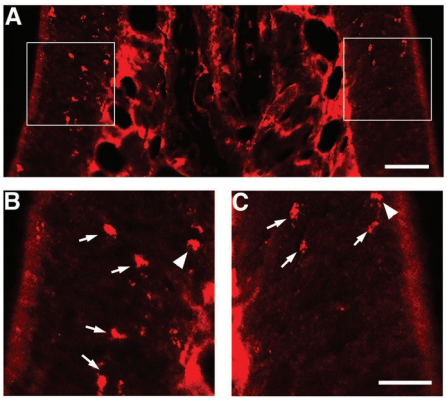

Fig. 5. Prolonged survival of OSNs in naris-closed animals. (**A**) An example of BrdU-positive cells in the septum detected by immunostaining at PD20. The animal was injected with BrdU, and the left naris was closed at PD10. (**B** and **C**) Enlarged views of left (closed side) and right (open side) boxes in (A), respectively. BrdU-immunopositive profiles, indicated by arrows in the OSN layer, were of the type included in our analysis. BrdU profiles indicated by arrowheads in the basal cell layer (B) and supporting cell layer (C) were excluded from our analysis. Scale bar in (A), 50 μm; in (C) for (B) and (C), 25 μm.

OSNs could underlie the preservation of heterogeneous glomeruli in the bulb[X].

Taken together, our results establish four principles of olfactory system development. First, the absence of sensory activity perturbs glomerular maturation; second, there is a sensitive period during which activity influences the maturation of glomerular organization; third, sensitive periods occur asynchronously, with the specific timing dependent on the OR; fourth, glomeruli may be heterogeneously innervated by more than one OSN population early in development or in the absence of activity.

Molecular determinants are necessary for the initial formation of glomeruli. Evidence for activity-independent formation of the glomerular array has emerged from studies using genetic ablations of components in the transduction pathway to reduce odor-driven activity. However, these manipulations typically leave one or more residual processes intact. In contrast, naris closure reduces all activity associated with normal passage of air through the nasal cavity—biochemical, electrical, and toxic—providing a more revealing challenge to the system. The increased survival of OSNs on the closed side suggests that the dynamics of cell turnover in the epithelium provides a mechanism for the refinement of OSN axon projections. A decrease in OSN turnover, such as occurs in an epithelium that is deprived of activity, may provide less opportunity for the removal of cells that project axons to heterogeneous glomeruli. Unlike in the visual system (*30*), selective pruning of axon branches is less likely to operate in the olfactory system because axons do not have interglomerular axon collaterals. Further, we deem it unlikely that coordinated activity evoked by natural odors, which are typically complex mixtures of odorants, over relatively short preadult stages would provide enough discrimination as the driving force to untangle 1000 populations of axons into discrete glomeruli. A more complex chain of events may underlie the dynamic effects observed here.

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31. We thank K. Yazdanbakhsh and C. Kaliszewski for technical support, E. Ruthazer and H. Cline for discussion and comments on the manuscript, and members of the Firestein, Greer, and Mombaerts labs for valuable input. Supported by grants from the National Institute on Deafness and Other Communication Disorders.

Supporting Online Material
www.sciencemag.org/cgi/content/full/1093468/DC1
Materials and Methods
References

10 November 2003; accepted 11 May 2004
Published online 3 June 2004;
10.1126/science.1093468
Include this information when citing this paper.

Regulation of Dendritic Protein Synthesis by Miniature Synaptic Events

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We examined dendritic protein synthesis after a prolonged blockade of action potentials alone and after a blockade of both action potentials and miniature excitatory synaptic events (minis). Relative to controls, dendrites exposed to a prolonged blockade of action potentials showed diminished protein synthesis. Dendrites in which both action potentials and minis were blocked showed enhanced protein synthesis, suggesting that minis inhibit dendritic translation. When minis were acutely blocked or stimulated, an immediate increase or decrease, respectively, in dendritic translation was observed. Taken together, these results reveal a role for miniature synaptic events in the acute regulation of dendritic protein synthesis in neurons.

