

# Synaptic activity modulates presynaptic excitability

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**Synaptic activity modulates synaptic efficacy and is important in learning and development. Here we show that development of excitability in presynaptic motor neurons required synaptic activation of postsynaptic muscle cells. Synaptic blockade broadened action potentials and decreased repetitive firing of presynaptic neurons. Consistent with these findings, synaptic blockade also decreased potassium-current density in the presynaptic cell. Application of neurotrophin-3, but not related neurotrophins, prevented these changes. Recordings from patches of somatic membrane indicated that modifications of presynaptic potassium and sodium currents occurred in a remote, nonsynaptic compartment. Thus, activity-dependent postsynaptic signals modulated presynaptic excitability, potentially regulating transmission at all synapses of the presynaptic cell.**

Electrical activity regulates cell death, neurite outgrowth, neuronal differentiation and synaptic competition in the developing nervous system<sup>1-4</sup>. Although developmental changes in postsynaptic activity during synapse formation have been examined<sup>5</sup>, regulation of excitability in presynaptic cells during development and other forms of plasticity is not well understood. Moreover, it is completely unknown whether activation of postsynaptic cells regulates presynaptic excitability. This lack of understanding is particularly striking in light of the huge body of data on synapse-specific plasticity<sup>6-10</sup>, which cannot explain the spread of synaptic modulation that occurs in many neural systems<sup>11-14</sup>.

Studies suggest that the intrinsic excitability of a single neuron may be regulated according to its history of firing action potentials<sup>15-17</sup>. Real and model neurons can respond actively to experimentally induced alterations in input frequency by changing expression of voltage-dependent ion channels. The mechanism underlying a neuron's ability to calculate how these properties should be altered and what aspect of its activity is important in this calculation are not known. Brain-derived neurotrophic factor (BDNF) is proposed to be involved<sup>18</sup>, although the source of this factor (pre-, post-, auto- or non-synaptic) and the mechanism underlying its regulation of excitability are unclear. Others propose that the neuron averages over time a sequence of 'guesses' regarding how it should respond to given inputs<sup>16</sup>. Alternatively, a neuron may respond to signals relayed from the postsynaptic cell that indicate successful synaptic transmission. Here we report that one aspect of activity important in regulating neuronal excitability is the extent to which such activity induces synaptic transmission and subsequent retrograde signaling from activated targets.

Although synaptic transmission critically depends on the shape and frequency of presynaptic action potentials, nothing is known concerning the role of synaptic activity in their regulation. Here we present evidence that presynaptic action potentials can be modulated by synaptic activation of efferent targets. Control of network properties through feedback regulation of presynaptic

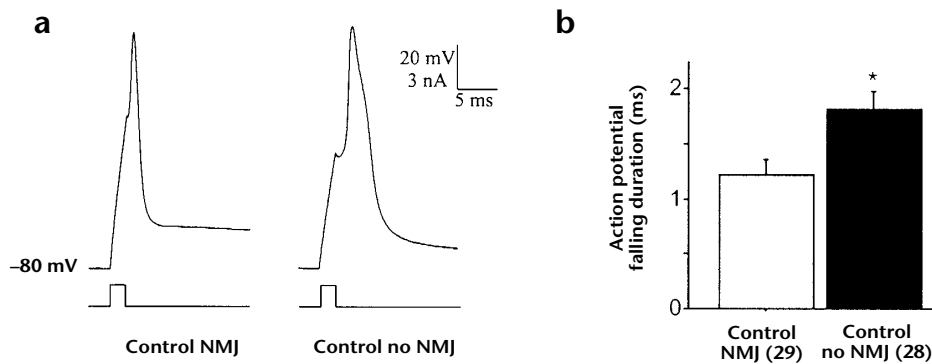
excitability by postsynaptic cells would allow targets to modulate the responsiveness of their presynaptic neurons to afferent inputs. Such regulation could have profound implications for the function of neural networks in development and learning. Aspects of this work have previously appeared in abstract form (T.A.N. and A.B.R., Soc. Neurosci. Abstr. 25, 406.18, 1999).

## RESULTS

We used neuromuscular junctions (NMJs) in *Xenopus* neuron/myocyte co-cultures<sup>19</sup> to investigate the role of the postsynaptic cell in the differentiation of presynaptic excitability. These cultures are essentially homogeneous during the initial differentiation of excitability, when duration of action potentials dramatically decreases<sup>20,21</sup>. However, these cultures contain a variety of neuronal subtypes<sup>22</sup>. We hypothesized that, as neurons mature in these co-cultures, they become heterogeneous with regard to electrical excitability. In addition, we proposed that synaptic activation induces myocytes to release a retrograde signal that alters motor neuron excitability. Myocytes were the ideal postsynaptic cell for this investigation because they are morphologically distinguishable from other cells and contain a variety of factors that affect motor neurons<sup>23-27</sup>. To test our hypotheses, we first compared the excitability of neurons that formed NMJs with excitability of those without NMJs. We then examined neuron excitability following blockade of synaptic activity with the nicotinic acetylcholine receptor blocker  $\alpha$ -bungarotoxin ( $\alpha$ -BgTx) during NMJ formation and differentiation.

