findings from the world of *Drosophila* neurobiology and convey them to the neuroscience community at large, let alone the lay public. How does the elegant work of Saitoe and colleagues help us in understanding dementia? First of all, they do not suggest that a run on the D-serine aisle at the health food store is in order for the forgetful; there are far too many differences between the fly system and mechanisms of human cognitive impairment to warrant its use as a supplement. What is fascinating to consider, however, is that NMDA receptor activation that is crucial for memory in the healthy adult animal may be deliberately downregulated by neuronal-glial crosstalk in aging. According to this model, in aging, PKA-associated neuronal activation augments glial PC, which puts a brake on D-serine synthesis. This may in turn feed back to inhibit NMDA receptor activity as a neuroprotective mechanism. While the precise mechanisms whereby this occurs are not set forth in this issue of *Neuron*, Yamazaki et al. (2014) do provide the background for further experiments defining the details of this and related interactions and clarifying their role in AMI in contexts apart from olfactory memory in the fly. If indeed glia are playing a modulatory role in dementia, rather than acting as passive nursemaids of sick neurons, then the paper in this issue of *Neuron* may represent the advance guard of work that defines the nature of the neuronal-glial conversation that regulates memory in health and aging and, ideally, exploits this knowledge to generate novel or improved therapies for age-related cognitive disorders.

REFERENCES

Dudai, Y., Jan, Y.N., Byers, D., Quinn, W.G., and Benzer, S. (1976). Proc. Natl. Acad. Sci. USA *73*, 1684–1688.

Kalderon, D., and Rubin, G.M. (1988). Genes Dev. *2* (12A), 1539–1556.

Murray, M.E., Kouri, N., Lin, W.L., Jack, C.R., Jr., Dickson, D.W., and Vemuri, P. (2014). Alzheimers Res. Ther. *6*, 1.

Nagai, M., Re, D.B., Nagata, T., Chalazonitis, A., Jessell, T.M., Wichterle, H., and Przedborski, S. (2007). Nat. Neurosci. *10*, 615–622.

Skoulakis, E.M., Kalderon, D., and Davis, R.L. (1993). Neuron *11*, 197–208.

Yamazaki, D., Horiuchi, J., Miyashita, T., and Saitoe, M. (2010). J. Neurosci. *30*, 15573–15577.

Yamazaki, D., Horiuchi, J., Ueno, K., Ueno, T., Saeki, S., Matsuno, M., Naganos, S., Miyashita, T., Hirano, Y., Nishikawa, H., et al. (2014). Neuron *84*, this issue, 753–763.

TRPV1 Channels: Not So *Inactive* on the ER

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In this issue of *Neuron*, Wong et al. (2014) report a remarkable evolutionarily conserved role for the *Drosophila* TRPV1 homolog *Inactive* controlling synaptic growth at larval neuromuscular junctions by facilitating Ca^{2+} release from the endoplasmic reticulum.

Transient receptor potential (TRP) channels constitute an extremely versatile family of cation-conducting proteins that are expressed in numerous excitable and nonexcitable cells. TRP channels are unique multimodal cell sensors that sense the outside world but also the extra- and intracellular environment facilitating diverse functions. TRP channels are also multimodal signal integrators, such that the response to one input is modified by another. TRP-mediated Ca^{2+} signaling is used in multiple ways; it triggers specific intracellular Ca^{2+} signaling cascades, changes the membrane potential modulating the driving force for Ca^{2+} entry pathways, or activates tightly voltage-dependent Ca^{2+} channels (Gees et al., 2010).

The main dogma has been that most TRP channels exert their functional effects as plasma membrane channels. However, many TRP channels are also found on diverse intracellular membranes. TRP vanilloid 1 channels (TRPV1) represent one example for the potentially divergent roles of an individual TRP channel found on both the plasma membrane and an endomembrane.