Although it is now well established that dendrites are capable of mRNA translation, the synaptic events that regulate dendritic protein synthesis are still largely unknown. We examined the activity-dependent regulation of dendritic protein synthesis in cultured hippocampal neurons using time-lapse microscopy and a green fluorescent protein (GFP)–based reporter (*1*). In initial experiments, we determined the effects of either the blockade of action potentials [by tetrodotoxin (TTX)] or the blockade of both action potentials and miniature excitatory synaptic events [by TTX, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and D-(-)-2-amino-5-phosphonovaleric acid (APV)]. After 9.5 hours of treatment, we began time-lapse imaging of neurons that expressed the protein synthesis reporter (*2*). We analyzed the levels of fluorescence in the distal dendrites (those >125 μm from the soma), where the fluorescent signal is primarily derived from local translation (*1*). Control dendrites exhibited moderate levels of reporter expression that increased modestly over the next two hours (Fig.

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1, A to C). Dendrites that experienced prolonged action potential blockade exhibited significantly less reporter expression in the distal dendrites, relative to controls (Fig. 1, A to E).

Although TTX prevents neurotransmitter release by action potentials, it spares the spontaneous release of synaptic vesicles from presynaptic terminals (*3*). At glutamatergic synapses, these spontaneous release events give rise to miniature excitatory postsynaptic currents (mEPSCs or "minis") that can be blocked by glutamate receptor antagonists. Dendrites that experienced a blockade of both action potentials and minis demonstrated significantly higher initial levels of reporter expression relative to controls (Fig. 1, A and B); these levels continued to increase over the next two hours (Fig. 1, C to E), suggesting that minis can inhibit dendritic protein synthesis. We also repeated the same experiment in the presence of the protein synthesis inhibitor anisomycin (40 μM). With reporter synthesis prevented, the rate of reporter degradation in dendrites was similar among all groups (fig. S1), indicating that the differences in reporter expression observed when minis are permitted or blocked reflect differences in protein synthesis rather than degradation.