## Neuronal properties correlate with synaptic contact

We found that neurons that did not contact muscle (solitary) and those that contacted muscle but did not form an NMJ (no NMJ) were significantly different from cells that formed functional NMJs (Table 1; Figs. 1 and 2). Compared with neurons lacking NMJs (solitary, no NMJ analyzed separately), cells with NMJs had higher membrane capacitance ( $p < 0.0001$ ), hyperpolarized resting potential ( $p < 0.0002$ ), shorter falling phases



**Fig. 1.** Neurons with NMJs had shorter-duration action potentials than other neurons. Traces show sample whole-cell current-clamp recordings of motor neurons with functional NMJs (**a**) or myocyte contact but no NMJ (**b**). Action potentials had significantly shorter falling durations in neurons with NMJs compared with cells lacking NMJs. \*Significantly different from control NMJ.

of the action potential ( $p < 0.04$ ; Fig. 1), shorter refractory periods (compared only with no NMJ;  $p < 0.005$ ; Fig. 2) and increased potassium-current ( $I_K$ ) density (stepped from  $-80$  to  $0$  mV;  $p < 0.04$ ). These findings indicate that neurons that form

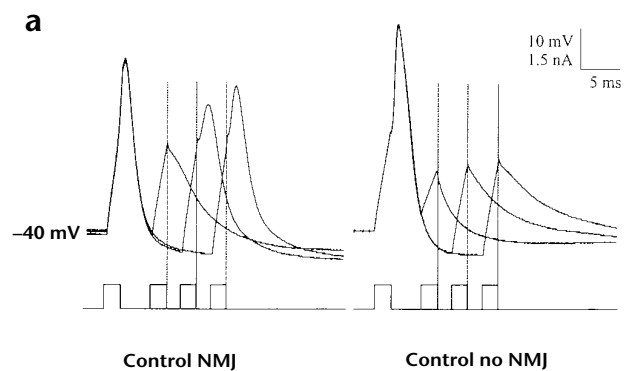
NMJs fundamentally differ from cells that do not contact muscle and from those that contact muscle but do not form functional synapses. We next investigated whether these differences were due to intrinsic, cell-autonomous mechanisms of motor

**Table 1. Properties of neurons vary with synaptic contact and function.**

	Control, with NMJ	Control, no NMJ	Control, solitary	$\alpha$ -BgTx, NMJ	NT-3 + $\alpha$ -BgTx, NMJ
Soma diameter ( $\mu$ m)	23.0 $\pm$ 0.3 <i>n</i> = 97	23.2 $\pm$ 0.3 <i>n</i> = 85	23.2 $\pm$ 0.3 <i>n</i> = 85	22.5 $\pm$ 0.3 <i>n</i> = 136	23.9 $\pm$ 0.4 <i>n</i> = 54
Membrane capacitance (pF)	64 $\pm$ 4 <i>n</i> = 126	30 $\pm$ 1* <i>n</i> = 136	25 $\pm$ 1* <i>n</i> = 29	50 $\pm$ 3* <i>n</i> = 175	47 $\pm$ 5* <i>n</i> = 50
Input resistance (M $\Omega$ )	723 $\pm$ 64 <i>n</i> = 28	836 $\pm$ 132 <i>n</i> = 13	689 $\pm$ 137 <i>n</i> = 6	681 $\pm$ 61 <i>n</i> = 36	646 $\pm$ 75 <i>n</i> = 15
Resting potential (mV)	-51 $\pm$ 2 <i>n</i> = 96	-41 $\pm$ 2* <i>n</i> = 54	-31 $\pm$ 2* <i>n</i> = 12	-43 $\pm$ 1* <i>n</i> = 111	-45 $\pm$ 2* <i>n</i> = 36
Mean rheobase (nA)					
at -40 mV	0.65 $\pm$ 0.03 <i>n</i> = 28	0.60 $\pm$ 0.04 <i>n</i> = 27	0.54 $\pm$ 0.05 <i>n</i> = 9	0.80 $\pm$ 0.05* <i>n</i> = 45	0.68 $\pm$ 0.04 <i>n</i> = 13
at -80 mV	1.64 $\pm$ 0.05 <i>n</i> = 30	1.52 $\pm$ 0.04 <i>n</i> = 28	1.55 $\pm$ 0.07 <i>n</i> = 10	1.57 $\pm$ 0.03 <i>n</i> = 54	1.48 $\pm$ 0.05* <i>n</i> = 20
Action potential falling duration					
at -40 mV (ms)	1.2 $\pm$ 0.1 <i>n</i> = 28	1.7 $\pm$ 0.2* <i>n</i> = 27	1.7 $\pm$ 0.2* <i>n</i> = 9	1.7 $\pm$ 0.1* <i>n</i> = 45	1.2 $\pm$ 0.1 <i>n</i> = 13
at -80 mV (ms)	1.2 $\pm$ 0.1 <i>n</i> = 29	1.8 $\pm$ 0.2* <i>n</i> = 28	1.8 $\pm$ 0.3* <i>n</i> = 10	2.1 $\pm$ 0.2* <i>n</i> = 54	1.1 $\pm$ 0.1 <i>n</i> = 20
Action potential amplitude					
at -40 mV (mV)	58 $\pm$ 2 <i>n</i> = 28	58 $\pm$ 3 <i>n</i> = 27	49 $\pm$ 4* <i>n</i> = 9	53 $\pm$ 2* <i>n</i> = 45	53 $\pm$ 2 <i>n</i> = 13
at -80 mV (mV)	102 $\pm$ 1 <i>n</i> = 29	103 $\pm$ 2 <i>n</i> = 28	99 $\pm$ 2 <i>n</i> = 10	96 $\pm$ 1* <i>n</i> = 54	96 $\pm$ 2* <i>n</i> = 20
Refractory period (ms)	13.2 $\pm$ 1.0 <i>n</i> = 27	20.4 $\pm$ 2.7* <i>n</i> = 15	17.0 $\pm$ 2.1 <i>n</i> = 6	17.7 $\pm$ 1.5* <i>n</i> = 32	12.2 $\pm$ 1.3 <i>n</i> = 10
$I_K$ density at 0 mV (step from -80 mV; A/F)	89 $\pm$ 13 <i>n</i> = 17	50 $\pm$ 9* <i>n</i> = 17	51 $\pm$ 9* <i>n</i> = 11	59 $\pm$ 7* <i>n</i> = 30	109 $\pm$ 15 <i>n</i> = 17

Abbreviations: NMJ, neuromuscular junction;  $\alpha$ -BgTx,  $\alpha$ -bungarotoxin; NT3, neurotrophin-3. \*Significantly different from control NMJ.

