Partitioning the Synaptic Landscape: Distinct Microdomains for Spontaneous and Spike-Triggered Neurotransmission

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Science Signaling **2** (65), pe19. [DOI: 10.1126/scisignal.265pe19]

NEUROSCIENCE

Partitioning the Synaptic Landscape: Distinct Microdomains for Spontaneous and Spike-Triggered Neurotransmission

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Published 7 April 2009, Volume 2 Issue 65 pe19

For over 50 years, it has been recognized that two distinct modes of neurotransmission are operative at synapses: the release of neurotransmitter triggered by the invasion of action potentials into presynaptic terminals and spontaneous neurotransmitter release that occurs independently of action potentials. In the past, spontaneous neurotransmitter release has been dismissed as mere synaptic noise, but recent studies have suggested that spontaneous release has important functional roles at synapses. New evidence indicates that spontaneous release and action potential–evoked release preferentially activate distinct subsets of postsynaptic receptors, suggesting that synapses use physically segregated pathways to decode spontaneous and evoked neurotransmission.

Our ability to sense and respond to the external world is critically dependent on the rapid relay of information across synapses in the brain. The description of the neuronal action potential (AP) by du Bois-Reymond (*1*), together with the elucidation of its underlying ionic basis by Hodgkin and Huxley (*2*) and of the principles of AP-triggered neurotransmitter secretion at synapses by Katz, Miledi, and their many colleagues (*3*), laid down an elegant conceptual framework for understanding how this rapid signal propagation occurs. Given this framework, it has always seemed odd that neurotransmitter might be secreted at a low rate in the absence of APs. Nevertheless, after the classic work of Fatt and Katz (*4*) at the frog neuromuscular junction, we now know that the spontaneous (in other words, AP-independent) release of synaptic vesicles is a ubiquitous property of all synapses in the nervous system. These "miniature events" or "minis," as they are affectionately referred to today, are one of the cornerstones of the quantal theory of neurotransmission. Still, the small amplitude, low frequency, and (presumably) stochastic nature of minis, compared to the precision offered by AP-triggered events, led neurobiologists to dismiss any role of these events in shaping synaptic function.

Work over the past decade, however, has changed this view. First, individual minis can influence firing rates in electrically compact neurons (*5*), and spontaneous release rates can be elevated to levels that will trigger spiking in large hippocampal CA3 pyramidal neurons (*6*). In addition, minis can stabilize excitatory synaptic function (*7*, *8*) and may stabilize structural integrity in hippocampal circuits (*9*). Finally, minis can regulate the activity of postsynaptic signaling pathways in a manner that is qualitatively distinct from that controlled by AP-triggered neurotransmission (*10*, *11*). Together, these observations suggest that although AP-triggered neurotransmission is critical for rapid information flow, miniature synaptic events have their own separate functional roles in neural circuits.

A recent paper by Kavalili and colleagues (*12*) adds an important new element to this emerging story. Through a number of convincing experiments, Atasoy *et al*. (*12*) demonstrated that spontaneous neurotransmitter release and AP-evoked release appear to target distinct populations of postsynaptic receptors at the same synapse. These authors took advantage of the pharmacological properties of MK-801: a use-dependent irreversible blocker of *N*-methyl-D-aspartate receptors (NMDARs), one class of postsynaptic receptor for the neurotransmitter glutamate. Atasoy and colleagues exploited the use-dependent irreversible nature of MK-801 blockade to functionally isolate NMDAR pools activated by spontaneous neurotransmitter release from those activated by AP-triggered release at synapses. If these receptor pools are largely overlapping, as had been widely assumed, then the block of NMDAR current conferred by eliciting AP-evoked release should be evident in subsequent recordings of spontaneous AP-independent NMDAR currents, and vice versa. Surprisingly, however, Atasoy and colleagues found that this was not the case. In cultured hippocampal neurons, the authors measured evoked NMDAR-mediated excitatory postsynaptic currents (EPSCs) by eliciting a single AP by means of field stimulation. Then they isolated spontaneous neurotransmission by applying tetrodotoxin (TTX), which blocks voltage-gated Na^+ channels, and found that MK-801 treatment rapidly abolished miniature NMDAR currents (within ~ 1 min). They then washed out TTX and again elicited AP-triggered NMDAR EPSCs using field stimulation. Remarkably, although MK-801 effectively abolished NMDAR miniature neurotransmission under these conditions, there was virtually no effect on the evoked NMDAR EPSCs. Continued stimulation in the presence of MK-801, however, did produce the expected use-dependent block of evoked NMDAR-mediated currents. In a complementary set of experiments, the authors showed that, when the order of blockade was reversed (first blocking evoked NMDAR currents with MK-801), miniature NMDAR currents were largely unaffected by a previous block of NMDARs activated by AP-triggered neurotransmitter release. These results nicely extended to recordings in autaptic hippocampal cultures and those from acutely prepared hippocampal slices, where the intrinsic hippocampal circuitry is largely preserved. In all of these systems, near-complete blockade of NMDARs under one mode of neurotransmission spared NMDAR current observed under the alternate mode, suggesting that the receptor pools activated by AP-triggered and miniature neurotransmission are distinct.

