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Review article

How to maintain active zone integrity during high-frequency transmission

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ABSTRACT

In the central nervous system, the frequency at which reliable synaptic transmission can be maintained varies strongly between different types of synapses. Several pre- and postsynaptic processes must interact to enable high-frequency synaptic transmission. One of the mechanistically most challenging issues arises during repetitive neurotransmitter release, when synaptic vesicles fuse in rapid sequence with the presynaptic plasma membrane within the active zone (AZ), potentially interfering with the structural integrity of the AZ itself. Here we summarize potential mechanisms that help to maintain AZ integrity, including arrangement and mobility of release sites, calcium channel mobility, as well as release site clearance via lateral diffusion of vesicular proteins and via endocytotic membrane retrieval. We discuss how different types of synapses use these strategies to maintain high-frequency synaptic transmission.

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1. Introduction

Signal transmission via chemical synapses is integral to the function of the nervous system. During synaptic transmission, a presynaptic action potential (AP) causes brief opening of voltage-gated calcium (Ca^{2+}) channels. The resulting influx of Ca^{2+} ions then triggers the fusion of neurotransmitter-filled synaptic vesicles with the presynaptic membrane within the so-called active zone (AZ; Kaeser and Regehr, 2014; Schoch and Gundelfinger, 2006; Südhof, 2012). As a result, the membrane and proteins of synaptic vesicles are incorporated into the plasma membrane within the AZ. Assuming a diameter of 200 nm for the AZ (Holderith et al., 2012; Schikorski and Stevens, 1997) and a diameter of 40 nm for a synaptic vesicle (Hu et al., 2008), the fusion of only three vesicles would enlarge the AZ area by 50%. Based on larger estimates of vesicle size (80 aF/vesicle; Sakaba, 2006; and 1 $\mu F\,cm^{-2}$; Gentet et al., 2000), the fusion of only two vesicles would be sufficient to increase the AZ area by even 60%. Thus, vesicle fusion instantaneously alters AZ structure, thereby potentially impairing the integrity of AZ constitution and function. For example, the coupling between Ca^{2+} channels and Ca^{2+} sensor for vesicle fusion could be impaired or

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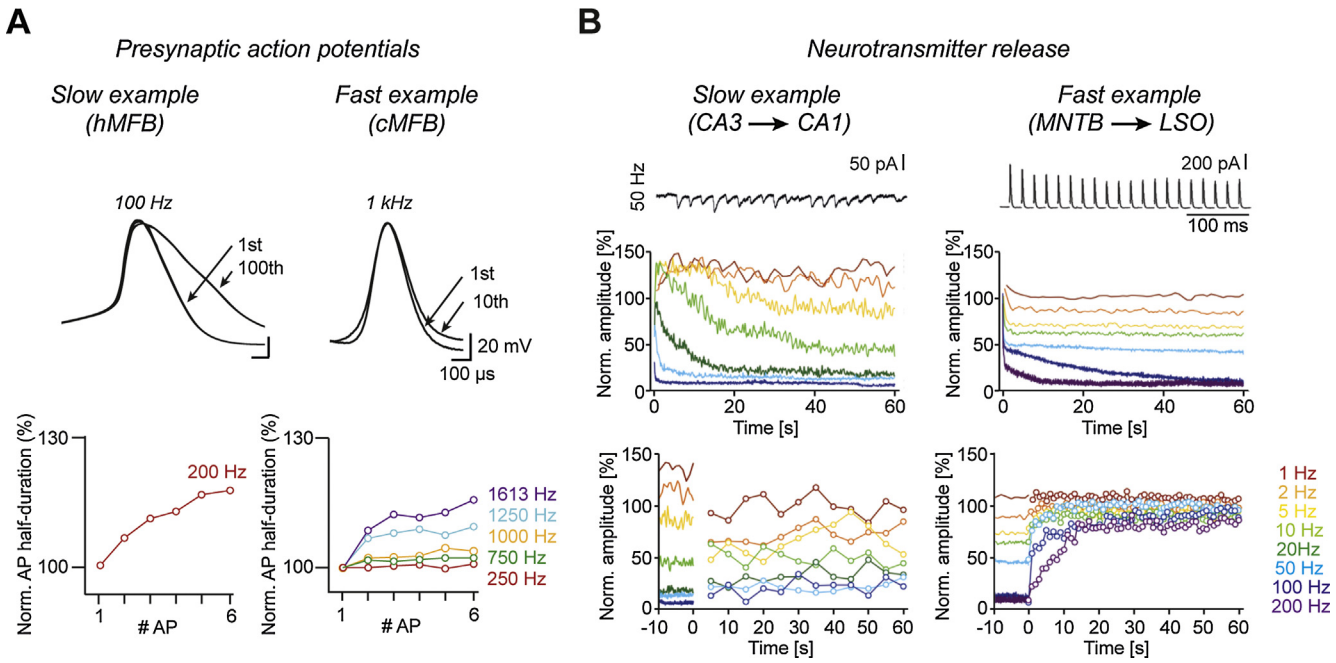