**Fig. 2.** Neurons with NMJs showed enhanced ability to fire repetitively. (a) Refractory period was measured with a twin-pulse protocol. Three superimposed episodes from a sample cell are shown for each condition. Dotted lines indicate the end of the second current pulse for each episode. (b) Refractory period (minimum time between two pulses that each evoke an action potential) was shorter in neurons with NMJs compared with those lacking NMJs. \*Significantly different from control NMJ.



neurons or whether signals from the postsynaptic muscle cell induce a specific set of changes in innervating neurons.

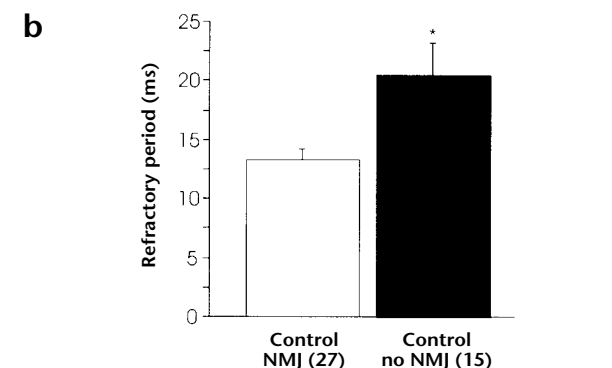
### Synaptic blockade alters motor neuron excitability

Chronic blockade of the NMJ with rhodamine-conjugated  $\alpha$ -BgTx changed passive (Table 1) and active (Table 1; Figs. 3–7) electrophysiological properties of motor neurons. Compared with untreated controls, motor neuron resting potential was significantly depolarized by chronic  $\alpha$ -BgTx treatment ( $p < 0.0001$ ). In addition, synaptic blockade with  $\alpha$ -BgTx increased the duration ( $p < 0.006$ ; Fig. 3) and decreased the amplitude ( $p < 0.003$ ) of the action potential. Similar changes in resting potential and action potentials are observed during NMJ formation and differentiation *in vivo*<sup>28,29</sup>. Thus, synaptic blockade seems to prevent normal electrophysiological development of motor neurons.

Capacitance of motor neurons was also decreased by  $\alpha$ -BgTx treatment ( $p < 0.0001$ ; Table 1). Decreased capacitance seemed to reflect changes in the process and/or synaptic terminal, as soma diameter was unaffected. NMJ blockade with curare modestly reduces the number of synapses formed at the earliest stages examined<sup>30</sup>. Because we examined the initial stages of NMJ formation, our results parallel these previous findings.

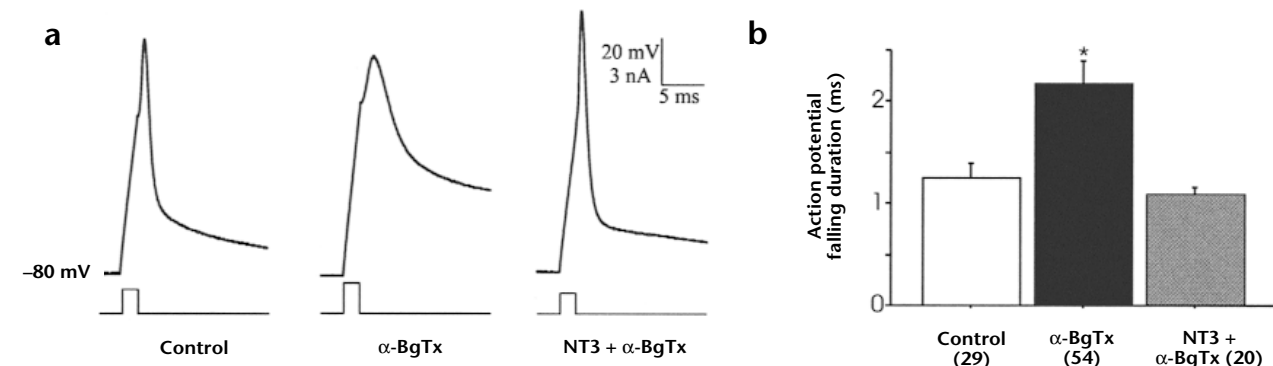
### NT-3 prevents effects of synaptic blockade

If synaptic blockade prevents release of a retrograde signal and subsequent differentiation of presynaptic excitability, then co-application of the putative signal to  $\alpha$ -BgTx-treated junctions should overcome the effects of synaptic inactivity. Because this neurotrophin alters presynaptic secretion<sup>23,25,26,31</sup> and is upregulated by myocyte depolarization<sup>31</sup>, NT-3 was the first candidate retrograde signal investigated. Co-application of NT-3 blocked effects of  $\alpha$ -BgTx on resting potential (versus  $\alpha$ -BgTx alone;  $p < 0.002$ ) and action potential falling duration (versus  $\alpha$ -BgTx alone;  $p < 0.008$ ; Table 1; Fig. 3) but not capacitance or action potential amplitude. Thus NT-3, a potential retrograde signal released from myocytes, alters a specific subset of presynaptic electrophysiological properties.



