Department of Physiology and Biophysics, School of Life Sciences, Fudan University, Shanghai, China

# Levetiracetam inhibits endocytosis and augments short-term depression

Li-Meng, Lei-Xue*

*Received July 5, 2018, accepted August 5, 2018*

**Corresponding author: Lei-Xue, Department of Physiology and Biophysics, School of Life Sciences, Fudan University, Shanghai, China, 200438*
*15210700017@fudan.edu.cn*

*Pharmazie 73: 643–646 (2018)* *doi: 10.1691/ph.2018.8634*

Levetiracetam (LEV) is an anti-epileptic drug with demonstrated efficacy against generalized seizures. Recent studies have found that LEV affects the release of neurotransmitters by binding to synaptic vesicle protein 2A (SV2A). However, the details of LEV regulation of synaptic transmission remain poorly understood. Here, we used the whole-cell patch-clamp technique to further characterize the effects of LEV on synaptic transmission at a large mammalian central synapse, the calyx of Held. Our results showed that two common forms of vesicle endocytosis, including slow and rapid endocytosis, were dramatically inhibited when slices were incubated in 100 μM LEV for 1 h, however, the action potential (AP), calcium influx and exocytosis were not affected. Furthermore, by measuring the level of steady-state depression induced by 100 Hz stimulus trains, we found that the steady state level of depression was significantly stronger after LEV treatment, indicating that LEV enhanced short-term depression (STD). Thus, these findings suggested that the mechanisms of the antiepileptic of LEV seem to be mediated, at least partly, by regulating endocytosis and STD.

## 1. Introduction

Epilepsy is a symptom due to abnormal excessive neuronal activity in the brain (Thurman et al. 2011) and antiepileptic drugs (AED) are the main form of symptomatic treatment for patients with epilepsy (Kwan and Brodie 2000). It is well established that most antiepileptic drugs act on voltage-gated sodium channels or GABA receptors to reduce seizures (Margineanu and Klitgaard 2003; Niespodziany et al. 2004: Zona et al. 2001).

Levetiracetam (LEV) has a unique but yet not fully understood mechanism of action (Moshe et al. 2015). Previous studies have found that LEV does not directly regulate voltage-gated sodium channels or GABA receptors, but binds to synaptic vesicle glycoprotein 2A (SV2A), ubiquitously expressed in the central nervous system, which might mediate neurotransmitter release (Lynch et al. 2004; Meehan et al. 2011). Studies have shown that the anti-seizure efficacy of LEV is reduced in SV2A$^{-/-}$ mice (Kaminski et al. 2009). By patch-clamp recording and two-photon imaging, LEV has been determined to inhibit excitatory neurotransmitter release in hippocampal slices (Yang et al. 2007; Lee et al. 2009; Yang and Rothman 2009). However, the downstream details of LEV binding to SV2A remain poorly understood.

Exocytosis and endocytosis are important for maintaining normal brain functions, including synaptic transmission and plasticity. Vesicle exocytosis, upon entry of calcium, releases neurotransmitter mediate synaptic transmission. Efficient endocytosis plays a crucial role in recycling vesicles, and it is also helpful to inhibit swelling of the plasma membrane (Xue and Mei 2011; Gordon and Cousin 2016). Previous studies found that LEV has an effect on vesicle recycling in rat hippocampal slices, implying that LEV might impair vesicle endocytosis (Yang et al. 2015a; Bartholome et al. 2017). However, there is no direct presynaptic evidence of LEV regulation of endocytosis.

STD, which occurs at almost all central nervous system synapses during repetitive firing, is a form of short term plasticity (Regehr 2012). Electrophysiological experiments indicated that LEV causes a mild acceleration of supply rate depression, a form of short-term plasticity caused by the supply of new vesicles during initial epileptic activity, in hippocampal slices and cultured neurons from SV2A$^{-/-}$ mice (García-Pérez et al. 2015). This modulation of neuronal activity indicated that interaction of LEV with SV2A is likely to explain the phenomenon. Therefore, increasing the effects of depression might suppress epilepsy (García-Pérez et al. 2015; Yang et al. 2015a,b). However, the mechanism of LEV affected STD remains to be elucidated.

