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研究課題名（英文）Molecular targets of volatile anesthetics isoflurane at the calyx of Held synapse.

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研究成果の概要（和文）：全身麻酔のシナプス機構は未確認のままです。 ラット脳幹スライスでは、イソフルランはシナプス前カルシウムチャネルとエキソサイトーシスの機械をブロックすることにより興奮性伝達物質の放出を阻害し、後者のメカニズムは高周波伝達に対するその阻害効果が支配的です。 スライスとin vivoの両方で、イソフルランは高周波シナプス前入力によって誘発されるスパイク伝達を優先的に抑制します。 イソフルランのこのローパスフィルタリング動作は、全身麻酔で重要な役割を果たす可能性があります。

研究成果の学術的意義や社会的意義
We discovered isoflurane preferentially inhibits high-frequency neurotransmission, therefore, selective inhibition by volatile anesthetics of high-frequency transmission will effectively attenuate such integral neuronal functions, with minimal inhibition of basal neuronal functions.

研究成果の概要（英文）：Synaptic mechanisms of general anesthesia remain unidentified. In rat brainstem slices, isoflurane inhibits excitatory transmitter release by blocking presynaptic calcium channels and exocytic machinery, with the latter mechanism predominating in its inhibitory effect on high-frequency transmission. Both in slice and in vivo, isoflurane preferentially inhibits spike transmission induced by high-frequency presynaptic inputs. This low-pass filtering action of isoflurane likely plays a significant role in general anesthesia.

研究分野：Anesthesiology

キーワード：Isoflurane　calyx of Held　capacitance measurement　exo-endocytosis　neurotransmission

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１．研究開始当初の背景

Volatile anesthetics have been widely utilized for surgery since the nineteenth century. Sherrington (1906) predicted that the synapse is the main target of volatile anesthetics. At inhibitory synapses, volatile anesthetics prolong postsynaptic responses (Nicoll, 1972; Mody et al., 1991), and at both excitatory and inhibitory synapses, they inhibit neurotransmitter release (Takenoshita and Takahashi, 1987; Kullmann et al., 1989; Wu et al., 2004; Baumgart et al., 2015). Various mechanisms have been postulated to explain presynaptic inhibitory effects of volatile anesthetics. These include (i) inhibition of voltage-gated Na^+ channels (Haydon and Urban, 1983; Rehberg et al., 1996; Ouyang and Hemmings, 2005), (ii) inhibition of voltage-gated Ca^{2+} channels (Study, 1994; Kamatchi et al., 1999), and (iii) activation of voltage-independent K^+ channels (Patel et al., 1999; Ries and Puil, 1999; Franks and Honore, 2004). Volatile anesthetics are also proposed to (iv) directly block vesicle exocytosis via inhibiting vesicle fusion machineries (van Swinderen et al., 1999; Nagele et al., 2005; Herring et al., 2009; Xie et al., 2013). However, the primary target of anesthetics remains unidentified.

２．研究の目的

Recently, at hippocampal synapses in culture, Baumgart *et al* (2015) reported that isoflurane inhibits presynaptic Ca^{2+} influx without changing the Ca^{2+}-release relationship. Hence, they postulated that a reduction of Ca^{2+} influx fully explains presynaptic inhibitory effect of isoflurane. Since this conclusion is based on experiments using single action potential (AP) stimulation, the target of the anesthetics on repetitive neurotransmission remain open. It also remains unidentified whether the reduction of Ca^{2+} influx is caused by direct inhibition of Ca^{2+} channels or indirectly caused by a reduction of presynaptic AP amplitude. At the calyx of Held in brainstem slices from pre-hearing rats, Wu et al. (2004) did not observe consistent inhibition of presynaptic Ca^{2+} channel currents by isoflurane, but found that isoflurane reduces presynaptic AP amplitude, thereby proposing that the latter mechanism may mediate inhibition of transmitter release by isoflurane.

