Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

The N-Type Voltage-Gated Calcium Channel: When a Neuron Reads a Map

Norbert Weiss

Laboratoire Physiologie Intégrative, Cellulaire et Moléculaire, Unité Mixte de Recherche 5123, Centre National de la Recherche Scientifique, Université Claude Bernard Lyon 1, F-69622 Villeurbanne cedex, France

Review of Sann et al. (http://www.jneurosci.org/cgi/content/full/28/10/2366)

Neuronal voltage-gated calcium (Ca^{2+}) channels (VGCCs) are multimeric protein complexes that are localized to the presynaptic membrane of neurons, where they serve several functions. VGCCs are composed of the $Ca_v2.x$ pore-forming subunit surrounded by β, γ, and $\alpha_2\delta$ auxiliary subunits, which control channel trafficking and activity (Walker and De Waard, 1998). These channels allow functional coupling between action potentials and Ca^{2+} entry into nerve terminals, where they control evoked neurotransmitter release. They also play an important role in the control of neurite outgrowth at early developmental stages and are involved in the organization of functional neuromuscular synapses during the development of motor nerve terminals (Nishimune et al., 2004). The latter function is mediated by a direct interaction of the 11th extracellular loop of $Ca_v2.1$ (P/Q-type Ca^{2+} channel) and $Ca_v2.2$ (N-type Ca^{2+} channel) subunits of VGCCs with a leucine-arginine-glutamic acid (LRE) motif of the $\beta2$ chain of laminin, a component of the extracellular matrix. In a recent issue of *The Journal of Neuroscience*, Sann et al. (2008) provide new insights into the functional importance of the interaction between the $Ca_v2.2$ subunit and laminin $\beta2$ (Sann et al., 2008). They demonstrate *in vitro* and *in vivo* in *Xenopus laevis* embryos that $Ca_v2.2$–laminin $\beta2$ interaction acts as a stop signal for neurite outgrowth during sensory innervation of the skin, allowing appropriate innervation of the target tissue.

Using immunohistochemistry on tailbud (stage 26) *X. laevis* embryos, the authors show that $Ca_v2.2$ channels are expressed in growth cones of extending commissural and Rohon-Beard sensory axons innervating the skin [Sann et al. (2008), their Fig. 1*D*,*E* (http://www.jneurosci.org/cgi/content/full/28/10/2366/F1)], as well as in growth cones of spinal neurons grown *in vitro* [Sann et al. (2008), their Fig. 1*G*,*H* (http://www.jneurosci.org/cgi/content/full/28/10/2366/F1)]. This observation suggests that $Ca_v2.2$ channels could be involved in cellular processes other than the control of synaptic activity, such as neurite outgrowth and guidance. In parallel, the authors show that the skin expression pattern of laminin $\beta2$ is restricted to hexagonal cells in 2-d-old embryos [Sann et al. (2008), their Fig. 2 (http://www.jneurosci.org/cgi/content/full/28/10/2366/F2)]. Hence, $Ca_v2.2$ channels of sensory neurons and laminin $\beta2$ are expressed in *X. laevis* embryos, where they may interact, as previously shown for $Ca_v2.1$ channels at the neuromuscular junction (Nishimune et al., 2004).

To investigate the functional significance of the $Ca_v2.2$–laminin $\beta2$ interaction in neurite outgrowth, *X. laevis* spinal neurons were grown *in vitro*, and extending processes were quantified. Whereas neurons had many processes when they were grown on a substrate of laminin-111 (devoid of the $\beta2$ chain), the addition of the C-terminal fragment of laminin $\beta2$ (containing the LRE domain) specifically inhibited neurite outgrowth [Sann et al. (2008), their Fig. 3*A–C* (http://www.jneurosci.org/cgi/content/full/28/10/2366/F3)]. Neurite outgrowth was rescued by the addition to the culture medium of a peptide corresponding to the 11th extracellular loop of the $Ca_v2.1$ subunit, which also binds to the LRE motif of the laminin $\beta2$ [Sann et al. (2008), their Fig. 3*D*,*E* (http://www.jneurosci.org/cgi/content/full/28/10/2366/F3)]. These results suggest that inhibition of neurite outgrowth by laminin $\beta2$ is mediated by direct interaction of the LRE motif with the 11th extracellular loop of VGCCs.

