Is replenishment of the readily releasable pool associated with vesicular movement?

Loc Bui · Mladen I. Glavinović

Received: 14 February 2013 / Revised: 19 June 2013 / Accepted: 23 July 2013 / Published online: 30 July 2013
© Springer Science+Business Media Dordrecht 2013

Abstract At the excitatory synapse of rat hippocampus the short-term synaptic depression observed during long high-frequency stimulation is associated with slower replenishment of the readily-releasable pool. Given that the replenishment rate is also not $[Ca^{++}]_o$ sensitive this puts into question a widely held notion that the vesicles—constrained by the cytoskeleton and rendered free from such constraints by Ca^{++} entry that renders them more mobile—are important in the replenishment of the readily-releasable pool. This raises a question—Is vesicular replenishment of the readily releasable pool associated with significant movement? To answer this question we evaluated how okadaic acid and staurosporine (compounds known to affect vesicular mobility) influence the replenishment rate. We used patterned stimulation on the Schaffer collateral fiber pathway and recorded the excitatory post-synaptic currents (EPSCs) from rat CA1 neurons, in the absence and presence of these drugs. The parameters of a circuit model with two vesicular pools were estimated by minimizing the squared difference between the EPSC amplitudes and simulated model output. $[Ca^{2+}]_o$ did not influence the progressive decrease of the replenishment rate during long, high frequency stimulation. Okadaic acid did not significantly affect any parameters of the vesicular storage and release system, including the replenishment rate. Staurosporine reduced the replenishment coupling, but not the replenishment rate, and this is owing to the fact that it also reduces the ability of the readily releasable pool to contain quanta. Moreover, these compounds were ineffective in influencing how the replenishment rate decreases during

long, high frequency stimulation. In conclusion at the excitatory synapses of rat hippocampus the replenishment of the readily releasable pool does not appear to be associated with a significant vesicular movement, and during long high frequency stimulation $[Ca^{++}]_o$ does not influence the progressive decrease of vesicular replenishment.

Keywords Synaptic depression · Replenishment rate · Vesicular mobility · Time dependent system · Model fitting · Hippocampus · Okadaic acid · Staurosporine

Introduction

Short-term synaptic plasticity—a term describing reversible modulation of synaptic efficacy observed during and following intervals of high-frequency stimulation—consists of several synaptic processes operating on the time-scales ranging from millisecond-to-minute. If the stimulation leads to greater synaptic efficacy we have either facilitation or augmentation, and if we have a decrease then short-term depression is present (Zucker and Regehr 2002). In this study we focus on the short-term depression. A variety of different roles have been suggested for short-term depression in synaptic computations and information processing in central nervous system. It can serve as a dynamic filter allowing transmission of specific input frequencies or patterns, i.e. it can act as a low-pass, band-pass or high-pass filter. Whereas inhibitory mechanisms reduce synaptic efficacy to all inputs, synaptic depression is input-specific. It modulates the sensitivity of a neuron to subtle changes in the firing patterns of its inputs (Abbott and Regehr 2004). Short-term depression may also be involved in processing of sensory information (Cook et al. 2003;

L. Bui · M. I. Glavinović (✉)
Department of Physiology, McGill University,
3655 Sir William Osler Promenade, Montreal H3G 1Y6, Canada
e-mail: mladen.glavinovic@mcgill.ca

MacLeod and Horiuchi 2011) or in decision making (Bourjaily and Miller 2012). There have been significant advances in understanding of the roles of short term depression in synaptic computation and information processing, but there is still a partial disconnect between the studies at the level of cognitive neuroscience and more basic electrophysiological, biophysical and biochemical studies, which would be highly desirable to bridge (Soltani and Wang 2008).

It has been known long ago that the neurotransmitter is stored in small vesicles in the presynaptic terminal (del Castillo and Katz 1954a; Fatt and Katz 1952), and that all presynaptic processes either directly or indirectly, involve synaptic vesicles (Sudhof 2004). Progressive decrease of synaptic efficacy during high frequency stimulation is mainly due to the depletion of vesicles in the readily releasable pool (RRP) (Birks and MacIntosh 1961; del Castillo and Katz 1954b). As the synaptic depression progresses the replenishment of the readily releasable pool becomes more important in maintaining the synaptic output. However, the biophysical picture of the replenishment process is still unclear. A widely held view that vesicles, which are slow at rest due to the cytoskeletal constraints, become free as Ca^{++} enters, and thus more mobile (Shakiryanova et al. 2007), is now in question. At the excitatory synapses of rat hippocampus at room temperature the replenishment of RRP is largely $[\text{Ca}^{2+}]_o$ independent, and the replenishment rate does not increase during prolonged stimulation, which leads to $[\text{Ca}^{2+}]_i$ accumulation, but decreases instead (Bui and Glavinovic 2013a). In this study we further explore this problem and try to answer an even more basic question—Is vesicular replenishment of the RRP associated with significant vesicular movement? We investigated how okadaic acid and staurosporine (compounds which have been shown to influence vesicular mobility) affect the replenishment rate. Although the details are still unclear, phosphorylation plays an important role in regulating vesicular mobility, and okadaic acid, an inhibitor of phosphatases 1 and 2A, has been shown to increase vesicular trafficking (Gaffield et al. 2006). Conversely, staurosporine, a protein kinase inhibitor, curtails movement and reduces their mobility (Gaffield and Betz 2007; Kraszewski et al. 1996).

We estimated the parameters of a two pooled vesicular storage and release system, as described previously (Aristizabal and Glavinovic 2004), and not only commonly used ‘derived’ parameters (fractional release and replenishment rate), but also ‘basic’ parameters (release and replenishment coupling, size of vesicular pools). Moreover, we determined how these parameters change during long, high frequency stimulation. The depletion model was used as the basis for the analysis of short-term synaptic depression (Birks and MacIntosh 1961; Glavinovic and Narahashi

1988; Zucker and Regehr 2002), since other factors that can contribute to depression, such as lower quantal size due to receptor desensitization or presynaptic changes appear to be minimal. The quantal sizes before and after stimulation were not significantly different. Finally, Ca^{++} channel inactivation or conduction failure do not appear to be significant contributing factors since the fractional release did not change with stimulation (Bui and Glavinovic 2013a).