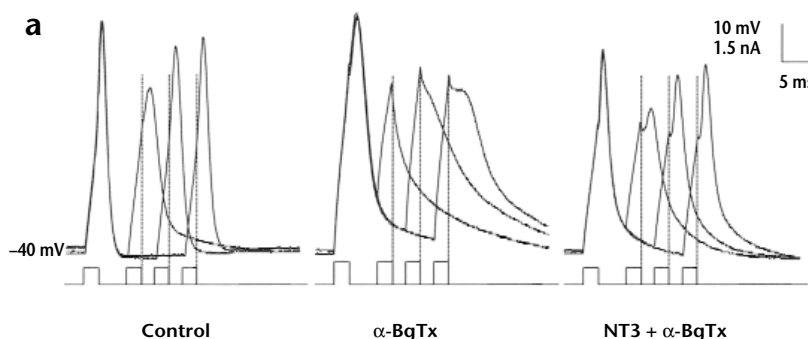
The increase in action potential falling duration resulting from  $\alpha$ -BgTx-induced synaptic blockade (Fig. 3) revealed a regulatory mechanism that may explain previous reports of synaptic depression upon caged-calcium release in the myocyte<sup>14,32</sup>. During normal NMJ development without  $\alpha$ -BgTx or with the putative retrograde signal NT-3 in addition to  $\alpha$ -BgTx, the spike substantially narrows, decreasing the amount of transmitter released per action potential. Upregulation of calcium in the myocyte increases NT-3 transcription<sup>31</sup> and, presumably, release<sup>26,31</sup>. Thus, caged-calcium release in the myocyte may induce release of NT-3, which would tend to narrow the presynaptic action potential and seem to produce synaptic depression.

Ability of motor neurons to fire repetitively increases during development<sup>33,34</sup>. Spike narrowing may allow neurons to recover from action potentials faster and thus enhance repetitive firing. Consistent with this hypothesis, we found that synaptic blockade



**Fig. 3.** Chronic synaptic blockade with  $\alpha$ -BgTx induced broadening of action potentials that was prevented with neurotrophin-3. (a) Whole-cell current-clamp recordings of motor neurons with functional NMJs following chronic  $\alpha$ -BgTx revealed dramatic broadening of action potentials that was prevented with simultaneous application of NT-3. (b) Action potential broadening induced by  $\alpha$ -BgTx was due to an increase in the falling duration. NT-3 prevented the  $\alpha$ -BgTx-induced increase in falling duration (indistinguishable from controls). \*Significantly different from controls.

**Fig. 4.** Synaptic blockade reduced the ability to fire repetitively, whereas co-application of NT-3 increased repetitive-firing capacity to control levels. (a) Refractory period was measured with a twin-pulse protocol. Three episodes from a sample cell are superimposed for each condition. The dotted lines indicate the end of the second current pulse for each episode. Note that action potentials overshoot the stimulus artifact in control and NT-3 +  $\alpha$ -BgtTx conditions. (b) Refractory period was increased by  $\alpha$ -BgtTx but unchanged by co-applied  $\alpha$ -BgtTx and NT-3. \*Significantly different from controls.



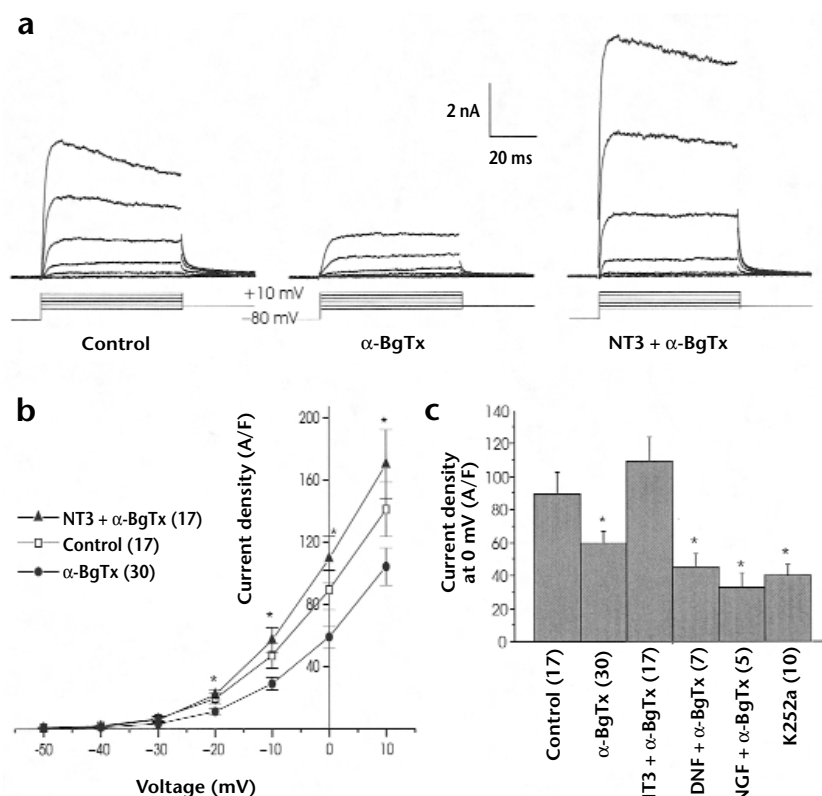
with  $\alpha$ -BgtTx, which produces spike broadening, does indeed decrease capacity for repetitive firing as measured by an increase in refractory period (Fig. 4,  $p < 0.02$ ). NT-3, which prevents  $\alpha$ -BgtTx-induced spike broadening, also prevents the increase in refractory period (versus  $\alpha$ -BgtTx;  $p < 0.05$ ). These changes in repetitive firing globally regulate neuronal output. Thus, a change initiated by postsynaptic feedback at one synapse may modulate the efficacy of all synapses of a given presynaptic neuron.