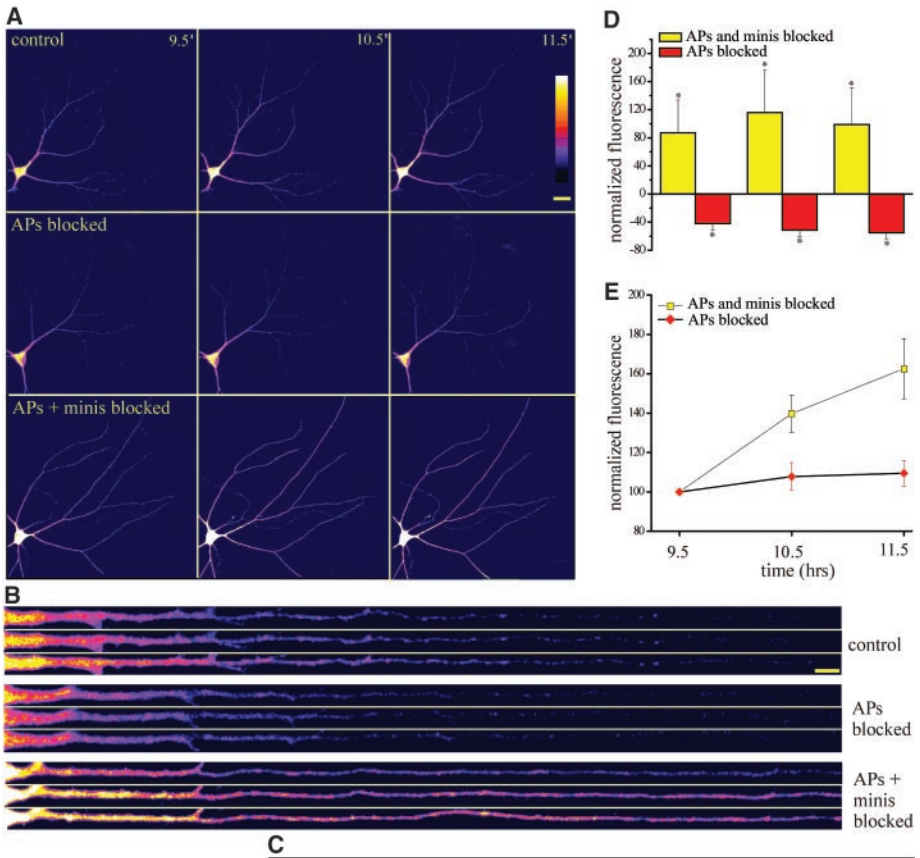

Fig. 1. The integrity of miniature synaptic transmission determines changes in dendritic protein synthesis during chronic activity blockade. (**A**) Whole cell images and (**B**) straightened dendrites from a time-lapse experiment in which cultured hippocampal neurons expressing the protein synthesis reporter were treated with vehicle (control), TTX (0.5 μM, APs blocked) or TTX plus CNQX (40 μM) and APV (20 μM) (APs and minis blocked) for 9.5 hours before the first image was obtained. The color look-up bar indicates the intensity of GFP signal from lowest (black) to highest (white). Scale bars, (A) 20 μm and (B) 10 μm. (**C**) Analysis of fluorescence ($F_{t=11.5} - F_{t=9.5}/F_{t=9.5}$, where F is fluorescence and t is time in hours) for the dendrites shown in (B) as a function of time and distance from the soma. (**D**) Analysis of group data, normalized to control levels for the three time points shown in (A). At all time points, the action potentials (APs) blocked group and the APs and minis blocked group showed significant (*, $P < 0.05$) decreases or increases, respectively, in reporter expression in distal dendrites when compared to controls from sister cultures. $n = 15$, 14, and 15 neurons for the control, blocked APs, and blocked APs and minis groups, respectively. (**E**) Analysis of group data showing the change in dendritic protein synthesis over time for each group, as labeled.

When postsynaptic cells experience long-term (24- to 48-hour) global activity blockade, a homeostatic increase in glutamate receptor currents (*4*–*6*) or presynaptic release probability (*7*, *8*) gradually develops. To determine whether the increase in dendritic protein synthesis observed after mini blockade represents a long-term homeostatic response or, alternatively, an acute form of regulation, we monitored protein synthesis immediately before and after mini blockade (Fig. 2A). All neurons were treated chronically (for 9 hours) with TTX alone before imaging; after a baseline image was acquired, neurons were acutely challenged with either glutamate receptor (GluR) antagonists or vehicle (TTX alone) (Fig. 2A). As before (Fig. 1), neurons that experienced action potential blockade alone exhibited stable levels of reporter expression in dendrites over the course of the experiment (Fig. 2, A to E). In contrast, neurons acutely challenged with GluR antagonists demonstrated a rapid increase in reporter synthesis in distal dendrites (Fig. 2, A to E), indicating that in the absence of evoked synaptic transmission, minis can acutely inhibit dendritic protein synthesis. A prolonged exposure to TTX was not required for the enhancement of protein synthesis induced by mini blockade (fig. S2). Although the increase in protein synthesis induced by mini blockade tended to be strongest in dendrites, we also observed (with some exceptions) increases in reporter expression in the cell bodies (Fig. 2B), presumably due to the presence of excitatory synapses on the somata of cultured hippocampal neurons. Thus, the impact of miniature synaptic events on protein synthesis in neurons is rapid and can occur in both dendritic and somatic compartments.

To examine a potential contribution of *N*-methyl-D-aspartate (NMDA) receptor–mediated signaling to the inhibition of protein synthesis by minis, we conducted an additional set of experiments in which only NMDA-mediated mEPSCs were blocked (by APV) during action potential blockade (Fig. 2A). We found that exposure to APV alone was sufficient to significantly increase protein synthesis in distal dendrites, although this increase was less than that observed in the presence of both CNQX and APV (Fig. 2F). This suggests a contribution of Ca^{2+} signaling (*9*–*11*) to the regulation of local protein synthesis by minis.

