Journal Club

Editor's Note: These short reviews of a recent paper in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to mimic the journal clubs that exist in your own departments or institutions. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Synapses Fight Over Glutamate Receptor 1

Gavin Rumbaugh

Department of Neuroscience, The Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Review of Harms et al. (http://www.jneurosci.org/cgi/content/full/25/27/6379)

Activity shapes the input–output relationships of neuronal circuits in several ways. Rewiring of discrete circuits can occur after disruption of normal input (Wiesel, 1982). However, networks can maintain plasticity without widespread changes in connectivity by regulating synaptic function within existing pathways. For example, synaptic scaling, or homeostatic plasticity, results after a global change in activity throughout an entire network (Turrigiano and Nelson, 2000). In addition, Hebbian plasticity can occur after coincident activity in presynaptically and postsynaptically connected neurons and leads to fine tuning of distinct synaptic inputs. A hallmark of both homeostatic and Hebbian plasticity is a change in postsynaptic glutamate receptor (GluR) function. AMPA receptors (AMPARs) carry the majority of fast synaptic transmission at excitatory synapses. Therefore, understanding how activity can modify the accumulation of these receptors, in addition to rewiring of neuronal circuits, is critical to the elucidation of basic neural function.

The recent article by Harms et al. in *The Journal of Neuroscience* (http://www.jneurosci.org/cgi/content/full/25/27/6379) addresses the nature of neurotransmitter release in the fate of individual synapses. What role does neurotransmitter release play in the generation or maintenance of inputs, the structural and morphological characteristics of the synapse, and glutamate receptor accumulation? The authors used a clever combination of chronically overexpressed proteins to measure properties of active and inactive synapses in cultured hippocampal neurons. When present in neurons, the light chain of the tetanus toxin (TNT) cleaves vesicle-associated membrane protein 2 (VAMP2)/VAMP3, inhibiting vesicular neurotransmitter release. Synaptophysin specifically targets presynaptic vesicles containing neurotransmitter. The authors coexpressed TNT fused to cyan fluorescent protein (TNTCFP) and synaptophysin fused to yellow fluorescent protein (SYNYFP) and then visualized individual synapses in which they expected to see disrupted neurotransmitter release. Because a single postsynaptic cell contains inputs from multiple neurons, inhibited synapses (SYNYFP plus TNTCFP) should be surrounded by actively releasing presynaptic terminals originating from untransfected neurons (detected by endogenous synapsin). To test the validity of this system, the authors demonstrated that terminals positive for SYNYFP and TNTCFP contain lower levels of VAMP2, suggesting that TNTCFP was indeed functional [Harms et al. (2005), their Fig. 1 (http://www.jneurosci.org/cgi/content/full/25/27/6379/FIG1)]. Importantly, neighboring synapses positive for synapsin, but negative for SYNYFP, had normal levels of VAMP2. Did neurons that expressed TNTCFP have deficits in neurotransmitter release? To address this issue, the investigators recorded evoked EPSCs from reciprocally connected pairs of neurons grown on isolated microislands. Indeed, when a TNTCFP-expressing cell was the presynaptic neuron, the EPSC failure rate was nearly 100%, confirming that the toxin had eliminated vesicular release [Harms et al. (2005), their Fig. 2 (http://www.jneurosci.org/cgi/content/full/25/27/6379/FIG2)].

Once the system was validated, the authors probed inhibited synapses for morphological and compositional changes. Surprisingly, many of the usual suspects appeared to be unaffected. Total synaptic density was unchanged in neurons expressing TNTCFP and SYNYFP (compared with SYNYFP plus CFP) [Harms et al. (2005), their Fig. 3 (http://www.jneurosci.org/cgi/content/full/25/27/6379/FIG3)]. Using immunocytochemistry on fixed "Banker sandwich" neurons, the authors demonstrated that accumulation of postsynaptic signaling and scaffolding molecules, including PSD-95, was also unaltered by terminal inactivation [Harms et al. (2005), their Fig. 4 (http://www.jneurosci.org/cgi/content/full/25/27/6379/FIG4)]. The key discovery came when they probed for various subunits comprising AMPARs. Nearly all of the active synapses accumulated both GluR1 and GluR2 subunits. Surprisingly, however, SYNYFP plus TNTCFP synapses that were adjacent to active synapses exhibited a significant (~25%) reduction in GluR1 accumulation (Fig. 1). Did inputs compete for GluR1-containing AMPA receptors? To address this question, the authors grew hippocampal cultures in the pres-

Received Aug. 1, 2005; accepted Aug. 9, 2005.

Correspondence should be addressed to Gavin Rumbaugh, Department of Neuroscience, The Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, 930 Preclinical Teaching Building, Baltimore, MD 21205. E-mail: grumbaug@jhmi.edu.

DOI:10.1523/JNEUROSCI.3209-05.2005

 0270-6474/05/258347-02$15.00/0

ence of soluble tetanus toxin. This treatment blocks nearly all vesicular neurotransmitter release, including miniature EPSCs (mEPSCs) (Capogna et al., 1997), thus eliminating activity-dependent competition between synapses. When the authors analyzed TNTCFP plus SYNYFP synapses in the presence of chronically applied TNT, there was no effect on GluR1 accumulation relative to control synapses [Harms et al. (2005), their Fig. 5 (http://www.jneurosci.org/cgi/content/full/25/27/6379/FIG5)]. These data indicate that, in a competitive environment, GluR1-containing receptors are preferentially targeted to active synapses at the expense of less-active neighboring synapses.