To further investigate this inhibition pathway and analyze the importance of Ca^{2+} signaling, neurons were grown in the presence of ω-conotoxin GVIA, a specific inhibitor of N-type Ca^{2+} channels. In these experimental conditions, laminin $\beta2$ did not affect the number of processes, suggesting that Ca^{2+} influx into neuronal

Received April 9, 2008; revised April 22, 2008; accepted April 23, 2008.

Correspondence should be addressed to Norbert Weiss, Laboratoire Physiologie Intégrative, Cellulaire et Moléculaire, Unité Mixte de Recherche 5123, Centre National de la Recherche Scientifique, Université Claude Bernard Lyon 1, Bâtiment Raphaël Dubois, 43 boulevard du 11 Novembre 1918, 69622 Villeurbanne cedex, France. E-mail: norbert.weiss@univ-lyon1.fr.

DOI:10.1523/JNEUROSCI.1538-08.2008

 0270-6474/08/285621-02$15.00/0

growth cones through $Ca_v2.2$ channels is required for laminin-$\beta2$-mediated inhibition of neurite outgrowth [Sann et al. (2008), their Fig. 4*A–C* (http://www.jneurosci.org/cgi/content/full/28/10/2366/F4)].

To examine the involvement of Ca^{2+} influx in the laminin-$\beta2$-mediated stop signal, neurons were grown *in vitro* on a native or denaturated laminin-$\beta2$-coated strip, and the intracellular Ca^{2+} level ($[Ca^{2+}]_i$) in the growth cones was monitored by confocal microscopy. Fascinatingly, neurites, initially grown on the denaturated substrate, stalled or stopped when their growth cones encountered native laminin $\beta2$. This change in neurite growth was associated with a long-lasting increase of $[Ca^{2+}]_i$ [Sann et al. (2008), their Fig. 5 (http://www.jneurosci.org/cgi/content/full/28/10/2366/F5)]. Together, these results suggest that $Ca_v2.2$–laminin $\beta2$ interaction mediates Ca^{2+} entry into growth cones through N-type Ca^{2+} channels, initiating an inhibition pathway of neurite outgrowth.

To extend these observations to *in vivo* neurite outgrowth during sensory innervation of the skin, an agarose bead releasing ω-conotoxin GVIA was implanted in the developing *X. laevis* embryo from the time of neural tube formation until a late larval stage when sensory terminals have been formed. This method allows efficient delivery of the toxin *in vivo*, at least 200 μm around the bead. *In vivo* inhibition of Ca^{2+} influx through N-type Ca^{2+} channels resulted in a twofold increase in the number of sensory nerve terminal clusters in the skin [Sann et al. (2008), their Fig. 6 (http://www.jneurosci.org/cgi/content/full/28/10/2366/F6)]. Similar results were obtained using a bead releasing the peptide of the 11th extracellular loop of the $Ca_v2.1$ channel, suggesting that the increase in nerve terminal clusters is likely to be mediated by the disruption of $Ca_v2.2$–laminin $\beta2$ interaction, as observed *in vitro* [Sann et al. (2008), their Fig. 7 (http://www.jneurosci.org/cgi/content/full/28/10/2366/F7)].

In summary, Sann et al. (2008) provide evidence that $Ca_v2.2$–laminin $\beta2$ interaction in *X. laevis* embryos mediates a Ca^{2+}-dependent stop signal for neurite outgrowth during sensory innervation of the skin. The expression pattern of laminin $\beta2$, restricted to hexagonal cells, acts as a map for neurite outgrowth, which is read by the N-type Ca^{2+} channel expressed in the growth cone, ensuring precise innervation of the target tissue. This function is very different from that observed in the case of the mouse neuromuscular junction, where $Ca_v2.1$ binding to laminin $\beta2$ plays a scaffolding role (and not a Ca^{2+} signaling function) for precise presynaptic anchoring and enrichment of P/Q-type Ca^{2+} channels in active zones of motor nerve terminals (Nishimune et al., 2004).