These data suggest that minis provide tonic inhibition of protein synthesis in neurons, as monitored with a fluorescent protein synthesis reporter (*1*). To examine whether minis affect

the translation of endogenous proteins, we conducted metabolic labeling experiments in which total protein synthesis was examined in hippocampal neurons exposed to a blockade of action potentials (by TTX) with or without an acute block of minis (15 min before labeling). Lysates prepared from neurons exposed to a block of action potentials and minis consistently showed enhanced total protein synthesis relative to lysates prepared from neurons exposed to action potential blockade alone (fig. S3), indicating that minis can exert a global inhibitory effect on protein synthesis, rather than selectively affecting the translation of a single mRNA.

In these experiments, we assessed the impact of minis by blocking their postsynaptic detection. If minis truly regulate protein synthesis, then blocking the presynaptic release of neurotransmitter should produce similar effects. We therefore examined the effects of blocking presynaptic release of minis with botulinum toxin A (BoNT/A) (*12*) during chronic activity blockade (Fig. 3A). In whole-cell voltage clamp recordings (Fig. 3B), 2 to 3 hours of pretreatment with BoNT/A (100 nM) was sufficient to block the majority of spontaneous neurotransmitter release (mean ± SEM mini frequency: vehicle, 6.38 ± 0.64 Hz; BoNT/A, 0.10 ± 0.02 Hz; $n = 6$ neurons for each group). In imaging experiments, neurons were pretreated with TTX, and then a subset of neurons were exposed to BoNT/A for 120 min before and throughout imaging (Fig. 3A). Dendrites that experienced action potential blockade exhibited stable levels of reporter expression over the course of imaging (Fig. 3, C to E). However, when mini release was blocked (with TTX and BoNT/A), the rate of reporter synthesis in the dendrites was markedly enhanced (Fig. 3, C to E). We also examined the effects of BoNT/A treatment on neurons treated chronically with TTX and GluR antagonists. Blocking the impact of minis postsynaptically with GluR antagonists occluded the acute effects of BoNT/A (Fig. 3F), providing strong evidence that the increase in dendritic protein synthesis observed in each case is due to a disruption of miniature synaptic transmission.

We next asked if enhancing mini release would produce the opposite effect, namely an inhibition of dendritic protein synthesis. To acutely enhance mini release, we used a low concentration of α-latrotoxin (α-LTX, 100 pM) (*13*) in the presence of 10 μM $LaCl_3$ to limit formation of α-LTX pores (*14*) (Fig. 4A). Under these conditions, α-LTX increased mini frequency by roughly 60% (Fig. 4B) (mean ± SEM mini frequency: baseline, 6.25 ± 0.56 Hz; 5 to 10 min after exposure to α-LTX, 10.3 ± 1.2 Hz; $n = 6$ neurons). In imaging experiments, neurons maintained in TTX alone exhibited a stable level of reporter expression throughout the experiment (Fig. 4, C to E). Neurons treated acutely with α-LTX demonstrated decreasing levels of reporter expression in the dendrites (Fig. 4, C to E), indicating that increasing mini frequency can enhance the tonic inhibition of protein synthesis. This decrease in reporter expression was due to a slower rate of synthesis, because the rate of degradation in the presence of anisomycin over the same time period was not altered by α-LTX (fig. S1). Taken together, these data show that minis can bidirectionally regulate dendritic protein synthesis during chronic activity blockade.