Hence, using the calyx of Held in post-hearing rat brainstem slices, we systematically addressed the target of isoflurane. In variance-mean analysis, isoflurane attenuated both the release probability (p_r) and the number of functional release sites (N), suggesting that multiple mechanisms likely underlie the isoflurane effect. In presynaptic recordings, we consistently found that isoflurane inhibited voltage-gated Ca^{2+} channels (VGCCs) of P/Q type, thereby reducing Ca^{2+} influx. Isoflurane also inhibited voltage-gated Na^+ channels and reduced presynaptic AP amplitude, but in contrast to previous proposal (Wu et al., 2004), this effect could not explain the reduction of EPSC amplitude by isoflurane because of the wide safety margin of AP amplitude for transmitter release (Hori and Takahashi, 2009). When vesicle exocytosis was triggered by Ca^{2+} through VGCCs activated by a short depolarizing pulse, isoflurane inhibited exocytosis via inhibiting Ca^{2+} influx. However, when more massive exocytosis was induced by a long presynaptic depolarization, isoflurane directly inhibited exocytic machinery downstream of Ca^{2+} influx. In simultaneous recordings of pre- and postsynaptic APs, isoflurane preferentially impaired the fidelity of transmission at higher frequencies. Likewise, in unit recordings from cerebral cortical neurons in mice *in vivo*, isoflurane preferentially inhibited monosynaptic transmission evoked by a higher frequency stimulation. Thus, isoflurane inhibits excitatory transmission by dual mechanisms, of which its direct inhibitory effect on exocytic machinery significantly contributes to general anesthesia by low-pass filtering excitatory spike transmission.

３．研究の方法

All animal experiments were performed in accordance with guidelines of the Physiological Society of Japan, and institutional regulations of animal experiments at Okinawa Institute of Science and Technology and Nagoya University Research Institute of Environmental Medicine.

Slice preparation and solutions.

Wistar rats (postnatal day [P] 13-15) of either sex were killed by decapitation under isoflurane anesthesia. Transverse brainstem slices (175-200 μm in thickness) containing the medial nucleus of the trapezoid body (MNTB) were cut in ice-cold solution containing (in mM): 200 sucrose, 2.5 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 6 $MgCl_2$, 10 glucose, 3 *myo*-inositol, 2 sodium pyruvate, and 0.5 sodium ascorbate (pH 7.4 when bubbled with 95% O_2 and 5% CO_2, 310–320 mOsm) by using vibroslicer (VT1200S, Leica, Germany). Before recording, slices were incubated for 1 h at 36-37°C in standard artificial cerebrospinal fluid (aCSF) containing (in mM): 125 NaCl, 2.5 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 3 *myo*-inositol, 2 sodium pyruvate, and 0.5 sodium ascorbate (pH 7.4 when bubbled with 95% O_2 and 5% CO_2, 310–320 mOsm), and maintained thereafter at room temperature (24–26 ℃). For recordings aCSF routinely contained bicuculline methiodide (10 μM) and strychnine hydrochloride (0.5 μM) to block inhibitory synaptic transmission, unless otherwise mentioned. Tetrodotoxin (TTX, 1 μM) was add to aCSF for recording miniature EPSCs. For postsynaptic EPSC recordings, pipette solution contained (mM): 110 CsF, 30 CsCl, 10 HEPES, 5 EGTA, 1 $MgCl_2$ and 5 QX-314-Cl (pH adjusted to 7.3–7.4 with CsOH, 300–320 mOsm). For recording postsynaptic APs, pipettes contained (mM): 120 potassium gluconate, 30 KCl, 5 EGTA, 12 disodium phosphocreatine, 1 $MgCl_2$, 3 Mg-ATP, 0.3 Na_2-GTP, 1 L-arginine (pH 7.3–7.4 adjusted with

KOH, 315–320 mOsm). For presynaptic K^+ current recording, TTX (1 μM) was added to aCSF. For recording presynaptic Ca^{2+} currents or membrane capacitance, NaCl in the aCSF was replaced with tetraethylammonium chloride (TEA-Cl, 10 mM), and TTX (1 μM) and 4-aminopyridine (4-AP, 0.5 mM) were added. For presynaptic Na^+ current recording, the extracellular Na^+ concentration was reduced to 5%, being replaced by TEA-Cl (119 mM), to optimize voltage-clamp control. 4-AP (0.5 mM) and $CdCl_2$ (200 μM) were added to aCSF to block K^+ and Ca^{2+} conductance, respectively. In most experiments, presynaptic pipette solutions contained (mM): 105 cesium gluconate, 30 CsCl, 10 HEPES, 0.5 EGTA, 1 $MgCl_2$, 12 disodium phosphocreatine, 3 Mg-ATP, 0.3 Na-GTP (pH 7.3–7.4 adjusted with CsOH, 315–320 mOsm). For presynaptic AP recording, potassium gluconate concentration was reduced to 110 mM from the postsynaptic pipette solution and 10 mM L-glutamate was supplemented. In experiments for testing the effect of presynaptic AP amplitude on EPSCs (Fig. 3E), we added kynurenic acid (1 mM) to aCSF to minimize saturation of postsynaptic AMPA receptors (Koike-Tani et al., 2008).

