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Spontaneous neurotransmission: a form of neural communication comes of age

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Since its discovery by Bernard Katz and colleagues at the frog neuromuscular junction spontaneous neurotransmitter release has never ceased to fascinate neurophysiologists. The initial observations of spontaneous events were considered within the general context of biological noise and their functional implications were only guessed (Fatt and Katz, 1950). However, even during initial experiments these random miniature synaptic release events were shown to trigger occasional action potentials in the muscle, suggesting a potential physiological impact of this form of neurotransmitter release (Fatt and Katz, 1950; Fatt and Katz, 1952). In the intervening decades, the function and mechanism of these seemingly random neurotransmitter release events have continued to be a subject of extensive speculation. Yet, the question of why synapses — dedicated to information transfer and processing with high temporal and spatial precision —would give rise to random outbursts of neurotransmitter release and activity, has remained unanswered.

The advent of high resolution electrophysiology uncovered the ubiquity of this form of neurotransmission in multiple species, from invertebrates to vertebrates, including the mammalian brain. However, direct examination of spontaneous neurotransmission was hindered by the ability to manipulate it, due to limited insight into the properties of the release machinery and synaptic transmission in single synapses. This critical insight was gained during initial molecular manipulations of the release machinery via clostridial toxins or genetic models, which provided the first hints at a diversity of mechanisms which could give rise to spontaneous and action potential evoked neurotransmission. In these studies, evoked release could be readily impaired by disruption of the SNARE machinery or Ca^{2+} sensors while spontaneous release was more resilient to these manipulations (see Kavalali, 2015 for review). This suggested that spontaneous and evoked release could be regulated independently and spontaneous release might serve its own function.

In the last decade, our group, as well as others, has initiated a systematic analysis of spontaneous neurotransmission in order to uncover its molecular regulation and synaptic function. This work has been enabled by the development of new methods for optical imaging of synaptic vesicle trafficking and visualization of synaptic transmission at single synaptic terminals. In this way, we could focus on examination of synaptic transmission at the level of individual synaptic contacts, single synaptic vesicles, and postsynaptic receptor

CONFLICT OF INTEREST STATEMENT

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clusters. When combined with these advanced functional tools, our increasing insight into the molecular organization of synaptic terminals brought this peculiar form of neuronal communication from the realm of speculation and assumptions into the territory of direct experimental inquiry. This endeavor led to several unexpected findings. Importantly, cross-depletion experiments —where one form of neurotransmission can be selectively impaired — provided evidence for the independence of the two forms of neurotransmission and the potential diversity of vesicle populations giving rise to spontaneous and action potential evoked neurotransmitter release. The findings from this work showed that spontaneous neurotransmitter release is driven by a vesicle population that preferentially recycles at rest independently of the vesicle pool that drives action potential evoked release (Sara et al., 2005; Fredj and Burrone, 2009; Chung et al., 2010). Several molecular mechanisms appear to work in parallel to make this apparent vesicle pool diversity possible. For instance, vesicular SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) molecules vti1a, VAMP7 and to some extent VAMP4 selectively impact spontaneous release (Hua et al., 2011; Ramirez et al., 2012; Raingo et al., 2012; Bal et al., 2013), whereas complexins that interact with SNARE complexes augment evoked release but suppress spontaneous release in multiple systems (Huntwork and Littleton, 2007; Yang et al., 2013). Recent studies show that active zone proteins such as RIM1 or RIMBP show some selectivity towards regulation of evoked events (Calakos et al., 2004; Acuna et al, 2015; Li and Kavalali, 2015). Finally Ca^{2+} sensors such as synaptotagmin-1 suppress spontaneous release but promote and synchronize evoked release (Maximov and Südhof, 2005; Xu et al., 2009), whereas another Ca^{2+} sensor Doc2 (soluble calcium-binding double C2 domain protein) appears to selectively regulate spontaneous release (Groffen et al., 2010; Pang et al., 2011).

Identification of presynaptic molecular release machinery components that are largely dedicated to the maintenance and regulation of spontaneous release provided new opportunities to probe the functions of spontaneous release events in isolation. For instance, as stated above, earlier studies have suggested that alternative SNAREs vti1a and VAMP7 selectively contribute to spontaneous neurotransmission (Hua et al., 2011; Ramirez et al., 2012; Bal et al., 2013). In a recent study, targeting these vesicular SNAREs could elicit selective regulation of spontaneous neurotransmission and downstream postsynaptic signaling without significantly altering evoked neurotransmission (Crawford et al., 2017). In subsequent work, targeting the Doc2-like protein family selectively reduced spontaneous neurotransmission in neurons while leaving action potential-evoked responses unaltered (Ramirez et al., 2017). This molecularly specific manipulation of spontaneous release, in return, was sufficient to increase the amplitudes of miniature quantal excitatory events augmenting synaptic efficacy. Taken together, these findings suggest that molecularly distinct pathways selectively maintain spontaneous neurotransmission and enable these events to regulate synaptic efficacy independent of evoked neurotransmission.