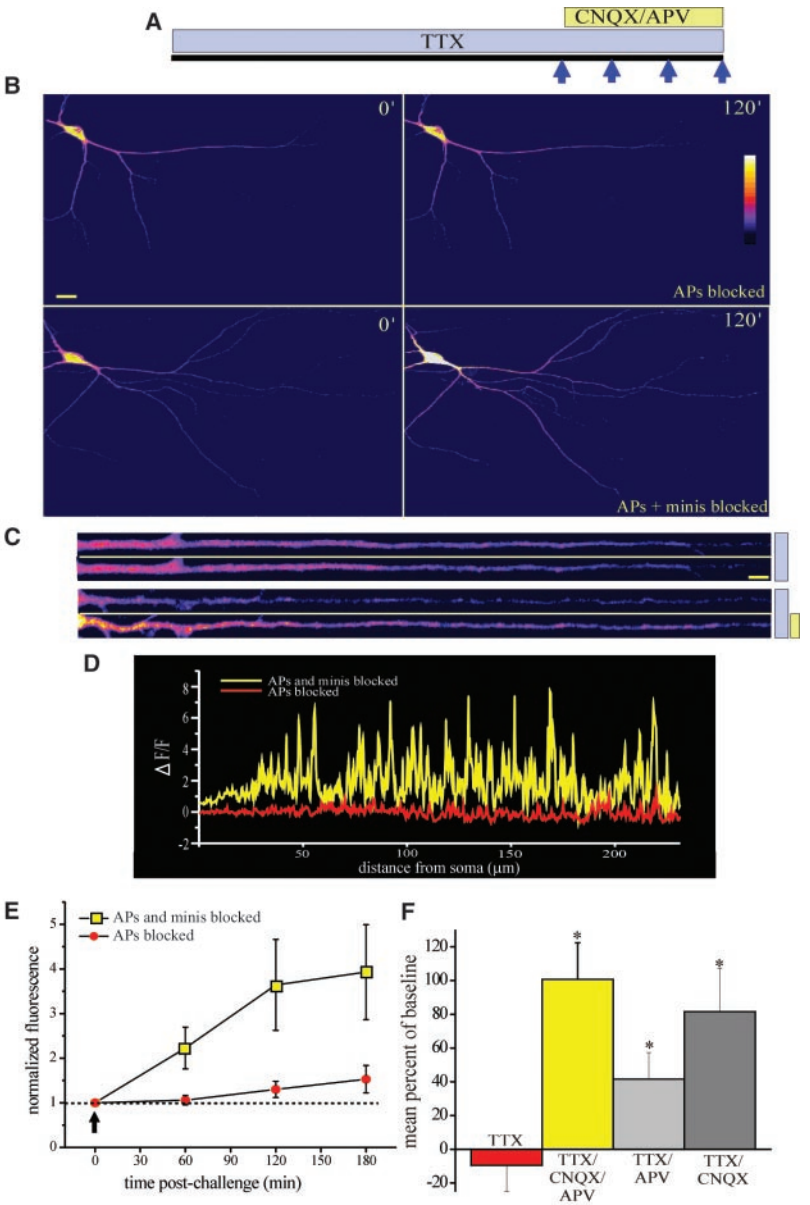

Fig. 2. Miniature excitatory synaptic events tonically inhibit dendritic protein synthesis. (**A**) Cultured hippocampal neurons were treated with TTX (1 μM) for 9 hours, and after the acquisition of a baseline image, a subset of neurons were then exposed to CNQX and APV (APs + minis blocked), to block the postsynaptic detection of miniature synaptic events. Arrows indicate the times at which images were acquired. (**B**) Whole cell images and (**C**) straightened dendrites from a time-lapse experiment in which neurons were acutely challenged with CNQX or APV. Shown are the first images (0′) and images obtained two hours (120′) after mini blockade. Scale bars, (B) 20 μm and (C) 10 μm. (**D**) Analysis for the straightened dendrites shown in (C) as a function of distance from the soma. (**E**) Analysis of group data showing the change in dendritic protein synthesis over time for each group, as labeled ($n = 10$ neurons for both groups). The acute blockade of minis resulted in a significant ($P < 0.05$) increase in dendritic protein synthesis relative to TTX alone. (**F**) Analysis of group data showing the relative contributions of NMDA- or α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA)–mediated minis. Treatment with either APV ($n = 16$ neurons) or CNQX ($n = 13$ neurons) alone or with both ($n = 15$ neurons) resulted in a significant (*, $P < 0.05$) increase in reporter expression, relative to TTX-alone controls ($n = 13$ neurons) measured at 120 min post-treatment. The increase observed with APV alone, however, was significantly ($P < 0.05$) less than that observed with both CNQX and APV. Scale bar, 10 μm.

Since their initial observation (*3*), the functional significance of minis has continued to intrigue neurobiologists. Our results add to recent studies that have suggested functional roles for minis in regulating both neuronal firing behavior (*15*) and postsynaptic signaling events important for synaptic plasticity (*16*). In addition, minis are sufficient to maintain dendritic spine density (*17*) and to induce glutamate receptor clustering (*18*) in the face of long-term chronic activity blockade. Our data suggest that mini-induced inhibition of protein synthesis could contribute to the postsynaptic integrity observed in these studies. Our data also show that a complete loss of input leads to a stimulation of protein synthesis, the products of which could contribute to structural remodeling at synapses (*19*, *20*). Although the mechanism by which minis regulate dendritic translation is unknown, the potentially low frequency of spontaneous release events at a given synapse puts constraints on the biochemical pathways responsible for coupling mini detection to the translation machinery.

