Inhibitory synaptic transmission tuned by Ca\(^{2+}\) and glutamate through the control of GABA\(_{A}\)R lateral diffusion dynamics

Hiroko Bannai\(^{1,2,3,4}\) | Fumihiro Niwa\(^{4,5}\) | Shigeo Sakuragi\(^{1,6}\) | Katsuhiko Mikoshiba\(^{4,7,8}\)

Abstract
The GABAergic synapses, a primary inhibitory synapse in the mammalian brain, is important for the normal development of brain circuits, and for the regulation of the excitation-inhibition balance critical for brain function from the developmental stage throughout life. However, the molecular mechanism underlying the formation, maintenance, and modulation of GABAergic synapses is less understood compared to that of excitatory synapses. Quantum dot-single particle tracking (QD-SPT), a super-resolution imaging technique that enables the analysis of membrane molecule dynamics at single-molecule resolution, is a powerful tool to analyze the behavior of proteins and lipids on the plasma membrane. In this review, we summarize the recent application of QD-SPT in understanding of GABAergic synaptic transmission. Here we introduce QD-SPT experiments that provide further insights into the molecular mechanism supporting GABAergic synapses. QD-SPT studies revealed that glutamate and Ca\(^{2+}\) signaling is involved in (a) the maintenance of GABAergic synapses, (b) GABAergic long-term depression, and GABAergic long-term potentiation, by specifically activating signaling pathways unique to each phenomenon. We also introduce a novel Ca\(^{2+}\) imaging technique to describe the diversity of Ca\(^{2+}\) signals that may activate the downstream signaling pathways that induce specific biological output.
1 | INTRODUCTION

In the central nervous system (CNS), the chemical synapse is a highly specialized structure for neuronal transmission. There are two types of synapses, i.e., the excitatory synapse that leads to depolarization of neurons, and the inhibitory synapse that balances neuronal excitation. The balance between the excitatory and inhibitory synapses is important, as it determines neuronal output and ultimately brain activity. In CNS, GABA\(_A\) receptor (GABA\(_A\)R), a pentameric ion channel, is essential for inhibitory GABAergic synaptic inputs and plays a key role in maintaining this excitation-inhibition balance. GABA\(_A\)Rs are required for the onset of the critical period (Fagiolini et al., 2004) that is a fundamental process of visual development in newborn mammals, and “Synapse pruning” for normal development of the cerebral neural circuit (Nakayama et al., 2012). Furthermore, abnormalities in GABAergic synaptic transmission are known to cause various mental/neurological diseases such as epilepsy, anxiety disorder, Huntington’s disease, Angelman syndrome, Fragile X syndrome, schizophrenia, alcoholism, and postpartum depression etc. (Jacob, Moss, & Jurd, 2008; Maguire & Mody, 2008) For this reason, elucidating the regulatory mechanism of GABAergic synaptic transmission is essential, not only for understanding the function of the brain, but also for determining the pathogenesis and diagnosing and developing treatments for mental/neurological diseases.

In this review, we discuss novel regulatory mechanisms of GABAergic synaptic transmission revealed using cutting-edge bioimaging techniques including single-molecule imaging and Ca\(^{2+}\) imaging at subcellular resolution. We introduce the ingenious regulation of GABAergic transmission by Ca\(^{2+}\) and glutamate, discovered for the first time using “Quantum dot-single particle tracking (QD-SPT) (Bannai, Levi, Schweizer, Dahan, & Triller, 2006)”, i.e., a super-resolution imaging technique to analyze the dynamics of membrane molecules.

2 | EXCHANGE OF SYNAPTIC GABA\(_A\)R BY LATERAL DIFFUSION IS A NOVEL FACTOR TO DETERMINE GABAERGIC SYNAPTIC TRANSMISSION

GABA\(_A\)R is a Cl\(^-\) ion channel that plays an important role in rapid inhibitory synaptic transmission in the mammalian CNS. A typical GABA\(_A\)R exists as a pentamer consisting of two \(\alpha\)-subunits, two \(\beta\)-subunits, as well as a \(\gamma\) or \(\delta\) subunit. There are six isoforms of the \(\alpha\)-subunit, three of \(\beta\), three of \(\gamma\) and one of \(\delta\), and when combined with the minor subunits \(\epsilon\), \(\theta\), and \(\pi\), 19 types of GABA\(_A\)R subunits have been identified.

