A Three-Pool Model Dissecting Readily Releasable Pool Replenishment at the Calyx of Held

Jun Guo1*, Jian-long Ge1*, Mei Hao1*, Zhi-cheng Sun1*, Xin-sheng Wu2, Jian-bing Zhu1, Wei Wang1, Pan-tong Yao1, Wei Lin3 & Lei Xue1

1State Key Laboratory of Genetic Engineering, Collaborative Innovation Center of Genetics and Development, Department of Physiology and Biophysics, School of Life Sciences, Fudan University, Shanghai, P.R. China, 200433, 2Synaptic Transmission Section, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland, 20892, 3School of Mathematical Sciences, Centre for Computational Systems Biology and Shanghai Centre for Mathematical Sciences, Fudan University, P.R.China, 200433.

Although vesicle replenishment is critical in maintaining exo-endocytosis recycling, the underlying mechanisms are not well understood. Previous studies have shown that both rapid and slow endocytosis recycle into a very large recycling pool instead of within the readily releasable pool (RRP), and the time course of RRP replenishment is slowed down by more intense stimulation. This finding contradicts the calcium/calmodulin-dependence of RRP replenishment. Here we address this issue and report a three-pool model for RRP replenishment at a central synapse. Both rapid and slow endocytosis provide vesicles to a large reserve pool (RP), 42.3 times the RRP size. When moving from the RP to the RRP, vesicles entered an intermediate pool (IP), 2.7 times the RRP size with slow RP-IP kinetics and fast IP-RRP kinetics, which was responsible for the well-established slow and rapid components of RRP replenishment. Depletion of the IP caused the slower RRP replenishment observed after intense stimulation. These results establish, for the first time, a realistic cycling model with all parameters measured, revealing the contribution of each cycling step in synaptic transmission. The results call for modification of the current view of the vesicle recycling steps and their roles.

Repetitive firing causes short-term depression (STD) at many synapses1, which plays an important computational role in neuronal circuits2. A major mechanism underlying STD during intense stimulation is depletion of the readily releasable pool (RRP)1. The degree of RRP depletion and its subsequent replenishment may determine the degree and the time course of STD, and thus the synaptic strength and neuronal circuit function1. Given such important roles, however, the source of vesicles that are mobilised to replenish the RRP and the mechanism that determines the rate of the RRP replenishment remain poorly understood.

Studies several decades ago revealed that intense stimulation slows the recovery of STD3. Accordingly, a vesicle cycling model composed of two pools, the RRP and the reserve pool (RP: containing all vesicles except those in the RRP) was proposed, in which fused vesicles are retrieved via endocytosis to the RP, which then supplies vesicles to the RRP. Depletion of the RP could thus account for the slower recovery of STD1,3. Consistent with this model, blocking endocytosis at the neuromuscular junction of shibire mutants led to a progressive decline in transmitter release during repetitive stimulation4,5. However, this model has not been tested rigorously by measuring all model parameters, including pool sizes, RP-RRP kinetics, and endocytosis rates, and by determining whether the model with measured parameters matches the observed STD. More importantly, many additional factors have been reported since this model was proposed, including various forms of endocytosis, vesicle pools, and various rates of the RRP replenishment, which necessitates a rethinking the model.

Studies in the last 15 years have revealed that endocytosis can be rapid (1–2 s) or slow (10–30 s)6,7. Rapid endocytosis is often considered to be kiss-and-run fusion and retrieval8, which involves rapid fusion pore opening and closure at the same site9–12. It has been proposed that kiss-and-run locally recycles vesicles within the RRP at cultured hippocampal synapses13–15, though whether rapid kiss-and-run exists at this synapse is still debated16.

