Opinion

Cell PRESS

The low synaptic release probability *in vivo*

J. Gerard G. Borst

Department of Neuroscience, Erasmus MC, University Medical Center Rotterdam, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands

The release probability, the average probability that an active zone of a presynaptic terminal releases one or more vesicles following an action potential, is tightly regulated. Measurements in cultured neurons or in slices indicate that this probability can vary greatly between synapses, but on average it is estimated to be as high as 0.5. *In vivo*, however, the size of synaptic potentials is relatively independent of recent history, suggesting that release probability is much lower. Possible causes for this discrepancy include maturational differences, a higher spontaneous activity, a lower extracellular calcium concentration and more prominent tonic inhibition by ambient neurotransmitters during *in vivo* recordings. Existing evidence thus suggests that under physiological conditions *in vivo*, presynaptic action potentials trigger the release of neurotransmitter much less frequently than what is observed in *in vitro* preparations.

Synaptic release probability

The core business of chemical presynaptic terminals is the well-timed release of neurotransmitter. At all times, terminals have to be ready to relay the presence of the presynaptic neuron to the many neurons it innervates. Terminals are often relatively remote from the soma, and this long distance increases the delay before new supplies arrive. To minimize their dependence on the remote soma, terminals can locally recycle their vesicles. Much of the elaborate machinery that is in place in terminals is dedicated to attaining the availability of a sufficient supply of vesicles at all times. Terminals have high cytoplasmic concentrations of neurotransmitter, rapid uptake mechanisms and pumps to concentrate the neurotransmitter into vesicles and a large set of proteins to deliver the vesicles to the active zone and retrieve them upon exocytosis [1,2]. Despite this dedicated effort, the supply of releasable vesicles is not inexhaustible. The pool of vesicles that can be immediately released by a large stimulus, for example a high-frequency train of presynaptic action potentials, is generally called the readily releasable pool (RRP) [3]. It takes as much as a few seconds to fully replenish this RRP [4], or to retrieve released vesicles from the plasma membrane [2]. As a result, the release probability, defined here as the probability that an active zone releases at least one vesicle following an action potential, can decrease when terminals are stimulated at high frequencies. The resulting short-term depression (STD) thus primarily represents the depletion of vesicles of the RRP, although other factors can also contribute [4]. Release of vesicles following a single action potential is probabilistic, but the average release probability is under tight control [5]. Release probability depends in a complex way on recent activity, not only STD but also several other forms of short-term plasticity (STP) can be observed at most synapses. These different forms of STP are thought to increase the computational abilities of neuronal networks considerably [6,7], allowing the synapses to act as low-pass, band-pass or high-pass filters with adjustable corner frequencies [8].

The amount of STD can be used to estimate release probability, and these estimates have been corroborated using optical or pharmacological tools [5]. Although it can vary considerably within the same preparation, the available *in vitro* evidence suggests that release probability is often around 0.5 [5]. This value, in combination with the long time needed to fully replenish all released vesicles, suggests that at stimulation frequencies as low as a few Hz, the vesicle cycle might already have problems to keep up with release. Because the average firing frequency of neurons in the rat brain is estimated to be as high as 4 Hz [9], this would mean that most terminals in the brain are operating close to maximum most of the time. This conclusion is not very appealing, and recent evidence summarized below indeed suggests that *in vivo* the release probability is clearly lower than suggested by *in vitro* studies, and that synapses whisper instead of shout.

Measuring release probability *in vivo*

Many of the techniques that have been used in slices or in culture to measure release probability cannot be easily transferred to the *in vivo* situation [5]. *In vivo*, it is difficult to control firing rates, to estimate the concentration of drugs at the synapse, to do imaging or to record from two connected neurons. Even if it is possible to activate a single input to a neuron, it can be quite difficult to discriminate between changes in release probability at this synapse and concurrent changes that result from the (indirect) activation of other inputs to this cell. Excitation and inhibition are especially tightly coupled in the cortex [10], making it difficult to discriminate between a decrease in release probability or an increase in inhibition. However, an estimate of STD has nevertheless been obtained at some well-characterized and identifiable synapses during *in vivo* recordings. In the following sections, I will compare synaptic transmission *in vivo* and *in vitro* at five 'model' synapses,

Corresponding author: Borst, J.G.G. (g.borst@erasmusmc.nl).

0166-2236/$ – see front matter doi:10.1016/j.tins.2010.03.003 Trends in Neurosciences 33 (2010) 259–266

Table 1. A comparison of *in vivo* and *in vitro* recordings at five synapses

Synapse type	Number of release sites/ AZs[a]	P_r (slices)	STD (slices)[b]	STD (*in vivo*)	Best calcium[c]	Spontaneous frequency[d]	Refs
Endbulb of Held synapse	Up to 150	0.6 @ P11	40% @ 100 Hz	Little or none	n.t.	Typically >20 Hz	[12,13,71–74]
Calyx of Held synapse	~600	0.35 @ P13	80% @ 500 Hz	Little or none	1.2 mM	30 Hz (range <1–200)	[19,21,75–77]
Retinogeniculate synapse	Up to 250	~0.4	60% @ 10 Hz	Little or none	n.t.	~15 Hz	[24–26,78–80]
Visual thalamocortical synapse	1–10	>0.45	>25% @ 20 Hz	40% @ 100 Hz	1–1.2 mM	~8 Hz	[29–33,40,81]
Cerebellar mossy fiber synapse	5	~0.5	65% @ 100 Hz	0–40% @ ~100 Hz	1.2 mM	~4 Hz	[34,35,38,39,82]