Methods

Solution and recordings

All procedures were performed in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee of McGill University. Post-natal (100–125 g) male Sprague–Dawley rats (Charles River Ltd., Canada) were decapitated under isoflurane anaesthesia (Baxter Corporation, Canada; Dingleline 1984). The brain was rapidly (~ 1 min) removed from the cranial vault and placed in oxygenated (96 % O_2 , 5 % CO_2) ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl 125, KCl 3, CaCl_2 2, MgCl_2 1.3, NaHCO_3 26, NaH_2PO_4 1.25 and glucose 10, and gassed with 95 % O_2 and 5 % CO_2 (pH 7.2–7.4). The brain was subsequently glued onto a mounting block in a cutting chamber, filled with ice-cold oxygenated ACSF. Hippocampal slices (400–450 μm thick) were cut using a vibrating microtome (Campden, UK) and then transferred to a container with oxygenated ACSF and incubated at room temperature (20–22 $^\circ\text{C}$) for at least 1 h before the recordings. In the recording chamber they were kept fully submerged in ACSF, flowing at 3–4 ml/min. Patch pipettes (0.8–1.1 $\times$ 100 mm; Kimble Chase, USA) were filled with (mM): CsCl 130, NaCl 10, ATP-Mg 3, guanosine-5'-triphosphate (GTP) 0.3, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid HEPES 10, ethyl-enebis (okonitrib) tetracetate 10; pH was adjusted to 7.2–7.3 with NaOH. Cesium was chosen as the main cation to block potassium conductances to make the neuron (soma and dendrites) electrotonically more compact and to have better spatial clamp. Bicuculline methiodide (10 μM) was added at least 10 min before the recordings, to suppress inhibitory (g-aminobutyric acid type A) synaptic currents. DL-2-amino-5-phosphonovaleric acid (10 μM) was added to reduce NMDA-activated channels (Taniike et al. 2008), to ensure no induction of the long-term potentiation, although such an induction was not expected to occur given comparatively low frequency of stimulation (5–10 Hz). The difference in quantal size of miniature excitatory postsynaptic currents prior to and after long patterned stimulation was indeed not statistically significant (Bui and

Glavinovic 2013a). Note that it is also simpler to study the short-term depression due to the activation of AMPA receptors, if EPSCs contain only such a contribution, and are not ‘contaminated’ by an NMDA component.

EPSCs were recorded “blindly” at 20–22 °C, in the deeper areas of the pyramidal cell layer of the CA1 region with whole-cell patch-clamp electrodes and an Axopatch-1D amplifier (Axon Instruments, USA) and displayed on an oscilloscope (BK Precision, USA). The holding potentials were –110 to –70 mV. The series resistance was 3–10 MΩ. If the resistance changed by more than 15 % the cell was discarded. EPSCs were anti-alias filtered with a Bessel filter whose corner frequency was set at 5 kHz, and were digitized and recorded in real time on a computer, using a BNC-2120 interface card (National Instruments, USA) with a sampling rate of 10 kHz. Same interface card was also used to provide the timing pulses for the stimulus isolation unit (Digitimer Ltd., UK), and subsequent activation of the Schaffer collateral/commissural pathway. Stimulation pulse duration was 0.2 ms, whereas the voltage was chosen such that the initial EPSC amplitude was approximately half of the maximum response. Various stimulation inputs were used—a pair of high-low frequency trains (10 Hz train followed by a 5 Hz train), each lasting 5 s, patterned stimulation (an alternating sequence of 10 Hz and 5 Hz trains, each lasting 5 s and repeated 10 times).

EPSC amplitude was determined as a difference between the baseline (determined as the mean value of 5–10 sample points just before an individual EPSC) and the peak value of the same EPSC (in this case the peak is calculated as the mean value of 3 points; the peak value plus 2 values, 1 on each side of the peak). This was compared with the amplitudes of EPSCs obtained by multi-exponential fits to individual EPSCs using the least squares fitting method. The values were very similar, as were the parameters of the vesicular dynamics evaluated using model-fitting (see below). This is not surprising given the large variation of EPSC amplitudes relative to that of the estimation errors.

Electrical circuit model of the vesicular storage and release system

The dynamics of vesicular storage system was simulated using an electrical circuit model (Bielecki et al. 2008) with two vesicular pools—the readily releasable pool (C_1) and resting pool (C_2) (Fig. 1a, b). The resistor R controls the vesicular flux, the capacitance C shows the ability of the pool to store vesicles, whereas the voltage U across the capacitor gives the vesicular density in the pool. The synthesis of new vesicles is indicated by the battery E . If

one vesicle corresponds to a charge q , then $(C*U)/q$ gives the number of vesicles in that pool.

The differential equations which describe the dynamics of the vesicular system during release (i.e. when the switch is closed) are:

$$\frac{dU_1}{dt} = -\left(\frac{1}{R_1 \cdot C_1} + \frac{1}{R_0 \cdot C_1}\right) \cdot U_1 + \frac{1}{R_1 \cdot C_1} \cdot U_2 \quad (1)$$

and

$$\frac{dU_2}{dt} = \frac{1}{R_1 \cdot C_2} \cdot U_1 - \left(\frac{1}{R_2 \cdot C_2} + \frac{1}{R_1 \cdot C_2}\right) \cdot U_2 + \frac{1}{R_2 \cdot C_2} \cdot E \quad (2)$$

The vesicular concentration difference between two pools (RP and RRP) drives the replenishment of the RRP and is controlled by the replenishment rate of RRP. Likewise, the vesicular synthesis (shown in Fig. 1b as E) drives the replenishment of the RP and is controlled by the replenishment rate of RP. All parameters used to characterize vesicular storage and release by the rate-based models are also defined in the electrical model of vesicular storage and release. The release from the RRP (assuming no replenishment of the pool) at any time t is governed by the equation $I_0 \cdot \exp(-t/(R_0 \cdot C_1))$, where I_0 is the release at time zero. The fractional release is equal to $dt/(R_0 \cdot C_1)$, whereas the replenishment rate of the readily releasable pool is $1/(R_1 \cdot C_1)$ and the replenishment rate of the resting pool is $1/(R_2 \cdot C_2)$. dt is the time interval during which an individual stimulus (i.e. an action potential) causes the vesicles to release their content and to produce a post-synaptic response, and was chosen to be 1 ms. Simulink was used for simulation of the vesicular storage and release system in all model-fitting of our experimental data.

Finally note that the model of the vesicular storage and release system we use is based on the average properties of the system. Having the stochastic models of the vesicular storage and release, especially if combined with the model-fitting methods for the estimation of the stochastic parameters of such systems, may provide new insights into the nature of vesicular storage and release and would probably significantly advance our ability to understand the processes involved in short-term synaptic depression. However, there is presently very little work based on such models (see however Matveev and Wang 2000). This approach is clearly beyond the scope of this paper.