The present study was designed to determine the presynaptic regulations of LEV at a large mammalian central synapse, the calyx of Held (Sun et al. 2004; Wu et al. 2005; Schneggenburger and Forsythe 2006; Borst and Soria van Hoeve 2012; Yue and Xu 2014). Here, we found that LEV inhibits slow and rapid endocytosis with high time-resolved membrane capacitance measurement. Furthermore, we report that LEV leads to an augmented steady-state neurotransmission, indicating that LEV enhances STD. However, the RRP replenishment, along with other properties of synaptic transmission, remains intact after LEV treatment. Mechanisms, which indicate LEV might alter the downstream protein of SV2A, are discussed.

## 2. Investigations and results

### *2.1. LEV does not affect presynaptic action potential*

Transmitter release is triggered by calcium influx through calcium channels, which are activated by AP. To clarify the presynaptic mechanisms of the reduction of synaptic transmission of LEV, in terms of AP involved, we successfully induced a train of APs (success rate: 98±2 %, n = 6; Fig. 1A) by injecting ten 3-ms pulses of 400 pA current at 100 Hz. After treatment with 100 μM LEV, the induction of AP trains was also successful (success rate: 97±2 %, n=8, p=0.7; Fig. 1B). We further investigated the AP waveform in a single current injection. Application of LEV did not affect the half-width (control: 0.61±0.04 ms, n=6; LEV: 0.62±0.06 ms, n=8, p=0.9, Fig. 1D) and amplitude of the action potential (control: 99.5±3.3 mV, n=6; LEV: 96.2±2.7 mV, n=8, p=0.5, Fig. 1E). These results suggest that LEV does not affect presynaptic AP firing and the AP waveform.

### *2.2. LEV inhibits both slow and rapid endocytosis*

Since LEV did not affect presynaptic AP, we further measured calcium influx, vesicle exocytosis and endocytosis at the

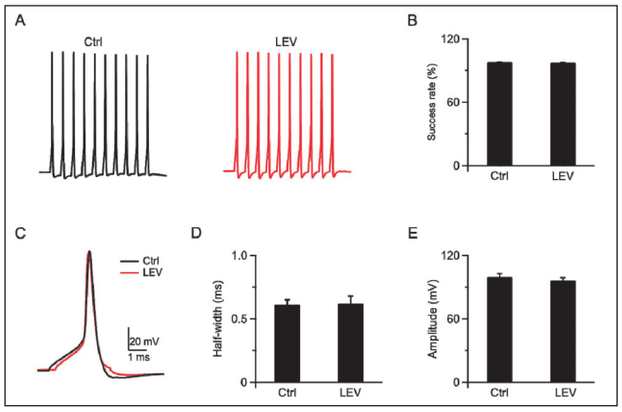


Fig. 1: LEV does not affect presynaptic action potential. (A) Sampled action potentials induced by current injection (3-ms step current of 400 pA at 100 Hz) in the control and LEV group. (B) Statistics for the action potential success rates in the control and LEV group. (C) Sampled action potential waveform induced by single current injection (3-ms step current of 400 pA in the control and LEV group. (D) Statistics for action potential half-width in the control and LEV group. (E) Statistics for action potential amplitude in the control and LEV group.

presynaptic nerve terminals. Here we determined whether LEV modulates calcium influx and two common observed forms of endocytosis, slow and rapid endocytosis. We applied a 20 ms depolarization pulse (depol$_{20ms}$, depolarized from -80 mV to +10 mV), which can deplete the readily releasable pool (RRP) and induce a following clathrin-dependent, dynamin-dependent slow endocytosis (Fig. 2A). In controls, depol$_{20ms}$ evoked calcium current (ICa) of 2.1±0.1 nA (n=5), followed by an increase in membrane capacitance (ΔCm) of 518±71 fF (n = 5), following exocytosis, endocytosis decayed at the rate (Rate$_{endo}$) of 54±6 fF/s (n=5). After incubation of 100 µM LEV, calcium current (ICa: 1.9±0.1 nA, n=6, p=0.3, Fig. 2B) and exocytosis (ΔCm: 534±51 fF, n=6, p=0.9, Fig. 2C) were not affected. However, the Rate$_{endo}$ was dramatically decreased to 22±3 fF/s (n=6, $p < 0.01$, Fig. 2D). We also measured the net capacitance increase 15 s after the stimulation (ΔCm$_{15s}$). Our results showed ΔCm$_{15s}$ increased to 317±34 fF (n=6, $p < 0.05$, Fig. 2E) compared to controls (213±17 fF, n=5). These effects suggested that LEV inhibits slow endocytosis.

