Vesicular GABA Uptake Can Be Rate Limiting for Recovery of IPSCs from Synaptic Depression

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Highlights

- Vesicular GABA uptake time constant is 40 s at physiological temperature
- Recovery rate of IPSCs from depression coincides with vesicular GABA uptake rate
- Vesicular GABA uptake can be rate limiting for recovery of IPSCs from depression

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In Brief

Recovery of inhibitory synaptic transmission from activity-dependent depression requires refilling of vesicles with GABA. Yamashita et al. find that vesicular uptake rate of GABA is a slow process, limiting the recovery rate of IPSCs from depression.
Vesicular GABA Uptake Can Be Rate Limiting for Recovery of IPSCs from Synaptic Depression

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SUMMARY

Synaptic efficacy plays crucial roles in neuronal circuit operation and synaptic plasticity. Presynaptic determinants of synaptic efficacy are neurotransmitter content in synaptic vesicles and the number of vesicles undergoing exocytosis at a time. Bursts of presynaptic firings depress synaptic efficacy, mainly due to depletion of releasable vesicles, whereas recovery from strong depression is initiated by endocytic vesicle retrieval followed by refilling of vesicles with neurotransmitter. We washed out presynaptic cytosolic GABA to induce a rundown of IPSCs at cerebellar inhibitory cell pairs in slices from rats and then allowed fast recovery by elevating GABA concentration using photo-uncaging. The time course of this recovery coincided with that of IPSCs from activity-dependent depression induced by a train of high-frequency stimulation. We conclude that vesicular GABA uptake can be a limiting step for the recovery of inhibitory neurotransmission from synaptic depression.

INTRODUCTION

At chemical synapses, neurotransmitters are stored in synaptic vesicles (SVs) and released by exocytosis. After exocytosis, SVs are retrieved by endocytosis, refilled with neurotransmitters, and recycled to be reused for a subsequent round of transmission (Heuser and Reese, 1973; Rizzoli, 2014). Vesicular uptake of glutamate (Maycox et al., 1988; Carlson et al., 1989) or GABA (Kiš et al., 1989; Hell et al., 1990) takes 5–10 min in isolated vesicles. However, glutamate uptake measured at the calyx of Held presynaptic terminal, using caged glutamate, revealed a much faster time constant of 7 s at physiological temperature (PT) (Hori and Takahashi, 2012). This estimation was made from the recovery rate of excitative postsynaptic currents (EPSCs) after glutamate uncaging, based on the fact that vesicular transmitter content is in dynamic equilibrium with its cytosolic concentration (Ishikawa et al., 2002; Yamashita et al., 2009). This uncaging method established at giant presynaptic terminals is widely applicable to neuronal pairs, where the presynaptic endogenous neurotransmitter can be replaced by caged transmitter compounds. At brainstem inhibitory neuronal pairs, the recovery time of inhibitory postsynaptic currents (IPSCs) was measured using caged glutamate photolysis (Apostolidis and Trussell, 2013). This recovery time comprises both the time for GABA synthesis from glutamate by glutamate decarboxylase (GAD) and the time of GABA uptake into vesicles. Since the rate of GABA synthesis from cytosolic glutamate is undetermined, the vesicular GABA uptake rate remains unknown. Hence, in cerebellar basket cell (BC)-Purkinje cell (PC) pairs, we directly measured vesicular GABA uptake rate using caged GABA. Our results indicated that the vesicular GABA uptake rate is 5–6 times slower than the glutamate uptake rate (Hori and Takahashi, 2012).

Given that the vesicular GABA uptake rate is slow, it might limit the recovery rate of IPSCs from short-term depression (STD). During high-frequency stimulations, IPSCs undergo STD, mainly due to the depletion of releasable SVs. Recovery of neurotransmission from STD is initiated by the endocytic retrieval of SVs, followed by their refilling with neurotransmitter and transport to release sites. At excitatory synapses, vesicular transmitter refilling is not considered as rate limiting for the recovery of neurotransmission from STD (e.g., Zucker and Regehr, 2002). In fact, vesicular glutamate uptake (Hori and Takahashi, 2012) is faster than the recovery of EPSC amplitudes after strong STD (Liu and Tsien, 1995; Ryan et al., 1993). However, at GABAergic inhibitory synapses, we found here that the recovery rate of IPSCs from STD is close to the vesicular GABA uptake rate. Furthermore, the rates of GABA uptake and recovery of IPSCs from STD showed a similar dependence on presynaptic cytosolic GABA concentrations. Thus, at inhibitory synapses, the vesicular GABA uptake rate can serve as the rate-limiting step for recovery of neurotransmission from STD.

