Dynamic Factors for Transmitter Release at Small Presynaptic Boutons Revealed by Direct Patch-Clamp Recordings

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Small size of an axon and presynaptic structures have hindered direct functional analysis of axonal signaling and transmitter release at presynaptic boutons in the central nervous system. However, recent technical advances in subcellular patch-clamp recordings and in fluorescent imagings are shedding light on the dynamic nature of axonal and presynaptic mechanisms. Here I summarize the functional design of an axon and presynaptic boutons, such as diversity and activity-dependent changes of action potential (AP) waveforms, Ca\textsuperscript{2+} influx, and kinetics of transmitter release, revealed by the technical tour de force of direct patch-clamp recordings and the leading-edge fluorescent imagings. I highlight the critical factors for dynamic modulation of transmitter release and presynaptic short-term plasticity.

Keywords: axon, patch-clamp, transmitter release, presynaptic terminal, action potential, short-term plasticity

INTRODUCTION

Direct patch-clamp recording from an axonal compartment tells a lot about the axon physiology and mechanisms of synaptic transmission. Unfortunately an axon and presynaptic terminals are usually too small to apply patch-clamp technique, except for unusually large structures, such as synapses of calyx of Held in the auditory brainstem and presynaptic boutons of hippocampal mossy fiber (Forsythe, 1994; Borst et al., 1995; Geiger and Jonas, 2000). Most conventional synapses in the central nervous system have much smaller presynaptic boutons (∼1 \( \mu \text{m} \)) containing only 1–2 active zones, from which direct recording has been almost impossible to perform. However, in this decade remarkable technical advances have been accomplished in recording even from conventional small presynaptic structures in primary culture system, such as boutons of hippocampal pyramidal cells (∼1 \( \mu \text{m} \), Novak et al., 2013), cerebellar Purkinje cell (PC) axon terminals (∼2–3 \( \mu \text{m} \), Kawaguchi and Sakaba, 2015), and cerebellar granule cell (GC) axon varicosities (∼1 \( \mu \text{m} \), Kawaguchi and Sakaba, 2017). These advancements have provided detailed information about the mechanisms of presynaptic function, such as dynamics of presynaptic membrane excitability, Ca\textsuperscript{2+} influx and buffering, pool size of readily releasable synaptic vesicles, and functional coupling between Ca\textsuperscript{2+} and release machinery. In this article, I like to overview these recently clarified presynaptic mechanisms for transmitter release, particularly focusing on small synapses.
FACTORS TO DETERMINE PRESYNAPTIC TRANSMITTER RELEASE

Fluorescent labeling of axons and presynaptic boutons makes it possible to precisely position a thin tip of glass pipette at a small structure for patch-clamp recordings. Voltage-clamp of an axon terminal allows to study quantitative relationship between the Ca$^{2+}$ influx to a presynaptic bouton and the amount of exocytosis of synaptic vesicles (Figure 1). For example, a long-duration of depolarization pulse applied to a bouton triggers fusion of almost all release-ready synaptic vesicles to the cytoplasmic membrane, resulting in an increase of surface membrane area size which can be recorded as a sudden jump of membrane capacitance ($C_m$) (Lindau and Neher, 1988; Figure 1). The amount of $C_m$ increase, that is, the number of synaptic vesicles in readily releasable pool (RRP) changes depending on the type of synapses. For example, calyx of Held synapse (diameter, $\sim$10 $\mu$m) shows about 400 fF of $C_m$ increase upon a large Ca$^{2+}$ influx as $\sim$2 nA upon a presynaptic depolarization pulse to 0 mV (Sun and Wu, 2001). On the other hand, $\sim$100 fF of $C_m$ increase is caused by $\sim$200 pA of Ca$^{2+}$ currents at mossy fiber boutons in hippocampus ($\sim$5 $\mu$m) and those in the cerebellum ($\sim$6 $\mu$m) (Hallemann et al., 2003; Delvendahl et al., 2013). Recently, an inhibitory neuronal presynaptic terminal, a PC bouton with a size of 2$\sim$3 $\mu$m was patched, and a $C_m$ increase of 80 fF was observed upon depolarization-induced Ca$^{2+}$ influx of $\sim$300 pA (Kawaguchi and Sakaba, 2015; Figure 1). Thus, given that a single synaptic vesicle has a $C_m$ of $\sim$0.1 fF; about 1,000 synaptic vesicles are exocytosed from a single presynaptic bouton of a mossy fiber or a PC within several hundred milliseconds. On the other hand, a much smaller conventional presynaptic structure ($\sim$1 $\mu$m), an axon varicosity of a cerebellar GC, exhibited a $\sim$20 fF of $C_m$ increase with $\sim$60 pA of Ca$^{2+}$ currents upon the identical depolarization pulse (Kawaguchi and Sakaba, 2017; Figure 1). Thus, even at a small axon terminal about 200 synaptic vesicles rapidly undergo fusion, which surprisingly corresponds to one third of total synaptic vesicles in a single bouton, based on the estimation by an electron microscopic analysis (Xu-Friedman et al., 2001). It should be noted that the quality of patch-clamp recording (i.e., physical access to the cytosol reflected by the series resistance) is critical for precise activation and measurement (in the extent and time course) of Ca$^{2+}$ currents and vesicular release. These measurements of presynaptic $C_m$ increase upon strong Ca$^{2+}$ influx suggests that substantial number of synaptic vesicles rapidly undergo exocytosis at most synapses irrespective of their being excitatory or inhibitory.