Parameter estimation using optimization

All parameters of the vesicular storage and release system (R_0 , R_1 , R_2 , C_1 , C_2 , E , U_1 and U_2) were determined by minimizing the sum of squared errors (residuals) between

Fig. 1 The effect of calcium on the fractional release and replenishment rate during long, high frequency stimulation.

a–b Diagram and equivalent electrical circuit of a secretory cell with two vesicular pools, readily releasable and resting pool. **c–d** EPSC amplitudes with model fits at 2 and 10 mM calcium. **C₁–D₁** Fit versus EPSC amplitudes scatterograms for *C* and *D*, with best-fitted lines (Fit = $0.6 \cdot A_{\text{EPSC}} + 15.4$; $r = 0.77$ and Fit = $0.7 \cdot A_{\text{EPSC}} + 25.5$; $r = 0.86$, respectively). **e** Fractional release estimates of *C* and *D*, with best-fitted lines (FR = $0.01 \cdot t + 10.15$; $r = 0.10$ and FR = $-0.02 \cdot t + 19.42$; $r = 0.36$, respectively). **f** Replenishment rate estimates of *C* and *D*, with best-fitted exponential curves (RR = $-0.01 + 0.99 \cdot \exp(-t/33.73)$; $r = 0.98$ and RR = $0.06 + 1.30 \cdot \exp(-t/14.12)$; $r = 0.95$, respectively). Patterned stimulation consisted of 10 repeating trains of brief high (10 Hz, 5 s)—low (5 Hz, 5 s) frequencies

the experimental data points (EPSC amplitudes) and the simulated data points predicted by the Simulink model (the i_0 current amplitudes, when the switch is closed).

We used the MATLAB Optimization Toolbox function `lsqnonlin` for optimization. `lsqnonlin` uses a subspace trust region method and is based on the interior-reflective Newton method (Coleman and Li 1996a, b). Briefly, a trust region method approximates the function to be minimized by a quadratic model within a region around the current search point. Within this region the quadratic model for minimization is “trusted” to be valid (i.e. it approximates the function for minimization adequately well). During search the size of the region is modified, based on how well the model agrees with the evaluations of the function, but choosing the steps to remain within this region of trust. If quadratic model approximates well the function for

minimization within the trust region then the region is expanded. However, if the approximation is poor, the region is contracted. The procedure is repeated until convergence. The algorithm is taken to converge when one of the following criteria is met: (a) the cost function changes less between iterations than a tolerance value, or (b) a direction of negative curvature cannot be found. The parameters of the vesicular dynamics that produce minimal error—the ‘best’ parameters—are taken to be ‘true’ parameters. Note that all parameters of the vesicular storage and release system are estimated from 10 s data segments.

‘Open system’ contribution

The model of vesicular storage and release we used rests on the assumption that the vesicular fluxes are driven by the

differences in pool vesicular densities (Kruckenberg and Sandweg 1968). However, the fluxes that may move against their concentration gradient (such as directed movement) were not neglected, but were captured as the ‘open system’ contribution. In a ‘closed’ type model, the estimate of the number of vesicles in each pool is taken as an initial condition for the next window until the end of stimulation, and vesicular synthesis is the only source of new quanta. In our model, however, the initial number of vesicles in each pool (i.e. the charges Q_1 and Q_2) was estimated for every window, but also the capacitors C_1 and C_2 and the initial voltages U_1 and U_2 across these capacitors. The ‘open system’ contribution is then assessed by subtracting the release of a simulated ‘closed’ system from the release of an ‘open system’. Note that over 10 s windows used for the estimation of parameters of vesicular dynamics the ‘open system’ contribution is considered constant, but may change from one window to another. If the model of the vesicular storage and release system is considered ‘open’, it also takes into account the possibility that vesicle pools may not initially be in equilibrium. The parameter estimates of the first 1–3 windows are sometimes quite inaccurate (data not shown) if an equilibrium assumption is made, not surprisingly since vesicular pools do not have to be necessarily in equilibrium initially. Finally, note that regardless of whether the traditionally used rate-based models or our electrical model are used, the estimated rate values are the same (Bui and Glavinovic 2013a).

All graphs and all regression fits through the data were performed using Origin 8.0 software (Origin Lab, USA). Comparisons between two means were performed using Student’s paired or unpaired t test, as appropriate.

Results

Stimulation-induced change of vesicular dynamics is not affected by $[Ca^{++}]_o$

The vesicular storage and release model used in this study to fit the EPSC amplitudes consists of two pools, where C_1 and C_2 depict the readily releasable pool (RRP) and resting pool (RP), respectively (Fig. 1a, b). $1/R_0$ gives the release coupling, and $1/R_1$ the replenishment coupling between the two pools. The fractional release [calculated as $dt/(R_0 \cdot C_1)$] represents the fraction of the RRP that is released per impulse, and the replenishment rate [calculated as $1/(R_1 \cdot C_1)$] is the rate at which the RRP is refilled.

As shown recently the fractional release is $[Ca^{++}]_o$ dependent (it increases steeply as $[Ca^{++}]_o$ rises), whereas the replenishment rate is $[Ca^{++}]_o$ independent. In contrast during long high frequency stimulation, the fractional

release remains unaltered, whereas the replenishment rate decreases markedly and rapidly (Bui and Glavinovic 2013a). No change (increase) of the fractional release during long stimulation is puzzling given that the fractional release steeply rises as $[Ca^{++}]_o$ increases, since the stimulation should lead to $[Ca^{++}]_o$ accumulation. Considering that the replenishment rate is $[Ca^{++}]_o$ independent, but also that it decreases during stimulation raise an issue of whether $[Ca^{++}]_i$ plays any role in replenishment, and whether the evoked release is under any control of cytoskeleton, which is $[Ca^{++}]_i$ regulated. We thus evaluate to what extent the change of vesicular dynamics is influenced if $[Ca^{++}]_o$ rises.

Figure 1c, d depicts EPSC amplitudes during patterned stimulation together with the best model fits at 2 and 10 mM $[Ca^{++}]_o$, respectively. The corresponding EPSC amplitudes versus fit values scatterograms and best-fitted lines are shown as insets (Fig. 1c₁, d₁). Figure 1e, f is the fractional release and replenishment rate estimates (taken every 10 s) of the above data. To characterize how the fractional release and replenishment rate estimates change with stimulation they were fit with a line and exponential curve, respectively.

Figure 2 shows the pooled results of the effects of $[Ca^{++}]_o$ on the changes of the fractional release and the replenishment rate during long, high frequency stimulation. The intercepts indicate the fractional release at 0 s (Fig. 2a₁), and the slopes describe how the fractional release changes with stimulation (Fig. 2a₂). Slope estimates below zero (dotted line) mean that the fractional release decreased with stimulation, whereas values above zero represent an increase. As $[Ca^{++}]_o$ increases, the intercepts of the fractional release fits increase, but the slopes remain close to zero. The fractional release is thus initially (i.e. at 0 s) greater at higher $[Ca^{++}]_o$, but remains unchanged during stimulation regardless of $[Ca^{++}]_o$. At physiological $[Ca^{++}]_o$ (2 mM), the replenishment rate was initially at $\sim 1 \text{ s}^{-1}$, and during stimulation it decreased to $\sim 0.3 \text{ s}^{-1}$ with a decay time of $\sim 20 \text{ s}$ (Fig. 2b₁, b₂). Surprisingly, large increase in $[Ca^{++}]_o$ had no significant effect on either the extent of change of the replenishment rate or on its time course. The amplitudes and decay times of the exponential fits did not change significantly. Overall, $[Ca^{++}]_o$ thus affects the fractional release initially, but not during long, high frequency stimulation, whereas the replenishment rate is not affected either initially or during stimulation.