In general, neurotransmitter receptors are accumulated at the postsynaptic membrane to efficiently receive the neurotransmitter released from the presynaptic terminal. Synaptic accumulation of GABA\(_A\)Rs can be visualized by immunocytochemistry as a cluster at the postsynaptic membrane (Figure 1). However, immunoelectron microscopy has shown that approximately 60% of all GABA\(_A\)R are present outside the synapse (Kasugai et al., 2010), suggesting that a considerable number of GABA\(_A\)Rs are located outside the synapse, without forming clusters. The \(\alpha1\)- and \(\gamma2\)-subunit containing GABA\(_A\)Rs localize primarily within the synapse, whereas the GABA\(_A\)Rs which contain the \(\alpha4\), \(\alpha5\), \(\alpha6\), and \(\delta\)-subunits primarily localize outside the synapse (Farrant & Nusser, 2005). The number of GABA\(_A\)R present in the synapse is the main factor that determines GABAergic synaptic transmission efficiency. The number of synaptic GABA\(_A\)R depends on the total number of GABA\(_A\)R present on the cell surface. The number of GABA\(_A\)R on the cell surface is a result of the dynamic equilibrium among GABA\(_A\)R synthesis, exocytosis that presents GABA\(_A\)R onto the cell membrane, endocytosis that takes GABA\(_A\)R into the cell, and degradation of GABA\(_A\)R in the cell (Figure 2). Furthermore, because the endocytosis and exocytosis of GABA\(_A\)R occur outside the synapse (Bogdanov et al., 2006), the movement of GABA\(_A\)R to the post-synaptic membrane by lateral diffusion has received much attention in recent years as another
important factor that determines the number of GABA$_A$R in the synapse.

The presence of a considerable proportion of GABA$_A$R outside the synapse has important implications for neuron function. One important role of extrasynaptic GABA$_A$Rs is long-term inhibition of neural activity, called tonic inhibition. GABA spilled over from the inhibitory synaptic cleft binds to extrasynaptic GABA$_A$R and induces sustained neural inhibition. Even GABA$_A$R containing $\alpha_1$, ...
α2, and β3-subunit, which is considered as "synaptic" GABA<sub>A</sub>R, carries out this tonic inhibition function when outside the synapse (Kasugai et al., 2010). The other role of extrasynaptic GABA<sub>A</sub>Rs could be replaced with the inactivated synaptic GABA<sub>A</sub>R to constantly maintain the GABAergic synapse in its functional state. It has also been shown that "inactivated" GABA<sub>A</sub>Rs translocate to another neighboring synapse by lateral diffusion and inhibits transmission in that synapse (de Luca et al., 2017). In light of these discoveries, understanding the mechanism for controlling the diffusion movement of GABA<sub>A</sub>R on the cell membrane has become an important challenge.

3 | QUANTUM DOT-SINGLE PARTICLE TRACKING (QD-SPT), AN IMAGING TECHNIQUE TO VISUALIZE THE BEHAVIOR OF MEMBRANE MOLECULES AT SINGLE-MOLECULE RESOLUTION

Lateral diffusion dynamics of GABA<sub>A</sub>R has been analyzed using quantum dot-single particle tracking (QD-SPT) techniques. In QD-SPT, quantum dots (QDs), i.e., semiconductor fluorescent nanocrystals, are used as a marker to track a molecule of interest (Bannai et al., 2006). QDs are targeted to membrane molecules through specific antibodies to the extracellular domain, biotin, and streptavidin, and observed under a fluorescent microscope (Figure 3). There are several advantages to use QD-SPT for the study of GABA<sub>A</sub>R dynamics in the neuron. Firstly, the QD-SPT technique allows the tracking of endogenous proteins, without overexpression of exogenous recombinant proteins tagged with reporter (e.g., fluorescent proteins). Secondly, the fluorescence signals from a QD is brighter and more resistant to photo-bleach; therefore, QD-SPT enables to track the target molecule for a longer period with better signal to noise ratio, compared with chemical dyes and fluorescent proteins. Thirdly, QD has a broad absorption spectrum and a narrow emission spectrum (Michalet et al., 2005), facilitating multicolor imaging with other fluorescent markers (such as fluorescent Ca<sup>2+</sup> indicators, fluorescent dyes, and marker proteins tagged with fluorescent proteins) (Bannai, Inoue, Hirose, Niwa, & Mikoshiba, 2020; Bannai et al., 2006). Finally, relatively small hydrodynamic radius of QDs (4–7 nm) (Swift & Cramb, 2008) enables QD-antibody to enter into the synaptic cleft (Dahan et al., 2003; Heine et al., 2008). However, QD-SPT has a few limitations that cannot be ignored, for instance, the hydrodynamic volume of QD-antibody complex affects the diffusion properties of membrane molecules.