Slow endocytosis, mediated by a clathrin-dependent mechanism16,17, is considered a major endocytic pathway17. This pathway is hypothesised to retrieve vesicles into a small recycling pool8. The concept of a small recycling pool was based on the finding that only a small fraction (~5–20%) of vesicles can be stained with the
styril dye (e.g., FM1-43) during low and intermediate frequency stimulation that releases all recycling vesicles.15 If this concept holds, two predictions can be made: 1) the majority of vesicles residing in the RP are irrelevant to synaptic transmission under many physiological conditions, and 2) interference of this small recycling pool will have a crucial and rapid influence on the RRP replenishment. However, our previous studies challenged the presence of a small recycling pool. First, by simultaneously recording presynaptic capacitance and postsynaptic EPSC in the absence or presence of glutamate at rat calyces, we suggest a large recycling pool ~46 times the size of RRP, which is close to the total vesicle amount measured by electron microscopy.9,20 Such a large recycling pool confirms that almost all vesicles are mobilised to maintain synaptic transmission upon high frequency stimulation.20 Second, we have shown that blocking of both rapid and slow endocytosis does not affect the rate of RRP replenishment22, which rules out the possibility that endocytosed vesicles recycle within the RRP or a small recycling pool because it predicts slower RRP replenishment when endocytosis is blocked. Therefore, our previous results suggest that endocytosed vesicles are retrieved into a large recycling pool instead of a small one.

The RRP replenishment time course is often bi-exponential with a calcium/calmodulin-dependent rapid component of ~1 s or less.22,23 However, the source of vesicles responsible for rapid and slow RRP replenishment is unclear. Original candidates that were considered include vesicles formed by rapid endocytosis and the small recycling pool.15 However, both candidates have been ruled out based on our previous studies.20,21 Furthermore, we found that the RRP replenishment time course was slowed down by more intense stimulation.21 Therefore, a mechanism other than calcium/calmodulin-dependent rapid component of RRP replenishment is unclear. Original candidates that were considered included them back using MATLAB Simbiology toolbox (v2014a, the Mathworks, USA) to generate the vesicle cycling in each time step. The simplified scheme is shown below:

![Diagram](1) [Endocytosed vesicles enter only the RP, not the IP or RRP, and the percentage of rapid and slow endocytosis was from our previous study.](2) As blocking endocytosis does not affect RRP replenishment, to simplify the mathematical reasoning, we did not include the endocytosed vesicles when calculating all of the parameters and included them back using MATLAB Simbiology toolbox (v2014a, the Mathworks, USA) to generate the vesicle cycling in each time step. The simplified scheme is shown below:

![Diagram](2)

This model could explain the bi-exponential time course of replenishment by assuming a rapid rate constant for k1 and k2, a slow rate constant for k3 and k4, and a small IP size. The small IP could be depleted more by a more intense stimulus, explaining why the replenishment amplitude was decreased, instead of increased, after more intense stimulation. In the following, we performed quantitative calculations to determine the parameters (initial size of IP, RP, and k1, k2, k3, and k4) that fit our data.

From scheme 2,

\[ dRP/dt = k_2 * RP - k_{-2} * IP - k_1 * IP + k_{-1} * RRP, \]

where RP, IP, and RRP are the corresponding amount at time t after stimulation.

\[ dRRP/dt = k_1 * IP - k_{-1} * RRP, \]

From the law of conservation of mass,

\[ \text{RP}_0 - \text{RP} + \text{IP}_0 - \text{IP} = \text{RRP}, \]

where \( \approx \) denotes the steady state, or the resting condition. (We assumed them the same here to simplify calculation.)

Based on equations 3–5, we obtained the second order differential equation of RRP:

\[ \frac{d^2\text{RRP}}{dt^2} + (k_1 + k_{-1} + k_2 + k_{-2}) \frac{d\text{RRP}}{dt} + (k_1 + k_2 + k_1 + k_{-1} + k_{-2} + k_2) \text{RRP} = -k_2 \cdot \text{IP}_0 \]

\[ \frac{d\text{RRP}}{dt} = k_1 * \text{IP} - k_{-1} * \text{RRP} = 0. \]