Interestingly, comparison of various neuronal terminals implies a relationship that the maximal $C_m$ increase and presynaptic Ca$^{2+}$ current amplitude likely depend on the first to second power of presynaptic diameter. Thus, we are tempted to assume that the RRP size estimated by $C_m$ measurement simply reflects the surface area size of presynaptic structure, implying that the release machinery downstream of activation by Ca$^{2+}$ may operate in a similar manner at various neuronal boutons. However, it should be noted that the vesicular release estimated by $C_m$ increases upon depolarization for tens of ms sometimes reflects the fusion of multiple states of synaptic vesicles, including those already primed at and those loosely coupled to the release sites (Sakaba and Neher, 2001), and even newly recruited ones at an extremely rapid rate ($\sim$ several ms) (Saviane and Silver, 2006; Kawaguchi and Sakaba, 2017). Recent elegant glutamate imaging coupled with super-resolution analysis of active zone proteins and electron microscopic analysis suggested that the exact number of release sites, which corresponds to synaptic vesicles maximally ready for immediate exocytosis upon Ca$^{2+}$ influx, is defined by the number of protein complex clusters consisting of Ca$^{2+}$ channels, and some active zone protein like Munc-13 (Miki et al., 2017; Sakamoto et al., 2018). Thus, the molecular organizations of presynaptic active zone and the vesicular recruitment mechanisms toward empty release sites determine the strength and sustainability of presynaptic transmitter release.
Different neuronal terminals show diversity in the process from \( \text{Ca}^{2+} \) channel activation to triggering of vesicular release. First, \( \text{Ca}^{2+} \) current is rapidly activated at a PC bouton (Kawaguchi and Sakaba, 2015) like a calyx of Held synapse (Borst et al., 1995), whereas a GC bouton exhibits slow activation of \( \text{Ca}^{2+} \) current upon the identical depolarization pulses (Kawaguchi and Sakaba, 2017; Figure 1). In addition, the \( \text{Ca}^{2+} \)-release coupling estimated by the sensitivity to \( \text{Ca}^{2+} \) chelators like EGTA varies in different neuronal boutons: a PC bouton shows tight coupling with high EGTA sensitivity (Kawaguchi and Sakaba, 2017), in a similar manner to hippocampal mossy fiber boutons (Vyleta and Jonas, 2014). Furthermore, even action potential (AP) waveforms are quite different in axon terminals of PCs and GCs (see Figure 2).