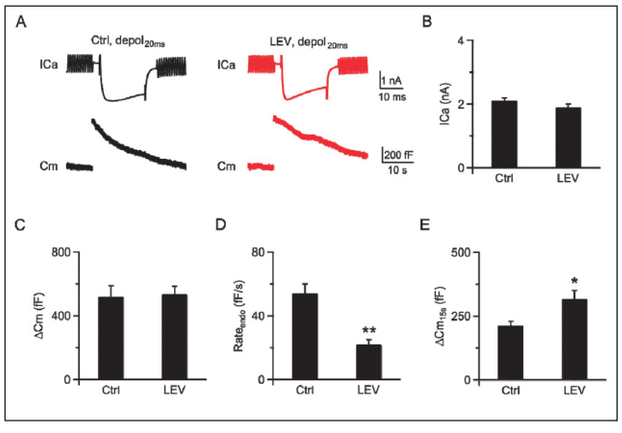


Fig. 2: LEV inhibits slow endocytosis. (A) Sampled presynaptic calcium current (ICa, upper) and membrane capacitance (Cm, lower) induced by depol$_{20ms}$ in the control and LEV group. (B-E) Statistics for ICa, ΔCm, Rate$_{endo}$, and ΔCm$_{15s}$ in the control and LEV group, * $p < 0.05$, ** $p < 0.01$.

Next, we investigated whether LEV also affected the rapid endocytosis, which occurs under intense stimulation. By applying 10 depolarization pulses of 20 ms at 10 Hz (depol$_{20msx10}$), we induced a larger amount of exocytosis and an additional rapid form of endocytosis, which depends on dynamin but not clathrin (Fig. 3A). In controls, depol$_{20msx10}$ induced a calcium influx (QICa) of 299±27 pC (n=6) and a total capacitance jump (ΔCm) of 1540±75 fF (n=6). After incubation of 100 µM LEV, depol$_{20msx10}$ induced a QICa of 309±28 pC (n=5, p=0.8, Fig. 3B) and a ΔCm of 1417±68 fF (n=5, p=0.3, Fig. 3C), which were similar to controls. However, the endocytosis rate (Rate$_{endo}$) was significantly smaller compared to controls (control: 248±28 fF/s, n=6; LEV: 134±18 fF/s, n=5, $p < 0.01$, Fig. 3D). Furthermore, the increase in net capacitance 30 s after stimulation in the LEV treatment group was much larger than in the control group (control: 117±33 fF, n=6; LEV: 264±58 fF, n=5, $p < 0.05$, Fig. 3E), which also confirmed the inhibition of rapid endocytosis after LEV incubation.

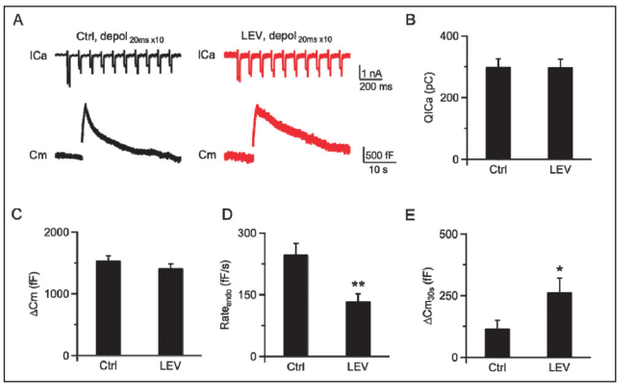


Fig. 3: LEV inhibits rapid endocytosis. (A) Sampled presynaptic calcium current (ICa, upper) and membrane capacitance (Cm, lower) induced by depol$_{20ms\ x10}$ in the control and LEV group. (B-E) Statistics for QICa, ΔCm, Rate$_{endo}$, and ΔCm$_{30s}$ in the control and LEV group, * $p < 0.05$, ** $p < 0.01$.

### 2.3. *LEV enhances STD*

STD, which is induced by brief bursts of activity, has been reported at the calyx of Held synapse as a form of short-term plasticity (von Gersdorff and Borst 2002; Schneggenburger and Forsythe 2006; Friauf et al. 2015; Baydyuk et al. 2017). A series of experiments indicated that LEV decreases vesicular release during high-frequency, this modulation of neuronal activity represents a form of STD (Yang et al. 2007; Yang and Rothman 2009; Meehan et al. 2011). Thus, we continued to explore the regulation of LEV on STD. At 100 Hz stimulation frequency, our results showed depression that reached a steady-state after ~ 10 stimuli (Fig. 4A). We found the steady state level of depression, average from 11$^{th}$–20$^{th}$ stimuli, was significantly different for the two groups (Fig. 4B). In controls, steady-state depression was of 31.8±6.4 % (n=7) of the initial EPSC magnitude, whereas LEV incubation groups depressed to 17.4±1.2 % of the initial EPSC magnitude (n=5, $p < 0.05$, one-way ANOVA, Fig. 4C). Thus, the smaller steady-state level of transmission suggested LEV augments STD dramatically.