TRPV1 channels sense diverse signals including temperature (>43°C), protons, some endogenous lipids, and capsaicin, the spicy compound of chili peppers (Gees et al., 2010; Venkatachalam and Montell, 2007). Inflammation shifts the activation threshold of TRPV1 channels downward causing an inflammation-induced hypersensitivity, making them a potential target treating chronic pain (Basbaum et al., 2009). TRPV1 knockout (KO) mice confirmed their critical role for the detection and integration of painful chemical and thermal stimuli (Caterina et al., 1997). TRPV1 channels are highly expressed in neurons of the dorsal root ganglion (DRG) and trigeminal ganglion

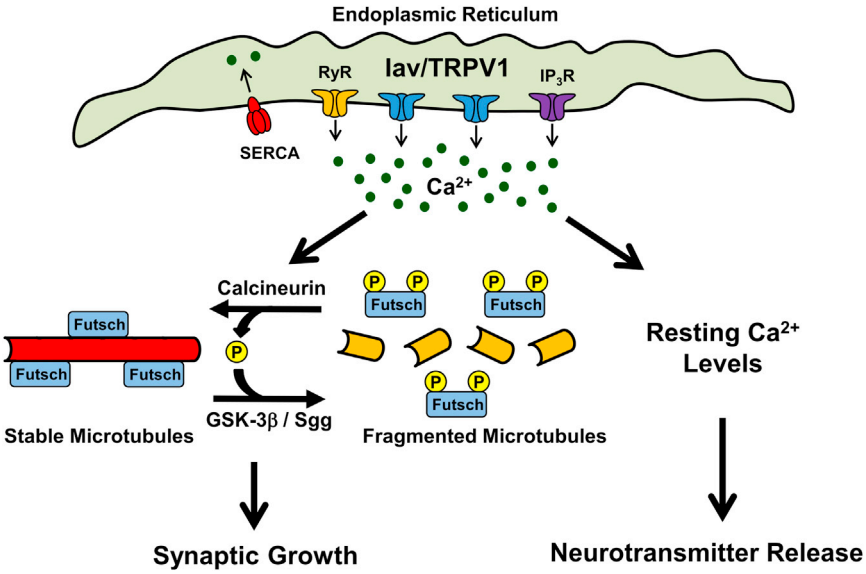

Figure 1. Proposed Models of *iav*/TRPV1-Mediated ER Ca^{2+} Release Controlling Synaptic Growth

Iav controls the stability of microtubules (MTs) through the phosphorylation state of Futsch. MTs are stabilized by binding dephosphorylated Futsch. The phosphorylation state of Futsch is controlled by GSK-3β/Sgg and calcineurin. Other ER Ca^{2+} transducers such as ryanodine receptors (RyRs), IP_3 receptors (IP_3Rs), and the Ca^{2+} pump SERCA can also affect synaptic growth. Iav also controls presynaptic resting Ca^{2+} levels, which may fine tune the probability of neurotransmitter release.

(TG) but are also present in axon terminals of peripheral nerves, the brain, and multiple nonneuronal cell types (Gees et al., 2010; Venkatachalam and Montell, 2007). In the brain, TRPV1 is a key mediator of various forms of synaptic plasticity, like LTP and LTD in the hippocampus (Gibson et al., 2008; Marsch et al., 2007) and presynaptic facilitation of glutamatergic transmission at solitary tract afferents (Peters et al., 2010).

A role of TRPV1 for Ca^{2+} release from intracellular Ca^{2+} stores emerged from early studies showing that activation of native TRPV1 in DRG neurons increased intracellular Ca^{2+} in the absence of extracellular Ca^{2+} (Olah et al., 2001). Further experiments revealed that a large amount of TRPV1 localizes to the ER, where it forms functional ER Ca^{2+} release channels (Gallego-Sandín et al., 2009). However, whether TRPV1 channels on the ER are critical for neuronal function is poorly understood.

In this issue of *Neuron*, Wong et al. (2014) report that ER Ca^{2+} release by the *Drosophila* TRPV channel Inactive (Iav) modulates synaptic growth and presynaptic Ca^{2+} resting levels. The authors genetically screened most *Drosophila* TRP channels for a potential synaptic role at glutamatergic larval neuromuscular junctions (NMJs) and found that mutations in *iav* impaired synaptic growth. This defect was restored by expression of a genomic *iav* transgene, suggesting that Iav is required for synaptic growth of larval NMJs.