Rapid changes in local protein synthesis at synaptic sites is an important mechanism contributing to use-dependent changes in synaptic strength (*19*, *21*–*25*) that may ultimately underlie long-term memory storage. The tonic inhibition of dendritic protein synthesis by miniature synaptic events may prime stimulus-induced local protein synthesis by maintaining a pool of available (i.e., nontranslating) ribosomes and translation factors, allowing for rapid recruitment of this translation machinery to recently activated synapses during plasticity. The translational regulation conferred by minis could also alter which specific mRNA transcripts are likely to be translated under particular patterns of synaptic activity. Recent studies have suggested that a transient inhibition of overall protein synthesis in neurons may enhance the activity-dependent translation of some mRNAs (*26*, *27*), results that are consistent with work in other eukaryotic systems that has established that transient repression of translation can alter the complement of mRNAs in the actively translating pool (*28*, *29*). In addition to regulating plasticity, the impact of minis on protein synthesis will likely also be altered by plasticity; the coordinate (*5*, *6*, *30*) or independent (*4*) scaling of minis with evoked synaptic transmission could change the relative impact of miniature events on local synthesis. Taken together, these results suggest a mechanism by which ongoing miniature synaptic transmission can alter the gain for local translational regulation on a rapid time scale.

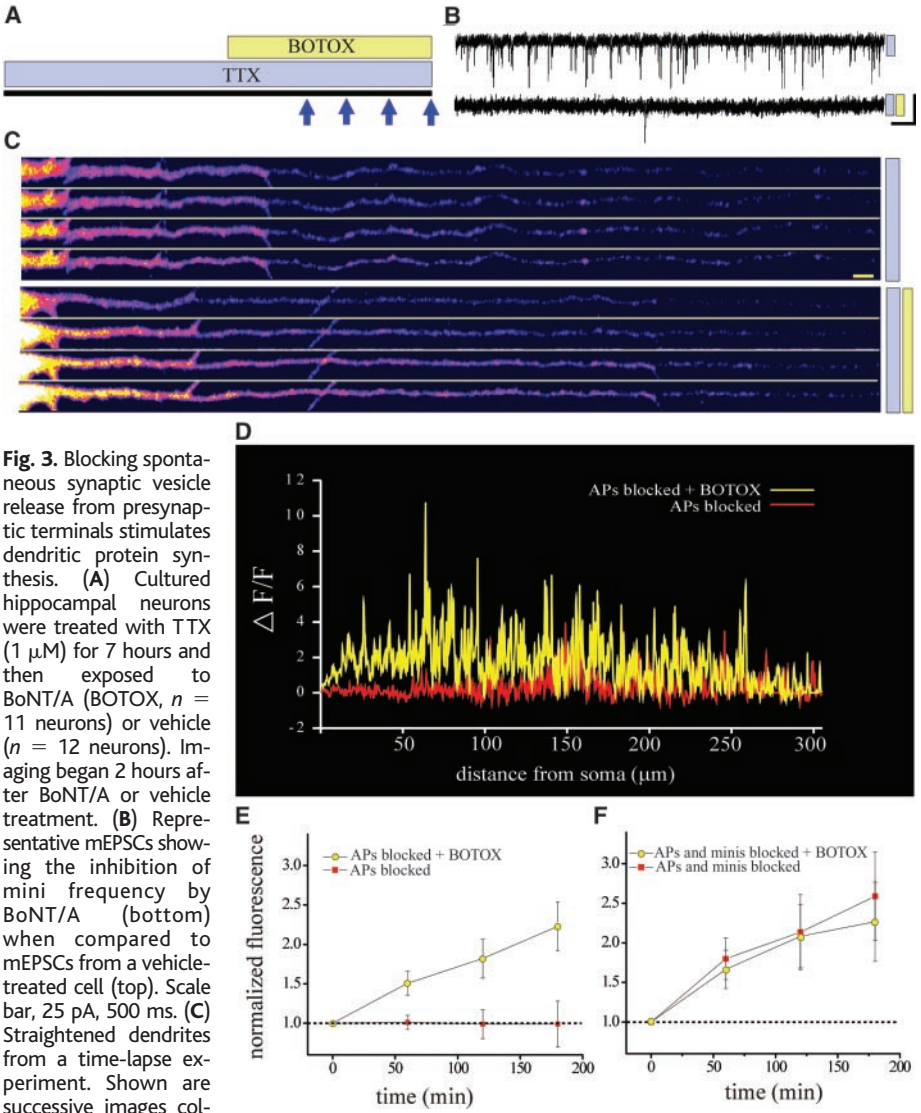