The solution of this differential equation is:

\[ \text{RRP} = A_1 \left(1 - e^{-\ell} \right) + A_2 \left(1 - e^{-\ell} \right), \]

where

\[ t_1 = \left[\frac{1}{k_1} \cdot k_2 \cdot k_3 \cdot k_4 \cdot \frac{1}{k_1 + k_2 + k_3 + k_4} \left(\frac{1}{k_1} \cdot k_2 \cdot k_3 \cdot k_4 - 1\right) \right]^{-1} \]

\[ t_2 = \left[\frac{1}{k_1} \cdot k_2 \cdot k_3 \cdot k_4 \cdot \frac{1}{k_1 + k_2 + k_3 + k_4} \left(\frac{1}{k_1} \cdot k_2 \cdot k_3 \cdot k_4 - 1\right) \right]^{-1} \]

\[ A_1 = -\left(\text{A}_2 \cdot k_2 \cdot k_3 \cdot k_4 \cdot \frac{1}{k_1 + k_2 + k_3 + k_4} \right) \]

Equation 7 indicates that the RRP replenishment time course is bi-exponential. Equations 8–9 indicate that the time constants for both rapid and slow RRP are independent of the stimulation intensity but depend on only four kinetic constants, k1, k2, k3, and k4. These features are consistent with our experimental result that the RRP replenishment time course is bi-exponential, and that the time constants for rapid and slow RRP replenishment did not change significantly after 1–10 pulses of 20 ms depolarisation (Fig. 1). Such a consistency further strengthened our model.

**Methods**

**Slice preparation and electrophysiology.** Slice preparation was similar as described previously. Briefly, Postnatal day 7–10 (p7–p10) old Wistar rats of either sex were decapitated and the brain stem slices of ~200 μm thick containing the medial nucleus of the trapezoid body (MNTB) were prepared using a vibratome (VT 1200s, Leica, Germany). Recordings were made at room temperature (22–24°C). Whole-cell capacitance measurements were made with the EPC-10 amplifier together with the software lock-in amplifier (PatchMaster, HEKA, Lambrecht, Germany). Endocytosis and endocytosis are represented by capacitance changes after conditioning stimulation.22,23 Measurements of the RRP size and RRP replenishment time course were similar to previous reports.21–23 The presynaptic pipette (3.5–5 MΩ) solution contained (in mM): 125 Cs-glucuronate, 20 CsCl, 10 NaCl, 0.3 gTP, 10 HEPES, 0.05 BAPTA (pH 7.2, adjusted with CsOH). Measurements of the AMPA receptor-mediated EPSC were made by whole-cell patch at the postsynaptic principle neurones. The postsynaptic pipette (2.5–4 MΩ) solution contained (in mM): 125 K-glucose, 20 KCl, 4 MgATP, 10 Na2-phosphocreatine, 0.3 GTP, 10 HEPES, and 0.5 EGTA (pH 7.2, adjusted with KOH). The series resistance (<10 MΩ) was compensated by 90% (lag 10 μs). For recordings of the EPSC, kynurenic acid (1 mM) was added in the bath solution to relieve saturation and desensitisation of postsynaptic AMPA receptors. The holding potential was ~80 mV for both presynaptic and postsynaptic recordings if not mentioned. Statistical analysis used a t test unless otherwise noted, and means are presented as ± SE. All the methods were carried out in accordance with the approved guidelines and all animal experimental protocols were approved by the Animal Care and Use Committee of Fudan University.
Figure 1 | More intense stimulation slows the RRP replenishment. (A) Left: Sampled presynaptic calcium current (ICa, upper) and membrane capacitance (Cm, lower) induced by a 20 ms depolarisation followed by a conditioning pulse of 20 ms depolarization with a 0.5 s interval. Right: Similar to Left, except that the stimulus interval is 20 s. (B) Upper: The protocol used to measure the RRP replenishment after a 20 ms depolarisation pulse. Lower: Cm induced by a 20 ms depolarisation applied at various intervals after the conditioning stimulus (n = 8). Data were normalised to the Cm induced by the conditioning pulse, and fit with a bi-exponential function (solid line) where A1 = 0.71, \( t_1 = 0.26 \) s, A2 = 0.29, \( t_2 = 9.5 \) s. (C) Similar to A, except that the conditioning stimulus was 10 pulses of 20 ms depolarisation at 10 Hz. (D) Similar to B, except that the conditioning stimulus was 10 pulses of 20 ms depolarisation at 10 Hz (n = 11). Data were normalised to the Cm induced by a 20 ms depolarisation applied at >30 s after the conditioning stimulus, and fit with a bi-exponential function (solid line) where A1 = 0.33, \( t_1 = 0.38 \) s, A2 = 0.67, \( t_2 = 7.8 \) s. The fitting curve of single pulse was also plotted for comparison (dotted line). (E) Similar to A, except that the conditioning stimulus was 10 pulses of 20 ms depolarisation at 1 Hz. (F) Similar to D, except that the conditioning stimulus was 10 pulses of 20 ms depolarisation at 1 Hz (n = 6). Data were fit with a bi-exponential function where A1 = 0.29, \( t_1 = 0.25 \) s, A2 = 0.71, \( t_2 = 7.9 \) s. (G) The plot of the normalised RRP replenishment amplitude versus calcium influx (QICa) in a 20 ms depolarisation pulse and 10 pulses of 20 ms depolarisation at 1–10 Hz (QICa: 38.9 ± 2.6 pC, n = 8, single pulse; 312 ± 34 pC, n = 11, 10 pulses at 10 Hz; 323 ± 15 pC, n = 6, 10 pulses at 1 Hz).
In the steady state ($t = \infty$), or resting conditions,
\[ \text{IP}_5 \times k_1 = \text{RRP}_\infty \times k_{-1}, \]  
\[ \text{IP}_5 \times k_{-2} = \text{RRP}_\infty \times k_2. \]  