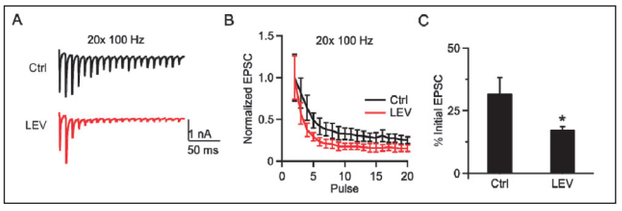


Fig. 4: LEV enhances STD. (A) Sampled EPSC in the control and LEV group. (B) Normalized EPSC in the control and LEV group. (C) Statistics for steady-state EPSC amplitude in the control and LEV group, * $p < 0.05$.

### 2.4. *LEV does not affect RRP replenishment*

To further clarify the mechanism underlying the augmented STD by LEV, we examined whether RRP replenishment influences STD.

At various times (Δt = 0.05–20 s) after a conditioning 20 ms depolarisation (-80 to +10 mV), which depleted the RRP, we applied a 20 ms depolarisation to measure the resulting capacitance jump (ΔCm), which reflected the recovery of the RRP (Fig. 5A). We also used different intensities of conditioning stimuli, including a single 20 ms depolarisation, and 10 pulses of 20 ms depolarisation delivered at 10 Hz (Fig. 5C). After a 20 ms depolarisation, the RRP recovery could be fitted with a bi-exponential function. We found that the RRP replenishment was unchanged ($\tau_{fast}$ =0.20 s, 56%; $\tau_{slow}$=6.18 s, 35 %, n=5~7, Fig. 5B) compared with control ($\tau_{fast}$=0.21 s, 60 %; $\tau_{slow}$ =5.83 s, 31%, n = 5~9). Thus, LEV did not affect RRP replenishment after a 20 ms depolarization.

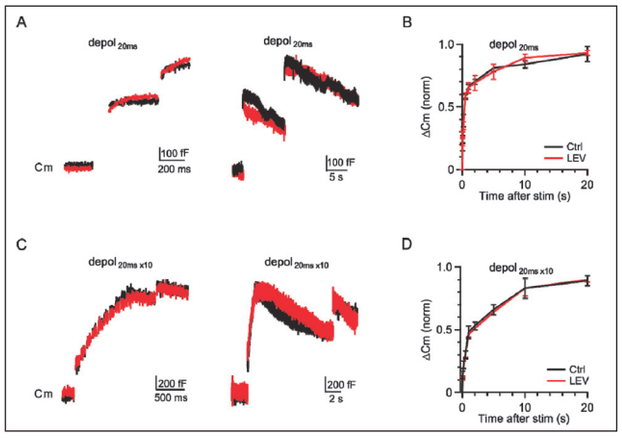


Fig. 5: LEV does not affect RRP replenishment. (A) Sampled Cm induced by a 20 ms depolarization applied at 0.5 (left) or 10s (right) after a conditioning pulse in the control and LEV group. (B) ΔCm induced by a 20 ms depolarization applied at various intervals after a conditioning 20 ms depolarization in the control LEV group. Data were normalized to the ΔCm and fit with a bi-exponential function. (C) Similar to A, except that the conditioning stimulus was 10 pulses of 20 ms depolarization at 10 Hz. (D) Similar to B, except that the conditioning stimulus was 10 pulses of 20 ms depolarization at 10 Hz in the control and LEV group.

After 10 pulses of 20 ms depolarisation at 10 Hz, the RRP replenishment could fit the bi-exponential function, with no significant difference between control ($\tau_{fast}$=0.34 s, 35 %; $\tau_{slow}$=6.25 s, $A_2$ = 53%, n=5~7) and LEV group ($\tau_{fast}$ =0.33 s, 34 %; $\tau_{slow}$=6.89 s, 57 %, n=5~6, Fig. 5D). Thus, LEV did not affect RRP replenishment after10 pulses of 20 ms depolarisation at 10 Hz.