Effect of $[Ca^{++}]_o$ on the change of ‘basic’ parameters during stimulation

Nevertheless, it is also of interest to find out how $[Ca^{++}]_o$ affects the change of ‘basic’ parameters during stimulation,

Fig. 2 Calcium affects the initial fractional release, but not its change during long, high frequency stimulation, whereas the replenishment rate is not affected at all. **a**₁–**a**₂ The intercepts of the fractional release versus time fits increase with $[Ca^{2+}]_o$, but the slopes are $[Ca^{2+}]_o$ independent. **b**₁–**b**₂ The time course of the replenishment rate versus time exponential fits are not affected by $[Ca^{2+}]_o$, as the amplitude and decay times remained unchanged. Patterned stimulation consisted of 10 repeating trains of brief high (10 Hz, 5 s)—low (5 Hz, 5 s) frequencies. Each bar or symbol represents the mean and standard error ($n \geq 6$). * $p < 0.05$, significant compared with the parameter estimate at 2 mM $[Ca^{2+}]_o$ (Student's t test)

as that can provide additional insight into the dynamics of the vesicular storage and release system. Release coupling ($1/R_0$) and replenishment coupling ($1/R_1$) control how vesicles are released and replenished into the RRP, respectively, whereas RRP size (C_1) describes its ability to contain vesicles (see “Methods”). The change of the fractional release can be due to alterations in release coupling, RRP size, or both. Likewise, both the replenishment coupling and RRP size influence the change of the replenishment rate of RRP. Figure 3 depicts how the change of ‘basic’ parameters during stimulation is influenced by $[Ca^{2+}]_o$. As in Fig. 2a₁, a₂, the changes of ‘basic’ parameters were fit with lines or exponential curves. The intercepts of the release coupling versus time fits were higher at high $[Ca^{2+}]_o$, but the slopes remained close to zero (Fig. 3a₁, a₂). Neither the amplitudes nor the time

Fig. 3 The effects of calcium on the ‘basic’ parameters of vesicular dynamics during long, high frequency stimulation. **a**–**b** Parameters were estimated in 10 s segments, and they were fit with lines (release coupling) or exponential curves (replenishment coupling) to characterize their change with stimulation. The initial values of the release coupling (intercepts; **a**₁) are affected by calcium, but the slopes of the release coupling versus time linear fits are not (**a**₂). Slopes above the dashed line indicate that the parameter (release coupling) increased during stimulation and values below mean that it decreased. Neither the amplitude (**b**₁) nor the time constant (**b**₂) of the best exponential fits to the replenishment coupling versus time fits are affected by calcium. Patterned stimulation consisted of 10 repeating trains of brief high (10 Hz, 5 s)—low (5 Hz, 5 s) frequencies. Each bar or symbol represents the mean and standard error ($n \geq 6$). * $p < 0.05$, significant compared with the parameter estimate at 2 mM $[Ca^{2+}]_o$ (Student's t test)

constants of the replenishment coupling versus time fits change with $[Ca^{2+}]_o$ (Fig. 3b₁, b₂). Thus, release coupling, but not replenishment coupling, is affected by changes in $[Ca^{2+}]_o$.

Okadaic acid does not affect vesicular dynamics parameters, whereas staurosporine does, but only to a limited extent

Okadaic acid has been shown to promote vesicular mobility, whereas staurosporine has a functionally the opposite effect. In this study we evaluate their effects on all

parameters of the vesicular storage and release system using a pair of brief high–low frequency stimulation trains. The changes of EPSC amplitudes due to a single pair of such brief trains of stimulation are sufficient to estimate all of the parameters (Bui and Glavinovic 2013a). Following the evaluation at the control conditions (but with DMSO; see “Methods”) the synapses were exposed to the drugs at least 30–60 min prior to recordings. The okadaic acid however does not significantly affect either the fractional release or the replenishment rate (Fig. 4b, c), and the ‘basic’ parameters are also unaffected (Fig. 4d, f).

Staurosporine had also no influence on either the fractional release or the replenishment rate (Fig. 5b, c). However, there is a modest and statistically significant decrease in the replenishment coupling and RRP size with the drug (Fig. 5e, f). Overall, the results thus do not support the idea

that the replenishment of the RRP is much influenced by the changes of vesicular mobility.

Neither okadaic acid nor staurosporine influence the change of vesicular dynamics parameters during long, high frequency stimulation

Although okadaic acid and staurosporine do not influence, or only modestly so, the vesicular dynamics parameters estimated by a pair of brief trains of stimulation, their effects may become more apparent during long, high frequency stimulation, where synaptic depression becomes more pronounced. Figure 6a, b shows the change of the EPSC amplitudes during long patterned stimulation, under control conditions (but with DMSO) and okadaic acid (500 nM), and Fig. 6c, d depict the change of the fractional

Fig. 4 Okadaic acid does not affect the parameters of the vesicular dynamics. **a** EPSC amplitudes during brief high–low frequency trains in control conditions (DMSO) and in presence of okadaic acid (500 nM). **b–c** The fractional release and replenishment rate do not significantly change with okadaic acid. **d–f** The release coupling ($1/R_0$), the replenishment coupling ($1/R_1$) and the readily releasable pool size are also unaffected. Each bar represents the mean and standard error ($n \geq 4$). Stimulation consisted of pairs of brief high (10 Hz, 5 s) and low (5 Hz, 5 s) frequency trains, repeated ≥ 7 times, with 1 min in between to allow for recovery

Fig. 5 Effect of staurosporine on vesicular dynamics is limited. **a** EPSC amplitudes in control conditions (DMSO) and in the presence of staurosporine (500 nM). **b–c** As staurosporine concentration rises, fractional release and replenishment rate remain unchanged. **d–f** The release coupling also remains unaltered, but both the replenishment coupling and the ability of the readily releasable pool to contain quanta diminish, though only modestly. Each bar represents the mean and standard error ($n \geq 4$). Stimulation consisted of pairs of brief high (10 Hz, 5 s) and low (5 Hz, 5 s) frequency trains, repeated ≥ 7 times with 1 min for recovery. * $p < 0.05$, significant compared with the control (DMSO) parameter estimate (Student’s t test)

release and replenishment rate (same data). Figure 6e, f provides pooled results. Similar to Fig. 1e, f, the fractional release versus time and replenishment rate versus time estimates were fit with a line and an exponential curve, respectively. The slope of the fractional release versus time fits (Fig. 6e) remains unaltered, as do the decay times and the steady-state values of the replenishment rate versus time fits over the range of okadaic acid concentrations (Fig. 6f₁, f₂). Overall thus the okadaic acid has no effect on the change of the vesicular dynamics during prolonged stimulation.