The newly proposed synaptic role of Iav is rather different from its known role in proprioception, hearing, and locomotion (Venkatachalam and Montell, 2007). Loss of sensory input could alter locomotion and motor circuitry such that it indirectly affects synaptic growth at NMJs. However, expression of Iav in the respective sensory organs of *iav* mutants restored the defects in somatosensation and locomotion but failed to restore synaptic growth of NMJs. Vice versa, expression of Iav in motor neurons (MNs) restored the growth defect but had no effect on locomotion, suggesting that Iav mediates synaptic growth at larval NMJs in a cell-autonomous manner.

Surprisingly, transgenic expression of human TRPV1, but not TRPV4, restored the synaptic growth defect of *iav* mutant NMJs. Feeding flies the TRPV1 antagonist capsazepine suppressed the genetic rescue of TRPV1, indicating that a functional channel is required mediating synaptic growth. Considering the significant evolutionary divergence of sensory modalities between Iav and TRPV1 channels—Iav mediates mechanosensation and hearing while TRPV1 has a prominent role in nociception (Venkatachalam and Montell, 2007)—the TRPV1-mediated rescue of the synaptic defects at *iav* mutant NMJs indicates a most remarkable evolutionarily conserved synaptic role of Iav and TRPV1.

How does Iav promote synaptic growth? Loss of Iav decreases the number of synaptic boutons while it increases their size without affecting evoked neurotransmitter release—a defect that is mirrored by mutants that affect the stability of microtubules (MTs). Indeed, *iav* mutants exhibit fragmented MT and a reduced number of MT loops in synaptic boutons, which are robust indicators of a mature and stable synaptic bouton (Roos et al., 2000).

The stability of MT loops is regulated to a large part by the phosphorylation state of Futsch (Figure 1), the fly ortholog of the MT-binding protein MAP1B (Roos et al., 2000). Shaggy (Sgg), the fly glycogen synthase kinase-3β, phosphorylates Futsch, causing its dissociation from MT and their fragmentation (Franco et al., 2004). Reciprocally, Iav-mediated Ca^{2+} release could promote Futsch binding to MT after dephosphorylation by calcineurin, a Ca^{2+}/calmodulin-sensitive protein phosphatase, as it has been shown for mammalian MAP1B (Gong et al., 2000). Testing this possibility genetically by decreasing phosphorylated Futsch levels, using either expression of constitutively active calcineurin or dominant-negative Sgg, suppressed the growth deficit of *iav* mutant NMJs. These results indicate that calcineurin and Futsch act downstream of Iav in the same signaling pathway. Furthermore, expression of constitutively active calcineurin at futsch mutant NMJs failed to suppress their synaptic growth deficit, indicating that calcineurin indeed acts upstream of Futsch and not in a parallel pathway.

Iav/TRPV1 function in sensory cells has been associated with Ca^{2+} entry across the plasma membrane (Gees et al.,

2010; Zhang et al., 2013). However, endogenous Iav protein was not detectable at all in fly MNs and NMJs, even though endogenous Iav was detected in cilia of chordotonal organs that are required for hearing in flies. Overexpressed Iav was found on the ER in muscles and MN somas but was still not detectable at NMJs.

The ER localization of overexpressed Iav is unlikely due to an unspecific accumulation on the ER for three reasons. First, transgenically expressed TRPV1 also associated with the ER while TRPV4 did not, which correlates well with the ability of TRPV1 but not TRPV4 to restore the synaptic growth defects of *iav* mutant NMJs. Second, expression of Iav or TRPV1 in Neuro2A cells showed that both can form a functional ER Ca^{2+} release channel, while TRPV4 cannot. Third, genetic alterations reducing ER Ca^{2+} release by reduced protein levels of IP_3-receptors, Ryanodine receptors (RyRs), or the ER Ca^{2+} pump SERCA all decreased synaptic growth, suggesting that Iav's synaptic growth function is based on ER Ca^{2+} release. Consistently, increasing ER Ca^{2+} release by overexpressing RyRs restored the synaptic growth defects of *iav* mutants. Together, these findings clearly support a critical role of Iav/TRPV1 for ER Ca^{2+} release and link it to synaptic growth at larval NMJs.