RESULTS

IPSC Rundown by Presynaptic Cytosolic GABA Washout and Its Recovery by GABA Loading

In whole-cell pair recordings from a BC and a PC in cerebellar slices of rats, we evoked IPSCs by a train of 5 action potentials
Figure 1. IPSC Rundown after GABA Washout and Recovery after GABA Reloading

(A) Schematic illustration of a pair recording from a presynaptic BC (B) and a postsynaptic PC (P), S, stellate cell.

(B) Mean amplitude of IPSCs recorded from a PC after whole-cell washout of cytosolic GABA in a BC, normalized to IPSC amplitude at time 0. Sample traces on the right show IPSCs evoked by a train of 5 stimuli at 50 Hz, at different time periods after membrane rupture with a whole-cell pipette. Data points and error bars indicate means and SEM of IPSC amplitude (n = 4 pairs). Experiments were performed at RT.

(C–F) Amplitudes of IPSCs evoked at 0.1 Hz by presynaptic APs, measured from 3 to 4 min after whole-cell rupture. After IPSCs ran down to a low steady level, GABA was infused into a presynaptic BC via pipette perfusion: n = 5 at 1 mM (C), n = 5 at 2 mM (D), n = 6 at 5 mM (E), and n = 5 at 10 mM (F). Experiments were performed at PT.

(G) Relationship between presynaptic cytosolic GABA concentrations ([GABA]) and percentage of recovery of IPSCs (IPSC amplitude after GABA loading relative to that before washout). The curve fit represents the Hill equation

\[
\text{IPSC amplitude} = \text{IPSC amplitude after GABA washout} + \left(\frac{[\text{GABA}]}{\text{EC}_{50}}\right)^n
\]

where n and EC50 represents the Hill coefficient (1.5) and 50% effective concentration of GABA (3.0 mM), respectively. The curve fit was constrained at 0 mM [GABA] determined from the mean IPSC amplitude 1 min before GABA loading. Maximal IPSC amplitude was determined (legend continued on next page)
(APs) every 5 min (Figures 1A and 1B). When the presynaptic pipette contained neither GABA nor glutamate, IPSCs underwent a rundown, with an exponential time constant of 9.8 ± 1.2 min at room temperature (RT) (n = 4; Figure 1B), suggesting a passive leak of GABA from vesicles. We also blocked vesicular GABA uptake using a vacuolar ATPase blocker, bafilomycin A1 (Baf). After bath application of Baf (5 μM), IPSCs ran down with a time constant of 11.4 ± 2.3 min (n = 5; Figure S1A) like that after GABA washout (Figure 1B; p = 0.57, Student’s t test). The IPSC rundown was faster during higher frequency stimulation (Figure S1A), suggesting an additional contribution of empty vesicles accumulated by recycling (Takami et al., 2017). The blocking effect of Baf was irreversible (Figure S1B), confirming that vesicular transmitter refilling is indispensable for recovery from depression.

Since rundown of EPSCs by glutamate washout is rescued by loading glutamate into the presynaptic cytosol (Hori and Takahashi, 2012), we likewise infused GABA into BCs using patch pipette perfusion (Hori et al., 1999) at different concentrations (1–10 mM) to let them recover (Figures 1C–1G). Full recovery of IPSCs was observed with GABA above 5 mM (Figure 1G), suggesting that endogenous GABA concentrations in BCs are ~5 mM, as in other inhibitory neurons (Apostolides and Trussell, 2013; Otsuka et al., 1971). After a 10-mM GABA infusion, recovery of IPSCs significantly exceeded the initial control level (Figure 1G), suggesting that postsynaptic GABA receptors are not saturated by vesicular GABA, as at glutamatergic calyceal synapses (Ishikawa et al., 2002).