#### NT-3 effects on $I_K$ are specific

Changes in action potential duration suggest changes in  $I_K$ , which is the ionic current primarily responsible for repolarization during an action potential. We found that chronic synaptic blockade with  $\alpha$ -BgtTx decreased  $I_K$  density in motor neurons (measured from  $-20$  to  $0$  mV;  $p < 0.04$ ; Fig. 5). Moreover, NT-3 prevented this decrease in current density (versus  $\alpha$ -BgtTx,  $-20$  to  $+10$  mV;  $p < 0.007$ ).

To further test the hypothesis that  $I_K$  was modulated by neurotrophins, we exposed cultures to K252a ( $0.2 \mu\text{M}$ ), which, at this concentration, is a relatively specific inhibitor for the neurotrophin-receptor protein-tyrosine kinases (Trks)<sup>25,35</sup>. In the absence of synaptic blockade, K252a decreased  $I_K$  compared with untreated controls ( $p < 0.01$ ; Fig. 5c). This further indicates the role of neurotrophins in the modulation of  $I_K$ .

To investigate potential non-specific neurotrophic effects of NT-3, we attempted to prevent  $\alpha$ -BgtTx effects on  $I_K$  with two related neurotrophins, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). Neither of these factors was effective in preventing  $\alpha$ -BgtTx-induced changes in  $I_K$  (NGF versus control at  $0$  mV,  $p < 0.04$ ; BDNF,  $p < 0.05$ ; Fig. 5c). Thus, NT-3 was the only neurotrophin tested that was capable of reversing the effects of synaptic blockade on  $I_K$ .



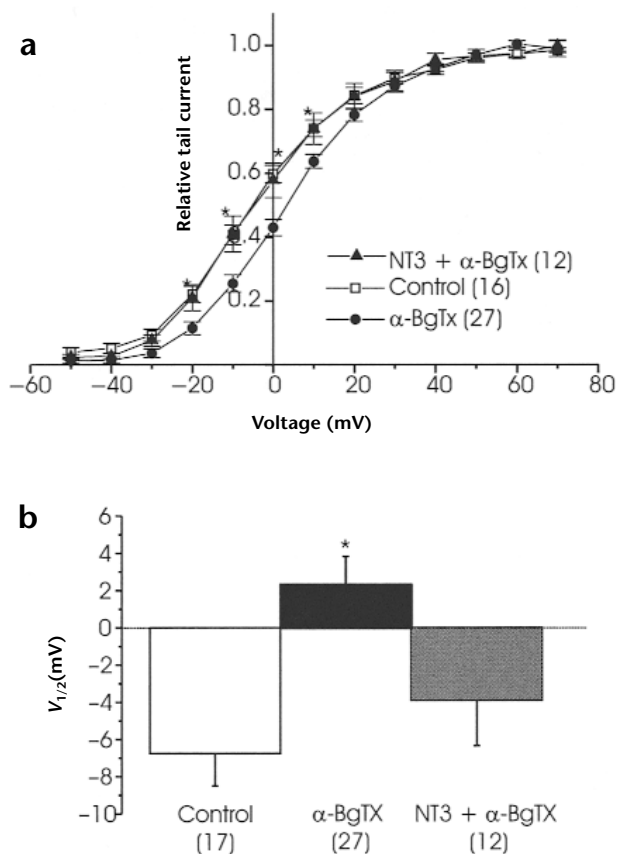
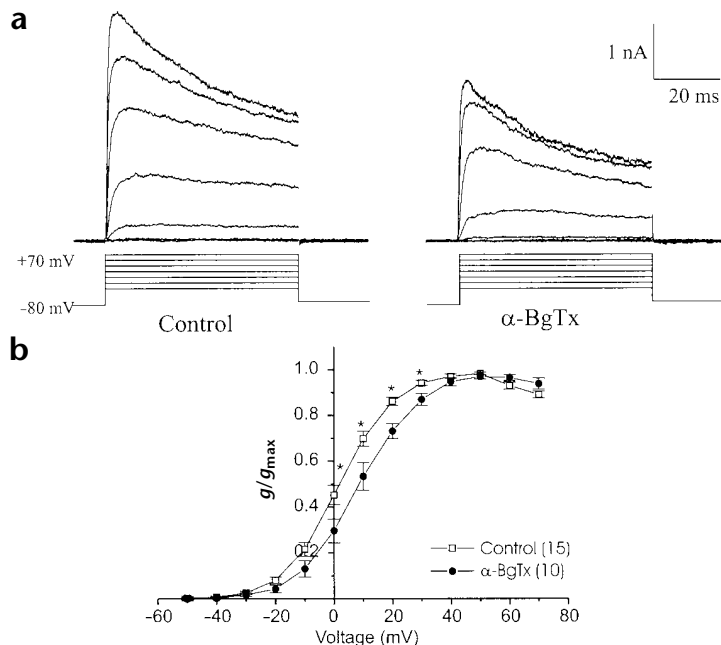
**Fig. 5.** Decreases in delayed-rectifier  $I_K$  density induced by synaptic blockade were prevented by co-application of NT-3, but not other neurotrophins. (a) Sample whole-cell patch-clamp recordings of  $I_K$  in motor neurons with NMJs revealed a substantial decrease in  $I_K$  induced by chronic  $\alpha$ -BgtTx that was prevented by NT-3. (b) Current-density-voltage plots show that at several voltages  $\alpha$ -BgtTx induced a current decrease that was prevented by NT-3. (c) Although prevented by NT-3, decreases in current density induced by  $\alpha$ -BgtTx were unaffected by BDNF or NGF. K252a alone decreased current densities to levels comparable to those of motor neurons treated with  $\alpha$ -BgtTx alone. \*Significantly different from controls.