Fig. 3. Blocking spontaneous synaptic vesicle release from presynaptic terminals stimulates dendritic protein synthesis. (**A**) Cultured hippocampal neurons were treated with TTX (1 μM) for 7 hours and then exposed to BoNT/A (BOTOX, n = 11 neurons) or vehicle (n = 12 neurons). Imaging began 2 hours after BoNT/A or vehicle treatment. (**B**) Representative mEPSCs showing the inhibition of mini frequency by BoNT/A (bottom) when compared to mEPSCs from a vehicle-treated cell (top). Scale bar, 25 pA, 500 ms. (**C**) Straightened dendrites from a time-lapse experiment. Shown are successive images collected at 60-min intervals beginning 2 hours after BoNT/A addition. Scale bar, 10 μm. (**D**) Analysis ($F_{t=120} - F_{t=0}/F_{t=0}$) for the straightened dendrites shown in (C) as a function of distance from the soma. (**E**) Analysis of group data showing the change in dendritic protein synthesis over time for groups treated with TTX alone or with TTX plus BoNT/A. (**F**) Analysis of group data for experiments in which BoNT/A or vehicle (n = 10 and 11 neurons, respectively) was administered to cells treated chronically with TTX plus CNQX and APV. The rate of reporter synthesis in distal dendrites was significantly enhanced ($P < 0.05$) by BoNT/A relative to TTX alone. Prior blockade of minis with GluR antagonists also produced a significant ($P < 0.05$) increase in reporter synthesis relative to TTX alone and completely occluded the effects of BoNT/A on dendritic protein synthesis (nonsignificant).

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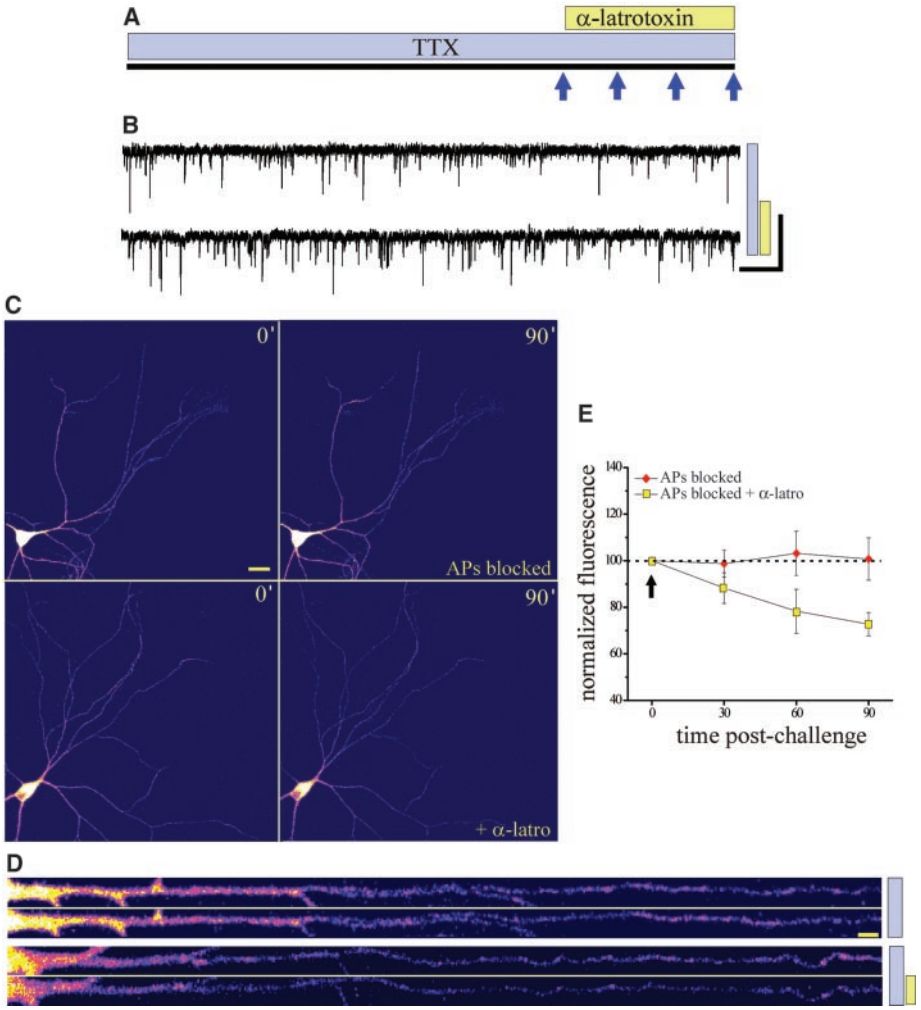