Figure 7a, b shows the EPSC amplitudes with best model fit under control conditions (DMSO) and with staurosporine (500 nM), whereas Fig. 7c, d depict how staurosporine affects the change of the fractional release and replenishment rate during stimulation (same data). Figure 7e, f gives pooled data. The fractional release does not change (overall) during stimulation and this is not changed by staurosporine, as the rate of change (slope) during stimulation remains close to zero (Fig. 7e). The change of the replenishment rate during stimulation, which is fast and pronounced, is also unaffected, as both the decay times and steady-state values remain unchanged (Fig. 7f₁, f₂). This clearly shows, that neither the okadaic acid nor staurosporine alter the change of the vesicular dynamics during long, high frequency stimulation.

Quantal analysis of release during continuous high frequency stimulation

Our measurements are not the result of the activity of one synapse, but of greater and possibly variable number of synapses. We now explore using quantal analysis whether this may influence the parameter estimates of vesicular dynamics. Quantal analysis based on binomial theorem enables one to determine the basic parameters of release— p (probability of release) and n (number of vesicles in the RRP) (Chuhma and Ohmori 1998; Martin 1955). Figure 8a shows the changes of the amplitudes of the EPSCs (A_{EPSC}) together with the bi-exponential curve fit, whereas Fig. 8b shows the frequency histogram of the amplitudes of the miniature excitatory post-synaptic currents (A_{mEPSC}), and two frequency histograms of A_{EPSC} (hatched histograms), one from the first 10 s of stimulation (Beginning), and another from the last 10 s of stimulation (End). In both cases the amplitudes were corrected for the overall decrease of A_{EPSC} during the time interval over which they were recorded, and which was determined by the bi-exponential curve fit. Figure 8c–e shows the change of the QC, p and n respectively estimated from 100 values of A_{EPSC} from consecutive time intervals. The p , which is quite high, changes but only marginally with stimulation, whereas the n and the QC decrease.

Fig. 6 The change of fractional release and the replenishment rate during long, high frequency stimulation is not affected by okadaic acid. **a–b** EPSC amplitudes during patterned stimulation together with the model fits at control conditions (DMSO) and in presence of okadaic acid (500 nM). **c** Fractional release changes only slightly during stimulation and the change is affected only marginally by okadaic acid ($FR_{control} = 0.02 \cdot t + 7.50$; $r = 0.27$ and $FR_{drug} = 0.09 \cdot t + 6.07$; $r = 0.58$). **d** Replenishment rate decreases during stimulation, but the change is not significantly affected by okadaic acid ($RR_{control} = 0.20 + 1.17 \cdot \exp(-t/22.78)$; $r = 0.93$ and $RR_{drug} = 0.25 + 0.91 \cdot \exp(-t/18.36)$; $r = 0.93$). The arrows indicate the decay times. **e–f** The effect of okadaic acid on the parameters during stimulation (pooled data). Okadaic acid does not affect the fractional release and replenishment rate time course during stimulation. Slopes above the dashed line indicate that the fractional release increased during stimulation, and values below mean it decreased. Steady-state values are represented as a percentage of the replenishment rate at $t = 0$ s. Each bar or symbol represents the mean and standard error ($n \geq 4$). Patterned stimulation consisted of 10 repeating trains of brief high (10 Hz, 5 s)—low (5 Hz, 5 s) frequencies

Discussion

This study evaluates the importance of vesicular movement in the replenishment of the readily releasable pool (RRP). It is prompted by our recent findings that: (a) the replenishment rate of the readily releasable pool (RRP) does not increase, but decreases instead during long high-frequency stimulation, and (b) the replenishment rate is not $[Ca^{++}]_o$

Fig. 7 Staurosporine does not influence how the parameters of vesicular dynamics change during long high frequency stimulation. **a–b** EPSC amplitudes during patterned stimulation together with the model fits at control conditions (DMSO) and staurosporine (500 nM). **c** Fractional release changes only slightly during stimulation and the change is only marginally affected by staurosporine ($FR_{control} = -0.01 \cdot t + 4.84$; $r = 0.08$ and $FR_{drug} = 0.01 \cdot t + 4.34$; $r = 0.24$). **d** Replenishment rate decreases during stimulation, and staurosporine does not alter this change ($RR_{control} = 0.29 + 1.71 \cdot \exp(-t/12.99)$; $r = 0.94$ and $RR_{drug} = 0.21 + 1.64 \cdot \exp(-t/6.78)$; $r = 0.98$). The arrows indicate the decay times. **e–f** The effect of staurosporine on the parameters during stimulation (pooled data). Staurosporine does not alter the change of the fractional release during stimulation and the decay times and steady states values of the replenishment rate decrease are also unaffected. Slopes above the dashed line indicate that the fractional release increased during stimulation, and values below mean it decreased. Steady-state values are represented as a percentage of the replenishment rate at $t = 0$ s. Each bar or symbol represents the mean and standard error ($n \geq 4$). Patterned stimulation consisted of 10 repeating trains of brief high (10 Hz, 5 s)—low (5 Hz, 5 s) frequencies

sensitive (Bui and Glavinovic 2013a). These findings suggest that a significant re-formulation of the biophysical picture of the mechanism of evoked release may be needed.