One possible explanation for these results is that, at individual synapses, either evoked or spontaneous neurotransmission takes place in a dominant fashion to the exclusion of the other. In this case, the different NMDAR pools identified by Atasoy *et al*. might reside at different synapse populations, rather than within the same synapse. To explore this possibility, the

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authors turned to an optical approach that allowed them to assess the kinetics of evoked and spontaneous neurotransmission at the same synaptic sites. They used a variant of the synaptophluorin technique, in which a highly pH-sensitive green fluorescent protein variant (phluorin) is fused to a synaptic vesicle protein (in this case, synaptophysin) and can be used to identify release events at individual synaptic terminals (*13*). Using this approach, Atasoy *et al*. found that the vast majority (~80%) of synaptic sites identified showed both AP-triggered and AP-independent modes of neurotransmitter release, making it unlikely that their results are due to effects occurring at different synapses.

Taken together, the experimental data provided by Atasoy *et al*. suggest that there is a spatial separation of the NMDAR populations activated by AP-evoked and spontaneously released neurotransmitter. This idea was further supported by modeling NMDAR activation at the synapse, which showed that the probability of NMDARs opening in response to simulated quantal events falls with distance from the site of release, and that medium to large synapses (those greater than 0.2 μm^2 in area) could accommodate distinct pools of NMDARs that would be differentially activated in response to release at the center versus lateral regions of the synapse. Thus, if APs trigger release from a region of the active zone that is apposed to the central region of the postsynaptic density, and spontaneous events are released more laterally, these modeling results add credence to the idea that evoked and spontaneous neurotransmission may be physically segregated within the same synapse.