Because the recycling pool, which included RRP, IP$_5$, and IP$_5$, was 46 times the size of RRP, in which RRP, IP$_5$, and IP$_5$ were all normalised to RRP. (RRP = 1). Thus, RRP$_\infty = 45 - \text{IP}_5$. These calculations allow us to rewrite equations 12 and 13 as equations 14 and 15:

\[ \text{IP}_5 \times k_1 = 1 \times k_{-1}, \]
\[ \text{IP}_5 \times k_{-2} = (45 - \text{IP}_5) \times k_2. \]

In equations 8, 9, 14, and 15, $t_1$ and $t_2$ (the time constant for rapid and slow RRP replenishment) could be obtained by fitting experimental measurements after a single 20 ms depolarisation with equation 1 (0.26 and 9.5 s, Fig. 1A). The normalised RRP$_\infty$ was 1, and the normalised RRP$_\infty = 45 - \text{IP}_5$. The above parameters were obtained by comparing the model with the observed replenishment rate after a 20 ms depolarisation. The prediction was made by numerical simulation at 1 Hz, which matched the measurement well (Fig. 2B, black curve). To further determine whether these parameters were appropriate, we used the model with these parameters to replicate several predictions that were not related to the single 20 ms depolarisation data. The prediction was made by numerical simulation of the RRP replenishment using equations 3–4.

First, the model-predicted time course of the RRP replenishment after 10 pulses of 20 ms depolarisation at 10 Hz matched well with the observed time course (Fig. 2B, black curve, p = 0.93, K-S test). The predicted time course was fitted by a bi-exponential function with parameters (A$_1$ = 0.37, $\tau_1$ = 0.3 s, A$_2$ = 0.63, $\tau_2$ = 8.3 s, black curve) similar to those obtained from fitting the observed data (Fig. 1D). Second, the model-predicted RRP replenishment matched well with the measured one after 10 depolarising pulses at 1 Hz (Fig. 2C, black curve, p = 0.90, K-S test). The model-predicted parameters were also very close to those in observed ones (A$_1$ = 0.22, $\tau_1$ = 0.2 s, A$_2$ = 0.78, $\tau_2$ = 6.5 s). Third, the model-generated exocytosis during each step matched well with the experimental results. As the model was derived from the RRP replenishment data, it would be supportive if the model could also predict exocytosis. For 10 depolarising pulses at 10 Hz, it is difficult to accurately measure the capacitance jump after each stimulus, so we only compared the total capacitance jump. The model predicted a total ΔCm of 2.46 times the RRP evoked by 10 depolarising pulses at 10 Hz. By multiplying this value with the capacitance jump induced by a 20 ms depolarisation, which was the RRP size, it predicted a ΔCm of 1131 ± 61 fF (1127 ± 61 fF if endocytosed vesicles are not included, n = 11), which closely matched the measured ΔCm after 10 depolarising pulses at 10 Hz (1260 ± 72 fF, n = 11, p = 0.2, Fig. 2D). For 10 depolarising pulses at 1 Hz, we could accurately compare the capacitance jump after each stimulus. The model predicted a gradual decrease of the capacitance jump induced by each stimulus during 10 depolarising pulses at 1 Hz, which matched the measurement well (p = 0.7 with endocytosis, K-S test, Fig. 2E). The predicted total exocytosis amount (with endo: 2.3 ± 0.1 pF, without endo: 2.2 ± 0.1, n = 6) also closely matched the measured net exocytosis (2.3 ± 0.1 pF, n = 6, p = 0.8 with endocytosis, Fig. 2F). All these matches between predictions and experimental results (Figs. 2B–F) further strengthened our model with parameters described above.