Vesicular movement in secretory cells has been in the focus of interest of neurobiologists for a long time. It is now well established that the vesicles do not move freely in

Fig. 8 Quantal analysis of release during continuous high frequency stimulation. **a** A_{EPSCs} during stimulation [best-fitted curve: $A_{EPSC} = 21.7 (\pm 2.2) + 146.2 (\pm 20.2) \cdot \exp(-t/0.4 (\pm 0.1)) + 92.6 (\pm 2.5) \cdot \exp(-t/19.2 (\pm 1.6))$]. Two traces, each lasting 1 s are shown as insets, one at the beginning of stimulation, and another at the end (note that EPSCs are shown as downward deflections). **b** Frequency histograms of A_{mEPSCs} (filled) and A_{EPSCs} (hatched) (see text). **c** Best-fitted curve: $QC = 3.2 (\pm 1.0) + 14.2 (\pm 1.1) \cdot \exp(-t/18.3 (\pm 4.6))$; QC quantal content. **d** Best-fitted line: $p = 43.3 (\pm 7.6) + 0.2 (\pm 0.2) \cdot t$; $R = 0.37$, t time. **e** Best-fitted curve: $n = -1.5 (\pm 15.2) + 33.0 (\pm 12.7) \cdot \exp(-t/39.9 (\pm 36.6))$; n number of quanta in RRP. Stimulation was 10 Hz for 60 s. Arrows depict decay times. Note that both QC and n diminish, while p is moderately high and increases, but only marginally with stimulation

the secretory cells, but are hindered in their movement, and that this depends on many factors. Based on fluorescence recovery after photobleaching (FRAP) measurements, vesicle diffusion constants are highly variable and dependent on the type of cell. Vesicular mobility is high in retinal cells with ribbon synapses ($D = 0.11 \mu^2/s$; Rea et al. 2004), approaching the value of unhindered vesicles in free solution ($D = 0.15 \mu^2/s$; Luby-Phelps et al. 1987), low at frog neuromuscular junctions ($D = 0.0002 \mu^2/s$), and much lower in cultured hippocampal neurons ($D = 0.00005 \mu^2/s$; Jordan et al. 2005). Moreover, diffusion constants can also vary between different vesicular pools. At frog motor nerve terminals, vesicular mobility in the resting pool (sometimes also called reserve or large pool)

and the readily releasable pool (also called a recycling pool) can greatly differ (Gaffield et al. 2006).

The processes involved in the control of vesicular mobility have been much studied. They revealed that the synaptic vesicles are associated with a variety of structural and motor proteins (Dresbach et al. 2001; Morales et al. 2000; Ryan 1999), and it has been suggested that the vesicular mobility is regulated by synapsin (Deak et al. 2006; Llinas et al. 1985). Synapsin indeed binds to both synaptic vesicles (Benfenati et al. 1989) and to actin (Petrucchi and Morrow 1987), and is thus believed to keep vesicles anchored to the cytoskeleton (Gitler et al. 2004; Pieribone et al. 1995; Rosahl et al. 1995; Sun and Dobrunz 2006). In this model during stimulation and subsequent Ca^{++} entry synapsin becomes phosphorylated and releases vesicles from their cytoskeletal constraints (Chi et al. 2001), making the vesicles available to join the RRP. This is an attractive explanation, but some evidence now challenges synapsin's role in vesicular movement, since disruption of actin in frog motor nerve terminals showed no significant changes in vesicular mobility (Gaffield et al. 2006). Moreover, in knockout mice lacking synapsins, the neurons display little gross anatomical abnormalities (Rosahl et al. 1995) and there are no changes in synaptic vesicle trafficking (Gaffield and Betz 2007).

Phosphorylation however, clearly plays an important role in regulating vesicular mobility, and okadaic acid—an inhibitor of phosphatases 1 and 2A—increases vesicular mobility (Betz and Henkel 1994; Gaffield et al. 2006). Vesicles in the reserve pool (RP), which were immobile, and vesicles in the recycling pool (RRP), which were mobile, both increased their mobility to the same level following application of okadaic acid (Gaffield et al. 2006). Staurosporine, a protein kinase inhibitor, curtails movement and reduces their mobility (Gaffield and Betz 2007; Kraszewski et al. 1996). Regardless of the mechanism of action of okadaic acid and staurosporine, if the replenishment of the readily releasable pool is associated with significant vesicular movement, it should become faster in the presence of okadaic acid and slower in the presence of staurosporine.

We estimated the parameters of a two pooled vesicular storage and release system, as described previously (Aristizabal and Glavinovic 2004; Bui and Glavinovic 2013a). The depletion model was used as the basis for the analysis of short-term synaptic depression (Birks and MacIntosh 1961; Glavinovic and Narahashi 1988; Zucker and Regehr 2002), since other factors that can contribute to depression, such as lower quantal size due to receptor desensitization or presynaptic changes appear to be minimal. The quantal sizes before and after stimulation were not significantly different (Bui and Glavinovic 2013a). Finally, Ca^{++} channel inactivation (Brehm and Eckert 1978) or

conduction failure (Krnjevic and Miledi 1958) do not appear to be significant contributing factors since the fractional release also did not change with stimulation (Bui and Glavinovic 2013a).

What are the potential limitations of fitting the depletion model (which is based on average release) to the changes of EPSC amplitudes during depression to obtain the parameters of the vesicular dynamics? It may be argued that the number and position of active synapses change during stimulation influencing significantly the values of the estimated parameters of the vesicular dynamics. Note however that we already demonstrated that the vesicular dynamics remains the same, if the number of activated synapse changes (Bui and Glavinovic 2013a). To further address this problem we estimated the release parameters— p (probability of release) and n (number of vesicles in the RRP) using quantal analysis based on binomial theorem (Chuhma and Ohmori 1998; Martin 1955), although quantal analysis has its own limitations. The values of the probability of release thus obtained are much higher than those based on the kinetic analysis. This is not surprising given the previous report (Brown et al. 1976), that if vesicles positioned for release do not have equal probability of release the estimate of p will be biased to vesicles with high p (i.e. those that have been primed, or are positioned more closely to the Ca-channels or plasma membrane and/or have larger size that renders them more efficient barriers to calcium diffusion; Glavinovic and Rabie 2001). Nevertheless, the quantal analysis provides some insight into the probabilistic nature of release and its putative changes during stimulation. It can thus serve as a method, albeit an imperfect one for evaluating whether the number and position of active synapses changes during stimulation. Greater spatial variation of the release sites or active synapses (or probabilities of release) renders the probability of release of the whole system to be even more overestimated. In contrast greater temporal variation has the opposite effect, and the estimates of p are much more sensitive to temporal than to spatial variation (Brown et al. 1976). As shown in Fig. 8 the probability of release, which is initially quite high, does not decrease, but increases with stimulation though only marginally. This does not support the notion that the number and position of active synapses changes during stimulation.