One unresolved question in neurobiology relates to the ability of neuronal activity to regulate individual synapses. Many paradigms that induce plasticity, such as chronic activity blockade resulting in synaptic scaling, lead to global changes in synaptic strength and therefore lack specificity toward individual synapses. The induction of long-term potentiation (LTP), a type of Hebbian plasticity, is thought to be input-specific and should therefore be limited to activated synapses. However, it is believed that potentiation induced by LTP spreads to neighboring synapses, and the specificity breaks down at distances of <70 μM (Engert and Bonhoeffer, 1997). Recently, the application of two-photon uncaging of glutamate in hippocampal slice cultures revealed that single synapses can insert AMPA receptors after an "LTP-like" stimulus (Matsuzaki et al., 2004), suggesting that the synaptic machinery is capable of regulating strength at this resolution, although this study did not use unitary presynaptic inputs to induce changes in AMPA receptor function. In this context, the data presented by Harms et al. (2005) suggest that activity of a presynaptic terminal can regulate synaptic accumulation of AMPA receptors at the level of an individual synapse.

The data presented in this study are intriguing, although some fundamental questions should be addressed. Does silencing of an individual input change synaptic strength? The authors did not directly measure synaptic strength at inhibited synapses, although they did measure AMPA receptors by immunocytochemistry. They concluded that there was a downregulation of homomeric GluR1-containing channels at inhibited synapses, because they did not observe a parallel increase in GluR2 or GluR3 immunoreactivity. This method may not be sensitive enough to accurately measure the level of AMPA receptors at synapses, and therefore the effect on synaptic strength is unknown in this paradigm. In addition, would the authors see the same phenomenon if they used another means to disrupt activity at individual terminals? TNT essentially inhibits release of neurotransmitter vesicles, including spontaneous fusions leading to mEPSCs. Using an additional method that inhibits activity-dependent release, but preserves mEPSCs, could potentially give a different result. Perhaps quantal release of glutamate, or some other yet-to-be-discovered factor released from spontaneously fusing vesicles, is necessary for proper postsynaptic receptor accumulation in developing neurons. Finally, what role does inhibiting vesicle release have after the formation of stable networks? The methods used in this study involved blocking activity from day 1 in culture [day *in vitro* (DIV) 1]. It would be interesting to know whether the same phenomenon described by Harms et al. (2005) would occur if TNTCFP were transfected after the completion of synaptogenesis (i.e., DIV >14).

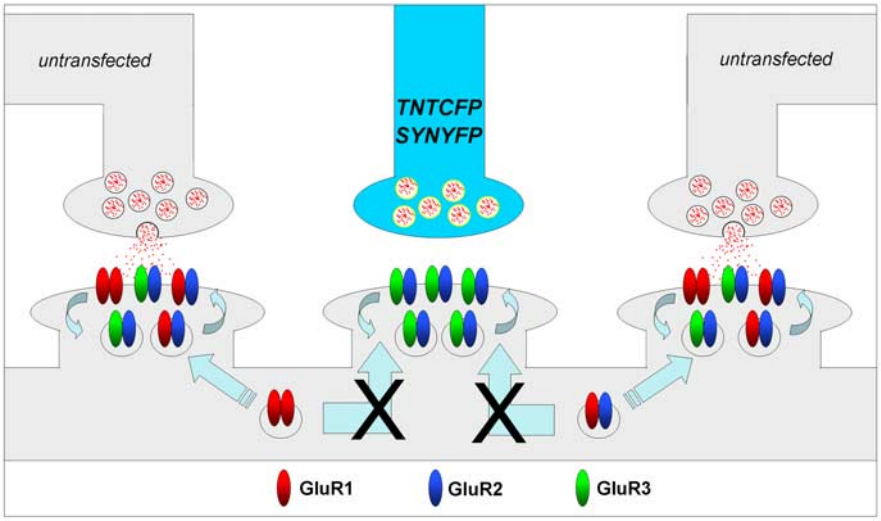

Figure 1. Dramatization of the results presented by Harms et al. (2005). A dendritic segment contains spines opposed to either normal, releasing terminals (untransfected) or activity inhibited terminals (TNTCFP/SYNYFP). Synapses normally acquire GluR1, GluR2, and GluR3 subunits of the AMPA receptor throughout development. However, Harms et al. (2005) report that inactive synapses acquire less GluR1 when flanked by active synapses.

References

Capogna M, McKinney RA, O'Connor V, Gahwiler BH, Thompson SM (1997) Ca^{2+} or Sr^{2+} partially rescues synaptic transmission in hippocampal cultures treated with botulinum toxin A and C, but not tetanus toxin. J Neurosci 17:7190–7202.

Engert F, Bonhoeffer T (1997) Synapse specificity of long-term potentiation breaks down at short distances. Nature 388:279–284.

Harms KJ, Tovar KR, Craig AM (2005) Synapse-specific regulation of AMPA receptor subunit composition by activity. J Neurosci 25:6379–6388.

Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H (2004) Structural basis of long-term potentiation in single dendritic spines. Nature 429:761–766.

Turrigiano GG, Nelson SB (2000) Hebb and homeostasis in neuronal plasticity. Curr Opin Neurobiol 10:358–364.

Wiesel TN (1982) Postnatal development of the visual cortex and the influence of environment. Nature 299:583–591.