**Fig. 6.**  $\alpha$ -BgTx and NT-3 shifted the voltage dependence of activation of  $I_K$ . (a) The relative conductance–voltage curve for motor neuron  $I_K$  shifted to more depolarized values with  $\alpha$ -BgTx-induced synaptic blockade. This shift was prevented by NT-3. (b) Boltzmann fits of  $G$ – $V$  plots revealed a significant change in  $V_{1/2}$  with  $\alpha$ -BgTx that was prevented by co-application of NT-3.

The observed changes in  $I_K$  density could be due to changes in the number of channels expressed, the properties of channels or both. If the number of channels in the plasma membrane changed, the maximum conductance (and, thus, maximum current density) of the neuron should also change. We found that the number of channels, as measured by maximum whole-cell tail-current density, was not significantly different across groups (control,  $56 \pm 6.2$  A/F,  $n = 16$ ;  $\alpha$ -BgTx,  $44 \pm 3.9$  A/F,  $n = 27$ ; NT-3 +  $\alpha$ -BgTx,  $53 \pm 6.4$  A/F,  $n = 12$ ). We next examined changes in channel properties through comparison of normalized conductance–voltage ( $G$ – $V$ ) relationships for control,  $\alpha$ -BgTx and NT-3 +  $\alpha$ -BgTx groups. Channel properties changed in response to  $\alpha$ -BgTx, as indicated by a shift in the  $G$ – $V$  curve compared with controls (measured from  $-20$  to  $+10$  mV;  $p < 0.02$ ; Fig. 6a). Moreover, NT-3 prevented this shift (versus  $\alpha$ -BgTx,  $-10$  to  $+10$  mV;  $p < 0.05$ ). To determine whether this shift was due to a change in the voltage that gave a half-maximal conductance ( $V_{1/2}$ ) and/or a change in the slope ( $k$ ) of the  $G$ – $V$  curve, we fit data from each neuron with the Boltzmann equation. Slope values were not significantly different (control,  $15 \pm 1.2$  mV;  $\alpha$ -BgTx,  $13 \pm 0.6$  mV; NT-3 +  $\alpha$ -BgTx,  $14 \pm 1.2$  mV). In contrast, we found that  $V_{1/2}$  was shifted to a more depolarized potential by  $\alpha$ -BgTx ( $p < 0.0005$ ; Fig. 6b) and that this shift was prevented by co-application of NT-3 (versus  $\alpha$ -BgTx;  $p < 0.03$ ). The finding that  $\alpha$ -BgTx and NT-3 both affected the same  $I_K$  parameters further indicates that they act upon the same pathway.

#### Synaptic blockade alters channels in a cell-wide manner

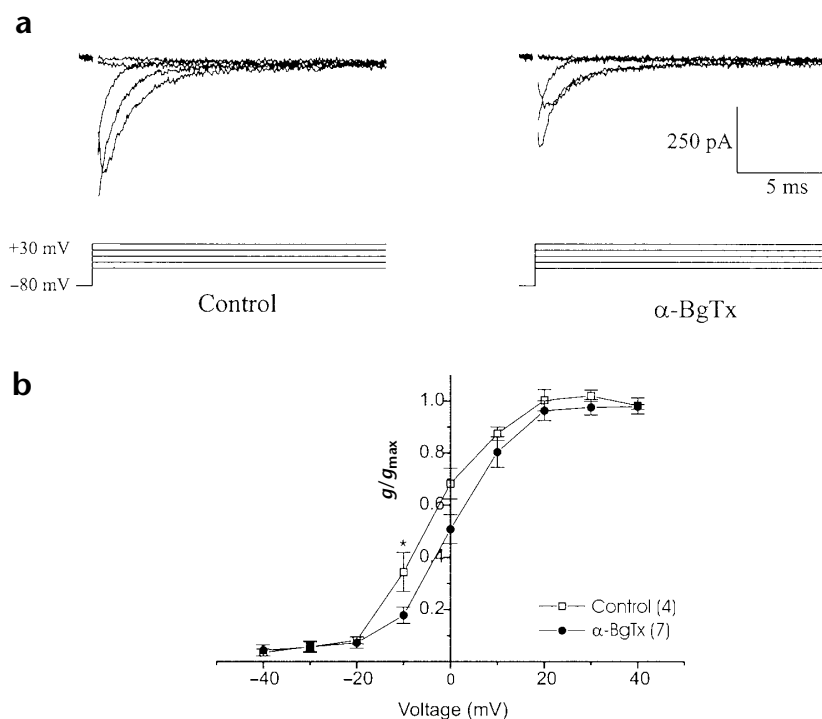
To determine whether the effects of synaptic blockade extend beyond the synapse, we examined  $I_K$  properties in outside-out macropatches pulled from somata. We found that, comparable



to whole-cell experiments,  $\alpha$ -BgTx shifted the  $G$ – $V$  curve in macropatches to more depolarized potentials (measured from  $0$  to  $+30$  mV;  $p < 0.03$ ; Fig. 7b). In these experiments, we also noted an overall depolarizing shift in both groups compared to whole-cell data, most likely due to a difference in C-type inactivation between excised membrane patches and whole-cell patches<sup>36,37</sup>. The finding that macropatches pulled from the soma show an effect initiated at the synapse suggests that this particular postsynaptic effect on presynaptic differentiation is cell-wide.