We find that the okadaic acid does not significantly affect any parameters of the vesicular storage and release system, including the replenishment rate, when tested by a pair of brief high-low frequency trains. Staurosporine reduced the replenishment coupling, but not the replenishment rate owing to the fact that it also reduces the ability of the readily releasable pool to contain quanta. Moreover, these compounds were ineffective in influencing how the replenishment rate decreases during long, high frequency

stimulation. Note that the okadaic acid was also not effective in altering the recovery of the replenishment rate following long, high frequency stimulation (Bui and Glavinovic 2013b). Drug uptake is unlikely to be an issue, as the synapses were exposed to drug concentrations ranging between 0.25 and 2 μM for at least 30–60 min prior to recordings, i.e. under conditions similar to previous studies that demonstrated the effects of these compounds on vesicle mobility (Issa et al. 1999; Jordan et al. 2005). Okadaic acid at far lower concentrations (1–5 nM) interfered with vesicular recycling at goldfish retinal bipolar cells (Guatimosim et al. 2002). The temperature at which the experiments were performed at is also unlikely a source of discrepancy, as others have demonstrated the effects of okadaic acid (Shtrahman et al. 2005) and staurosporine (Kavalali et al. 1999) on vesicular mobility at room temperature (21–23 °C) in cultured rat hippocampal synapses. Nevertheless, some experiments were done at higher temperatures (30–35 °C) and the vesicular dynamics were also not altered by either compound (data not shown). Finally, $[\text{Ca}^{2+}]_o$ also did not influence how much and how fast the replenishment rate of the RRP decreased during long high frequency stimulation. Taken together, these findings suggest that at the excitatory synapses of rat hippocampus during short-term synaptic depression the replenishment of the RRP is not associated with significant vesicular movement.

If replenishment of the RRP is not associated with vesicular movement, what is the biophysical basis of replenishment? The vesicles can replenish the RRP without movement, if they are positioned close to the membrane, but are not releasable. As shown by the electron microscope studies of chromaffin cells the RRP measured electrophysiologically is much smaller than the pool of docked vesicles, which turns over slowly (Steyer et al. 1997). Many vesicles may be positioned for release, but are not in the RRP, and to replenish it they do not have to move. The replenishment rate may in such a case simply represent the rate at which such vesicles become releasable by for example being docked and/or primed (Burgalossi et al. 2010). The level of their releasability may also be a function of free $[\text{Ca}^{++}]_i$ near the membrane that accumulates underneath the vesicles (Glavinovic and Rabie 2001). Finally, the replenishment of the RRP may be due to the re-deployment of the same vesicles that have recently undergone a complete or a partial fusion (“kiss-and-run” mechanism) (Fesce et al. 1994; Aravanis et al. 2003). Note also that at rat neuromuscular junction false transmitter is incorporated very quickly and released preferentially for evoked quanta, although not for spontaneously released quanta induced by high potassium, which are released much more slowly (Large and Rang 1978). It is thus possible that the replenishment of RRP emptied by high potassium is associated with vesicular

movement, but this awaits experimental verification. If this is demonstrated it would show that both types of replenishment of RRP may exist in the same secretory system, which can switch from one mode of replenishment to another depending on the stimulation. Finally note that this study does not address the issue whether the replenishment of the resting pool is associated with vesicular movement. This seems more likely, but remains speculative, because we know very little about the dynamics of replenishment of the resting pool, how it changes during stimulation or whether it is Ca-sensitive.

Acknowledgments This work was supported by the grant from the Natural Sciences and Engineering Research Council of Canada and Canadian Heart and Stroke Foundation to M.I.G.

References

- Abbott LF, Regehr WG (2004) Synaptic computation. *Nature* 431:796–803
- Aravanis AM, Pyle JL, Tsien RW (2003) Single synaptic vesicles fusing transiently and successively without loss of identity. *Nature* 423:643–647
- Aristizabal F, Glavinovic MI (2004) Simulation and parameter estimation of dynamics of synaptic depression. *Biol Cybern* 90:3–18
- Benfenati F, Bahler M, Jahn R, Greengard P (1989) Interactions of synapsin I with small synaptic vesicles: distinct sites in synapsin I bind to vesicle phospholipids and vesicle proteins. *J Cell Biol* 108:1863–1872
- Betz WJ, Henkel AW (1994) Okadaic acid disrupts clusters of synaptic vesicles in frog motor nerve terminals. *J Cell Biol* 124:843–854
- Bielecki A, Kalita P, Lewandowski M, Skomorowski M (2008) Compartment model of neuropeptide synaptic transport with impulse control. *Biol Cybern* 99:443–458
- Birks R, MacIntosh FC (1961) Acetylcholine metabolism of a sympathetic ganglion. *Can J Biochem Physiol* 39:787–827
- Bourjaily MA, Miller P (2012) Dynamic afferent synapses to decision-making networks improve performance in tasks requiring stimulus associations and discriminations. *J Neurophysiol* 108:513–527
- Brehm P, Eckert R (1978) Calcium entry leads to inactivation of calcium channel in paramecium. *Science* 202:1203–1206
- Brown TH, Perkel DH, Feldman MW (1976) Evoked neurotransmitter release: statistical effects of nonuniformity and nonstationarity. *Proc Natl Acad Sci USA* 73:2913–2917
- Bui L, Glavinovic MI (2013a) Synaptic activity slows vesicular replenishment at excitatory synapses of rat hippocampus. *Cogn Neurodyn* 7:105–120. doi:10.1007/s11571-012-9232-y
- Bui L, Glavinovic MI (2013b) Recovery of vesicular storage and release parameters after high frequency stimulation in rat hippocampus. *Cogn Neurodyn* 7:311–323. doi:10.1007/s11571-012-9240-y
- Burgalossi A, Jung S, Meyer G, Jockusch WJ, Jahn O, Taschenberger H, O'Connor VM, Nishiki T, Takahashi M, Brose N, Rhee JS (2010) SNARE protein recycling by alphaSNAP and betaSNAP supports synaptic vesicle priming. *Neuron* 68:473–487
- Chi P, Greengard P, Ryan TA (2001) Synapsin dispersion and recluster during synaptic activity. *Nat Neurosci* 4:1187–1193