Further confirming the independent functional relevance of spontaneous events, an increasing number of studies have revealed that these events alone trigger biochemical signaling. For instance, relatively small Ca^{2+} signals triggered by activation of NMDA receptors by spontaneous release events can be amplified by Ca^{2+}-induced Ca^{2+} transients in postsynaptic neurons (Reese and Kavalali, 2015). Biochemical signaling triggered by

spontaneous events such as this has been shown to lead to maturation and stability of synaptic networks, regulation of local dendritic protein synthesis, and control of postsynaptic responsiveness during homeostatic or other forms of synaptic plasticity in several species (for review see Kavalali, 2011). Electrophysiological experiments have also revealed that spontaneous glutamate release events activate a distinct set of postsynaptic receptors compared to action potential evoked release (Atasoy et al., 2008; Sara et al., 2011; Melom et al, 2013; Peled et al., 2014; Reese and Kavalali, 2016). The segregation of receptor populations activated by spontaneous release may explain why these miniature events are able to elicit signaling pathways that maintain synaptic homeostasis. A clear example of this form of neuronal signaling emerged from studies which showed that blockade of spontaneous NMDA receptor-mediated synaptic events, but not evoked neurotransmitter release, deactivates eukaryotic elongation factor 2 (eEF2) kinase, resulting in reduced eEF2 phosphorylation and de-suppression of dendritic protein translation (Sutton et al., 2007; Autry et al., 2011; Nosyreva et al., 2013). This same mechanism was shown to be responsible for the increase in amplitudes of miniature quantal excitatory events seen after reducing spontaneous neurotransmission by decreasing the Doc2-like protein family in the study mentioned previously. The increase in spontaneous event amplitude was accordingly occluded in eEF2 kinase deficient synapses (Ramirez et al., 2017). Furthermore, parallel studies demonstrated that this mechanism also plays a key role in mediating the rapid antidepressant action of the NMDA receptor blocker ketamine in vivo (Autry et al., 2011; Nosyreva et al., 2013). Although a recent study has suggested that the antidepressant effect of ketamine may be mediated via one of its metabolites independent of NMDA receptor block, albeit via activation of the same eEF2 kinase dependent pathway (Zanos et al., 2016), our subsequent studies have demonstrated that this ketamine metabolite also blocks NMDA receptors in manner similar to ketamine but with lower affinity and this block is required for the activation of eEF2 signaling (Suzuki et al., 2017). Additional experiments from our group showed that the effect of NMDA receptor blockers on synaptic plasticity can be mimicked by acute selective suppression of spontaneous release via application of a vacuolar ATPase blocker at rest to deplete neurotransmitter from spontaneously recycling vesicles (Nosyreva et al., 2013). This result suggested that selective presynaptic impairment of spontaneous release, without alterations in evoked neurotransmission, is sufficient to elicit postsynaptic signaling leading to synapse stability.

Given this growing literature on the dedicated mechanisms and function of spontaneous release events, in this focus issue, we sought to assemble a set of articles authored by three leading laboratories that contributed richly to our emerging understanding of the spontaneous neurotransmission process. The articles focus on three areas, namely Ca^{2+} regulation of spontaneous release, regulation of homeostatic synaptic plasticity by spontaneous release, and finally the impact of spontaneous release events on synapse development and maturation.

In their review article, Williams and Smith present a thorough discussion of the Ca^{2+} dependence of spontaneous neurotransmitter release (Williams and Smith, 2017). Despite their seemingly random nature, spontaneous release events can be regulated by Ca^{2+} signaling as well as several other signal transduction pathways. This fact prompted investigators to question whether spontaneous release is indeed “spontaneous” (Glitsch,

2008). A leading example for the non-spontaneity of these miniature fusion events is their facilitation by voltage-gated Ca^{2+} channel openings that occur at resting membrane potentials. A surprising feature of this activity is the coordinated opening of multiple Ca^{2+} channels akin to triggering of evoked release but which gives rise to a lower Ca^{2+} cooperativity consistent with a distinct Ca^{2+} sensor (Williams et al., 2012). In this context, Williams and Smith also address the apparent discrepancies in the literature on the role of resting voltage-gated Ca^{2+} channel openings in regulation of spontaneous release from inhibitory and excitatory synapses with an attempt to reconcile observations from diverse preparations (Tsintsadze et al., 2017). In addition, they present an overview of G-protein coupled mechanisms; in particular pathways that are activated by extracellular Ca^{2+}, in neuromodulation of spontaneous release (Vyleta and Smith, 2011).