Simultaneous recordings both from a presynaptic bouton and a postsynaptic cell provide more quantitative information about synaptic transmission (Sakaba and Neher, 2001; Sun and Wu, 2001). Figure 2 illustrates time courses of presynaptic APs and \( \text{Ca}^{2+} \) influx recorded from a PC or GC bouton, together with the kinetics of vesicular release estimated from postsynaptic responses (Kawaguchi and Sakaba, 2015, 2017). The remarkably different AP waveforms, when applied to the voltage-clamped bouton as voltage commands, cause clearly distinct kinetics of \( \text{Ca}^{2+} \) influxes. This difference in \( \text{Ca}^{2+} \) influx might be partly due to the slower activation of \( \text{Ca}^{2+} \) channels in a GC than in a PC bouton (see Figure 1), in addition to the distinct AP kinetics (Figure 2). Furthermore, the initiation of transmitter release delays more in a GC axon varicosity after the \( \text{Ca}^{2+} \) influx (~500 \( \mu \text{s} \)) than in a PC bouton (~300 \( \mu \text{s} \)), in spite that once started the release kinetics are similar (Figure 2). This distinct delay of release onset may partially reflect the different \( \text{Ca}^{2+} \)-release coupling: tight coupling at a PC bouton, whereas loose coupling in a GC bouton. On the other hand, similar time course of release rate by itself implies that release machinery may operate in a similar manner at these neurons. Thus, AP waveforms, the resultant activation of \( \text{Ca}^{2+} \) channels, and the \( \text{Ca}^{2+} \)-driven activation of release machinery, seem to change depending on the neuronal type, and likely become as key factors to impact the transmitter release. Interestingly, recent imaging studies demonstrated that \( \text{Ca}^{2+} \)-release coupling becomes tighter after the chemical induction of presynaptic long-term potentiation (LTP) in hippocampal mossy fiber boutons (Midorikawa and Sakaba, 2017).

### DIGITAL AND ANALOG SIGNALING IN AN AXON

Traditionally AP has been regarded as an all-or-none type of digital signal, reliably conveying information toward terminals in the central nervous system of vertebrates. However, direct recordings of APs by axonal patch-clamp methods and fluorescent imagings of membrane potential with a genetically encoded or chemical voltage indicator, have demonstrated activity-and/or location-dependent dynamic changes of AP waveforms in axon terminals (Kole et al., 2007; Hoppa et al., 2014; Kawaguchi and Sakaba, 2015; Rowan et al., 2016). In addition, sub-threshold electrical signals affect AP waveforms by modulating axonal K\(^+\) channels and/or by passively propagating into an axon over hundreds \( \mu \text{m} \) even around distal regions (Pouzat and Marty, 1999; Alle and Geiger, 2006; Shu et al., 2006; Kole et al., 2007; Paradiso and Wu, 2009; Trigo et al., 2010; Pugh and Jahr, 2013; Zbili et al., 2016), which results in the modulation of transmitter release from presynaptic terminals (Rama et al., 2015; Rowan and Christie, 2017; Zorrilla de San Martin et al., 2017). Thus, the axonal AP signaling is modified in a manner like a hybrid of analog and digital transmission (Clark and Häusser, 2006; Debanne et al., 2011).

Because all \( \text{Ca}^{2+} \) channels at a presynaptic structure are not necessarily activated by individual APs, change of an AP waveform potentially impacts the \( \text{Ca}^{2+} \) influx into presynaptic cytoplasm, leading to modulation of transmitter release. In a cerebellar PC, high frequency AP firing results in attenuation of AP amplitude at a bouton, decreasing peak \( \text{Ca}^{2+} \) influx,

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**FIGURE 2** Kinetics of APs, \( \text{Ca}^{2+} \) influx, and transmitter release in a PC and GC bouton. Peak-scaled representative waveforms of APs, presynaptic \( \text{Ca}^{2+} \) currents (\( I_{\text{Ca}} \)), and transmitter release rate obtained by simultaneous recordings from a representative pair of presynaptic bouton (A, PC; B, GC) and postsynaptic cell. Spontaneous APs (blue), recorded from an EGFP-labeled axon varicosity of a PC (A, peak of +30 mV) and of a GC (B, peak of +37 mV), were used as voltage commands for stimulation to the voltage-clamped bouton, and presynaptic \( I_{\text{Ca}} \) (red, average of 5 traces from a pair) and postsynaptic currents were simultaneously recorded. Time courses of transmitter release rate (black, average of 5 traces recorded from a pair) were calculated by the deconvolution of the postsynaptic responses evoked by the presynaptic \( I_{\text{Ca}} \), based on the miniature postsynaptic responses. Presynaptic recordings from a bouton of PC axon and that of GC axon had similar recording conditions, precluding the possibility of the distinct kinetics being due to the quality of patch-clamp recordings. All traces are adapted from Kawaguchi and Sakaba (2015, 2017) with permission from Elsevier.
and hence synaptic outputs (Kawaguchi and Sakaba, 2015). On the other hand, at hippocampal mossy fiber synapses, activity-dependent attenuation of AP is accompanied with the slower decay and hence causes larger net influx of Ca$^{2+}$, leading to augmented transmission (Geiger and Jonas, 2000). Distinct Ca$^{2+}$-release coupling in PC boutons and mossy fiber boutons might be partially responsible for the apparently opposite effects of activity-dependent AP changes on transmitter release (Vyleta and Jonas, 2014; Kawaguchi and Sakaba, 2015). The synapses with tighter Ca$^{2+}$-release coupling like PC boutons would show shallower dependence of transmitter release on the number of activated Ca$^{2+}$ channels rather than the total amount of Ca$^{2+}$ influx. Then, the AP amplitude controlling the maximal number of activated Ca$^{2+}$ channels could be more influential for the release in such tightly coupled synapses than the decay time course affecting the duration of Ca$^{2+}$ influx. In contrast, the total Ca$^{2+}$ influx would more preferentially control transmitter release in loosely coupled synapses. Thus, the impact of AP waveform modulation is determined how the synapse is functionally designed at the molecular level.