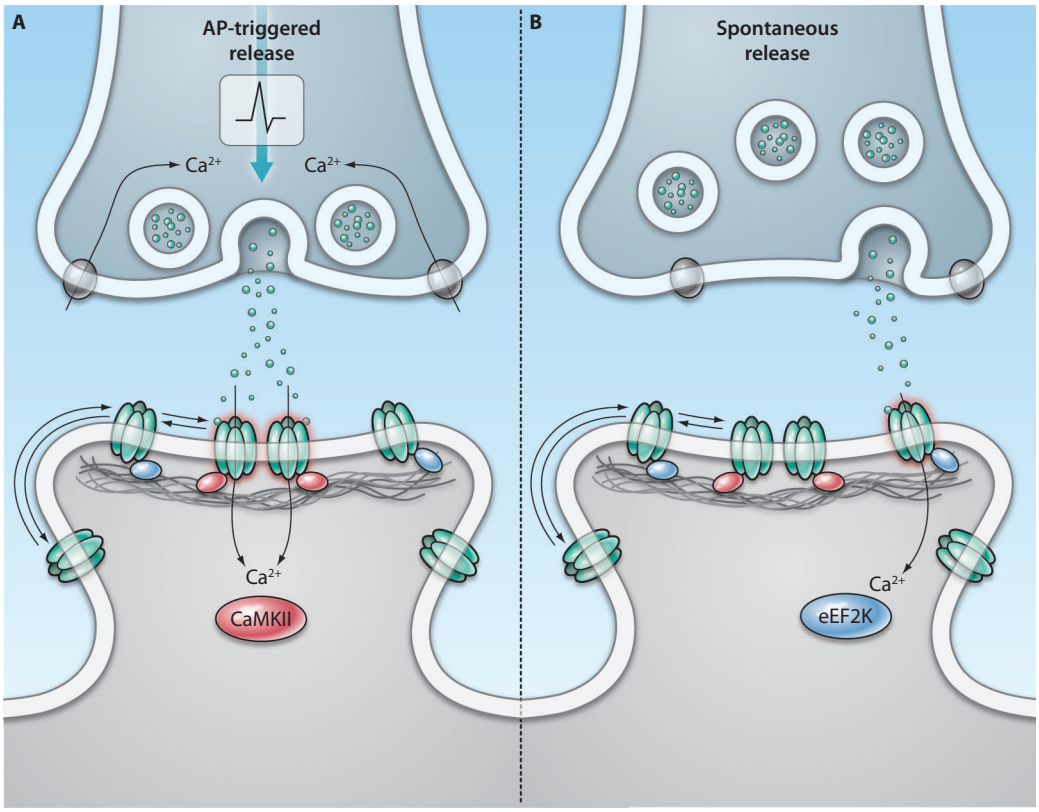

Fig. 1. Distinct synaptic microdomains for detecting evoked and miniature neurotransmission. At glutamatergic synapses, a clear distinction has previously been made between extrasynaptic and synaptic receptor populations. The data from Atasoy *et al.* suggest the existence of a third, functionally distinct spatial domain. In response to APs (**A**), Ca^{2+} influx through voltage-gated Ca^{2+} channels triggers release from the central presynaptic zone, whereas AP-independent release (**B**) occurs more laterally. Hence, separate populations of NMDARs (for clarity, AMPA receptors are not shown) are activated by evoked and miniature neurotransmission. (It is also possible that spontaneous release is organized centrally and evoked transmission laterally.) The physical segregation of these distinct synaptic NMDAR pools may allow for the differential decoding of evoked and spontaneous synaptic input, by coupling each receptor population to discrete signaling pathways [for instance, Ca^{2+}-calmodulin–dependent protein kinase II (CAMKII) for evoked transmission and Ca^{2+}-calmodulin–dependent eukaryotic elongation factor 2 kinase (eEF2K, also known as CAMKIII) for miniature neurotransmission (*11*)]. How these receptors are trafficked to and from these different synaptic NMDAR pools, and whether they are stabilized there through distinct molecular scaffolds, remain to be determined.

In the past few years, electrophysiological analyses (*14*) and single-particle tracking experiments (*15*) have revealed distinct synaptic and extrasynaptic behaviors of NMDARs and documented bidirectional trafficking of receptors between these compartments. The results of Atasoy *et al.* describe a further subdivision of receptors within the synapse, raising the possibility that lateral trafficking of receptors might also occur between these distinct pools. Consistent with this notion, Atasoy *et al.* showed that whereas relatively brief periods (2 to 10 min) of MK-801 treatment during spontaneous release had virtually no effect on evoked NMDAR currents, blocking NMDAR miniature EPSCs for longer periods (up to 40 min) progressively reduced evoked NMDAR currents. These results support the possibility that the differential localization of these receptors is not fixed, and suggest that NMDARs localized for the detection of spontaneous events may exhibit lateral trafficking to sites responsive to AP-triggered release. The authors did not demonstrate the reverse scenario: whether extended use-dependent MK-801 blockade during evoked synaptic transmission would eventually block receptors preferentially activated by spontaneous release. However, given the abundant evidence for lateral diffusion of neurotransmitter receptors in the plasma membrane, it would be surprising if these two compartments, the spontaneous release–detecting pool and AP-evoked-release–detecting pool, did not exchange receptors.

One implication of the findings by Atasoy *et al.* is that miniature and AP-elicited

neurotransmitter release may best be thought of as distinct modes of synaptic signaling. Previous studies have demonstrated that miniature events engage postsynaptic signaling pathways distinct from those stimulated by evoked events (*11*). The data provided by Atasoy *et al*. suggest that the receptor populations that are coupled to these signaling events are physically segregated, which could enable their physical association with distinct messenger systems. This raises the provocative idea that single quantal spontaneous or AP-derived events could be decoded differently by the postsynaptic neuron, depending on the location of the activated receptor (Fig. 1).

In recent years, there has been great interest in the trafficking of neurotransmitter receptors to synapses from both internal and extrasynaptic surface pools. These new data reinforce the idea that regulation of neurotransmitter receptors by lateral diffusion in the various plasma membrane compartments (the flux between the extrasynaptic compartment and the two synaptic compartments differentiated in this paper) may be at least as important as regulation by bulk exo- and endocytosis (*16*). Many open questions remain, however. For example, are the trafficking and posttranslational modification of these distinct synaptic receptor pools regulated coordinately or independently? Is the physical segregation of receptor pools a general property of all synapses, or is it unique to glutamatergic synapses? Do different scaffolds stabilize these distinct pools of receptors at synapses? Do receptors ultimately destined for detecting evoked synaptic transmission first transit through a synaptic region designated for the reception of spontaneous events? If so, does ongoing miniature neurotransmission influence where the receptor goes next? Although answers to these questions will not come easily, Atasoy and colleagues have cast new light on an area that is likely to fundamentally change our view of information processing at synapses.

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10.1126/scisignal.265pe19

Citation: M. A. Sutton, E. M. Schuman, Partitioning the synaptic landscape: Distinct microdomains for spontaneous and spike-triggered neurotransmission. *Sci. Signal.* **2**, pe19 (2009).