Slice experiments.

Recordings from slices were made mostly at room temperature (RT, 24-26 ℃), but AP recordings from presynaptic terminals and postsynaptic MNTB neurons were made at near physiological temperature (PT, 31-33ºC). Simultaneous pre-and postsynaptic AP recordings (Fig. 7) were performed at PT to improve synaptic fidelity (Kushmerick et al., 2006; Piriya Ananda Babu et al., 2020). MNTB principal neurons and calyx of Held presynaptic terminals were visually identified using a x40 water immersion objective (Olympus, Japan) attached to an upright microscope (BX51WI, Olympus, Japan). Whole-cell recordings were made from MNTB principal neurons and presynaptic terminals using an EPC-10 patch-clamp amplifier controlled by PatchMaster software (HEKA, Germany) after on-line low-pass filtering at 5 kHz and digitizing at 50 kHz. EPSCs were evoked by stimulation using a bipolar tungsten electrode (FHC, USA) positioned between the midline and the MNTB region. MNTB neurons were voltage-clamped at a holding potential of -70 mV. The postsynaptic pipette was pulled to a resistance of 2–3 MΩ and had a series resistance of 4–10 MΩ, which was compensated by 40-70% for a final value of 3 MΩ. For variance-mean analysis (Clements and Silver, 2000), EPSCs were evoked at 0.05 Hz in the presence of kynurenic acid (1 mM) under aCSF with various extracellular $[Ca^{2+}]$ / $[Mg^{2+}]$ (Fig. 2). Fifteen successive EPSCs were collected for constructing a variance-mean plot. To acquire EPSCs at high release probability (p_r), 4-AP (10 μM) was added to the aCSF (Koike-Tani et al., 2008). Plots of variance as a function of mean were fit by using the simple parabola equation:

$$\sigma^2 = qI - \frac{I^2}{N} \quad ,$$

where σ^2 and I represent the variance and mean amplitude of EPSCs respectively. The parameters q and N denote the mean quantal size and the number of release sites respectively. q can be estimated from the initial slope of the parabola. Nq can be estimated from the ~~large~~ X intercept of the parabola, p_r at 2 mM Ca^{2+} was calculated as I/Nq.

For presynaptic recordings, pipettes were pulled at a resistance of 5-7 MΩ and had a series resistance of 9-25 MΩ, which was compensated by up to 80% for a final value of ~7 MΩ. For measuring presynaptic Na^+ and K^+ currents, membrane potential was stepped up by 10 mV with a 20-ms pulse from -80 to +60 mV. For presynaptic Ca^{2+} current recordings, membrane potential was stepped up by 10 mV with a 20-ms pulse from -80 to +40 mV. The P/4 method was used for correcting leak and capacitance currents. For monitoring presynaptic membrane capacitance (C_m, Fig. 4, 5), pipette tips were coated with dental wax to reduce stray capacitance. A sinusoidal voltage command was applied with a peak-to-peak amplitude of 60 mV at 1 kHz. Samples of C_m were plotted as average values of 50 data points within 50 ms (short time scale) or 500 ms (long time scale). Presynaptic membrane capacitance changes (ΔC_m) were induced by Ca^{2+} currents as described previously (Taschenberger et al., 2002; Sun et al., 2004; Yamashita et al., 2005; Yamashita et al., 2010). The amplitude of exocytic capacitance changes was measured between the baseline and the maximal value of C_m at 450-500 ms after depolarization to avoid contamination by artificial ΔC_m changes (Yamashita et al., 2005). For testing the fidelity of synaptic transmission, simultaneous presynaptic and postsynaptic whole-cell recordings in current-clamp mode were made at near PT.