The presence of receptors for neurotransmitters in an axon has been demonstrated at various brain regions and influences axonal excitability and neurotransmitter release (Debanne et al., 2011). Because of the substantial passive traveling of electrical signals in an axon, even a limited number of receptors in individual boutons could cooperatively contribute to changing the local membrane potential in an axon. For example, the axonal GABA$_A$Rs have been reported at various excitatory and inhibitory neurons, such as those in calyx of Held, posterior pituitary, cerebral cortex, hippocampus, and cerebellum (MacDermott et al., 1999; Trigo et al., 2008; Zorriilla de San Martin et al., 2017). Axonal GABA$_A$Rs may monitor the level of GABAergic inhibition around the postsynaptic target, locally adjusting the amount GABA release. Perforated patch-clamp technique using gramicidin clarified the reversal potential of GABA-mediated current (EGABA) as $\sim-50$ mV in a terminal of the calyx of Held synapse (Price and Trussell, 2006) and $\sim-45$ mV in a PC bouton (Zorriilla de San Martin et al., 2017). Because of the depolarized EGABA, presynaptic GABA$_A$R activation depolarizes presynaptic boutons, facilitating Ca$^{2+}$ influx upon the subsequent AP arrival and hence synaptic transmission. In contrast, axonal GABA$_A$Rs also exert inhibitory effects in different neurons (Xia et al., 2014).

**SHORT-TERM PLASTICITY**

Activity-dependent short-term plasticity of synaptic transmission lasting for milliseconds to minutes is an important element in the neuronal computation (Abbott and Regehr, 2004; Regehr, 2012). A lot of central synapses exhibit increase or decrease of strength, termed short-term synaptic facilitation, or depression, respectively, upon repetitive activity at short time intervals (Zucker and Regehr, 2002; Fioravante and Regehr, 2011). Dynamic changes of presynaptic Ca$^{2+}$ concentration and downstream processes of transmitter release mediate short-term plasticity. Residual Ca$^{2+}$ hypothesis is the simplest candidate mechanism for facilitation (Katz and Miledi, 1968). Temporal summation of residual Ca$^{2+}$ remaining in the cytoplasm after the first AP was suggested to facilitate the following AP-triggered transmitter release based on the $\sim$third power Ca$^{2+}$-dependence of release (Neher and Sakaba, 2008). However, the residual Ca$^{2+}$ increase is too small compared with the local Ca$^{2+}$ to explain synaptic facilitation in most cases.

P/Q-type of Ca$^{2+}$ channels, which predominantly mediates transmitter release at many synapses, exhibit remarkable facilitation of its current in a Ca$^{2+}$-dependent manner, resulting in facilitation of transmitter release (Catterall and Few, 2008; Ben-Johny and Yue, 2014). The association of Ca$^{2+}$ to C-lobe of calmodulin, which is basically bound with the P/Q-type channels, is thought to rapidly augment the Ca$^{2+}$ current by increasing the probability of channel opening. Exogenous expression of mutant Ca$^{2+}$ channels in superior cervical ganglion neurons in culture demonstrated a tight correlation between facilitation of Ca$^{2+}$ current and that of synaptic transmission (Mochida et al., 2008). Paired recordings from pre- and postsynaptic structures of a PC-PC pair showed that facilitation is almost exclusively mediated by the Ca$^{2+}$ current facilitation (Diaz-Rojas et al., 2015). Similar Ca$^{2+}$-dependent facilitation of presynaptic Ca$^{2+}$ influx takes place at glutamatergic boutons in the calyx of Held, which partly contributes to facilitation of synaptic transmission (Felmy et al., 2003; Müller et al., 2008; Hori and Takahashi, 2009). However, recently it was reported that a mutant mice lacking Ca$^{2+}$-facilitation of P-type channels still exhibit short-term facilitation at many synapses in a physiological condition, suggesting just a minor contribution of Ca$^{2+}$-current facilitation to short-term synaptic facilitation (Weyer et al., 2019).

