicuculline, with or without 100 μM d-APV. If NMDA activation is important for synaptic scaling under these conditions, then we would expect an additional decrease in mEPSC amplitude with intact NMDA receptor signaling. No significant decrease was observed; instead, mEPSCs in the NMDA block condition were slightly smaller than those grown with NMDA signaling intact (−12.5 ± 1.0 pA vs −16.0 ± 2.8 pA, respectively; NS, p ≤ 0.24). Together with our previous results, these data suggest that NMDA receptor activation does not contribute significantly to the reduction in mEPSC amplitude produced by depolarization. The effects of APV seen by others can instead be explained by reduced overall depolarization in the bicuculline plus APV condition, as opposed to any direct role for NMDA receptor activation in synaptic scaling.

**Blocking spiking does not block the effect of depolarization**

A high level of KCl has two major effects: it depolarizes neurons and it increases firing rates. Are spikes needed to scale down mEPSC amplitude, or is depolarization enough? To test the relative contribution of action potentials to this regulation, we treated cells with 12 mM KCl plus ionotropic receptor blockade, with or without 0.5 μM TTX. Blocking action-potential generation did not prevent the reduction in quantal amplitude produced by chronic depolarization (Fig. 4). In contrast, for sister cultures grown in control medium (5.4 mM KCl) with synaptic transmission intact, 48 hr of treatment with TTX increased mEPSC amplitude significantly (250% ± 30% of control values; p ≤ 0.01), as reported previously (Rutherford et al., 1998; Turrigiano et al., 1998; Watt et al., 2000). Hence, depolarization alone, in the absence of action-potential generation, is sufficient to induce a downregulation of mEPSC amplitude.

**Blocking TrkB signaling does not block the effect of depolarization**

BDNF can regulate quantal amplitude over part of the dynamic range (Rutherford et al., 1998). We reasoned that depolarization may result in the graded release of BDNF, causing a reduction in quantal amplitude at depolarized potentials. If this were the case, then blocking BDNF signaling under these conditions should block the reduction in quantal amplitude observed in the high potassium condition.

To explicitly test the role of BDNF in the depolarization-mediated regulation of mEPSC amplitude, we used a TrkB–IgG to scavenge extracellular BDNF and prevent activation of endogenous TrkB receptors. TrkB–IgG is a fusion protein of the extracellular domain of TrkB, the high-affinity receptor for BDNF, and the Fc domain of human IgG (Shelton et al., 1995). To verify that the TrkB–IgG fusion protein was effective, we treated cultures grown in control medium (5.4 mM KCl) without synaptic blockade with the TrkB–IgG for 2 d (at concentrations of 2 or 10 μg/ml). As reported previously (Rutherford et al., 1998), this treatment increased mEPSC amplitude significantly (173% ± 21% of control values; p ≤ 0.002; 2 and 10 μg/ml produced similar results so the data were combined). However, TrkB–IgG failed to block the decrease in mEPSC amplitude produced by chronic depolarization in ionotropic receptor blockade, either in the presence or in the absence of TTX (Fig. 4) (TrkB–IgG plus 12 mM KCl was not significantly different from 12 mM KCl alone; p = 0.4). This suggests that the reduction in quantal amplitude produced by depolarization is independent of TrkB signaling.

**Blocking mGlur signaling does not block the effect of depolarization**

It is possible that chronic depolarization of presynaptic terminals may release enough glutamate to activate metabotropic receptors, which might in turn reduce quantal amplitude. Metabotropic glutamate receptors have been shown to play a role in long-term depression (LTD) (Egger et al., 1999). If the reduction of quantal amplitude is indeed dependent on metabotropic receptor signaling (either presynaptic or postsynaptic), then blocking this signaling should prevent the decrease in quantal amplitude produced by elevated KCl. We used LY341495 (Bortolotto et al., 1999; Sawtell et al., 1999), a potent mGlur blocker (100 μM), to test this hypothesis. LY341495 did not prevent the reduction in mEPSC amplitude produced by 12 mM KCl (Fig. 4), and indeed, there was a nonsignificant trend toward smaller quantal amplitudes in the presence of LY341495 (LY341495 plus 12 mM KCl was not different from 12 mM KCl alone; p ≤ 0.09). Hence, metabotropic receptor activation is not needed for depolarization-induced scaling.