Although GABA-concentration-dependent rundown and recovery of IPSCs are likely caused by changes in vesicular GABA content, they are also proposed to result from changes in the number of releasable vesicles at hippocampal inhibitory synapses in culture (Wang et al., 2013). We tested the latter possibility using a direct whole-cell recording from PC axon terminals in culture (Kawaguchi and Sakaba, 2015). In paired recordings from a PC axon terminal and a cell from the deep cerebellar nuclei (DCN), presynaptic GABA washout caused a rundown of IPSCs (Figures 2A and 2B), as at BC-PC or other inhibitory synapses. Presynaptic membrane capacitance changes were then evoked by depolarizing pulses in the presence of GABA (5 mM) or in its absence (Figures 2C and 2D). No significant difference was found between them (Figure 2D; p = 0.99, repeated-measures ANOVA), indicating that presynaptic cytosolic transmitter concentration has no effect on SV availability at inhibitory synapses as at excitatory synapses (Hori and Takahashi, 2012; Takami et al., 2017).

from the mean IPSC amplitude 5–10 min after GABA loading. Compared with control before GABA infusion, mean IPSC amplitudes were significantly smaller at 1 mM [GABA] (p = 0.0014, paired t test) and significantly larger at 10 mM [GABA] (p = 0.033, paired t test). In a non-parametric Steel-Dwass test, there was also a significant difference in IPSC amplitudes between 1 mM and 5 mM [GABA] (p = 0.04). Values are given as mean ± SEM.
The Rate of GABA Uptake into Vesicles

Since cytosolic GABA concentration determines IPSC amplitude, primarily through vesicular GABA contents (Figures 1 and 2), we estimated the rate of GABA uptake into SVs from the recovery time of IPSCs from rundown. Whole-cell infusion of GABA at a given concentration provides a reliable measure for the magnitude of IPSC recovery (Figures 1C–1G), but its rate includes the diffusion time of GABA from pipettes to BC terminals. To obtain a more reliable measure for the GABA uptake rate, we replaced GABA with 1-(4-Aminobutanoyl)-4-[1,3-bis(di-hydroxyphosphoryloxy)propan-2-yloxy]-7-nitroindoline (DPNI)-caged GABA (10 mM) in the pipette solution and let IPSCs rundown. When IPSCs reached a minimal level, we applied a UV flash (1 s) through an objective lens, thereby photo-releasing GABA from caged GABA within a BC (Figure 3). UV photolysis of caged compounds in presynaptic terminals often induces toxic effects, presumably due to free radicals arising from uncaging. These effects can, however, be minimized by including glutathione in presynaptic pipettes (Hori and Takahashi, 2012). At BC-PC synapses, in the presence of 10 mM GABA and 20 mM glutathione in the presynaptic pipettes, UV uncaging of DPNI-GABA (10 mM) had no significant effect on the IPSC amplitude (p = 0.94, paired t test; Figure S2, n = 4). At BC-PC synapses, immediately after presynaptic GABA uncaging, IPSC amplitude started to recover and then reached a peak, but it was soon followed by a decline (Figures 3A and 3B), presumably because a bulk of DPNI-GABA remaining in the whole-cell pipette washed out newly uncaged GABA. Since the time constant of IPSC recovery after GABA uncaging (1.9 ± 0.2 min, n = 8; Figure 3B) was much faster than that of IPSC rundown during 0.1-Hz stimulation at PT (7.4 ± 1.3 min, n = 8; p = 0.003, paired t test), we utilized the initial slope of recovery to estimate the rate of recovery after GABA uncaging (Figure 3A). We also measured the percentage of recovery of IPSCs after GABA uncaging (Figure 3A). Because efficiency of GABA uncaging is variable and the amount of photo-released GABA is unpredictable (Trigo et al., 2009), we utilized DPNI-GABA at a fixed concentration (10 mM) and sampled data showing different initial slopes and peaks of recovery (Figure 3C). We then compared recovery percentages of IPSCs after GABA uncaging with those obtained by infusion of GABA of defined concentrations (Figures 1C–1G). This enabled us to estimate GABA concentrations after GABA uncaging (Figure 3D), as previously estimated for glutamate concentrations after uncaging at the calyx of Held (Hori...
These results suggest that vesicular GABA uptake can serve as a rate-limiting factor for the recovery of IPSCs from STD in physiological conditions.