The newly defined role of Iav for ER Ca^{2+} release raised the question whether Iav controls Ca^{2+} homeostasis at presynaptic terminals, and if so, is Ca^{2+} homeostasis functionally linked to synaptic growth? Interestingly, at near physiological levels of extracellular Ca^{2+}, *iav* mutant axon terminals exhibited normal cytosolic Ca^{2+} resting levels. However, when extracellular Ca^{2+} was lowered, presynaptic Ca^{2+} resting levels of *iav* mutant boutons exhibited within minutes an accelerated decline, leading to a reduced probability of neurotransmitter release. Accordingly, Iav may mediate the stability of MT through defined Ca^{2+} microdomains instead of large fluctuations on presynaptic Ca^{2+} resting levels. Such microdomains could be facilitated by binding directly to MT, as it has been shown for TRPV1 channels (Storti et al., 2012).

The study by Wong et al. (2014) significantly extends our mechanistic understanding of Iav/TRPV1 channels in the ER membrane and has probably important implications for the proposed roles of TRPV1 as a key mediator of synaptic plasticity. The signals activating and regulating Iav/TRPV1 activity on the ER membrane remain to be elucidated. It seems likely that the channels on the ER and plasma membrane are differently regulated. For example, TRPV1 channels are regulated by $PI(4,5)P_2$, which is not present on the ER membrane. Iav/TRPV1 must be certainly considered as targets of changes in intracellular Ca^{2+}, which could be mediated by diverse signaling pathways. One likely candidate is Ca^{2+}/calmodulin, which can effectively inhibit TRPV1-mediated ER Ca^{2+} release in HEK293T cells (Gallego-Sandín et al., 2009). Future work will also be needed to resolve whether Iav/TRPV1 channels may even act as scaffolding proteins forming signaling complexes that link the ER and MT.

REFERENCES

Basbaum, A.I., Bautista, D.M., Scherrer, G., and Julius, D. (2009). Cell *139*, 267–284.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., and Julius, D. (1997). Nature *389*, 816–824.

Franco, B., Bogdanik, L., Bobinnec, Y., Debec, A., Bockaert, J., Parmentier, M.L., and Grau, Y. (2004). J. Neurosci. *24*, 6573–6577.

Gallego-Sandín, S., Rodríguez-García, A., Alonso, M.T., and García-Sancho, J. (2009). J. Biol. Chem. *284*, 32591–32601.

Gees, M., Colsoul, B., and Nilius, B. (2010). Cold Spring Harb. Perspect. Biol. *2*, a003962.

Gibson, H.E., Edwards, J.G., Page, R.S., Van Hook, M.J., and Kauer, J.A. (2008). Neuron *57*, 746–759.

Gong, C.X., Wegiel, J., Lidsky, T., Zuck, L., Avila, J., Wisniewski, H.M., Grundke-Iqbal, I., and Iqbal, K. (2000). Brain Res. *853*, 299–309.

Marsch, R., Foeller, E., Rammes, G., Bunck, M., Kössl, M., Holsboer, F., Zieglgänsberger, W., Landgraf, R., Lutz, B., and Wotjak, C.T. (2007). J. Neurosci. *27*, 832–839.

Olah, Z., Szabo, T., Karai, L., Hough, C., Fields, R.D., Caudle, R.M., Blumberg, P.M., and Iadarola, M.J. (2001). J. Biol. Chem. *276*, 11021–11030.

Peters, J.H., McDougall, S.J., Fawley, J.A., Smith, S.M., and Andresen, M.C. (2010). Neuron *65*, 657–669.

Roos, J., Hummel, T., Ng, N., Klämbt, C., and Davis, G.W. (2000). Neuron *26*, 371–382.

Storti, B., Bizzarri, R., Cardarelli, F., and Beltram, F. (2012). J. Biol. Chem. *287*, 7803–7811.

Venkatachalam, K., and Montell, C. (2007). Annu. Rev. Biochem. *76*, 387–417.

Wong, C., Chen, K., Lin, Y.Q., Chao, Y., Duraine, L., Lu, Z., Yoon, W.H., Sullivan, J.M., Broadhead, G.T., Sumner, C.J., et al. (2014). Neuron *84*, this issue, 764–777.

Zhang, W., Yan, Z., Jan, L.Y., and Jan, Y.N. (2013). Proc. Natl. Acad. Sci. USA *110*, 13612–13617.