BRAIN RESEARCH 1398 (2011) 126–138

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Review

# Calcium cooperativity of exocytosis as a measure of $Ca^{2+}$ channel domain overlap

Victor Matveev[a], Richard Bertram[b], Arthur Sherman[c,*]

[a]Department of Mathematical Sciences, NJIT, University Heights, Newark, NJ 07102-1982, USA
[b]Department of Mathematics and Programs in Neuroscience and Molecular Biophysics, Florida State University, Tallahassee, FL, USA
[c]Laboratory of Biological Modeling, NIDDK, NIH, Bethesda, MD, USA

ARTICLE INFO

*Article history:*
Accepted 4 May 2011
Available online 12 May 2011

*Keywords:*
Presynaptic
Calcium cooperativity
Channel cooperativity
Current cooperativity
Synaptic transmission
Calcium buffer
Calcium diffusion
Modeling
Exocytosis
Active zone

ABSTRACT

The number of $Ca^{2+}$ channels contributing to the exocytosis of a single neurotransmitter vesicle in a presynaptic terminal has been a question of significant interest and debate, and is important for a full understanding of localized $Ca^{2+}$ signaling in general, and synaptic physiology in particular. This is usually estimated by measuring the sensitivity of the neurotransmitter release rate to changes in the synaptic $Ca^{2+}$ current, which is varied using appropriate voltage-clamp protocols or via pharmacological $Ca^{2+}$ channel block under the condition of constant single-channel $Ca^{2+}$ current. The slope of the resulting log–log plot of transmitter release rate versus presynaptic $Ca^{2+}$ current is termed $Ca^{2+}$ *current cooperativity* of exocytosis, and provides indirect information about the underlying presynaptic morphology. In this review, we discuss the relationship between the $Ca^{2+}$ current cooperativity and the average number of $Ca^{2+}$ channels participating in the exocytosis of a single vesicle, termed the $Ca^{2+}$*channel cooperativity*. We relate these quantities to the morphology of the presynaptic active zone. We also review experimental studies of $Ca^{2+}$ current cooperativity and its modulation during development in different classes of synapses.



## Contents



* *Corresponding author*. Fax: +1 301 402 0535.
E-mail address: asherman@nih.gov (A. Sherman).

0006-8993/$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.brainres.2011.05.011

## 1. Introduction

Synaptic neurotransmitter release and endocrine hormone secretion are fundamental physiological processes, and there has been sustained interest and active research aimed at understanding better the steps leading from $Ca^{2+}$ entry to exocytosis. Synaptic transmitter release occurs from active zones, which contain $Ca^{2+}$ channels and transmitter-filled vesicles docked at release sites. The arrangement of channels and vesicles is important in the release process, since exocytosis is evoked by $Ca^{2+}$ that enters the synaptic terminal through voltage-dependent $Ca^{2+}$ channels (Llinás et al., 1981; Stanley, 1993) and remains highly localized to the channels' $Ca^{2+}$ domains (Augustine et al., 2003; Chad and Eckert, 1984; Fogelson and Zucker, 1985; Neher, 1998a; Simon and Llinas, 1985). However, it is exceedingly difficult to determine active zone morphology due to the small size of the active zone. Even in cases where such morphological information has been determined in detail using freeze-fracture combined with electron or atomic force microscopy, for instance at the frog neuromuscular junction (Ceccarelli et al., 1979; Harlow et al., 2001; Heuser et al., 1979; Pumplin et al., 1981; Stanley et al., 2003), there remains a lack of complete knowledge of the number of functional channels that open per action potential per vesicle, and the contribution of individual channels to vesicle release. Given this limitation in the direct measurement of functional active zone morphology, indirect techniques are used to estimate the number of $Ca^{2+}$ channels contributing to an exocytotic event, which we will refer to below as the *$Ca^{2+}$channel cooperativity*. These techniques consist of varying the number of channels that open during a stimulus while measuring both the presynaptic $Ca^{2+}$ current and the release of transmitter through either presynaptic capacitance measurements or postsynaptic measurements. Typically, a log–log plot of the release variable and the presynaptic $Ca^{2+}$ current is made, and the slope of the plot is determined (see e.g. Bucurenciu et al., 2010; Fedchyshyn and Wang, 2005; Kochubey et al., 2009; Mintz et al., 1995; Quastel et al., 1992; Wu et al., 1999). This slope, the *$Ca^{2+}$current cooperativity*, provides indirect information about the mean number of channels contributing to each exocytotic event and the active zone morphology. A large $Ca^{2+}$ current cooperativity suggests that many channels contribute to exocytotic events, while a small $Ca^{2+}$ current cooperativity is usually understood to mean that release is gated by just a few proximal channels. In particular, a $Ca^{2+}$ current cooperativity near 1 is often taken as an indication that each exocytotic event is gated by the opening of a single channel (reviewed in Gentile and Stanley, 2005; Schneggenburger and Neher, 2005).