**Block of L-type calcium channels**

Chronic depolarization can downregulate quantal amplitude in a parametric manner, independently of BDNF and glutamate receptor activation. One obvious candidate for transducing mem-
brane depolarization is the L-type VGCC. These VGCCs have been implicated in LTD (Cummings et al., 1996) and are themselves partially regulated by chronic changes in membrane potential (Franklin et al., 1992; Fickbohm and Willard, 1999).

We used nifedipine (5 μM) to test the role of L-type VGCCs in this regulation. Nifedipine did not block the reduction in quantal amplitude induced by high levels of potassium in ionotropic receptor blockade with TTX (Fig. 4). This suggests that calcium influx through L-type VGCCs is not necessary for depolarization-mediated synaptic scaling.

**DISCUSSION**

We have shown that postsynaptic glutamate receptor activation might be important for scaling. Both Turrigiano et al. (1998) and O'Brien et al. (1998) found that AMPA receptor blockade resulted in an increase in synaptic strength in cortical cultures and spinal cord cultures, respectively. Lissin et al. (1998) observed that NMDA receptor blockade partially blocked the effects of activity enhancement associated with GABA<sub>A</sub> receptor antagonists in hippocampal cultures. However, here we have shown that chronic depolarization can parametrically scale mEPSC amplitude over a large part of the dynamic range despite the complete blockade of ionotropically mediated synaptic transmission. This result suggests that the observed effects of glutamate receptor antagonists on synaptic scaling can be explained by overall changes in average membrane potential instead of by any direct effect of ionotropic receptor activation.

It is currently unclear how depolarization is transduced into an intracellular signal that scales down mEPSC amplitudes. We have ruled out an essential role for activation of ionotropic and metabotropic glutamate receptors, TrkB receptors, and L-type VGCCs. A number of other possibilities exist, including activation of other classes of VGCCs or depolarization-induced release of an unidentified factor. It is also possible that there are redundant mechanisms activated by depolarization, each of which can scale down mEPSC amplitude even in the absence of the other signals. Given the likely importance of synaptic scaling for network function, such redundancy would not be surprising but could make identification of the relevant intracellular signaling pathways difficult.

Keeping track of average depolarization would allow a neuron to track its own postsynaptic activity, which would be useful for stability rules such as synaptic normalization (Turrigiano and Nelson, 2000) as well as for the Bienenstock, Cooper, and Munro sliding plasticity-threshold rule (Bienenstock et al., 1982; Bear, 1995). Although spike counting might seem to be a useful measure of activity, it is a potentially ambiguous measure because low levels of depolarization (that are below threshold for spike generation) and saturating levels of depolarization (that inactivate sodium channels) both result in the same signal: no action potentials. In contrast, membrane potential provides a robust signal that always allows the neuron to regulate synaptic strengths in the proper direction. Regulation based on average membrane potential has the added advantage of integrating across spikes, bursts, and subthreshold activity.

BDNF is known to be an important activity signal for regulating synaptic scaling over part of the dynamic range of quantal amplitude. Although activity-dependent release of BDNF can account for many of the effects of activity, even relatively high concentrations of BDNF (25 ng/ml) are unable to reduce mEPSC amplitudes in pyramidal neurons to below control values (Rutherford et al., 1998). This suggests that there must be some other activity-dependent signal for reducing mEPSC amplitudes when activity rises. Our data suggest that postsynaptic depolarization provides this missing component.

If postsynaptic depolarization can track neuronal activity, then why should quantal amplitude also depend on BDNF? BDNF may act as a diffusible factor that allows neurons to communicate with their neighbors about local levels of activity. This is clearly the case with inhibitory interneurons, which do not produce BDNF but are responsive to it (Isackson et al., 1991; Zafra et al., 1991; Castren et al., 1992; Ghosh et al., 1994; Rutherford et al., 1997; Vicario-Abejon et al., 1998; Huang et al., 1999; Marty et al., 2000; Seil and Drake-Baumann, 2000). One advantage of a diffusible factor is that it can act at different sites within the network to balance both excitation and inhibition (Rutherford et al., 1998). Pyramidal neurons may also respond to the BDNF released by their neighbors. This would allow individual neurons to gauge local activity and regulate themselves accordingly, and may be important for the regulation of functional groups of neurons such as columns in the visual system. Our results suggest that BDNF and postsynaptic depolarization collaborate to scale mEPSC amplitudes over a large dynamic range. This may provide a robust mechanism for the homeostatic regulation of both unit and network level activity.

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