Another candidate mechanism for synaptic facilitation is the Ca$^{2+}$ buffer saturation hypothesis. If Ca$^{2+}$ buffer molecules remain occupied by Ca$^{2+}$ that entered into a bouton upon the first AP, cytoplasmic free Ca$^{2+}$ becomes more abundant upon the following APs, and leading to larger transmitter release. Indeed, calbindin contributes to the facilitation at hippocampal mossy fiber-CA3 excitatory synapses and inhibitory synapses in the cerebral cortex (Blatow et al., 2003). The buffer saturation model relies on the loose coupling between Ca$^{2+}$ and release machinery (Neher, 1998; Rozov et al., 2001; Vyleta and Jonas, 2014). Recently, direct recordings from a cerebellar GC axon varicosity demonstrated that Ca$^{2+}$-release coupling is indeed loose there, and facilitation depends on the presence of low concentration of fast Ca$^{2+}$ buffer in the terminal (Kawaguchi and Sakaba, 2017), in line with the Ca$^{2+}$ buffer saturation model.

The next hypothesis for short-term facilitation is the Ca$^{2+}$-dependent facilitation of release sensors (Regehr, 2012). Recent accumulating results indicate the critical role of Syt7 as such a facilitation sensor at various synapses (Jackman et al., 2016; Turecek and Regehr, 2018). The mechanism how Syt7 contributes to facilitation of transmitter release remains to be clarified in the future. However, it should be noted that some facilitation remains even with the genetic ablation of Syt7, implying that different combination of several facilitation mechanisms described above would operate together at distinct synapses.
In addition to facilitation, short-term depression has also been studied, and the RRP depletion is thought to be the major mechanism (Zucker and Regehr, 2002; Fioravante and Regehr, 2011). Other mechanisms, such as Ca$^{2+}$ current inactivation and AP conduction failure at axonal branches (Brody and Yue, 2000; Xu and Wu, 2005) and inactivation of release sites (Neher and Sakaba, 2008; Pan and Zucker, 2009), could also contribute. GABAergic synapses on deep cerebellar nuclei neurons innervated from PCs also exhibit short-term depression upon high frequency activation (Telgkamp and Raman, 2002; Pedroarena and Schwarz, 2003). Recently, direct axonal patch-clamp recordings together with fluorescent imaging of synaptopHfluorin demonstrated frequency-dependent attenuation of AP propagation from axonal tract toward the terminals in PCs (Kawaguchi and Sakaba, 2015). Smaller number of Ca$^{2+}$ channels activated by a smaller AP is responsible for the depression of transmitter release at high-frequency activation in a PC bouton. Importantly, the AP conduction faithfulness seems to increase in PC axons along with the development, resulting in smaller frequency-dependent depression at this synapse in mature animals (Turecek et al., 2016).

CONCLUSION AND FUTURE DIRECTION

As overviewed here, recent technical advances to study axon physiology are shedding light on rich computational ability of an axon affecting synaptic outputs, rather than the classical view of an axon as a simple reliable digital signal conductor. Transmitter release from a presynaptic bouton is dynamically controlled by a variety of activity-dependent modulations of critical factors, such as AP waveforms, Ca$^{2+}$ channel activation, and Ca$^{2+}$-triggered release processes based on fine molecular organizations of release sites and vesicular replenishment systems. Different combinations of these dynamic elements would define the neuronal type-specific functional design of synapses and their short- and/or long-term plasticity. Rapid expansion of techniques for direct recording of axonal signaling with subcellular patch-clamp methods and/or fluorescent imagings is going to clarify neuron-specific and common functional designs of a variety of CNS synapses in the near future, which enriches our understanding of neuronal circuitry.