The function of spontaneous neurotransmitter release has been a long standing open question (Chung and Kavalali, 2006). However, studies demonstrating the existence of dedicated mechanisms for maintenance and regulation of spontaneous release and its segregation from evoked neurotransmission imply a distinct functional role for the spontaneous neurotransmission process. Indeed, in the last decade, a number of studies have proposed a critical role for spontaneous release in homeostatic synaptic plasticity wherein synaptic strength is adjusted in response to sustained changes in activity levels. During this form of synaptic plasticity, synaptic weights are scaled concurrently across the neuron by a multiplicative factor in a process commonly referred to as “synaptic scaling”. Importantly, synaptic weights are scaled up or down in a negative feedback manner to counteract the chronic activity levels detected in the cell. Although initial work uncovered cell wide activity as a key regulator, later studies in multiple systems —including hippocampal neurons, embryonic spinal cord, and drosophila neuromuscular junction —have revealed a critical role for the actual neurotransmitter input in this process. In their article, Wenner and colleagues (Gonzalez-Islas et al., 2017) discuss the specific role played by spontaneous release in synaptic scaling based on recent experiments performed in the spinal cord and drosophila neuromuscular junction as well as mammalian central synapse systems. Importantly, these experiments took advantage of novel approaches to isolate the contribution of spontaneous release events to this form of plasticity. These studies either targeted spontaneous release-specific regulatory mechanisms to elicit direct and selective molecular interference with the spontaneous release mediated signaling (Crawford et al., 2017; Ramirez et al., 2017), or they used optogenetic stimulation to clamp cell wide activity levels while manipulating neurotransmitter input, thus uncovering an autonomous role for spontaneous release in regulation of synaptic strength (Fong et al., 2015; Garcia-Bereguiain et al., 2016).

Finally, Andreae and Burrone present an insightful overview of the role of spontaneous neurotransmission in synapse and circuit development (Andreae and Burrone, 2017). Early stages of neuronal development comprise a period of high levels of dynamic plasticity that includes the growth of dendrites and axons, as well as extensive synaptogenesis. Interestingly, under these labile circumstances immature synapses can sustain spontaneous neurotransmitter release although these nascent contacts may not yet be capable of regulated evoked release (Polo-Parada et al., 2001; Mozhayeva et al., 2002; Andreae et al., 2012; Andreae and Burrone, 2015). These spontaneous release events, in return, have been shown

to be critical in shaping neuronal morphology as well as modulating the properties of new synaptic contacts in the nervous system. In their review, Andreae and Burrone discuss studies from diverse species demonstrating that the spontaneous neurotransmitter release-mediated signaling is an important player in the formation of synaptic circuits and controlling maturation of synaptic junctions during development. In this context, spontaneous release events convey permissive as well as instructive signals that maintain synaptic contacts (Huntwork and Littleton, 2007) or drive synapse maturation and growth (Choi et al., 2014).

Taken together, as this collection of articles attest, spontaneous neurotransmission is now being studied for its own sake and not necessarily as a simple proxy for action potential evoked neurotransmission. Our increasing molecular insight into dedicated mechanisms that mediate and maintain spontaneous synaptic transmission enables us to address the roles played by this form of neurotransmission in synapse development and plasticity. To date, this work has revealed roles in regulation of synaptic network assembly and maturation as well as critical tasks in homeostatic forms of synaptic plasticity possibly underlying certain forms of behavior and response to neuropsychiatric treatments (Kavalali and Monteggia, 2012). Nevertheless, clearly more studies are needed to map the whole landscape of signaling mechanisms targeted by spontaneous events. These include but are not limited to protein translation machinery, specific protein kinases, cytoskeletal scaffolds and gene transcription. However, the information we have so far depicts a compelling picture where spontaneous neurotransmission operates as an autonomous synaptic signaling pathway that can be regulated to alter synaptic function and structure in a manner that is independent of classical Hebbian forms of synaptic plasticity and canonical forms of synaptic information processing.

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