We next asked whether synaptic blockade might globally affect other ionic currents. One candidate was sodium current ( $I_{Na}$ ), as the observed decrease in action potential amplitude with  $\alpha$ -BgTx (Table 1) suggested modulation of this current. In macropatches pulled from the soma, we found that the voltage dependence of activation of  $I_{Na}$  was shifted to more depolarized potentials in neurons after synaptic blockade with  $\alpha$ -BgTx (measured at  $-10$  mV;  $p < 0.04$ ; Fig. 8). Whole-cell data obtained with curare blockade of the NMJ yielded similar results (T.A.N. and A.B.R., unpublished observations).

**Fig. 7.**  $G$ – $V$  relation of  $I_K$  shifted in response to  $\alpha$ -BgTx in macropatches pulled from the somata of neurons as in whole-cell recordings. (a) Sample recordings of  $I_K$  in macropatches pulled from motor neuron somata. (b)  $G$ – $V$  plots of  $I_K$  from control and  $\alpha$ -BgTx-treated motor neurons. \*Significantly different from controls.



**Fig. 8.** The  $G$ - $V$  relation of  $I_{Na}$  in macropatches pulled from the neuronal soma showed an  $\alpha$ -BgTx-induced shift. (a) Sample recordings of  $I_{Na}$  in macropatches pulled from motor neuron somata. (b)  $G$ - $V$  plots of  $I_{Na}$  in macropatches from control and  $\alpha$ -BgTx-treated motor neurons. \*Significantly different from controls.

ciently stimulates a given myocyte would become more excitable and, thus, more successfully activate other contacted myocytes. This type of regulation may be particularly important during target-dependent motor neuron death, during which neurons seem to compete for limited muscle-derived factors<sup>42</sup>.

Studies suggest that neurons alter their excitability in response to firing history<sup>15,16,43</sup>. A potential mechanism for this phenomenon is provided by our data, which indicate that a neuron changes its excitability based upon its recent success at synaptic output, which depends on action potentials. Cell-wide activity-dependent modulation of presynaptic excitability by postsynaptic targets would provide

a feedback mechanism through which the postsynaptic cell can modulate not only a given presynaptic neuron's responsiveness to inputs, but also its output efficiency across all synapses, thus altering output to other postsynaptic cells. This type of regulation would have important effects on neural processing. Blocking transmission at only a subset of synapses would test this hypothesis and might yield further information on potential underlying mechanisms.

## METHODS

**Animals and cell culture.** *Xenopus laevis* embryos were produced by standard *in-vitro* fertilization techniques<sup>44</sup> and staged according to Nieuwkoop and Faber<sup>45</sup>. Neuron/myocyte cultures were prepared as described<sup>20</sup>. The dorsal-posterior region, which contained the presumptive spinal cord and surrounding somites, was removed from neural tube stage (stage 17–19) embryos and dissociated in divalent cation-free medium (116.7 mM NaCl, 0.67 mM KCl, 0.4 mM EDTA and 4.6 mM Tris at pH 7.8). Motor neurons from these embryonic stages have never contacted muscle<sup>46</sup>. Co-cultures were plated on plastic dishes in a completely defined medium (116.7 mM NaCl, 0.67 mM KCl, 1.31 mM MgSO<sub>4</sub>, 10 mM CaCl<sub>2</sub> and 4.6 mM Tris at pH 7.8) and recorded 15–26 h after plating. Immediately after plating, 50 ng per ml NT-3, BDNF, NGF (Alomone, Jerusalem, Israel), 0.2  $\mu$ M K252a (Calbiochem, San Diego, California) and rhodaminated  $\alpha$ -BgTx (2  $\mu$ g per ml; Molecular Probes, Eugene, Oregon) were added. Cultures were thus exposed to these reagents for 15–26 h before recording.

**Electrophysiology.** Current- and voltage-clamp records were obtained by whole-cell patch clamp<sup>47</sup> with 1–3 M $\Omega$  electrodes. In control cultures, motor neurons with NMJs were identified by their ability to cause myocyte contraction upon stimulation with 6 voltage steps (60 ms each, in 10-mV increments from +10 to +60 mV) from a holding potential of -40 mV. Neurons that did not cause contraction were placed in the no-NMJ group. Cells that did not contact muscle were placed in the solitary group. Only neurons that clearly had no contact with other neurons were used. In rhodaminated- $\alpha$ -BgTx-treated cultures, neurons with NMJs were identified by their ability to cause postsynaptic clustering of nicotinic acetylcholine receptors (AChRs), observed as patches of rhodamine staining under the presynaptic terminal<sup>46</sup>. Synaptic blockade was confirmed

## DISCUSSION

Our data indicate that motor neuron excitability is functionally regulated by postsynaptic target cells during development. This is by no means a passive feedback circuit, as postsynaptic myocytes must be synaptically activated for modulation of excitability to occur. In contrast to well-described synapse-specific plasticity<sup>6–10</sup>, the changes in excitability we report are global. Global regulation of excitability that actively depends on feedback cues from target cells represents a mechanism whereby neural systems may self-organize and function. This mechanism would allow presynaptic neurons to modify all outputs simultaneously in response to signals from postsynaptic cells.

Interestingly, the process we describe may underlie the spread of retrograde modulation of presynaptic transmission reported in the *Xenopus* NMJ culture system<sup>14</sup>. However, this group investigated short-term modulation, whereas we investigated long-term effects. Thus, the studies cannot be directly compared. Further examination of the temporal aspect of neurotrophin efficacy should reveal another level of complexity in the neurotrophin–excitability relationship.