The fast IP$_5$-RRP kinetics (k$_1$ = 0.8892, k$_{-1}$ = 2.4008) and the slow RP$\cdot$IP kinetics (k$_2$ = 0.0093, k$_{-2}$ = 0.1546) explained why...
replenishment was bi-exponential with rapid ($t_1$) and slow ($t_2$) time constants being controlled mostly by $k_1$ and $k_{-1}$, and $k_2$ and $k_{-2}$, respectively. Our numerical solution to $t_1$ and $t_2$ also further illustrated why the time constants in different stimulation protocols are roughly the same (equations 8–9). Our numerical calculation (equations 3–4) also showed that the IP size was 2.7 immediately after a 20 ms depolarisation, but decreased to ~0.9–1.4 immediately after 10 pulses of depolarisation at 1 Hz. The IP size after stimulation controlled the replenishment rapid amplitude, which explains why the replenishment rapid component was reduced after 10 pulses of depolarisation at 1 Hz.

High temperatures have been shown to lead to accelerated endocytosis and faster recovery from synaptic depression at calyces. Recently, ultra-fast endocytosis was observed at hippocampal neurons at 34°C. To test whether our model could also be applied at physiological temperatures, we performed similar experiments at a more physiological temperature (34°C). At higher temperatures, RRP replenishment was dramatically accelerated after a 20 ms depolarisation or 10 pulses of 20 ms depolarisation at 1 Hz. If these endocytosed vesicles directly enter the IP, RRP replenishment should be accelerated (Fig. 2C). In contrast, RRP replenishment was slower compared to a single 20 ms depolarisation (Figs. 1D, F). Our model also confirmed that, if the endocytosed vesicles entered the IP, RRP replenishment would be accelerated, which was not consistent with the experimental observations (Figs. 2A–C). Therefore, we conclude that endocytosed vesicles enter the RP before being mobilised to the IP.

The impact of endocytosis on synaptic transmission. Endocytosis not affecting RRP replenishment raises doubts about the role of endocytosis. To determine the impact of endocytosis during repetitive firing, we mimicked the physiological firing frequency of 50 Hz by depleting the RRP by ~6–12% for each action potential in our model. We used 50 Hz because the calyx may fire spontaneously at a mean rate of ~50–60 Hz in vivo. We used 50 Hz because an action potential may deplete ~6% of the RRP, whereas the IP size was only 2.7. Furthermore, a significant amount of endocytosis occurred during 10 pulses of 20 ms depolarisation at 1 Hz. If these endocytosed vesicles directly enter the IP, RRP replenishment should be accelerated (Fig. 2C). In contrast, RRP replenishment was slower compared to a single 20 ms depolarisation (Figs. 1D, F). Our model also confirmed that, if the endocytosed vesicles entered the IP, RRP replenishment would be accelerated, which was not consistent with the experimental observations (Figs. 2A–C). Therefore, we conclude that endocytosed vesicles enter the RP before being mobilised to the IP.