## 3. Discussion

By whole-cell patch-clamp measurements, our experiments extended prior results showing impairment of LEV on synaptic transmission at the calyx of Held, a mammalian nerve terminal. We found LEV inhibited endocytosis and enhanced STD. These findings show crucial regulatory roles of LEV in synaptic transmission and plasticity in the central nervous system. To some extent, our results may offer an explanation for its efficacy as an antiepileptic drug.

When the AP arrived at the nerve endings, it triggers the release of presynaptic glutamate. Therefore, we examined whether LEV can affect presynaptic AP. Studies found that LEV has no effects on voltage-dependent sodium channels and T- type calcium channels in hippocampal neurons (Zona et al. 2001). According to our results, LEV does not regulate AP, which is consistent with previous studies (Fig. 1). APs depolarize the presynaptic membrane by which calcium influxes to trigger neurotransmitter release (Sudhof 2004, 2012). Neurotransmitters are released *via* exocytosis, and following exocytosis, endocytosis is initiated to retrieve vesicles within seconds to minutes. Although previous studies have revealed actions of LEV on ion channels and synaptic transmission, it is still unclear, whether LEV influences endocytosis to reduce excitability (Deshpande and Delorenzo 2014; Wakita et al. 2014; Zona et al. 2001). By real-time endocytosis measurements, we provided direct evidence for the significant inhibitory effects of LEV on slow endocytosis (Fig. 2) and rapid endocytosis (Fig. 3). Photo-affinity labeling experiments in the rat brain revealed that LEV labeled SV2A (Lynch et al. 2004). Consistent with SV2A being the unique binding site for LEV, LEV is likely to act by modulating the action of SV2A. Our work advances over this previous study. It seems that LEV somehow alters SV2A's interaction with one of its binding partners, which include synaptotagmin, multiple clathrin adapter proteins and calcium/calmodulin-dependent protein kinase II, leading to many possible downstream consequences (Yao et al. 2010). In addition, previous observation suggested mutation of a tyrosine-based endocytosis motif (Y46) in SV2A resulted in dominant effect on synaptic endocytosis (Yao et al. 2010). Besides, a series of works have found SV2A binds to and regulates synaptotagmin into vesicles and synaptotagmin has been proposed to be required for normal vesicle endocytosis (Jorgensen et al. 1995; Poskanzer et al. 2003; Nicholson-Tomishima and Ryan 2004). The results suggested LEV alters SV2A's ability to bind to proteins that influence protein trafficking or localization, for example synaptotagmin. Recent evidence suggested disruption of endocytosis in excitatory neurons suppresses seizures, which was also consistent with our study (Kroll 2015).

Increasing depression at excitatory synapses might be helpful for treating epilepsy, because if there are more inhibitory effects, it will weaken the excitatory feedback in the neural circuit, thereby converting the balance between excitatory and inhibitory synapses to the inhibit one (Galarreta and Hestrin 1998; von Gersdorff and Borst 2002, Kaplan et al. 2003; Fioravante and Regehr 2011). As discussed in the introduction, several studies have found LEV may alter short-term synaptic plasticity, but the details were still under debate. In this study, to explore the mechanism underlying LEV potentiated STD, we examined whether other factors known to regulate STD can explain the results (Schneggenburger et al. 2002; Dutta Roy et al. 2014). First, we excluded the part of the postsynaptic receptor saturation and desensitization in the calyx of Held (Taschenberger et al. 2002). Because we used 1mM KYN to reduce AMPA receptor saturation and desensitization and found similar effects in both control and LEV groups. Second, we excluded the role of the calcium influx (Xu and Wu 2005; Catterall and Few 2008; Nakamura et al. 2008). We performed direct presynaptic whole-cell recordings and found that there was no difference in calcium currents (Fig. 2B). Third, a major mechanism underlying STD during intense stimulation is RRP replenishment, which may determine the degree of STD (Regehr 2012). We measured the time constant and level of the RRP replenishment and found no significant difference between control and LEV groups (Fig. 5). In the LEV treatment group, this indicated a normal rate of vesicle recovery at the calyx of Held synapse, which has a large pool of recycling vesicles (Schneggenburger et al. 2002; de Lange et al. 2003; Qiu et al. 2015). Therefore, we excluded the involvement of the RRP replenishment. Forth, as mentioned above, LEV somehow altered SV2A's interaction with one of its binding proteins, which include synaptotagmin (Yao et al. 2010). LEV treatment of neurons overexpressing SV2A decreased the amount of both SV2 and synaptotagmin at synapses (Nowack 2011). Overexpression of synaptotagmin in developing spinal neurons led to a reduced depression (Morimoto 1998). Our results showed that LEV dramatically augmented STD (Fig. 2 and 3), which was consistent with the finding that LEV might imped the downstream action of SV2A (García-Pérez et al. 2015; Kaempf et al. 2015; Yang et al. 2015a).

