

growth cone form into a thin, needle-like appendage. Originally pioneered by Mu-Ming Poo and colleagues, the "Xenopus growth cone turning assay" has recently become a popular assay for assessing growth cone turning responses to gradients of guidance factors. Using this assay, Poo and colleagues had previously demonstrated that growth cones from young *Xenopus* retinal explants extend toward a gradient of Netrin-1 protein and away from a gradient of semaphorin 3A (Song et al., 1998; de la Torre et al., 1997). Campbell and Holt showed that both this turning response and collapse in response to semaphorin 3A requires local protein synthesis; in the presence of the protein synthesis inhibitors anisomycin and cyclohexamide, isolated growth cones both fail to turn away from a gradient of semaphorin 1 and are no longer collapses by application of the repellent. In the presence of translation inhibitors, growth cones also failed to turn toward the Netrin-1 gradient, suggesting that this effect is not specific to repulsive agents. The rate of neurite outgrowth per se is not affected, but, rather, growth cones exhibit a neutral turning response. Interestingly, collapse in response to LPA, a G-protein mediated collapsing reagent, was not affected by translation inhibitors, suggesting some specificity to the effect.

Reasoning that if growth cone responses are sensitive to local translation, they may also require counteracting programs to regulate protein degradation, Campbell and Holt explored whether proteasome-mediated proteolysis could also play a role in chemotropic responses of growth cones. Indeed, consistent with previous reports demonstrating the presence of proteasome components in the growth cone, immunocytochemical staining revealed components of the ubiquitination machinery and proteasome present in isolated *Xenopus* growth cones. Moreover, addition of the cell-permeable proteasome inhibitors LnLL and lactacysin abolished the turning of growth cones toward a gradient of either Netrin-1 or BDNF. Proteasome inhibitors also inhibited LPA-induced collapse. However, semaphorin collapse was unaffected. These data suggest that interplay of both protein synthesis and protein degradation is important for axonal growth cone guidance.

With these recent papers, the study of axonal molecular biology has entered into a new era. It is no longer possible to argue that RNAs are not transported into axons or that protein synthesis cannot occur in axons. Among the questions that these revelations suggest are: which mRNAs are localized to axons? What are the signals on RNA transport granules that distinguish dendritically targeted granules from those targeted to the axon? Does translation in axons occur only in the growth cone, or can it occur along the length of the axon? Is translation in axons different from that in the cell soma, as has been shown to be the case for dendrite versus the cell soma translation (Job and Eberwine, 2001)? And finally, while difficult to experimentally investigate, it is appropriate to ask whether the in vitro influences of chemotropic agents can be recapitulated in vivo. With this new era of axonal investigation upon us, it will be interesting to see what surprises the axon has in store for us.

James Eberwine
Departments of Pharmacology and Psychiatry
University of Pennsylvania Medical School

36th and Hamilton Walk
Philadelphia, Pennsylvania 19104

Selected Reading

- Bassell, G.J., Zhang, H., Byrd, A.L., Femino, A.M., Singer, R.H., Taneja, K.L., Lifshitz, L.M., Herman, I.M., and Kosik, K.S. (1998). *J. Neurosci.* 18, 251–265.
- Campbell, D.S., and Holt, C.E. (2001). *Neuron* 32, this issue, 1013–1026.
- Crino, P.B., and Eberwine, J. (1996). *Neuron* 17, 1173–1187.
- de la Torre, J.R., Hopker, V.H., Ming, G.L., Poo, M.M., Tessier-Lavigne, M., Hemmati-Brivanlou, A., and Holt, C.E. (1997). *Neuron* 19, 1211–1224.
- Eberwine, J., Miyashiro, K., Kacharina, J.E., and Job, C. (2001). *Proc. Natl. Acad. Sci. USA* 98, 7080–7085.
- Huber, K.M., Kayser, M.S., and Bear, M.F. (2000). *Science* 288, 1254–1257.
- Job, C., and Eberwine, J. (2001). *Proc. Natl. Acad. Sci. USA* 98, 13037–13042.
- Kang, H., and Schuman, E.M. (1996). *Science* 273, 1402–1406.
- Ross, A.F., Oleynikov, Y., Kislauskis, E.H., Taneja, K.L., and Singer, R.H. (1997). *Mol. Cell. Biol.* 17, 2158–2165.
- Song, H., Ming, G., He, Z., Lehmann, M., McKerracher, L., Tessier-Lavigne, M., and Poo, M. (1998). *Science* 281, 1515–1518.
- Spencer, G.E., Lukowiak, K., and Syed, N.I. (2000). *J. Neurobiol.* 44, 72–81.
- Steward, O., Wallace, C.S., Lyford, G.L., and Worley, P.F. (1998). *Neuron* 21, 741–751.
- Zhang, H.L., Singer, R.H., and Bassell, G.J. (1999). *J. Cell Biol.* 147, 59–70.
- Zhang, H.L., Eom, T., Oleynikov, Y., Shenoy, S.M., Liebelt, D.A., Dichtenberg, J.B., Singer, R.H., and Bassell, G.J. (2001). *Neuron* 31, 261–275.