**DISCUSSION**

The refilling of vesicles with neurotransmitter is an essential step in SV recycling for maintaining the efficacy of neurotransmission. The time required for SV refilling is determined for glutamate at the excitatory calyx of Held synapse (Hori and Takahashi, 2012) but remained unknown at inhibitory synapses. Using GABA uncaging at BC-PC inhibitory synapses, we have determined the time constant of IPSC recovery from STD at BC-PC inhibitory synaptic pairs in cerebellar slices of juvenile rats, we have determined the time constant of GABA uptake into SVs as 40 s at PT. This time constant is 5–6 times slower than that of vesicular glutamate uptake (Hori and Takahashi, 2012). In both glutamatergic and GABAergic synapses, uptake of neurotransmitter by SVs is driven by a proton gradient generated by the vacuolar ATPase. Glutamate uptake by vesicular glutamate transporters (VGLUTs) depends mainly upon the trans-vesicular voltage difference in electrochemical equilibrium with proton concentration gradient produced by vacuolar ATPase (Blakely and Edwards, 2012), whereas vesicular GABA uptake by VGATs is mediated by an H+/GABA anti-port (Farsi et al., 2016) following vesicle acidification (Egashira et al., 2016). Our results indicate that the latter uptake mechanism takes a much longer time to refill vesicles with neurotransmitter than the former mechanism. Using a pH indicator, mOrange, in hippocampal cell culture, Egashira et al. (2016) estimated the GABAergic vesicle re-alkalization time constant as 25 s at PT. This time constant is faster than that of GABA uptake into SVs estimated at inhibitory synapses in this study. The slow uptake rate of GABA into vesicles predicts that recycling reuse of SVs can be limited by the refilling rate unless there are enough SVs in terminals already filled. Filled SVs can be depolarized, at least partially, during bursts of presynaptic firing at cerebellar inhibitory neurons (Barmack and Yakhnitsa, 2008; Ruigrok et al., 2011; Häusser and Clark, 1997). In our experimental conditions, SVs in BC terminals were strongly depleted during presynaptic stimulation at 50 Hz and more mildly during 10 Hz stimulation. In both cases, presynaptic GABA concentrations affected the time constant of IPSC recovery from STD, as do they for the vesicular GABA uptake time constant. The time constants of IPSC recovery after 50-Hz stimulation and slow recovery time constants after 10-Hz stimulation showed inverse relationships with presynaptic GABA concentrations, with a slope similar to that between GABA concentrations and vesicular GABA uptake time constants. Recovery of synaptic responses from STD can be accelerated if postsynaptic receptors are saturated with vesicular neurotransmitter (Foster et al., 2002). However, our results from presynaptic GABA infusion indicate a significant potentiation of IPSC amplitude above baseline after 10-mM GABA infusion (Figure 1G; p = 0.033), suggesting that postsynaptic GABA receptors are not saturated with vesicular GABA at normal or lower GABA concentrations.

At excitatory synapses, multiple mechanisms are involved in STD (Zucker and Regehr, 2002; Schneggenburger et al., 2002), including depletion of releasable vesicles (Betz, 1970), reduction in the number of available release sites (Neher,
inactivation of presynaptic Ca\textsuperscript{2+} channels (Forsythe et al., 1998), or desensitization of postsynaptic receptors (Saviane and Silver, 2006). At calyx of Held synapses, recovery from STD has fast and slow components, with the former being dependent on Ca\textsuperscript{2+} (Wang and Kaczmarek, 1998) and calmodulin (Sakaba and Neher, 2001), whereas the latter reflects GTP-dependent SV replenishment (Takahashi et al., 2000) or recovery of presynaptic Ca\textsuperscript{2+} channels from inactivation (Forsythe et al., 1998). Whereas much less is known about the mechanisms underlying the recovery of inhibitory neurotransmission from STD, our present results indicate that vesicular GABA uptake rate can limit the recovery rate of inhibitory neurotransmission from STD.