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REFERENCES

Abbot, L. F., and Regehr, W. G. (2004). Synaptic computation. Nature 431, 796–803.
Alle, H., and Geiger, J. R. P. (2006). Combined analog and action potential coding in hippocampal mossy fibers. Science 311, 1290–1293. doi: 10.1126/science.1119035
Bet-Jozefy, M., and Yue, D. T. (2014). Calmodulin regulation (calmodulation) of voltage-gated calcium channels. J. Gen. Physiol. 143, 679–692. doi: 10.1085/jgp.201311153
Blatow, M., Caputi, A., Burnashev, N., Monyer, H., and Rozov, A. (2003). Ca$^{2+}$ buffer saturation underlies paired pulse facilitation in calbindin-D28K-containing terminals. Neuron 38, 79–88. doi: 10.1016/s0896-6273(03)00196-x
Borst, J. G., Helmen, F., and Sakmann, B. (1995). Pre- and postsynaptic whole-cell recordings in the medial nucleus of the trapezoid body of the rat. J. Physiol. 489, 825–840. doi: 10.1113/jphysiol.1995.sp021095
Brody, D. L., and Yue, D. T. (2000). Release-independent short-term synaptic depression in cultured hippocampal neurons. J. Neurosci. 20, 2480–2494. doi: 10.1523/eneurosci.20-07-02480.2000
Bucurenciu, I., Kulik, A., Schwaller, B., Frotscher, M., and Jonas, P. (2008). Nanodomain coupling between Ca$^{2+}$ channels and Ca$^{2+}$ sensors promotes fast and efficient transmitter release at a cortical GABAergic synapse. Neuron 57, 536–545. doi: 10.1016/j.neuron.2007.12.026
Catterall, W. A., and Few, A. P. (2008). Calcium channel regulation and presynaptic plasticity. Neuron 59, 882–901. doi: 10.1016/j.neuron.2008.09.005
Clark, B., and Häusser, M. (2006). Neural coding: hybrid analog and digital signalling in axons. Curr. Biol. 16, R585–R588.
Debanne, D., Campanac, E., Bialowas, A., Carlier, E., and Alcaraz, G. (2011). Axon physiology. Physiol. Rev. 91, 555–602. doi: 10.1152/physrev.00048.2009
Delvendahl, I., Weyhersmüller, A., Ritzau-Jost, A., and Hallermann, S. (2013). Hippocampal and cerebellar mossy fibre boutons - same name, different function. J. Physiol. 591, 3179–3188. doi: 10.1113/jphysiol.2012.248294
Díaz-Rojas, F., Sakaba, T., and Kawaguchi, S. Y. (2015). Ca$^{2+}$ current facilitation determines short-term facilitation at inhibitory synapses between cerebellar Purkinje cells. J. Physiol. 593, 4889–4904. doi: 10.1113/journal.jphysiol.201311153
Eggermann, E., Bucurenciu, I., Goswami, S. P., and Jonas, P. (2012). Nanodomain coupling between Ca$^{2+}$ channels and sensors of exocytosis at fast mammalian synapses. Nature Rev. Neurosci. 13, 7–21. doi: 10.1038/nrn3125
Felmy, F., Neher, E., and Schneggenburger, R. (2003). Probing the intracellular calcium sensitivity of transmitter release during synaptic facilitation. Neuron 37, 801–811. doi: 10.1016/s0896-6273(03)00855-0
Fioravante, D., and Regehr, W. G. (2011). Short-term forms of presynaptic plasticity. Curr. Opin. Neurobiol. 21, 269–274. doi: 10.1016/j.conb.2011.02.003
Forsythe, I. D. (1994). Direct patch recording from identified presynaptic terminals mediating glutamatergic EPSCs in the rat CNS, in vitro. J. Physiol. 479, 381–387. doi: 10.1113/jphysiol.1994.sp020303
Geiger, J. R., and Jonas, P. (2000). Dynamic control of presynaptic Ca$^{2+}$ influx by fast-inactivating K$^+$ channels in hippocampal mossy fiber boutons. Neuron 28, 927–939. doi: 10.1016/s0896-6273(00)00164-1
Hallermann, S., Pawlu, C., Jonas, P., and Heckmann, N. (2003). A large pool of releasable vesicles in a cortical glutamatergic synapse. Proc. Natl. Acad. Sci. U.S.A. 100, 8975–8980. doi: 10.1073/pnas.1432836100
Hoppa, M. B., Gouzer, G., Armbruster, M., and Ryan, T. A. (2014). Control and plasticity of the presynaptic action potential waveform at small CNS nerve terminals. Neuron 84, 778–789. doi: 10.1016/j.neuron.2014.09.038
Hori, T., and Takahashi, T. (2009). Mechanisms underlying short-term modulation of transmitter release by presynaptic depolarization. J. Physiol. 587, 2987–3000. doi: 10.1113/jphysiol.2009
Jackman, S. L., Turecek, J., Belinsky, J. E., and Regehr, W. G. (2016). Axon terminals. Neuron 84, 778–789. doi: 10.1016/j.neuron.2014.09.038
Horii, T., and Takahashi, T. (2009). Mechanisms underlying short-term modulation of transmitter release by presynaptic depolarization. J. Physiol. 587, 2987–3000. doi: 10.1113/jphysiol.2009
Kawaguchi, J., and Sakaba, T. (2015). Smaller number of Ca$^{2+}$ channels activated by a smaller AP is responsible for the depression of transmitter release at high-frequency activation in a PC bouton. Importantly, the AP conduction faithfulness seems to increase in PC axons along with the development, resulting in smaller frequency-dependent depression at this synapse in mature animals (Turecek et al., 2016).