Figure 2 | A three-pool model underlies rapid and slow RRP replenishment. (A) The model-predicted RRP replenishment curves with endocytosed vesicles recycling to RP (black), IP (red) and RRP (blue) after a 20 ms depolarisation pulse. The measured data are also plotted for comparison (circle, same as Fig. 1B). The legend also applies to B and C. (B) Similar to A, but with a conditioning stimulus of 10 pulses of 20 ms depolarisation at 10 Hz. (C) Similar to A and B, but with a conditioning stimulus of 10 pulses of 20 ms depolarisation at 1 Hz. (D) The total measured and predicted ΔCm with and without endocytosis induced by 10 pulses of 20 ms depolarisation at 10 Hz (n = 11). (E) The model-predicted (with endocytosis: black curve, without endocytosis: dotted curve) and the measured (circle) ΔCm induced by each depolarising pulse (20 ms depolarisation) during a 10-pulse train at 1 Hz. Similar to D, except that the conditioning stimulus was 10 pulses of 20 ms depolarisation at 1 Hz (n = 6).
retrieved rapidly with a time constant of ~1.5 s, and the remaining 30% were retrieved slowly with a time constant of ~15 s. With scheme (1), the model predicted that the release evoked by a single action potential reached a steady state of 6–11% of the first response (Fig. 4B), which was similar to the steady-state reduction of the EPSCs evoked by axonal fibre stimulation at 50 Hz in the presence of 1 mM kynurenic acid, which prevents postsynaptic AMPA receptor saturation (Figs. 4A–B, n = 4 synapses).

In the absence of endocytosis, the model (scheme 2) predicted a gradual decline in release gradually to 0 (Fig. 4C). Compared to the prediction in the presence of endocytosis (scheme 1), the decline was obvious only after stimulation for >10 s at 50 Hz (Fig. 4C, right). This effect was not trivial to the calyx of Held synapse. The calyx fires spontaneously at a mean rate of 50 Hz in vivo and, without any previous firing, an action potential releases several hundred vesicles that ensure firing of a postsynaptic action potential. With firing at 50 Hz, the release induced by an action potential decreases to ~6–11% (Fig. 4B), which is still sufficient to induce a postsynaptic action potential. Without endocytosis, release will eventually decrease to 0 (Fig. 4C), which abolishes postsynaptic firing.

Discussion
Based on our previous finding that both rapid and slow endocytosis recycled vesicles to a large recycling pool instead of within the RRP or a small recycling pool, we proposed a model composed of three pools, a large RP, 42.3 times the size of RRP, a RRP, and a small IP, 2.7 times the size of RRP in between. We found that rapid IP-RRP kinetics, slow RP-IP kinetics, and limited IP size are responsible for the rapid and slow components of RRP replenishment, and the slower RRP replenishment with more intense stimulation, as observed at many synapses. This realistic model reveals the contribution of each vesicle cycling step in the maintenance of synaptic transmission, and thus, in the generation and recovery of STD during repetitive stimulation.

There are other explanations for the two components of RRP replenishment. Garcia-Perez et al. reported similar RRP replenishment at hippocampal synapses and provided “delayed depression” as an explanation, which lacked experimental verification. Otsu et al. proposed two general models that accounted for the fast and slow time constants of RRP replenishment. However, neither model could explain why blocking endocytosis does not affect RRP replenishment. First, our previous results ruled out the possibility that fused vesicles can recycle within the RRP. Second, the relatively small RP size (8–12 times the RRP), which could be dramatically decreased during STD (more than 50% decrease), and the fast docking/undocking kinetics between RP and RRP suggest a significant role of endocytosis in RRP replenishment, but this is also ruled out by our results. Pyle et al. proposed a similar two-pool model, suggesting that the RRP could be refilled by either rapid retrieval within the RRP or new recruitment from the reserve pool at the hippocampus, which still contradicts our findings. A recent study showed that exocytosed vesicles are not generally reused within 40 s, which further strengthens our conclusion that rapid reuse may not contribute to rapid RRP replenishment. With all parameters experimentally