Making full use of advantages of QD-SPT, fundamental questions in the field of neuroscience, such as those regarding synaptic structures and the molecular mechanisms for learning and memory, have been addressed by QD-SPT (Choquet, 2018; Petrini & Barberis, 2014). QD-SPT has also highlighted abnormal dynamics of membrane proteins in the model cells for epilepsy and Alzheimer's disease. In the next section, we will review the regulatory mechanisms for GABAergic synapses revealed by QD-SPT, together with other cell biological studies.

4 | SYNAPTIC SCAFFOLD PROTEIN THAT CONTROLS THE LATERAL DIFFUSION MOVEMENT OF GABA<sub>A</sub>R

One of the most important elements that affects the lateral diffusion of the neurotransmitter receptor is the synaptic scaffold protein that clusters in the cytoplasm of the post-synaptic membrane (Choquet & Triller, 2013). In the GABAergic synapse, it has been discovered that the GABA<sub>A</sub>R α1-subunit interacts with the inhibitory synaptic scaffold protein gephyrin to inhibit the lateral diffusion movement of GABA<sub>A</sub>R in the synapse (Mukherjee et al., 2011). Accumulation of GABA<sub>A</sub>Rs at the postsynaptic membrane is considered to result from the slow diffusion of GABA<sub>A</sub>Rs in the synapse due to interaction with gephyrin. Inhibition of the lateral diffusion of GABA<sub>A</sub>R due to long-term potentiation (LTP) in the GABAergic synapse is reported to be dependent on the increase in gephyrin levels in the synapse (Petrini et al., 2014). These results suggest that gephyrin plays an important role in the regulation of GABAergic synaptic transmission by controlling the lateral diffusion of GABA<sub>A</sub>R.

Interestingly, the change of synaptic GABA<sub>A</sub> number induced by neuronal stimulation is known to occur faster than the change of synaptic gephyrin number (Niwa, Patrizio, Triller, & Specht, 2012; Petrini et al., 2014). These results indicate that lateral diffusion of GABA<sub>A</sub>R also affects the accumulation of its scaffold gephyrin, whereas gephyrin is necessary for the accumulation of GABA<sub>A</sub>R in...
the synapse. Simultaneously, these findings imply the existence of other unknown molecular mechanisms underlying the lateral diffusion of GABA$_A$R in the synapse, other than gephyrin. Indeed, radixin has been identified as another factor that controls the lateral diffusion of GABA$_A$R outside the synapse in a gephyrin-independent manner. The actin filament-bound protein radixin controls the lateral movement of α5 subunit-containing GABA$_A$R outside of the synapse (Hausrat et al., 2015). However, the factor that controls GABA$_A$R lateral diffusion has not been identified yet. Unlike excitatory synaptic scaffold proteins that are easy to isolate as "synaptosomes" GABAergic post-synaptic density proteins are difficult to isolate biochemically. For this reason, the discovery of a new scaffolding protein that accumulates GABA$_A$R in the synapse has been a difficult task for many years. Recently, proteomic studies have revealed over one hundred GABAergic post-synaptic proteins (Nakamura et al., 2016; Uezu et al., 2016). As an example, GIT1 and βPIX were reported to stabilize GABA$_A$R with stabilization of actin filament at synapse (Smith et al., 2014). Furthermore, auxiliary subunits of neurotransmitter receptors (Yan & Tomita, 2012) also contribute to the regulation of GABA$_A$R clustering and functions. GABA$_A$R regulatory Lhfl (GARLH) family protein that was discovered as an auxiliary subunit of the γ2-containing GABA$_A$R, was shown to play the role of accumulating GABA$_A$R to the GABA$_A$R synapse by neuronin2 binding to GABA$_A$R (Davenport et al., 2017; Yamasaki, Hoyos-Ramirez, Martenson, Morimoto-Tomita, & Tomita, 2017). Another auxiliary GABA$_A$R subunit Shisa7 has been also shown to control receptor abundance at the synapse, in addition to the regulation channel deactivation kinetics (Han et al., 2019). Whether these newly found proteins are also involved in modulating the lateral diffusion of GABA$_A$R needs to be clarified in the future.

