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Activity-dependent scaling of GABAergic synapse strength is regulated by brain-derived neurotrophic factor

Catherine Croft Swanwick [a,*], Namita R. Murthy [b], and Jaideep Kapur [c]

[a] *Neuroscience Graduate Program, University of Virginia, Charlottesville, VA 22908, USA*
[b] *College of Arts and Sciences, University of Virginia, Charlottesville, VA 22908, USA*
[c] *Department of Neurology, University of Virginia, Charlottesville, VA 22908, USA*

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The homeostatic plasticity hypothesis suggests that neuronal activity scales synaptic strength. This study analyzed effects of activity deprivation on GABAergic synapses in cultured hippocampal neurons using patch clamp electrophysiology to record mIPSCs and immunocytochemistry to visualize presynaptic GAD-65 and the γ2 subunit of the $GABA_A$ receptor. When neural activity was blocked for 48 h with tetrodotoxin (TTX, 1 μM), the amplitude of mIPSCs was reduced, corresponding with diminished sizes of GAD-65 puncta and γ2 clusters. Treatment with the NMDA receptor antagonist APV (50 μM) or the AMPA receptor antagonist DNQX (20 μM) mimicked these effects, and co-application of brain-derived neurotrophic factor (BDNF, 100 ng/mL) overcame them. Moreover, when neurons were treated with BDNF alone for 48 h, these effects were reversed via the TrkB receptor. Overall, these results suggest that activity-dependent scaling of inhibitory synaptic strength can be modulated by BDNF/TrkB-mediated signaling.
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Introduction

The homeostatic plasticity hypothesis predicts that neurons stabilize their firing rates in response to long-term changes in neuronal activity by scaling synaptic strength (Turrigiano, 1999). Turrigiano et al. (1998) first demonstrated this form of homeostatic plasticity at excitatory synapses by showing that their strength was increased by the deprivation of neuronal activity and reduced by the enhancement of neuronal activity, as measured by fluctuations in the amplitude of miniature excitatory postsynaptic currents (mEPSCs). This activity-dependent scaling of excitatory synaptic strength has since been shown to be dependent on postsynaptic depolarization (Leslie et al., 2001) and brain-derived neurotrophic factor (BDNF) (Rutherford et al., 1998), a protein whose expression is induced by neuronal activity (Isackson et al., 1991; Lindholm et al., 1994; Berninger et al., 1995) and that has been implicated in multiple forms of synaptic plasticity (for a review, Poo, 2001).

Neuronal activity can also regulate the strength of inhibitory synapses (Kilman et al., 2002), although the signals that mediate this process are unknown. Similar to excitatory synapses, BDNF is an attractive candidate to scale the strength of inhibitory synapses in response to long-term alterations in neuronal activity. Previous evidence has shown that BDNF modulates the level of functional inhibition in an activity-dependent manner by regulating the number of GABAergic interneurons (Rutherford et al., 1997). Neuronal activity also modulates inhibitory synaptogenesis (Seil and Drake-Baumann, 1994; Marty et al., 2000), and this synapse formation is regulated by BDNF (Seil and Drake-Baumann, 2000). However, the long-term effects of BDNF on the strength of inhibitory synapses are unclear.

This study further explored the mechanisms of the activity-dependent scaling of inhibitory synaptic strength and whether this scaling can be modulated by BDNF. Experiments were performed in cultured hippocampal neurons, which provided the advantages of visibility of and accessibility to individual synapses. Presynaptic and postsynaptic elements were visualized using immunocytochemistry, while synaptic function was measured using whole-cell patch clamp electrophysiology to record miniature inhibitory postsynaptic currents (mIPSCs). We found that 48 h of activity deprivation with tetrodotoxin (TTX) reduced the amplitude of mIPSCs, corresponding with decreased sizes of presynaptic and postsynaptic GABAergic markers. These effects could be mimicked by antagonists of NMDA and AMPA receptors. Moreover, these effects were rescued by co-application of BDNF and reversed by treatment with BDNF alone, suggesting that BDNF scales the strength of inhibitory synapses in an activity-dependent manner.

* Corresponding author. NIH/NIDCD, Building 50, Room 4146, 50 South Drive, Bethesda, MD 20892, USA. Fax: +1 301 480 3242.
E-mail address: swanwickc@nidcd.nih.gov (C.C. Swanwick).
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Results

Activity deprivation reduced the strength of GABAergic synapses

To establish the effects of activity deprivation on GABAergic synaptic transmission in these cultured hippocampal neurons, tetrodotoxin (TTX, 1 μM), a Na^+ channel antagonist that blocks action potentials, was applied in the medium of cultured hippocampal neurons from 13 to 15 DIV, a time at which GABAergic synapses have previously been characterized as relatively mature (Swanwick et al., in press). The function of GABAergic synapses was analyzed by whole-cell patch clamp electrophysiology to record miniature inhibitory postsynaptic currents (mIPSCs). In a neuron deprived of activity for 48 h, the strength of GABAergic synaptic transmission was diminished as compared to a control neuron cultured at the same time but not subjected to activity deprivation (Fig. 1). The amplitude of the averaged mIPSC trace recorded from activity-deprived neuron was smaller than that recorded from control neuron (Fig. 1A). The peak of the mIPSC amplitude distribution histogram recorded from an activity-deprived neuron was shifted leftward, towards smaller values compared to the histogram from a control neuron (Fig. 1B). When a group of activity-deprived neurons were compared to control neurons from parallel cultures, the average median amplitude of mIPSCs recorded from activity-deprived neurons was approximately 25% lower than that from control neurons (Table 1).