Proton Release as a Modulator of Presynaptic Function

In this issue of *Neuron*, DeVries (2001) describes experiments suggesting that acidification of the synaptic cleft can reduce Ca²⁺ channel activity and thereby act as a brake on tonic synaptic release of glutamate from cone cells. This work hints at a potentially important new facet to the regulation of synaptic transmission.

Protons are the workhorse of solution biochemistry, long appreciated for their central role in a host of enzymatic reactions. Indeed, protonation of key residues at catalytic centers, or on ionizable substrates, controls many reactions. Enzymologists have often been able to exploit protein purification to study the complex details of acid-base chemistry within enzymatic reaction kinetics. By contrast, neurobiologists, have been considerably more measured in their willingness to accept the pH dependence of processes as an indication of a physiological role for protons. This largely reflects the comparative complexity of the systems they study. For example, synaptic transmission by itself involves perhaps hundreds of proteins, in addition to those involved in membrane remodeling—a daunting and complex system in which to search for pH-dependent effects.

Yet, protonation of ionizable residues is now emerging

as a central feature of voltage-gated channel function (Tomabaugh and Somjen, 1998), neurotransmitter uptake systems (Billups and Attwell, 1996), and ligand-gated channel function (Traynelis, 1998). Moreover, rapid changes in pH, particularly in the extracellular domain, are known to accompany or precede channel activation (Kaila and Chesler, 1998). Extracellular alkaline shifts, with rise times of tens of milliseconds (Krishtal et al., 1987), may be generated by the activation of channels (Kaila and Voipio, 1987) or transporters (Schwiening et al., 1993), and have been implicated in the modulation of NMDA receptors (Taira et al., 1993; Gottfried and Chesler, 1994). A far faster proton-dependent modulation of function may occur in the context of vesicle fusion.

In a study of hippocampal slices, Krishtal et al. (1987) noted that synaptic transmission was accompanied by a short latency transient change in phenol red absorption, consistent with a brief (few ms) extracellular acidification. This observation led to the hypothesis that acidification of the synaptic cleft occurred by release of proton equivalents from synaptic vesicles (or by the action of vesicular uptake pumps delivered to the plasma membrane), which might regulate synaptic transmission by proton-activated conductances or modulation of proton-sensitive channels. However, these early data suggesting a brief transient acidification of the synaptic cleft in the time frame of synaptic transmission have never been verified. Moreover, the reasonable prediction that acidification of the synaptic cleft might in some way influence synaptic transmission has largely lied dormant. That is, until this issue of *Neuron*, in which DeVries (2001) describes an electrophysiological study of what he interprets to be a transient inhibition by proton release of a presynaptic Ca^{2+} current recorded in retinal cone photoreceptors from ground squirrels.

The author uses a variety of conventional electrophysiological techniques to build a case that a 2 ms transient inhibition of a presynaptic current coincides with the synaptic transmitter release profile, and reflects a proton-induced shift in the voltage dependence of presynaptic Ca^{2+} channels that is consistent with pH-induced shifts in Ca^{2+} channel properties in the same cell. The result of this shift in the voltage dependence is a transient inhibition of the channel, and presumably a reduction in the Ca^{2+} influx and release process. DeVries (2001) further shows that this transient inhibition of a Ca^{2+} current is sensitive to pH and to the buffering strength of the extracellular solution, suggesting that the inhibition reflects transient acidification of the synaptic cleft. These electrophysiological arguments all lead to a working hypothesis suggesting that inhibition of presynaptic Ca^{2+} channels by release of intravesicular acid equivalents may exert a transient inhibition on synaptic transmission at a tonically active synapse.