Figure 3 | Rapid and slow endocytosis do not recycle vesicles in a small recycling pool. (A) Left: Sampled presynaptic calcium current (ICa, upper) and membrane capacitance (Cm, lower) induced by a 20 ms depolarisation applied at 0.5 s after a conditioning pulse of 20 ms depolarization with 0.3 mM GTPγS in place of GTP in the pipette solution. Right: Similar to Left, except that the stimulus interval is 20 s. (B) The model-predicted RRP replenishment curves with (scheme 1, black) and without endocytosis (scheme 2, red) after a 20 ms depolarisation pulse. Data measured with 0.3 mM GTPγS in the pipette solution are also plotted for comparison (circle). (C) Similar to A, but with a conditioning stimulus of 10 pulses of 20 ms depolarisation at 10 Hz. (D) Similar to B, except that the conditioning stimulus was 10 pulses of 20 ms depolarisation at 10 Hz.
measured, our model is more quantitatively accurate and more capable of explaining RRP replenishment after different stimulations.

The rapid component of the RRP replenishment time course has been hypothesised to be caused by a calcium/calmodulin-dependent mechanism\cite{22,23}. A recent study also showed that Munc13-1, the downstream target of the calcium/calmodulin signaling pathway, controls synaptic vesicle replenishment\cite{44}. However, these studies still could not explain our observation of a decrease in the amplitude, but not the time constant, of replenishment\textsubscript{rapid} with higher calcium influx during more intense stimulation (Figs. 1D, F). Accordingly, our three-pool model solves this problem by adding a small IP, which is responsible for the rapid and slow components of the RRP replenishment time course. The IP controls the amplitude of the replenishment\textsubscript{rapid}. More intense stimulation caused greater depletion of the IP, which decreased the replenishment\textsubscript{rapid} amplitude. By ruling out the possibility that rapid RRP replenishment is provided by vesicles made from rapid endocytosis or from a small recycling pool, we conclude that the RRP is replenished from the IP. Although currently more detailed characteristics of this pool remain unclear, a recent study showed that in synapsin triple knock-out mice, the RRP replenishment was significantly slowed down and the number of synaptic vesicles distally from the active zones was strongly decreased, whereas those localised at the active zones remained unchanged\cite{46}. Furthermore, most of the synapsin-defined vesicle pool in that study presented as a part of the traditional RP, comprising \textasciitilde95\% of the total synaptic vesicles\cite{18,47,48}. Another report using synapsin I/II double knock-out mice proposed a local reserve pool\cite{95797808} in which our model may also be helpful. For example, at frog auditory hair cell synapses, recovery draws mainly from the preformed vesicles rather than the rapid, freshly endocytosed vesicles, which is consistent with our model\cite{11795}. At hippocampal synapses, several new findings, such as the synapsin-mediated vesicle interconnection\cite{50} and local reserve pool model\cite{49}, also imply the potential usefulness of our model.

Although our three-pool model was proposed from RRP replenishment, it could also be used to accurately dissect the endocytosis at each time step during stimulation (Figs. 2D–F). Furthermore, we evaluated the impact of endocytosis using our three-pool model and concluded that endocytosis is important in maintaining synaptic transmission during high frequency stimulation, which often happens in the central nervous system. Thus, we concluded that the three-pool model is a useful tool for revealing the contribution of each vesicle cycling step in the maintenance of synaptic transmission, and the generation and recovery of STD during repetitive stimulation.

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Erratum: A Three-Pool Model Dissecting Readily Releasable Pool Replenishment at the Calyx of Held

Jun Guo, Jian-long Ge, Mei Hao, Zhi-cheng Sun, Xin-sheng Wu, Jian-bing Zhu, Wei Wang, Pan-tong Yao, Wei Lin & Lei Xue

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In the original version of this Article, there were errors in Affiliation 1 which was incorrectly listed as ‘State Key Laboratory of Genetic Engineering, Collaborative Innovation Center of Genetics and Development, Department of Physiology and Biophysics, School of Life Sciences, Fudan University, Shanghai, P.R. China, 200433’. The correct affiliation is listed below:

State Key Laboratory of Medical Neurobiology, Department of Physiology and Biophysics, School of Life Sciences and Collaborative Innovation Centre for Brain Science, Fudan University, Shanghai, 200438, P.R. China.