- Chuhma N, Ohmori H (1998) Postnatal development of phase-locked high-fidelity synaptic transmission in the medial nucleus of the trapezoid body of the rat. *J Neurosci* 18:512–520
- Coleman TF, Li Y (1996a) A reflective Newton method for minimizing a quadratic function subject to bounds on some of the variables. *SIAM J Optim* 6:1040–1058
- Coleman TF, Li Y (1996b) An interior, trust region approach for nonlinear minimization subject to bounds. *SIAM J Optim* 6:418–445
- Cook DL, Schwindt PC, Grande LA, Spain WJ (2003) Synaptic depression in the localization of sound. *Nature* 421:66–70
- Deak F, Shin OH, Tang J, Hanson P, Ubach J, Jahn R, Rizo J, Kavalali ET, Sudhof TC (2006) Rabphilin regulates SNARE-dependent re-priming of synaptic vesicles for fusion. *EMBO J* 25:2856–2866
- del Castillo J, Katz B (1954a) Quantal components of the end-plate potential. *J Physiol* 124:560–573
- del Castillo J, Katz B (1954b) Statistical factors involved in neuromuscular facilitation and depression. *J Physiol* 124:574–585
- Dingledine R (1984) Brain slices. Plenum Press, New York
- Dresbach T, Qualmann B, Kessels MM, Garner CC, Gundelfinger ED (2001) The presynaptic cytomatrix of brain synapses. *Cell Mol Life Sci* 58:94–116
- Fatt P, Katz B (1952) An analysis of the end-plate potential recorded with an intra-cellular electrode. *J Physiol* 115:320–370
- Fesce R, Grohovaz F, Valtorta F, Meldolesi J (1994) Neurotransmitter release: fusion or ‘kiss-and-run’? *Trends Cell Biol* 4:1–4
- Gaffield MA, Betz WJ (2007) Synaptic vesicle mobility in mouse motor nerve terminals with and without synapsin. *J Neurosci* 27:13691–13700
- Gaffield MA, Rizzoli SO, Betz WJ (2006) Mobility of synaptic vesicles in different pools in resting and stimulated frog motor nerve terminals. *Neuron* 51:317–325
- Gitler D, Takagishi Y, Feng J, Ren Y, Rodriguiz RM, Wetsel WC, Greengard P, Augustine GJ (2004) Different presynaptic roles of synapsins at excitatory and inhibitory synapses. *J Neurosci* 24:11368–11380
- Glavinovic MI, Narahashi T (1988) Depression, recovery and facilitation of neuromuscular transmission during prolonged tetanic stimulation. *Neuroscience* 25:271–281
- Glavinovic MI, Rabie HR (2001) Monte Carlo evaluation of quantal analysis in the light of Ca^{2+} dynamics and the geometry of secretion. *Pflugers Arch* 443:132–145
- Guatimosim C, Hull C, Von GH, Prado MA (2002) Okadaic acid disrupts synaptic vesicle trafficking in a ribbon-type synapse. *J Neurochem* 82:1047–1057
- Issa AM, Gauthier S, Collier B (1999) Effects of calyculin A and okadaic acid on acetylcholine release and subcellular distribution in rat hippocampal formation. *J Neurochem* 72:166–173
- Jordan R, Lemke EA, Klingauf J (2005) Visualization of synaptic vesicle movement in intact synaptic boutons using fluorescence fluctuation spectroscopy. *Biophys J* 89:2091–2102
- Kavalali ET, Klingauf J, Tsien RW (1999) Properties of fast endocytosis at hippocampal synapses. *Philos Trans R Soc Lond B Biol Sci* 354:337–346
- Kraszewski K, Daniell L, Mundigl O, De Camilli P (1996) Mobility of synaptic vesicles in nerve endings monitored by recovery from photobleaching of synaptic vesicle-associated fluorescence. *J Neurosci* 16:5905–5913
- Krnjevic K, Miledi R (1958) Failure of neuromuscular propagation in rats. *J Physiol* 140:440–461
- Kruckenberg P, Sandweg R (1968) An analog model for acetylcholine release by motor nerve endings. *J Theor Biol* 19:327–332
- Large WA, Rang HP (1978) Factors affecting the rate of incorporation of a false transmitter into mammalian motor nerve terminals. *J Physiol (Lond)* 285:1–24
- Llinas R, McGuinness TL, Leonard CS, Sugimori M, Greengard P (1985) Intraterminal injection of synapsin I or calcium/calmodulin-dependent protein kinase II alters neurotransmitter release at the squid giant synapse. *Proc Natl Acad Sci USA* 82:3035–3039
- Luby-Phelps K, Castle PE, Taylor DL, Lanni F (1987) Hindered diffusion of inert tracer particles in the cytoplasm of mouse 3T3 cells. *Proc Natl Acad Sci USA* 84:4910–4913
- MacLeod KM, Horiuchi TK (2011) A rapid form of activity-dependent recovery from short-term synaptic depression in the intensity pathway of the auditory brainstem. *Biol Cybern* 104:209–223
- Martin AR (1955) A further study of the statistical composition on the end-plate potential. *J Physiol* 130:114–122
- Matveev V, Wang XJ (2000) Implications of all-or-none synaptic transmission and short-term depression beyond vesicle depletion: a computational study. *J Neurosci* 20:1575–1588
- Morales M, Colicos MA, Goda Y (2000) Actin-dependent regulation of neurotransmitter release at central synapses. *Neuron* 27:539–550
- Petrucchi TC, Morrow JS (1987) Synapsin I: an actin-bundling protein under phosphorylation control. *J Cell Biol* 105:1355–1363
- Pieribone VA, Shupliakov O, Brodin L, Hilfiker-Rothenfluh S, Czernik AJ, Greengard P (1995) Distinct pools of synaptic vesicles in neurotransmitter release. *Nature* 375:493–497
- Rea R, Li J, Dharia A, Levitan ES, Sterling P, Kramer RH (2004) Streamlined synaptic vesicle cycle in cone photoreceptor terminals. *Neuron* 41:755–766
- Rosahl TW, Spillane D, Missler M, Herz J, Selig DK, Wolff JR, Hammer RE, Malenka RC, Sudhof TC (1995) Essential functions of synapsins I and II in synaptic vesicle regulation. *Nature* 375:488–493
- Ryan TA (1999) Inhibitors of myosin light chain kinase block synaptic vesicle pool mobilization during action potential firing. *J Neurosci* 19:1317–1323
- Shakiryanova D, Klose MK, Zhou Y, Gu T, Deitcher DL, Atwood HL, Hewes RS, Levita ES (2007) Presynaptic ryanodine receptor-activated calmodulin kinase II increases vesicle mobility and potentiates neuropeptide release. *J Neurosci* 27:7799–7806
- Shtrahman M, Yeung C, Nauen DW, Bi GQ, Wu XL (2005) Probing vesicle dynamics in single hippocampal synapses. *Biophys J* 89:3615–3627
- Soltani A, Wang XJ (2008) From biophysics to cognition: reward-dependent adaptive choice behavior. *Curr Opin Neurobiol* 18:209–216
- Steyer JA, Horstmann H, Almers W (1997) Transport, docking and exocytosis of single granules in live chromaffin cells. *Nature* 388:474–478
- Sudhof TC (2004) The synaptic vesicle cycle. *Annu Rev Neurosci* 27:509–547. doi:10.1146/annurev.neuro.26.041002.131412
- Sun HY, Dobrunz LE (2006) Presynaptic kainate receptor activation is a novel mechanism for target cell-specific short-term facilitation at Schaffer collateral synapses. *J Neurosci* 26:10796–10807
- Tanike N, Lu YF, Tomizawa K, Matsui H (2008) Critical differences in magnitude and duration of *N*-methyl-D-aspartate (NMDA) receptor activation between long-term potentiation (LTP) and long-term depression (LTD) induction. *Acta Med Okayama* 62:21–28
- Zucker RS, Regehr WG (2002) Short-term synaptic plasticity. *Annu Rev Physiol* 64:355–405