This study provides some of the best evidence to date for proton-dependent modulation of ion channel function within the context of synaptic transmission. The efficacy of added buffers in reducing the putative proton inhibition constitutes the strongest data in support of the concept. Here it is noteworthy that addition of 20 mM HEPES, while less than half the total extracellular buffer, blocked up to 80% of the inhibitory effect. These data suggest that the extracellular buffering capacity provided by CO_2 and bicarbonate is minimal within the

rapid time frame of this study. This observation is consistent with the notion that normal interstitial buffering, reliant on catalysis by interstitial carbonic anhydrase, is limited in short time frames owing to regional absence or low concentration of this enzyme (Tong et al., 2000). For this reason, as the author notes, considerable proton-dependent modulation of Ca^{2+} channels is feasible within a few milliseconds despite high concentrations of bicarbonate buffer normally present in the extracellular fluid. The presumably tight spatial coupling between Ca^{2+} channels and vesicle release sites (Llinas et al., 1992) is also consistent with the idea that this channel is a target for synaptically released acid equivalents.

However, before the proposed mechanism of vesicle-mediated proton modulation can be considered established precedent, a number of further considerations must be weighed. For example, the author rules out many but not all other possible sources for this current—leaving open the possibility that an unexpected conductance could be shifting during synaptic transmission. Furthermore, independent methods need to be used to verify the underlying assumption—that synaptic transmission at this synapse creates a transient acidification of the synaptic cleft. This idea has required verification for more than a decade, and the present paper increases the urgency for completion of experiments that convincingly probe the pH of the intrasynaptic space with high temporal resolution. Advances in optical techniques in recent years should facilitate these experiments.

In addition, the current work needs to be extended into a physiological context in which the proposed pH-dependent feedback inhibition is shown to reduce neurotransmitter release. Without these additional experiments, it will be hard to accept the proposed mechanism as meaningful. However, the paper still stands on its own as showing, perhaps better than any previously published work, the potential role for protons in regulating synaptic transmission. Moreover, this work has accomplished another important goal all scientists should strive for in the design of experiments—this paper has created rationale for the pursuit of new experimental directions. Finally, this study by itself has advanced our understanding of the regulation of neurotransmission—no small feat for topics as complex as pH and synaptic transmission.

Stephen F. Traynelis¹ and Mitchell Chesler²

¹Department of Pharmacology
Emory University School of Medicine
Rollins Research Center
1510 Clifton Road
Atlanta, Georgia 30322

²Department of Physiology and Neuroscience
New York University Medical Center
550 First Avenue
New York, New York 10016

Selected Reading

Billups, B., and Attwell, D. (1996). *Nature* 379, 171–174.

DeVries, S.H. (2001). *Neuron* 32, this issue, 1107–1117.

Gottfried, J.A., and Chesler, M. (1994). *J Physiol.* 478, 373–378.

- Kaila, K., and Chesler, M. (1998). In *pH and Brain Function*, K. Kaila and B.R. Ransom, eds. (New York: John Wiley & Sons), pp. 309–338.
- Kaila, K., and Voipio, J. (1987). *Nature* 330, 163–165.
- Krishtal, O.A., Osipchuk, Y.V., Shelest, T.N., and Smirnov, S.V. (1987). *Brain Res.* 436, 352–356.
- Llinas, R., Sugimori, M., and Silver, R.B. (1992). *Science* 256, 677–679.
- Schwiening, C.J., Kennedy, H.J., and Thomas, R.C. (1993). *Proc. Royal Soc. Lond. B* 253, 285–289.
- Taira, T., Smirnov, S., Voipio, J., and Kaila, K. (1993). *Neuroreport* 4, 93–96.
- Tombaugh, G.C., and Somjen, G.C. (1998). In *pH and Brain Function*, K. Kaila and B.R. Ransom, eds. (New York: John Wiley & Sons), pp. 395–416.
- Tong, C.K., Brion, L.P., Suarez, C., and Chesler, M. (2000). *J. Neurosci.* 20, 8247–8253.
- Traynelis, S.F. (1998). In *pH and Brain Function*, K. Kaila and B.R. Ransom, eds. (New York: John Wiley & Sons), pp. 417–446.

Calcium/Calmodulin: A Synaptic Antidepressant?

Synaptic depression contributes to short-term changes in synaptic efficacy during sustained activity. Sakaba and Neher (2001) have characterized two kinetically distinct pools of releasable vesicles whose depletion underlies depression at a CNS synapse. Calcium/calmodulin dramatically accelerates replenishment of one of these pools—and hence recovery from depression.