Measurements of the $Ca^{2+}$ current cooperativity have been used to infer information about synaptic morphology in a wide variety of synapses, including the squid giant synapse (Augustine et al., 1991; Augustine and Charlton, 1986; Llinás et al., 1981), sensory ribbon synapses (Brandt et al., 2005; Coggins and Zenisek, 2009; Jarsky et al., 2010; Johnson et al., 2008; Keen and Hudspeth, 2006; Thoreson et al., 2004), motor nerve terminals (Quastel et al., 1992; Shahrezaei et al., 2006; Yoshikami et al., 1989), the rodent calyx of Held (Borst and Sakmann, 1996; Fedchyshyn and Wang, 2005; Kochubey et al., 2009; Sakaba and Neher, 2001; Wu et al., 1998; Wu et al., 1999) and other central synapses (Bucurenciu et al., 2010; Gentile and Stanley, 2005; Mintz et al., 1995). Theoretical studies have also explored this experimental assay (Bertram et al., 1999; Bucurenciu et al., 2010; Coggins and Zenisek, 2009; Matveev et al., 2009; Meinrenken et al., 2002; Quastel et al., 1992; Shahrezaei et al., 2006; Yoshikami et al., 1989; Zucker and Fogelson, 1986). The first aim of this review is to clarify what information is actually provided by the $Ca^{2+}$ current cooperativity, and to contrast this with the $Ca^{2+}$ channel cooperativity, which is only indirectly inferred from current cooperativity measurements. The second aim is to review $Ca^{2+}$ current cooperativity studies and focus on several cases in which the current cooperativity has been used to obtain important information on active zone morphology or changes in morphology.

## 2. Biochemical $Ca^{2+}$ cooperativity of exocytosis

Measurements of the $Ca^{2+}$ current cooperativity that reflects active zone morphology first arose in investigating the *biochemical* (intrinsic) *$Ca^{2+}$cooperativity* of release introduced by Dodge and Rahamimoff(1967), which is independent of synaptic morphology. The latter measure, which we denote by $n$, provides a lower bound on the number of $Ca^{2+}$ binding steps required to evoke vesicle fusion (Dodge and Rahamimoff, 1967). The most direct biochemical cooperativity measurement technique uses caged-$Ca^{2+}$ compounds to raise the $Ca^{2+}$ concentration almost uniformly throughout the synaptic terminal and $Ca^{2+}$ imaging to measure the internal $Ca^{2+}$ concentration (Beutner et al., 2001; Bollmann et al., 2000; Kochubey et al., 2009; Lando and Zucker, 1994; Schneggenburger and Neher, 2000). A less direct approach is to vary the extracellular $Ca^{2+}$ concentration $[Ca^{2+}]_{ext}$, which will affect $Ca^{2+}$ influx through all open $Ca^{2+}$ channels, and increase the intracellular $Ca^{2+}$ concentration (Augustine and Charlton, 1986; Borst and Sakmann, 1996; Dodge and Rahamimoff, 1967; Katz and Miledi, 1970; Lester, 1970; Llinás et al., 1981; Mintz et al., 1995; Stanley, 1986). The biochemical cooperativity is then obtained using the log–log slope of the $Ca^2$-secretion curve:

$$n = \frac{dlogR}{dlog[Ca]} \quad (1)$$

where [Ca] represents the concentration of either intracellular or extracellular $Ca^{2+}$, varied in a non-saturating range. Alternatively, some studies define $n$ as the parameter of the Hill-function fit to the entire saturating $Ca^{2+}$-release curve (Jarsky et al., 2010;