Our results, taken together with other reports, strongly support the particular mechanisms of LEV. The experiments described in this article illustrate that LEV enhances STD and inhibits endocytosis by binding to SV2A. But only a more comprehensive process of vesicle recycling can further clarify the precise molecular mechanism of this effect. Nonetheless, our study might provide an attractive target for designing new antiepileptic drugs.

## 4. Experimental

### *4.1. Slice preparation*

Sprague-Dawley rats (8-10 days old) of either sex were decapitated to obtain the medial nucleus of the trapezoid body (MNTB) in a low-$Ca^{2+}$ artificial cerebrospinal fluid (ACSF) solution. The ACSF included (in mM): 125 NaCl, 25 $NaHCO_3$,

3 myo-inositol, 2 Na-pyruvate, 2.5 KCl, 1.25 $NaH_2PO_4$, 0.4 ascorbic acid, 25 glucose, 3 $MgCl_2$, and 0.05 $CaCl_2$ (bubbled with 95% $O_2$ and 5% $CO_2$). Brain slices (180 ~ 200 μM thick) were sectioned using a vibratome (VT 1200s, Leica, Germany), then recovered in normal ACSF with 2 mM $CaCl_2$ at 37 °C for 1 h. After recovery, the slices were kept at room temperature 22-24 °C.

### *4.2. Electrophysiology*

The presynaptic membrane capacitance measurements were made using an EPC-10 amplifier (HEKA, Lambrecht, Germany). The presynaptic pipette (3 – 4 MΩ) solution contained the following (in mM): 125 Cs-gluconate, 20 CsCl, 4 Mg-ATP, 10 $Na_2$-phosphocreatine, 0.3 GTP, 10 HEPES, and 0.05 BAPTA. pH 7.2, adjusted with CsOH, osmolarity: 315–320 mOsm. The series resistance (< 10 MΩ) was compensated by 65% (lag 10 μs).

The postsynaptic EPSC was recorded by an EPC-10 amplifier. The pipette (2 – 3 MΩ) solution contained the following (in mM): 125 K-gluconate, 20 KCl, 4 Mg-ATP, 10 $Na_2$-phosphocreatine, 0.3 GTP, 10 HEPES, and 0.5 EGTA. pH 7.2, adjusted with KOH, osmolarity: 315–320 mOsm. The series resistance (< 10 MΩ) was compensated by 95% (lag 10 μs). The EPSC was induced by a 0.1 ms, 2-20 V pulse applied via a bipolar electrode placed at the midline of the trapezoid body. 1 mM kynurenic acid (KYN) was added to the bath solution to relieve AMPA receptor saturation and desensitization.

### *4.3. Data analysis*

The initial rate of endocytosis ($Rate_{endo}$) was measured within 2 s after depolarization with $depol_{20ms}$ or $depol_{20msx10}$. Measurements of the RRP replenishment time course were similar to previous reports (Wu and Wu 2009). Data were presented as mean±SE. and statistical analysis used a t test unless otherwise noted, and $p < 0.05$ indicated a significant difference. 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.

Conflicts of interest: None declared.

## References


Bartholome O, Van den Ackerveken P, Sanchez Gil J, de la Brassinne Bonardeaux O, Leprince P, Franzen R, Rogister B (2017) Puzzling out Synaptic vesicle 2 family members functions. Front Mol Neurosci 10:148-163.

Baydyuk M, Xu J, Wu L-G (2017) The calyx of Held in the auditory system: Structure, function, and development. Hearing Res 338: 22-31.

Borst JG, Soria van Hoeve J (2012) The calyx of Held synapse: from model synapse to auditory relay. Ann Rev Physiol 74: 199-224.

Catterall WA, Few AP (2008) Calcium channel regulation and presynaptic plasticity. Neuron 59: 882-901.

De Lange RP, De Roos AD, Borst JG (2003) Two modes of vesicle recycling in the rat calyx of Held. J Neurosci 23: 10164-10173.