It is one of the distinguishing features of a synapse that, unlike the relative constancy of the axon impulse, its “power of transmission” is subject to very large variations, many of which are caused by slowly subsiding aftereffects from previous impulses.

—Bernard Katz

Nerve, Muscle, and Synapse, 1966

When Katz wrote these words, it had already been clear for more than a decade—almost from the inception of his quantal hypothesis of neurotransmitter release—that changes in the number of quanta released are at the root of at least some aftereffects of prior stimulation. For instance, depression of synaptic efficacy during repetitive stimulation is due in part to fewer quanta being released by each presynaptic impulse (Del Castillo and Katz, 1954). Many presynaptic factors could potentially contribute to reduced quantal release during depression, including Ca^{2+} channel inactivation, presynaptic autoreceptors, changes in action potential waveform, altered sensitivity of the release machinery to Ca^{2+} , and depletion of releasable vesicles. In addition, postsynaptic factors such as receptor desensitization and receptor saturation play a role in synaptic depression. This multiplicity of relevant factors and their inaccessibility at most synapses make it difficult to unravel mechanisms of synaptic depression and recovery from depression. In this issue of *Neuron*, Sakaba and Neher (2001) were able to circumvent many of these problems by exploiting a combination of biological providence, analytical skill, and

pharmacology. Their work provides a detailed look at the depletion and replenishment of releasable vesicles at a CNS terminal and suggests that replenishment is in part regulated by elevated Ca^{2+} , acting via calmodulin. This result provides a mechanistic clue as to how increased presynaptic activity might both deplete vesicles and accelerate recovery from depletion at CNS synapses.

In sorting out the factors that alter synaptic efficacy, the ability to voltage clamp both the pre- and postsynaptic elements at the synapse is a considerable advantage. For this reason, Sakaba and Neher chose a synaptic preparation that allows simultaneous voltage clamp of both sides of the synapse: the calyx of Held. This giant synaptic terminal in the brainstem auditory system provides fast, highly reliable synaptic excitation of the postsynaptic cell, mediated by release of glutamate (Trussell, 1999). Although the postsynaptic response at the calyx of Held reflects the neurotransmitter output of a single giant terminal, the response consists of many superimposed quantal events, produced by vesicular release at numerous independent active zones scattered throughout the terminal. To decompose the overall response into its quantal components—and hence to estimate release rate—Sakaba and Neher used a modified form of deconvolution analysis (Neher and Sakaba, 2001). In this method, the EPSC is deconvolved with the waveform of the quantal event to yield the rate of release at each instant (after a couple of corrections for other factors, detailed in Neher and Sakaba, 2001). The accuracy of the deconvolution method hinges on the degree to which individual quantal responses sum linearly to produce the EPSC, but desensitization and saturation of postsynaptic AMPA receptors produce time-dependent changes in the quantal event that would distort the estimate of release rate. To prevent this, Sakaba and Neher treated the synapse with drugs that minimize desensitization and saturation of receptors (cyclothiazide and kynurenic acid, respectively). Under these conditions, deconvolution analysis accurately estimates quantal release rates at the calyx of Held (Neher and Sakaba, 2001).

The deconvolution method allows release rate to be tracked throughout the EPSC and thus permits resolution of multiple kinetic components of the release process. The essential findings are summarized in the Figure. Sakaba and Neher discerned two distinct populations of releasable vesicles that fuse on time scales differing about 10-fold (milliseconds versus tens of milliseconds, under the conditions of their experiments). These two pools of vesicles are replenished at different rates after depletion: the slow-release pool refills in <100 ms, but the fast-release pool requires seconds to refill. Further, the rate of refilling of the slowly recovering pool (i.e., the fast-release pool) is accelerated by elevated internal Ca^{2+} , and this acceleration is prevented by calmodulin blockers. These results lead to a scheme in which Ca^{2+} influx both triggers release by activating the release machinery and enhances refilling of the fast-release pool by activating calmodulin.

The idea that elevated intracellular Ca^{2+} speeds refilling of the releasable pool has been suggested previously from work on neuroendocrine cells (von Rüden and Neher, 1993) and synapses (Dittman and Regehr, 1998; Stevens and Wesseling, 1998; Wang and Kaczmarek, 1998). However, Sakaba and Neher refined this idea by