Fig. 4. Stimulating the frequency of miniature synaptic events with α-LTX inhibits dendritic protein synthesis. (**A**) Cultured hippocampal neurons were treated with TTX (2 μM) for 9 hours and then exposed to α-LTX or vehicle (n = 10 neurons for each group). (**B**) Representative mEPSCs before (top) and after (bottom) α-LTX application. Scale bar, 25 pA, 500 ms. (**C**) Whole cell images and (**D**) straightened dendrites from time-lapse experiments. Shown are images obtained before and 90 min after the addition of α-LTX (α-latro). Scale bars, (C) 20 μm and (D) 10 μm. (**E**) Analysis of group data showing the change in dendritic protein synthesis in distal dendrites over time for each group, as labeled. α-LTX produced a significant ($P < 0.05$) decrease in dendritic protein synthesis relative to TTX alone.

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32. We thank G. Patrick, C. Jiang, and C.-Y. Tai for critically reading the manuscript, H. Weld for making beautiful cultured hippocampal neurons, and all members of the Schuman lab. Supported by the Damon Runyon Walter Winchell Fund (M.A.S.) and the NIH (E.M.S.). E.M.S. is an associate investigator of HHMI.

Supporting Online Material
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Figs. S1 to S3
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29 January 2004; accepted 12 May 2004

Deficit in Attachment Behavior in Mice Lacking the μ-Opioid Receptor Gene

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Endogenous opioid binding to μ receptors is hypothesized to mediate natural rewards and has been proposed to be the basis of infant attachment behavior. Here, we report that μ-opioid receptor knockout mouse pups emit fewer ultrasonic vocalizations when removed from their mothers but not when exposed to cold or male mice odors. Moreover these knockout pups do not show a preference toward their mothers' cues and do not show ultrasonic calls potentiation after brief maternal exposure. Results from this study may indicate a molecular mechanism for diseases characterized by deficits in attachment behavior, such as autism or reactive attachment disorder.

The opioid system controls nociceptive and addictive behaviors, with a prominent role of μ-opioid receptors (*Orpm*) in these responses. Mice lacking the μ receptor gene ($Orpm^{-/-}$) show a loss of morphine-induced analgesia, reward, and dependence (*1*); increased sensitivity to painful stimuli (*2*, *3*); reduced reward to nonopioid drugs of abuse (*4*); and altered emotional responses (*5*). Endogenous opioid binding to μ receptors has been considered one of the neural mediators of infant attachment behavior (*6*–*10*), although the role of μ receptors in mediating the rewarding properties of mother-related stimuli has not been determined (*11*, *12*).

Attachment behavior entails the display of affiliative behaviors and the establishment of a special bond with the animal's

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