Fig. 1. Differential performance during high-frequency transmission.
(A) Comparison of AP waveforms during repetitive firing at two presynaptic boutons: The "slow-firing" hippocampal mossy fiber bouton (hMFB, left) and the "fast-firing" cerebellar mossy fiber bouton (cMFB, right). Top: Superposition of first and last presynaptic AP recorded in hMFBs and cMFBs during train-stimulation at 100 Hz (hMFB) and 1 kHz (cMFB). Bottom: Normalized AP half-durations during trains of six stimuli at indicated frequencies. (Left, from: Geiger and Jonas, 2000; right: unpublished data from I.D. (top) and from Ritzau-Jost et al., 2014 (bottom)).
(B) Differential synaptic performance during high-frequency transmission, exemplified at the "slow" glutamatergic CA3 to CA1 pyramidal cell synapse in the hippocampus (left) and at the "fast" glycinergic medial nucleus of the trapezoid body to lateral superior olive synapse (MNTB-LSO, right). Top: Postsynaptic inward (CA3-CA1) and outward (MNTB-LSO) currents evoked by 50 Hz stimulation indicate different levels of short-term synaptic plasticity. Middle: Normalized amplitudes of postsynaptic currents evoked at different frequencies (indicated by color code in lower panel). Bottom: Recovery time course of postsynaptic currents following train stimulation (see color code in right panel; from: Krächan et al., 2017).

the protein-protein interactions of the release machinery of the remaining vesicles could be disrupted. Furthermore, it has been suggested that the site where a vesicle fused is in a refractory state for many seconds, unable to offer "a competent fusion molecule" to the next approaching vesicle (Betz, 1970; Katz, 1996). Before a new vesicle can fuse at the site of a preceding fusion event, this site must consequently be cleared from, e.g., vesicular proteins – a process now referred to as release site clearance (Neher, 2010, 2017).

The frequency at which synapses operate varies strongly between different types of synaptic connections in the mammalian CNS (Delvendahl and Hallermann, 2016). For example, hippocampal mossy fiber boutons (hMFBs) usually operate at frequencies <100 Hz and show strong AP broadening, i.e. an activity-dependent prolongation of the AP duration due to, e.g., the inactivation of voltage-dependent potassium channels (Geiger and Jonas, 2000). On the other hand, cerebellar mossy fiber boutons (cMFBs) operate at frequencies up to 1 kHz and show only little AP broadening, most likely representing a special adaptation to the high frequencies that these presynaptic terminals operate at (Fig. 1A; Jörntell and Ekerot, 2006; Rancz et al., 2007; Ritzau-Jost et al., 2014).

The reliability of neurotransmitter release from synaptic vesicles, driven by the presynaptic release machinery, is even more variable across different types of synapses (Atwood and Karunanithi, 2002). For example, at synapses between hippocampal pyramidal cells, stimulation at frequencies exceeding 10 Hz leads to strong depression of synaptic transmission. In contrast, glycinergic synapses in the auditory brainstem maintain steady-state amplitudes during stimulation more effectively (Fig. 1B; Krächan et al., 2017). These synapses also recover faster from depression after cessation of the stimulus compared to the hippocampal ones (Fig. 1B, lower panels). Hence distinct types of synapses exhibit a strikingly different frequency dependence of synaptic transmission and consequently different bandwidths of synaptic transmission.

The structural and molecular differences underlying the functional diversity of synapses are currently poorly understood. Impairment of AZ integrity is likely a critical parameter determining the frequency-dependence of synaptic transmission. However, there are several other factors that can limit transmission frequency, such as onset kinetics of postsynaptic receptor saturation and receptor desensitization, the recovery from saturation/desensitization, and/or transmitter clearance from the synaptic cleft. Furthermore, a variety of presynaptic processes contribute to setting the maximum transmission frequency, such as intracellular Ca^{2+} accumulation (Delvendahl et al., 2015; Helmchen et al., 1997), vesicle mobility (Rothman et al., 2016), and vesicle recruitment (Hallermann and Silver, 2013). In this review, we focus on the integrity of the AZ during high-frequency synaptic transmission and identify five strategies that help to maintain AZ integrity. The discussion is based on full-collapse fusion of vesicles in order to limit the scope of this review. We note, however, that transient fusion-pore opening (kiss-and-run) of synaptic vesicles could represent a powerful strategy to maintain AZ integrity (Zhang et al., 2009).

2. Strategies to maintain AZ integrety

For the subsequent discussion, we would like to define the following four terms first (see Fig. 2).

1. We refer to a **synapse** as a chemical synapse, a single neuronal connection between a presynaptic en passant bouton or a ter-