Deshpande LS, Delorenzo RJ (2014) Mechanisms of levetiracetam in the control of status epilepticus and epilepsy. Front Neurol 5: 11.

Dutta Roy R, Stefan MI, Rosenmund C (2014) Biophysical properties of presynaptic short-term plasticity in hippocampal neurons: insights from electrophysiology, imaging and mechanistic models. Front Cell Neurosci 8: 141.

Fioravante D, Regehr WG (2011) Short-term forms of presynaptic plasticity. Curr Opin Neurobiol 21: 269-274.

Friauf E, Fischer AU, Fuhr MF (2015) Synaptic plasticity in the auditory system: a review. Cell Tissue Res 361: 177-213.

Galarreta M, Hestrin S (1998) Frequency-dependent synaptic depression and the balance of excitation and inhibition in the neocortex. Nat Neurosci 1: 587-594.

García-Pérez E, Mahfooz K, Covita J, Zandueta A, Wesseling JF (2015) Levetiracetam accelerates the onset of supply rate depression in synaptic vesicle trafficking. Epilepsia 56: 535-545.

Gordon SL, Cousin MA (2016) The iTRAPs: Guardians of synaptic vesicle cargo retrieval during endocytosis. Front Synaptic Neurosci 8:1.

Jorgensen EM, Hartwieg E, Schuske K, Nonet ML, Jin Y, Horvitz HR (1995) Defective recycling of synaptic vesicles in synaptotagmin mutants of Caenorhabditis elegans. Nature 378: 196-199.

Kaempf N, Kochlamazashvili G, Puchkov D, Maritzen T, Bajjalieh SM, Kononenko NL, Haucke V (2015) Overlapping functions of stonin 2 and SV2 in sorting of the calcium sensor synaptotagmin 1 to synaptic vesicles. Proc Natl Acad Sci USA 112: 7297-7302.

Kaminski RM, Gillard M, Leclercq K, Hanon E, Lorent G, Dassesse D, Matagne A, Klitgaard H (2009) Proepileptic phenotype of SV2A-deficient mice is associated with reduced anticonvulsant efficacy of levetiracetam. Epilepsia 50: 1729-1740.

Kaplan MP, Wilcox KS, Dichter MA (2003) Differences in multiple forms of short-term plasticity between excitatory and inhibitory hippocampal neurons in culture. Synapse 50: 41-52.

Kroll JR, Wong KG, Siddiqui FM, Tanouye MA (2015) Disruption of endocytosis with the dynamin mutant shibirets1 suppresses seizures in Drosophila. Genetics 201: 1087-1102.

Kwan P, Brodie MJ (2000) Early identification of refractory epilepsy. New Engl J Med 342: 314-319.

Lee CY, Chen CC, Liou HH (2009) Levetiracetam inhibits glutamate transmission through presynaptic P/Q-type calcium channels on the granule cells of the dentate gyrus. Br J Pharmacol 158: 1753-1762.

Lynch BA, Lambeng N, Nocka K, Kensel-Hammes P, Bajjalieh SM, Matagne A, Fuks B (2004) The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam. Proc Natl Acad Sci USA 101: 9861-9866.

Margineanu DG, Klitgaard H (2003) Levetiracetam has no significant gamma-aminobutyric acid-related effect on paired-pulse interaction in the dentate gyrus of rats. Eur J Pharmacol 466: 255-261.

Meehan AL, Yang X, McAdams BD, Yuan L, Rothman SM (2011) A new mechanism for antiepileptic drug action: vesicular entry may mediate the effects of levetiracetam. J Neurophysiol 106: 1227-1239.

Moshe SL, Perucca E, Ryvlin P, Tomson T (2015) Epilepsy: new advances. Lancet 385: 884-898.

Nakamura T, Yamashita T, Saitoh N, Takahashi T (2008) Developmental changes in calcium/calmodulin-dependent inactivation of calcium currents at the rat calyx of Held. J Physiol 586: 2253-2261.

Nicholson-Tomishima K, Ryan TA (2004) Kinetic efficiency of endocytosis at mammalian CNS synapses requires synaptotagmin I. Proc Natl Acad Sci USA 101: 16648-16652.

Niespodziany I, Klitgaard H, Margineanu DG (2004) Is the persistent sodium current a specific target of anti-absence drugs? Neuroreport 15:1049-1052.

Nowack A (2011) Levetiracetam reverses synaptic deficits produced by overexpression of SV2A. PLoSone 6: e29560.