Although neurotrophins alter synaptic efficacy and neuronal excitability<sup>38–40</sup>, the mechanisms through which physiological control of neurotrophic effects is functionally achieved are not well understood<sup>40</sup>. Our data indicate that synaptic activity critically regulates retrograde signaling between myocyte and neuron, and that the activity-dependent retrograde signal may be a specific neurotrophin, NT-3.

In the context of neuromuscular development, cell-wide changes in excitability due to postsynaptic activity may provide the soma and all synapses of the motor neuron with a measure of its success at competing in the periphery. Myocytes subject to polyneuronal innervation may differentially regulate the excitability of motor neurons based on synaptic efficacy, as previous studies suggest that retrograde signaling is localized to the site of synaptic activation<sup>32</sup> and that presynaptic depolarization facilitates neurotrophin-induced effects<sup>41</sup>. A motor neuron that effi-

by absence of myocyte contraction under motor neuron stimulation. To verify that differences in method of identifying motor neurons in control versus  $\alpha$ -BgTx-treated cultures did not affect our results, we repeated experiments with the AChR blocker curare, which could be washed out, thus allowing us to identify motor neurons by ability to produce myocyte contraction. Under these conditions, results were similar to those obtained with  $\alpha$ -BgTx (T.A.N. & A.B.R., *Soc. Neurosci. Abstr.* 24, 122.3, 1998).

Although previous studies conflict on whether AChRs on motor neurons are  $\alpha$ -BgTx-sensitive<sup>48,49</sup>, both studies used neurons older than those examined here. In contrast, neurons of the same age showed no acetylcholine response<sup>50</sup>. Because the motor neurons we examined did not express functional AChRs, they were probably not directly affected by  $\alpha$ -BgTx.

The pipet solution for current clamp and potassium current records contained 104 mM KCl, 3 mM MgCl<sub>2</sub> and 10 mM HEPES at pH 7.4. For current-clamp recording, the bath solution contained 125 mM NaCl, 3 mM KCl, 10 mM CaCl<sub>2</sub> and 5 mM HEPES at pH 7.4. Membrane potential was forced to -40 and -80 mV with injected current. Action potentials were initiated by 2-ms current injections, increased in 7 increments of 0.25 nA from 0.5 to 2.0 nA, with 5-s recovery periods between stimuli. Only action potentials that overshoot the stimulus artifact by at least 2.5 mV were included in analyses. Falling duration was measured as the time from the peak of the action potential to half-peak. The minimum current that could reliably evoke action potentials with a 5-s recovery period was used in a twin-pulse examination of relative refractory period at -40 mV. The interval between the two pulses was varied from 2 to 32 ms. Relative refractory period was defined as the shortest interval between pulses that allowed an action potential in response to the second pulse. Only depolarizations that overshoot the stimulus artifact by at least 2.5 mV were considered action potentials. Input resistance was determined by a 500-ms current pulse of -0.01 or -0.1 nA. Capacitance was determined from voltage-clamp recordings. The membrane potential was held at -40 mV and stepped to -90 mV in 6 steps of 10 mV. The area of the uncompensated capacitive current (charge) was plotted against voltage and fit with a linear regression. The slope of this line gave the capacitance. All data were analyzed with a two-tailed Student's *t*-test and reported as mean  $\pm$  standard error. Data were judged significant if *p* < 0.05. Numbers of observations are indicated in parentheses.

For *I<sub>K</sub>* records, the bath solution contained 80 mM NaCl, 3 mM KCl, 5 mM MgCl<sub>2</sub>, 10 mM CoCl<sub>2</sub> and 5 mM HEPES at pH 7.4. Tetrodotoxin (1  $\mu$ M; Calbiochem) was added to block voltage-gated sodium currents. Neurons were held at -80 mV and stepped from -50 to +100 mV in 16 steps (10 mV, 60 ms) with a 5-s recovery period between steps. To minimize the possibility of inadequate space clamp, cells with capacitances above 35 pF were not used for *I<sub>K</sub>* analyses; this cut-off was selected because current-capacitance plots show a decline in measured current density when capacitance is  $\geq$  40 pF (data not shown). The  $\alpha$ -BgTx-induced *G-V* shift found in whole-cell recordings was also found in macropatches, suggesting that poor control of membrane voltage in the whole-cell recordings was not responsible for the altered *G-V* relationship. Records were filtered at 5 kHz and sampled at 25 kHz with pClamp 6.2 (Axon, Foster City, California). For current-density measurements, mean steady-state current was measured 50–60 ms after the initiation of the voltage step. Tail current was measured 0.6 ms after the end of the pulse, which was beyond the compensated capacitive artifact for all neurons included. Relative tail current was used as a measure of whole-cell conductance because this measure obviates considerations of driving force. Calculations of conductance from steady-state current yielded similar results (data not shown). For macropatch recordings, the signal-to-noise ratio was not as high, and thus conductance was calculated from the steady-state current. Maximum tail-current density was used as a measure of channel number because the headstage of our amplifier (Axopatch 1C, Axon) was saturated by the large steady-state currents in control NMJs, and because this measure removes driving-force considerations. Inherent in the use of current density as a measure of channel number is the assumption that single-channel conductance does not change. For *I<sub>Na</sub>* records, the bath solution contained 105 mM NaCl, 20 mM TEA-Cl, 3 mM KCl, 10 mM CoCl<sub>2</sub> and 5 mM HEPES at pH 7.4.

The pipet solution contained 95 mM CsCl, 5 mM NaCl, 0.64 mM CaCl<sub>2</sub>, 2 mM EGTA and 10 mM HEPES at pH 7.4. Neurons were held at -80 mV and stepped from -40 to +40 mV in 9 steps (10 mV, 20 ms) with a 5-s recovery period between steps.

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