Since presynaptic cell firing activity varies from time to time and among different synapses, the magnitude of SV depletion from presynaptic terminals may also vary. When SV depletion is mild, recovery of IPSCs from depression is produced by a replenishment of release sites with already filled SVs, together with newly recycled and refilled SVs. In such cases,
the contribution of the refilling rate to the IPSC recovery rate is expectedly partial. In fact, after 10-Hz stimulation, recovery of IPSCs had a bi-exponential time course (Figures 4B–4E). The fast recovery time constant was much faster than GABA uptake time constant and showed no GABA-concentration dependence. In contrast, the slow time constant was similar to the vesicular GABA uptake time constant and, likewise, correlated with presynaptic cytosolic GABA concentrations (Figures 4F and 4G). At hippocampal inhibitory synapses, the recovery of IPSCs from STD, induced by a train of 20 Hz, follows a bi-exponential time course with time constants of 1.3 s and 32 s estimated at PT (Kraushaar and Jonas, 2000). This slow recovery time constant is comparable to that at BC-PC synapses, suggesting that it might be determined by vesicular GABA uptake rate at hippocampal inhibitory synapses. Like GABA, vesicular glycine uptake is mediated by VGAT (McIntire et al., 1997). Thus, the vesicular refilling rate of glycine may also be slow and may affect the recovery rate of glycineergic inhibitory transmission from STD.

The expression level of vesicular transporter is correlated with the rate of transmitter uptake (Wilson et al., 2005). The present study was made at BC-PC synapses in rat cerebellum at postnatal day (P)12–P16. At this age, expression levels of both VGAT (Minelli et al., 2003) and VGLUT (Billups, 2005; Blaesse et al., 2005), as well as the vesicular glutamate uptake rate (Hori and Takahashi, 2012), reach a plateau, suggesting that vesicular GABA uptake rate measured here probably persists into adult ages. However, the question remains open as to what extent the GABA uptake rate limits the recovery of IPSCs from STD in adult animals.

In the presence of both excitatory and inhibitory synaptic inputs, postsynaptic cell firing tends to be enhanced during STD of inhibitory transmission. In the cerebellar cortical molecular layer, BCs and stellate cells provide significant inhibitory drives to PCs (Andersen et al., 1963), mediating feed-forward inhibition from parallel fibers to PCs. BCs also provide tonic inhibition on PCs by firing spontaneously at high frequency to tens of hertz (Eccles et al., 1966; Häusser and Clark, 1997; Ruigrok et al., 2011). Thus, the slow recovery of IPSCs from STD, due to slow vesicular GABA uptake rate, may disinhibit PCs for a sustained period, thereby promoting the high excitability of PCs.

**EXPERIMENTAL PROCEDURES**

All experiments were performed in accordance with the guidelines of Doshisha University and the Physiological Society of Japan.

**Slice Preparation**

After decapitation, sagittal slices (200 µm thick) were cut from the cerebellar cortex of Wistar/ST rats (P 12–16) of either sex. Slices were superfused with artificial cerebrospinal fluid (aCSF) containing (in millimolar): 125 NaCl, 2.5 KCl, 26 NaH2PO4, 2 CaCl2, 6 MgCl2, 10 glucose, 3 myo-inositol, 2 sodium pyruvate, and 5 ascorbic acid (pH 7.3, when bubbled with 95% O2/5% CO2).

**Culture**

Primary cultures of cerebellar neurons were prepared from newborn Wistar rats of both sexes, as previously reported (Kawaguchi and Sakaba, 2015). For details, see the Supplemental Experimental Procedures.

**Electrophysiology and DPNI-GABA Uncaging**

Electrophysiological experiments were performed as in previous studies (Hori and Takahashi, 2012; Kawaguchi and Sakaba, 2015). Detailed information is available in the Supplemental Experimental Procedures.

**Data Analysis**

Data fitting was performed using the least-squares method (single or double exponential). All values are given as mean ± SEM; n represents number of experiments. A difference of p < 0.05, in Student’s t test, paired t test, repeated-measures ANOVA, comparison of two correlation coefficients, or Steel-Dwass test, was regarded as significant by Excel 2013 or JMP Pro 13.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures and two figures and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.02.080.

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**AUTHOR CONTRIBUTIONS**

M.Y., T.H., and T.T. designed experiments; M.Y., S.K., and T.H. performed experiments. A difference of p < 0.05, in Student’s t test, paired t test, repeated-measures ANOVA, comparison of two correlation coefficients, or Steel-Dwass test, was regarded as significant by Excel 2013 or JMP Pro 13.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

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**REFERENCES**