We applied isoflurane to slices as described previously (Wu et al., 2004). The gas mixture of 95% O_2 and 5% CO_2 was introduced by a flowmeter into a calibrated commercial vaporizer (MK-AT210, Muromachi, Japan) containing isoflurane (100%, 24.5 mM, Fig 1a). The gas mixture at various isoflurane concentrations (0.5%-5%) was then bubbled into the experimental solution in a tightly capped bottle. In most slice experiments, we used isoflurane at 3%, which was 0.72 ± 0.06 mM when examined using gas chromatography (Fig. 1B) and corresponded to twice the minimum alveolar concentration (2 MAC) of isoflurane (Mazze et al., 1985; Pal et al., 2012). For testing

neurotransmission fidelity (Fig. 7), we applied isoflurane at 1.5 % or 3% (1 or 2 MAC). The aqueous concentration of isoflurane, measured with gas chromatography-mass spectrometry (Pegasus 4D-C GCxGC-TOFMS, Saint Joseph, USA), was linearly proportional to the gaseous partial concentration of isoflurane (Fig. 1B). For i*n vivo* experiments, isoflurane (1.4 %) was applied by inhalation.

In vivo unit recordings.

For optogenetic stimulation (Fig. 8), we used double transgenic mice expressing channelrhodposin 2 (ChR2) in layer 5 (L5) pyramidal neurons of cerebral cortex (Rbp4-Cre; LSL-ChR2) that were obtained by crossing Rbp4-Cre (Gensat STOCK Tg(Rbp4-cre)KL100Gsat/Mmucd) mice with Ai32 (Jax #012569). Adult Rbp4-Cre; LSL-ChR2 mice were implanted with a light-weight metal head-holder and a recording chamber under isoflurane anesthesia, as previously described (Yamashita et al., 2013). A small craniotomy was opened over the left whisker primary somatosensory cortex (wS1, 1.65 mm posterior, 3.0 mm lateral from the bregma; Yamashita et al., 2018) and left whisker primary motor cortex (wM1, 1 mm anterior, 1 mm lateral from Bregma; Sreenivasan et al., 2016). In some recordings, the location of the left wS1-C2 barrel column was also identified using intrinsic optical imaging under light isoflurane anesthesia (Ferezou et al., 2007; Yamashita et al., 2013). Extracellular spikes were recorded using a silicon probe (A1x32-Poly2-10mm50s-177, NeuroNexus) with 32 recording sites along a single shank, covering 775 mm of the cortical depth in awake states or under isoflurane (1.4%) anesthesia (Miyazaki et al., 2019). The probe was lowered gradually until the tip was positioned at a depth of ~950 μm under the wS1 pial surface. Neural data were filtered between 0.5 Hz and 7.5 kHz and amplified using a digital head-stage (RHD2132, Intan technologies). The head-stage digitized the data with a sampling frequency of 30 kHz. The digitized signal was transferred to an acquisition board (Open Ephys) and stored on an internal HDD of the host PC for offline analysis. Photo-stimulation was carried out by applying 1-ms pluses of blue LED (19 mW) with an optical fiber (400 μm diameter) placed over the wM1 craniotomy. Two sweeps of 200 photo-stimuli at 0.2 Hz every 20 min were first applied in awake states and subsequently two sweeps of 0.2-Hz stimulation were applied under isoflurane anesthesia. Mice were then recovered by stopping isoflurane inhalation, and, after whisking behavior was observed as the mice awoke, five sweeps of 200 photo-stimuli at 2 Hz every 6 min were applied. Mice were again anesthetized by isoflurane inhalation and another five sweeps of 2-Hz stimulation were applied. Recordings with 0.2-Hz stimuli and 2-Hz stimuli were saved separately.

Spiking activity on each probe was detected and sorted into different clusters using Kilosort, an open source spike sorting software (https://github.com/cortex-lab/KiloSort). After an automated clustering step, clusters were manually sorted and refined. Only well-isolated single units (total 175 units) were included in the dataset. For analysis, we excluded units (32/175 units) that showed AP rates less than 5 Hz on average at 5-25 ms after photo-stimulation in awake states. Units that have reliably evoked APs with a low jitter (~1 ms) were tested for collisions in which we looked for absence of antidromic spikes when preceded by spontaneous spikes. Among these units, 11 units showed collisions between evoked and spontaneous APs (Fig. 8C) indicating putative wS1-to-wM1 projection neurons generating antidromic spikes. These data were excluded from analysis. In total, 143 units (83 units for 0.2 Hz stimulation and 60 units for 2 Hz stimulation) were selected for analysis as trans-synaptically activated units. Averaged spontaneous AP rates were measured for a period of 50 ms before photo-stimulation onset at 0.2 Hz. Evoked AP rates were calculated by subtracting averaged AP rates 0-50 ms before photo-stimulation from those during 5-25 ms after photo-stimulation.