Poskanzer KE, Marek KW, Sweeney ST, Davis GW (2003) Synaptotagmin I is necessary for compensatory synaptic vesicle endocytosis in vivo. Nature 426: 559-563.

Qiu X, Zhu Q, Sun J (2015) Quantitative analysis of vesicle recycling at the calyx of Held synapse. Proc Natl Acad Sci USA 112: 4779-4784.

Regehr WG (2012) Short-term presynaptic plasticity. Cold Spring Harbor Perspectives in Biology 4: a005702.

Schneggenburger R, Forsythe ID (2006) The calyx of Held. Cell Tissue Res 326: 311-337.

Schneggenburger R, Sakaba T, Neher E (2002) Vesicle pools and short-term synaptic depression: lessons from a large synapse. Trends Neurosci 25: 206-212.

Sudhof TC (2004) The synaptic vesicle cycle. Ann Rev Neurosci 27: 509-547.

Sudhof TC (2012) The presynaptic active zone. Neuron 75: 11-25.

Sun JY, Wu XS, Wu W, Jin SX, Dondzillo A, Wu LG (2004) Capacitance measurements at the calyx of Held in the medial nucleus of the trapezoid body. J Neurosci Methods 134: 121-131.

Morimoto T, Wang XH, Poo MM (1998) Overexpression of synaptotagmin modulates short-term synaptic plasticity at developing neuromuscular junctions. Neuroscience 969-978.

Taschenberger H, Leao RM, Rowland KC, Spirou GA, von Gersdorff H (2002) Optimizing synaptic architecture and efficiency for high-frequency transmission. Neuron 36: 1127-1143.

Thurman DJ, Beghi E, Begley CE, Berg AT, Buchhalter JR, Ding D, Hesdorffer DC, Hauser WA, Kazis L, Kobau R, Kroner B, Labiner D, Liow K, Logroscino G, Medina MT, Newton CR, Parko K, Paschal A, Preux PM, Sander JW, Selassie A, Theodore W, Tomson T, Wiebe S (2011) Standards for epidemiologic studies and surveillance of epilepsy. Epilepsia 52 Suppl 7: 2-26.

von Gersdorff H, Borst JG (2002) Short-term plasticity at the calyx of Held. Nature Rev Neurosci 3: 53-64.

Wakita M, Kotani N, Kogure K, Akaike N (2014) Inhibition of excitatory synaptic transmission in hippocampal neurons by levetiracetam involves $Zn^{2+}$-dependent GABA type A receptor-mediated presynaptic modulation. J Pharmacol Exp Ther 348: 246-259.

Wu W, Xu J, Wu XS, Wu LG (2005) Activity-dependent acceleration of endocytosis at a central synapse. J Neurosci 25: 11676-11683.

Wu XS, Wu LG (2009) Rapid endocytosis does not recycle vesicles within the readily releasable pool. J Neurosci 29: 11038-11042.

Xu J, Wu LG (2005) The decrease in the presynaptic calcium current is a major cause of short-term depression at a calyx-type synapse. Neuron 46:633-645.

Xue L, Mei YA (2011) Synaptic vesicle recycling at the calyx of Held. Acta Pharmacol Sin 32: 280-287.

Yang X, Bognar J, Jr., He T, Mohammed M, Niespodziany I, Wolff C, Esguerra M, Yang X, Meehan AL, Rothman SM, Dubinsky JM (2015b) Seletracetam enhances short term depression *in vitro*. Epilepsy Res 117: 17-22.

Yang XF, Rothman SM (2009) Levetiracetam has a time- and stimulation-dependent effect on synaptic transmission. Seizure 18: 615-619.

Yang XF, Weisenfeld A, Rothman SM (2007) Prolonged exposure to levetiracetam reveals a presynaptic effect on neurotransmission. Epilepsia 48: 1861-1869.

Yao J, Nowack A, Kensel-Hammes P, Gardner RG, Bajjalieh SM (2010) Cotrafficking of SV2 and synaptotagmin at the synapse. J Neurosci 30: 5569-5578.

Yue HY, Xu J (2014) Myosin light chain kinase accelerates vesicle endocytosis at the calyx of Held synapse. J Neurosci 34: 295-304.

Zona C, Niespodziany I, Marchetti C, Klitgaard H, Bernardi G, Margineanu DG (2001) Levetiracetam does not modulate neuronal voltage-gated Na+ and T-type calcium currents. Seizure 10: 279-286.