This error has now been corrected in the HTML version of this Article.

There are errors in Scheme (1),

should read:

There are errors in Scheme (2),

should read:

There are errors in Scheme (6),
should read:

\[
\frac{d^2\text{RRP}}{dt^2} + (k_1 + k_{-1} + k_2 + k_{-2}) \frac{d\text{RRP}}{dt} + (k_1 + k_2 + k_{-1} + k_{-2}) \text{RRP} - k_1 * k_2 * (\text{RP}_\infty + \text{IP}_\infty) = 0.
\]

There are errors in Scheme (10)

\[
A_1 = (-A_2 * k_2 * k_1 - A_2 * k_2 * k_{-1} - A_2 * k_{-2} * k_{-1} + k_2 * k_1 * \text{RP}_\infty + k_2 * k_1 * \text{IP}_\infty) * (k_2 * k_1 + k_2 * k_{-1} + k_{-2} * k_{-1})^{-1},
\]

should read:

\[
A_1 = (-A_2 * k_2 * k_1 - A_2 * k_2 * k_{-1} - A_2 * k_{-2} * k_{-1} + k_2 * k_1 * \text{RP}_\infty + k_2 * k_1 * \text{IP}_\infty) * (k_2 * k_1 + k_2 * k_{-1} + k_{-2} * k_{-1})^{-1},
\]

In the Results section under subheading 'The rate of the RRP replenishment was slower after more intense stimulation',

"At various times (\(\Delta t = 0.05–20 \text{ s}\)) after a conditioning 20 ms depolarisation (−80 to +10 mV, if not mentioned), which depleted the RRP (459 ± 29 fF; n = 11), we applied a 20 ms depolarisation to measure the resulting capacitance jump (\(\Delta C_m\)), which reflected the recovery of the RRP (Fig. 1A)."

should read:

"At various times (\(\Delta t = 0.05–20 \text{ s}\)) after a conditioning 20 ms depolarisation (−80 to +10 mV, if not mentioned), which depleted the RRP (459 ± 29 fF; from ref. 21), we applied a 20 ms depolarisation to measure the resulting capacitance jump (\(\Delta C_m\)), which reflected the recovery of the RRP (Fig. 1B)."

In the Results section under subheading 'Rapid and slow vesicle traffic among three pools underlie rapid and slow RRP replenishment',

"The above parameters were obtained by comparing the model with the observed RRP replenishment after a single 20 ms depolarization (Fig. 2A, black curve)."

should read

"The above parameters were obtained by comparing the model with the observed RRP replenishment after a single 20 ms depolarization (Fig. 2A)."

In the same section,

"The predicted total exocytosis amount (with endo: 2.3 ± 0.1 pF, without endo: 2.2 ± 0.1, n = 6) also closely matched the measured net exocytosis (2.3 ± 0.1 pF, n = 6, p = 0.8 with endocytosis, Fig. 2F)."

should read:

"The predicted total exocytosis amount (with endo: 2.3 ± 0.1 pF, without endo: 2.2 ± 0.1 pF, n = 6) also closely matched the measured net exocytosis (2.3 ± 0.1 pF, n = 6, p = 0.8 with endocytosis, Fig. 2F)."

The Acknowledgements section is incomplete,

"This work was sponsored by Shanghai Pujiang Program, National Natural Science Foundation of China (grant number: 31370828), Specialised Research Fund for the Doctoral Program of Higher Education (SRFDPF, grant number: 20120071120013) and the Shanghai Leading Academic Discipline Project (B111)."

should read:

"This work was sponsored by Shanghai Pujiang Program, National Natural Science Foundation of China (grant number: 31370828), National High-tech R&D Program of China (2015AA020512), Specialised Research Fund
for the Doctoral Program of Higher Education (SRFDPF, grant number: 20120071120013) and the Shanghai Leading Academic Discipline Project (B111).  

There is a typographical error in the Author Contributions statement,  

“J.-B. Z., W.W. and P.-t.Y. helped with experiments”  

should read:  

“J.-B. Z., W.W. and P.-T.Y. helped with experiments”