In vivo whole-cell recordings.

Adult Rbp4-Cre; LSL-ChR2 mice were implanted with a light-weight metal head-holder and a recording chamber under isoflurane anesthesia. After recovery, mice were habituated to head restraint (three sessions, one session per day) before recording. At the experimental day, a small craniotomy was opened over the left wS1 under isoflurane anesthesia. Recording pipettes (5-8 MΩ) were advanced into the cortex through the craniotomy with a positive pressure until the pipette resistance increased and then suction was applied to establish a giga-ohm seal followed by the whole-cell configuration using a patch-clamp amplifier (Multiclamp 700B, Molecular devices) (Margrie et al., 2002; Petersen et al., 2003). Pipettes were filled with a solution containing (in mM): 135 potassium gluconate, 4 KCl, 10 HEPES, 10 sodium phosphocreatine, 4 Mg-ATP, 0.3 Na_3GTP (adjusted to pH 7.3 with KOH). Recordings were made at the putative subpial depth of 250–400 μm in a dark environment to avoid ChR2 activation. The membrane potential, which was not corrected for liquid junction potential, was filtered at 8 kHz and digitized at 20 kHz.

Data analysis.

Data from slice experiments and *in vivo* whole-cell recordings were analyzed using IGOR Pro 6 (WaveMatrics), OriginPro 2015 (OriginLab), Sigmaplot 13 (Systat Software), and MS Excel 2016 (Microsoft). All values were expressed as mean ± SEM and 95% confidence intervals on the difference of the means were considered statistically significant in paired sample *t-tests* or one-way repeated measures *ANOVA* with a post-hoc Bonferroni test ($p < 0.05$), Mann-Whitney test ($p < 0.05$) or Kruskal-Wallis test with a post-hoc Dunn's test ($p < 0.05$).

４．研究成果

Dual presynaptic targets of isoflurane

At the calyx of Held in rat brainstem slices, we have systematically addressed targets of isoflurane action. Clinical doses of isoflurane attenuated evoked EPSC amplitude without affecting the mean quantal size, measured from spontaneous mEPSCs or variance-mean analysis, indicating that the site of its action is presynaptic. Isoflurane significantly lowered the release probability (p_r) and decreased the number of functional release sites (N). Presynaptic capacitance measurements revealed dual mechanisms underlying isoflurane action. Within a relatively low range of exocytosis, isoflurane reduces exocytosis via reducing Ca^{2+} influx without altering the Ca^{2+}-exocytosis relationship as reported previously (Baumgart et al., 2015), whereas for greater exocytosis, isoflurane directly blocks exocytic machinery downstream of Ca^{2+} influx. The former and latter mechanism explains a reduction of p_r and N, respectively, particularly since isoflurane had no effect on vesicle recycling. In agreement with inhibitory effect of isoflurane on release machinery, volatile anesthetics can reportedly bind to recombinant syntaxin (Nagele et al., 2005) and their inhibitory effects on neurosecretion can be eliminated by syntaxin mutant over-expressed in secretory cells (Herring et al., 2009).

Isoflurane reduced presynaptic AP amplitude at the calyx of Held as reported previously (Wu et al., 2004). However, low dose TTX-application experiments in simultaneous pre- and postsynaptic recordings indicated that EPSC amplitude is protected from a reduction of presynaptic AP amplitude with a wide safety margin in such a way that EPSCs remain unaffected when AP amplitude is reduced by isoflurane. Such a safety margin is absent in voltage-clamp experiments, where EPSCs are evoked by AP-waveform command pulses (Wu et al., 2004; Hori and Takahashi, 2009). This is likely because of limited space-clamp control of an AP-waveform command pulse. Thus, a reduction of Na^+ influx cannot be a mechanism for a reduction of transmitter release by isoflurane at the calyx of Held. However, this does not rule out the possibility that a reduction of AP amplitude might contribute to the inhibitory effect of isoflurane on transmitter release at other synapses having narrower safety margin or smaller presynaptic APs. Although isoflurane broadly inhibited presynaptic voltage-gated ion channels at the calyx of Held, only the inhibition of VGCCs could fully explained the inhibitory effect of isoflurane on transmitter release evoked by a single AP or a short depolarizing pulse.