These results leave some questions to be discussed, in particular regarding the molecular mechanism of Ca^{2+} influx in response to the contact of the growth cone with laminin $\beta2$. The authors suggest that laminin $\beta2$, by interacting with the $Ca_v2.2$ subunit, could induce a stretch activation of N-type Ca^{2+} channels, allowing Ca^{2+} influx into the growth cone, which in turn inhibits neurite outgrowth. However, although a mechanosensitivity of N-type Ca^{2+} currents has been demonstrated (Calabrese et al., 2002), there is no evidence that stretch alone is sufficient to activate N-type voltage-gated Ca^{2+} channels independently of any change in membrane potential. Hence, to discriminate between a stretch or voltage activation of the Ca^{2+} influx, it could be interesting to grow neurons on stripes of native and denaturated laminin $\beta2$ in the presence of tetrodotoxin (which blocks voltage-activated sodium channels), while monitoring $[Ca^{2+}]_i$ in the growth cone. A similar experiment using a soluble laminin $\beta2$ C-terminal fragment, which does not interact with the coating of the culture dish (and thus would not induce any stretch of the cell in interacting with the Ca^{2+} channel), could also provide important information regarding the mechanism of Ca^{2+} influx into the growth cone.

Another unanswered question concerns the molecular identity of the channel involved in this Ca^{2+} influx. It was previously shown that Ca^{2+} entry through stretch-activated Ca^{2+} channels inhibits neurite outgrowth of *X. laevis* spinal neurons, in contrast to other influx pathways (such as via voltage-gated Ca^{2+} channels) that would have opposite effects (Jacques-Fricke et al., 2006). Here, based on the observation that ω-conotoxin GVIA inhibits laminin-$\beta2$-mediated inhibition of neurite outgrowth, Sann et al. (2008) suggest that the N-type voltage-activated Ca^{2+} channel is the channel supporting Ca^{2+} influx involved in this inhibition. Nevertheless, ω-conotoxin GVIA binds to the 11th extracellular loop of the $Ca_v2.2$ subunit (Feng et al., 2003), that is, on the same molecular determinant as laminin $\beta2$. Thus, we cannot rule out the possibility that the binding of ω-conotoxin GVIA to the $Ca_v2.2$ subunit, in addition to inhibiting the Ca^{2+} permeability of the channel, could also alter, via a bulky effect, laminin $\beta2$ interaction with the channel. Such an effect was proposed (Nishimune et al., 2004) as a mechanism by which antibodies to the 11th loop of the $Ca_v2.1$ subunit found in the sera of Lambert-Eaton myasthenic patients could induce the linked neuromuscular disease (Takamori et al., 2000). Coimmunoprecipitation experiments of the $Ca_v2.2$ subunit with laminin $\beta2$ in the presence of ω-conotoxin GVIA could resolve this question.

Finally, it could be interesting to examine whether a Ca^{2+} release from intracellular stores is associated with the Ca^{2+} influx in the laminin-$\beta2$-induced neurite outgrowth inhibition. Monitoring neurite outgrowth on native/denaturated stripes in the presence of pharmacological inhibitors of ryanodine and inositol trisphosphate receptors could further detail the nature of the Ca^{2+} signaling in the control of the sensory innervation.

In conclusion, the study by Sann et al. (2008) provides important insights in the understanding of molecular mechanisms controlling sensory innervation. It also opens an interesting therapeutic strategy: blocking Ca^{2+} influx into nerve terminals via local administration of specific Ca^{2+} channel inhibitors could favor the recovery of sensory innervation after a trauma.

References

Calabrese B, Tabarean IV, Juranka P, Morris CE (2002) Mechanosensitivity of N-type calcium currents. Biophys J 83:2560–2574.

Feng ZP, Doering CJ, Winkfein RJ, Beedle AM, Spafford JD, Zamponi GW (2003) Determinants of inhibition of transiently expressed voltage-gated calcium channels by omega-conotoxins GVIA and MVIIA. J Biol Chem 278:20171–20178.

Jacques-Fricke BT, Seaow Y, Gottlieb PA, Sachs F, Gomez TM (2006) Ca^{2+} influx through mechanosensitive channels inhibits neurite outgrowth in opposition to other influx pathways and release from intracellular stores. J Neurosci 26:5656–5664.

Nishimune H, Sanes JR, Carlson SS (2004) A synaptic laminin-calcium channel interaction organizes active zones in motor nerve terminals. Nature 432:580–587.

Sann SB, Xu L, Nishimune H, Sanes JR, Spitzer NC (2008) Neurite outgrowth and *in vivo* sensory innervation mediated by a $Ca_v2.2$–laminin $\beta2$ stop signal. J Neurosci 28:2366–2374.

Takamori M, Maruta T, Komai K (2000) Lambert-Eaton myasthenic syndrome as an autoimmune calcium-channelopathy. Neurosci Res 36:183–191.

Walker D, De Waard M (1998) Subunit interaction sites in voltage-dependent Ca^{2+} channels: role in channel function. Trends Neurosci 21:148–154.