[a]Number of release sites were estimated by variance analysis or quantal analysis in slice recordings. The number of active zones (AZs) was determined by serial electron microscopy reconstruction.
[b]The amount of STD in slices indicates the percentage reduction of synaptic currents or potentials during brief trains at the indicated stimulus frequency at a calcium concentration of 2 mM. In the case of the visual thalamocortical synapse only two stimuli were given.
[c]Best calcium refers to the total calcium concentration in slice recordings that best mimicked the *in vivo* situation with regard to the response to trains of stimulation (n.t., not tested).
[d]Spontaneous frequency refers to the frequency of the inputs *in vivo*. In slices, these synapses are not spontaneously active.

which have in common that short-term synaptic plasticity has not only been studied extensively in slices but also *in vivo* (Table 1). Slice recordings described in this review were made in a Ringer solution containing 2 mM calcium and 1 mM magnesium if not noted otherwise.

Endbulb of Held synapse of the anteroventral cochlear nucleus (AVCN)

The anteroventral cochlear nucleus (AVCN) is part of the ventral auditory pathway, which is specialized in sound localization. The AVCN contains two types of bushy cells, the globular bushy cell (GBC) and the spherical bushy cell (SBC). Both provide well-timed information to cells in the superior olivary complex. The SBCs project to the medial superior olive, which contains binaurally innervated neurons that compare the arrival time of inputs from both ears. The GBCs innervate the contralateral principal cells of the medial nucleus of the trapezoid body (MNTB), forming the calyx of Held synapse, which will be discussed below. The SBCs are innervated by only a few auditory fibers, each forming a large terminal called the endbulb of Held. The GBCs are innervated by more auditory fibers than the SBCs, receiving smaller terminals called 'modified' endbulbs of Held. In slices, this synapse shows clear depression, which is reduced in the adult, but is still considerable (Table 1). *In vivo*, some bushy cells can show remarkably good phase locking and entrainment, allowing some cells to fire at every cycle of a tone burst with frequencies up to 700 Hz [11], but most bushy cells show frequent failures. This makes STD during high-frequency signaling an obvious mechanism underlying these failures, but, surprisingly, in a study that recorded simultaneously from both auditory nerve fibers and bushy cells, no evidence for the presence of STD was observed, although large endbulb inputs were not studied [12]. In addition, no evidence for the presence of STD was observed in an *in vivo* study of large inputs to the bushy cells [13]. In contrast, evidence has been presented that the main mechanism underlying these failures is synaptic inhibition, rather than synaptic depression [14].

Calyx of Held synapse of the medial nucleus of the trapezoid body (MNTB)

Each principal cell in the MNTB is contacted by a single, giant axosomatic terminal called the calyx of Held, formed by GBCs in the contralateral AVCN. The glycinergic principal cells provide well-timed inhibition to, among others, the lateral superior olive. The accessibility of the calyx of Held to direct patch-clamp recordings [15], and the possibility to study synaptic transmission by making simultaneous pre- and postsynaptic recordings at this synapse [16], has made it a popular model system for studying mechanisms of short-term synaptic plasticity [17]. In neonatal animals this synapse shows strong STD under standard slice conditions, but when the release probability is lowered, synaptic facilitation is uncovered (Box 1, [16]). Later during development, this synapse becomes more resistant to STD, as a result of, among other reasons, an increase in RRP, a decrease in release probability and the presence of a calcium-dependent speeding up of replenishment [4,18–20]. However, even in the young adult synapse, this synapse still depresses to 20% during a 500-Hz stimulus train under standard slice conditions (Figure 1a, [21]). As this frequency is in the same range as the firing frequencies reached during tone stimulation, the observed lack of evidence for the presence of STP during *in vivo* recordings was unexpected (Figure 1b; [21]). Quantal analysis during *in vivo* whole-cell recordings suggested a quantal content of only ~15, compared to ~50 obtained in slice recordings. The *in vivo* conditions could be approximated in slices by lowering the extracellular calcium concentration from 2 to 1.2 mM. This approximation improved in the presence of spontaneous activity [21], which is in line with the concept that the spontaneous GBC inputs tonically depress this synapse *in vivo* [22].

Retinogeniculate synapse of the lateral geniculate nucleus (LGN)

The synapse between retinal ganglion cells (RGCs) and relay neurons of the lateral geniculate nucleus (LGN) is used to transmit visual information from the retina to the cortex. In the mature animal, most neurons in the LGN are dominated by a single, large retinal input [23]. Similar to the endbulb and the calyx of Held synapses, the large size of these inputs makes it possible to record the excitatory postsynaptic potential (EPSP) extracellularly in the retinogeniculate synapse (Figure 1c). Despite the large size of these inputs, the relay neurons of the LGN do not faithfully follow the activity of the RGCs *in vivo*. In slice recordings from adult rats, this synapse depresses to 40% during