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Kawaguchi, S. Y., and Sakaba, T. (2015). Control of inhibitory synaptic outputs by low excitability of axon terminals revealed by direct recording. Neuron 85, 1273–1288. doi: 10.1016/j.neuron.2015.02.013

Kawaguchi, S. Y., and Sakaba, T. (2017). Fast Ca2+ buffer-dependent reliable but plastic transmission at small CNS synapses revealed by direct bouton recording. Cell Rep. 21, 3338–3345. doi: 10.1016/j.celrep.2017.11.072

Kole, M. H., Letzkus, J. J., and Stuart, G. I. (2007). Axon initial segment Kv1 channels control axonal action potential waveform and synaptic efficacy. Neuron 55, 633–647. doi: 10.1016/j.neuron.2007.07.031

Lindau, M., and Neher, E. (1988). Patch-clamp techniques for time-resolved capacitance measurements in single cells. Pflugers Arch. 411, 137–146. doi: 10.1007/bf00582306

MacDermott, A. B., Role, L. W., and Siegelbaum, S. A. (1999). Presynaptic ionotropic receptors and the control of transmitter release. Annu. Rev. Neurosci. 22, 443–485. doi: 10.1146/annurev.neuro.22.1.443

Midorikawa, M., and Sakaba, T. (2017). Kinetics of releasable synaptic vesicles and their plastic changes at hippocampal mossy fiber synapses. Neuron 96, 1033–1040. doi: 10.1016/j.neuron.2017.10.016

Miki, T., Kaufmann, W. A., Malagon, G., Gomez, L., Tabuchi, K., Watanabe, M., et al. (2017). Numbers of presynaptic Ca2+ channel clusters match those of functionally defined vesicular docking sites in single central synapses. Proc. Natl. Acad. Sci. U.S.A. 114, E5246–E5255. doi: 10.1073/pnas.1704470114

Mochida, S., Few, A. P., Scheuer, T., and Catterall, W. A. (2008). Regulation of presynaptic Ca(V)2.1 channels by Ca2+ sensor proteins mediates short-term synaptic plasticity. Neuron 57, 210–216. doi: 10.1016/j.neuron.2007.11.036

Müller, M., Felmy, F., and Schneggenburger, R. (2008). A limited contribution of Ca2+ current facilitation to paired-pulse facilitation of transmitter release at the rat calyx of Held. J. Physiol. 586, 5503–5520. doi: 10.1113/jphysiol.2008.155838

Neher, E. (1998). Vesicle pools and Ca2+ microdomains: new tools for understanding their roles in neurotransmitter release. Neuron 20, 389–399. doi: 10.1016/s0896-6273(00)80983-6

Neher, E., and Sakaba, T. (2008). Multiple roles of calcium ions in the regulation of transmitter release. Neuron 59, 861–872. doi: 10.1016/j.neuron.2008.08.019

Novak, P., Gorelik, J., Vivekananda, U., Shevchuk, A. I., Ermolyuk, Y. S., Bailey, R. J., et al. (2013). Nanoscale-targeted patch-clamp recordings of functional presynaptic ion channels. Neuron 79, 1067–1077. doi: 10.1016/j.neuron.2013.07.012