Activity deprivation did not affect the frequency of mIPSCs. One-minute traces from a control and an activity-deprived neuron showed a similar number of events, despite smaller amplitudes of activity-deprived neurons (Fig. 1C). Cumulative probability plots from an activity-deprived neuron and control demonstrated that approximately the same fraction of events from both conditions had similar inter-event intervals (Fig. 1D). These findings were confirmed with groups of activity-deprived and control neurons in which mean mIPSC frequency was similar (0.4 ± 0.1 Hz in 7 activity-deprived neurons and 0.2 ± 0.05 Hz in 6 control neurons, $P = 0.2$).

To visualize corresponding synaptic changes that occurred due to activity deprivation, double label immunocytochemistry was performed to analyze synapses between GABAergic interneurons and pyramidal neurons using the 65 kDa isoform of glutamic acid decarboxylase (GAD-65) as a presynaptic marker and the γ2 subunit of the $GABA_A$ receptor as a postsynaptic marker (Fig. 2). Similar to the descriptions of GAD-65 immunoreactivity in these cultures provided in detail in the past (Swanwick et al., 2004), large discrete GAD-65 puncta were distributed widely throughout the neuronal processes of control pyramidal neurons (Figs. 2A, C). In

Table 1
Measurements of mIPSC amplitude

mIPSC amplitude (pA)		
TTX	43.8 ± 1.6 (n = 6)	
Control	58.9 ± 3.6 (n = 7)	*P < 0.005
APV	49.9 ± 2.7 (n = 14)	
Control	60.1 ± 3.1 (n = 13)	*P < 0.05
DNQX	47.7 ± 2.7 (n = 14)	
Control	60.1 ± 3.1 (n = 13)	*P < 0.05
TTX + BDNF	45.5 ± 3.6 (n = 5)	
Control	45.2 ± 3.8 (n = 6)	P = 0.96
BDNF	62.6 ± 3.7 (n = 9)	
Control	50.1 ± 1.8 (n = 9)	*P < 0.01
BDNF + TrkB-Fc	46.2 ± 4.3 (n = 5)	
Control	47.7 ± 2.8 (n = 5)	P = 0.8

Due to variability between cultures, all treatments performed with parallel controls (n = neurons).

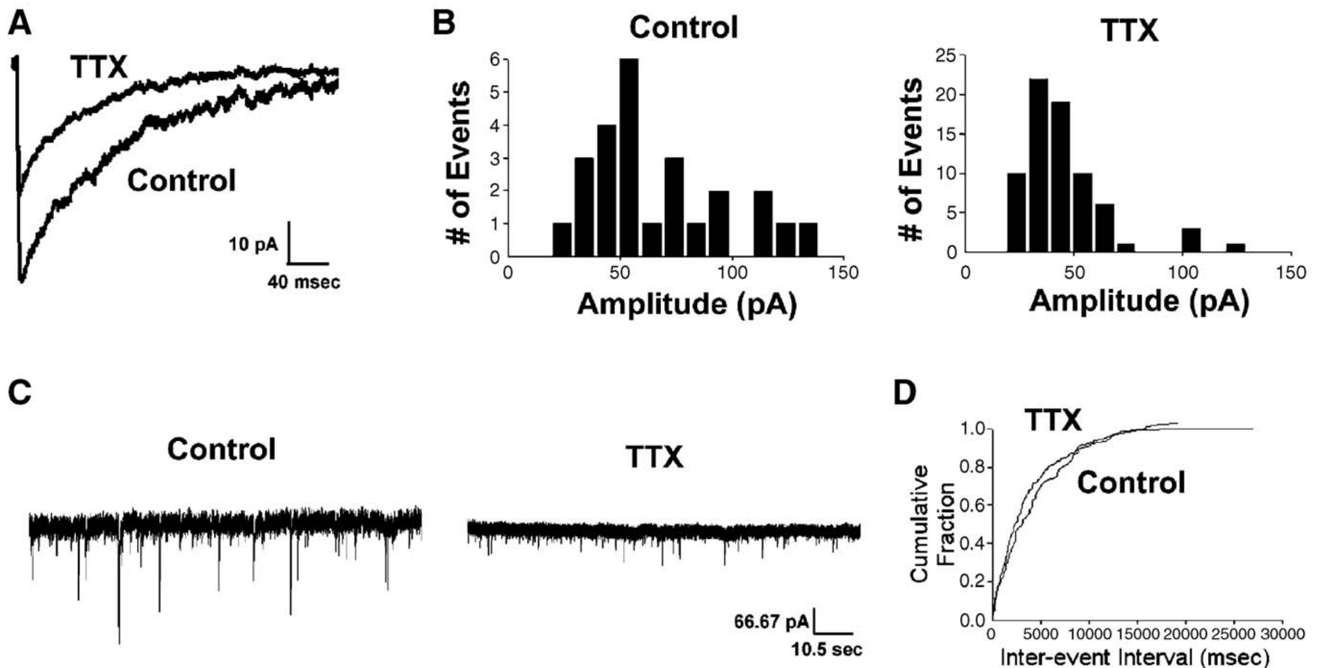

Fig. 1. Deprivation of neuronal activity with TTX altered mIPSC kinetics. (A) Average traces of mIPSCs from representative TTX-treated and untreated neurons, (B) distribution histograms of mIPSC amplitude from representative TTX-treated and untreated neurons, (C) 1-min traces from representative TTX-treated and untreated neurons, (D) cumulative probability plots of mIPSC frequency from representative TTX-treated and untreated neurons. Activity blockade reduced mIPSC amplitude but did not affect mIPSC frequency.

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