Frequency-dependent inhibitory effects of isoflurane on spike transmission in slice and *in vivo*

Excitatory neurotransmission is completed by a generation of postsynaptic AP. Even when transmitter release is reduced, as far as postsynaptic potentials (EPSPs) reach firing threshold, neurotransmission remains intact. In simultaneous pre- and postsynaptic recordings of AP trains, at the calyx of Held at near PT, isoflurane (1-2 Mac) had no effect on the initial or low-frequency transmission, but significantly inhibited high-frequency transmission in a frequency-dependent manner. Thus, a reduction of Ca^{2+} influx alone by isoflurane cannot inhibit spike transmission at this sensory relay synapse. In response to high-frequency inputs, EPSPs undergo STD, primarily due to a reduction in N (von Gersdorff et al., 1997; Schneggenburger et al., 2002). Isoflurane further decreased N by direct inhibition of exocytic machinery, thereby diminishing EPSPs below firing threshold. Therefore, with respect to the physiological excitatory transmission at the calyx of Held, the main target of isoflurane action is exocytic machinery rather than VGCCs. Unlike at the calyx of Held, however, at cortico-cortical synapses *in vivo*, isoflurane attenuated spike transmission evoked at low-frequency (0.2 Hz), suggesting that the inhibition of VGCCs may also operate for the effect of isoflurane. Nevertheless, like at the calyx of Held, inhibitory effect of isoflurane on cortico-cortical spike transmission was much stronger at higher frequency (2 Hz). Thus, both at the calyx of Held and cortico-cortical synapses, exocytic machinery is likely an important target of isoflurane for attenuating high-frequency neurotransmission.

Altogether, the inhibitory nature of isoflurane on excitatory neurotransmission can be characterized as a low-pass filter. Weaker effect of anesthetics on low-frequency transmission seems favorable for the maintenance of life-supporting basal neurotransmission. Low-pass filtering effect of isoflurane is also consistent with large-scale slow-wave synchronization of cortical neurons during anesthesia (Mohajerani et al., 2010; Kuroki et al., 2018). Since high-frequency neuronal activity plays essential roles in the maintenance of consciousness (Hermann et al., 2004; Larchaux et al., 2012), cognition (Sabatini and Regher, 1999; Buzsáki and Draguhn, 2004; Uhlhaas and Singer, 2010) and motor-control (Sugihara et al., 1993), selective inhibition by volatile anesthetics of high-frequency transmission will effectively attenuate such integral neuronal functions, with minimal inhibition of basal neuronal functions.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　1件）

１．著者名 Han-Ying Wang, Kohgaku Eguchi, Takayuki Yamashita and Tomoyuki Takahashi	４．巻 JN-RM-2946-19
２．論文標題 Frequency-dependent block of excitatory neurotransmission by isoflurane via dual presynaptic mechanisms	５．発行年 2020年
３．雑誌名 The Journal of Neuroscience	６．最初と最後の頁 JN-RM-2946-19
掲載論文のDOI（デジタルオブジェクト識別子） 10.1523/JNEUROSCI.2946-19.2020	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　2件）

１．発表者名 Han-Ying Wang, Kohgaku Eguchi, Tomoyuki Takahashi
２．発表標題 Presynaptic Inhibitory Mechanism of the Volatile Anesthetics Isoflurane.
３．学会等名 The 41st Annual Meeting of the Japan Neuroscience Society（国際学会）
４．発表年 2018年

１．発表者名 Han-Ying Wang, Kohgaku Eguchi, Tomoyuki Takahashi
２．発表標題 Isoflurane preferentially inhibits high-frequency excitatory neurotransmission via directly inhibiting vesicle fusion mechanism.
３．学会等名 Society for Neuroscience - Neuroscience 2018（国際学会）
４．発表年 2018年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考