Pan, B., and Zucker, R. S. (2009). A general model of synaptic transmission and short-term plasticity. Neuron 62, 539–554. doi: 10.1016/j.neuron.2009.03.025

Paradiso, K., and Wu, L.-G. (2009). Small voltage changes at nerve terminals travel up axons to affect action potential initiation. Nat. Neurosci. 12, 541–543. doi: 10.1038/nn.2301

Pedraza, C. M., and Schwarz, C. (2003). Efficacy and short-term plasticity at GABAergic synapses between Purkinje and deep cerebellar nuclei neurons. J. Neurophysiol. 89, 704–716.

Pouzat, C., and Marty, A. (1999). Somatic recording of gabaergic autoreceptor functionally defined vesicular terminal of pyramidal cells in layer 2/3 of the rat neocortex indicates a major cause of short-term depression at a calyx synapse. J. Physiol. 586, 5503–5520. doi: 10.1011/jphysiol.2008.155838

Price, G. D., and Trussell, L. O. (2006). Estimate of the chloride buffers in facilitating and depressing nerve terminals of pyramidal cells in layer 2/3 of the rat neocortex indicates a target cell-specific difference in presynaptic calcium dynamics. J. Physiol. 531, 807–826. doi: 10.1111/j.1469-7793.2001.00807.x

Sakaba, T., and Neher, E. (2001). Calmodulin mediates rapid recruitment of fast-releasing synaptic vesicles at a calyx-type synapse. Nature 392, 1119–1131. doi: 10.1038/35054388

Sakamoto, H., Aizyoshi, T., Kimpura, N., Sugao, K., Taiko, T., Takikawa, K., et al. (2018). Synaptic weight set by Munc13-1 supramolecular assemblies. Nat. Neurosci. 21, 41–49. doi: 10.1038/s41593-017-0041-9

Saviane, C., and Silver, R. A. (2006). Fast vesicle reloading and a large pool sustain high bandwidth transmission at a central synapse. Nature 439, 983–987. doi: 10.1038/nature04509

Shu, Y., Hasenstaub, A., Duque, A., Yu, Y., and McCormick, D. A. (2006). Modulation of intracortical synaptic potentials by presynaptic somatic membrane potential. Nature 441, 761–765. doi: 10.1038/nature04720

Sun, J. Y., and Wu, L. G. (2001). Fast kinetics of exocytosis revealed by simultaneous measurements of presynaptic capacitance and postsynaptic currents a central synapse. Neuron 30, 171–182. doi: 10.1016/s0896-6273(01)00271-9

Telkamp, P., and Raman, I. M. (2002). Depression of inhibitory synaptic transmission between Purkinje cells and neurons of the cerebellar nuclei. J. Neurosci. 22, 8447–8457. doi: 10.1523/neurosci.22-19-08447.2002

Trigo, F. F., Bouthou, B., Rostaing, P., Papageorgiou, G., Corrie, J. E. T., Triller, A., et al. (2010). Presynaptic miniature GABAergic currents in developing interneurons. Neuron 66, 235–247. doi: 10.1016/j.neuron.2010.03.030

Trigo, F. F., Marty, A., and Stell, B. M. (2008). Axonal GABA receptors. Eur. J. Neurosci. 28, 841–848. doi: 10.1111/j.1465-9968.2008.06404.x

Turecek, J., Jackman, S. L., and Regehr, W. G. (2016). Synaptic specializations support frequency-independent Purkinje cell output from the cerebellar cortex. Cell Rep. 17, 3256–3268. doi: 10.1016/j.celrep.2016.11.081

Zucker, R. S., and Regehr, W. G. (2002). Short-term synaptic plasticity. Neuron 39, 2105–2118. doi: 10.1016/s0896-6273(01)00271-9

Zylka, M. J., Trigo, F., and Kawaguchi, S. Y. (2017). Axonal GABA receptors depolarize presynaptic terminals and facilitate transmitter release in cerebellar Purkinje cells. J. Neurosci. 37, 6666–6672. doi: 10.1523/jneurosci.21-17-06666.2011

Zhang, Z., and Sakaba, T. (2016). Calcium signaling at depolarization-induced spillover of transmitter release from inhibitory axon terminals. J. Neurosci. 36, 1–12. doi: 10.1523/jneurosci.3605-15.2016

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