5 | CONTROL OF GABA$_A$R LATERAL DIFFUSION THROUGH Ca$_{2+}$ SIGNALING – AN INGENIOUS REGULATORY MECHANISM THAT MAKES FULL USE OF A LIMITED NUMBER OF SIGNALING MOLECULES

In addition to synaptic scaffolding proteins, the Ca$^{2+}$ signal, i.e. "the increase or decrease in intracellular Ca$^{2+}$ ion concentration", is another important factor that can control GABA$_A$R lateral diffusion. Ca$^{2+}$ signaling can be classified as "Ca$^{2+}$ influx" where Ca$^{2+}$ ions enter the cell from the extracellular space, and "Ca$^{2+}$ release" from the intracellular Ca$^{2+}$ storage endoplasmic reticulum (ER) (Figure 4). Intriguingly, mice lacking the ER Ca$^{2+}$-releasing channel "IP$_3$ receptor type 1 (IP$_3$R1)" exhibit epilepsy symptoms (Matsumoto et al., 1996), thereby suggesting the possibility that these animals may have abnormal inhibitory neurotransmission. Therefore, we examined whether Ca$^{2+}$ release from the IP$_3$R1 controls the lateral diffusion of GABA$_A$R, using QD-SPT (Bannai et al., 2006). Hippocampal neurons from IP$_3$R1-deficient mice showed increased lateral diffusion of GABA$_A$R in the synapse, compared to those in wild type mice. Through immunofluorescence staining, we found that the number of GABA$_A$R accumulating in the synapse were decreased in IP$_3$R1-deficient neurons. Furthermore, inhibition of the Ca$^{2+}$-release pathway by IP$_3$R antagonist in wild type hippocampal neurons for 1 hr using an inhibitor showed similar GABA$_A$R destabilization as that in IP$_3$R1-deficient neurons (Bannai et al., 2015) (Figure 5a). Furthermore, we showed that GABA$_A$R lateral diffusion is suppressed by Ca$^{2+}$-releasing signal downstream of the metabotropic glutamate receptor (mGluR), followed by the activation of protein kinase C (PKC) (Figure 5b). This result indicates that the ER-mediated Ca$^{2+}$-releasing signal pathway suppresses lateral diffusion of synaptic GABA$_A$R, and finally causes accumulation of GABA$_A$R inside the synapse. In other words, in addition to scaffolding proteins, Ca$^{2+}$ release from the ER, which starts from constitutive activation of mGluRs and causes activation of PKC, is necessary for continuous GABA$_A$R accumulation at the postsynaptic terminal.

On the contrary, Ca$^{2+}$ influx also controls the lateral diffusion of GABA$_A$R, and is involved in GABAergic synapse plasticity, i.e. the ability of neurons to change GABAergic synaptic transmission efficacy in response to neuronal input. In the rodent hippocampus, GABAergic synaptic transmission efficacy is weakened by the application of high-frequency stimulation (Wang & Stelzer, 1996). This phenomenon is a form of synaptic plasticity called "long-term depression (LTD)" in GABAergic synapse, and is known to be involved in learning, memory, and pathogenesis of epilepsy. GABAergic LTD requires Ca$^{2+}$ influx from the ionotropic glutamate receptor "NMDA receptors", activation of Ca$^{2+}$/calmodulin-dependent protein phosphatase "calcineurin" downstream of the Ca$^{2+}$ influx, and calcineurin-induced dephosphorylation of Serine-327 in the γ2 subunit of GABA$_A$R (Lu, Mansuy, Kandel, & Roder, 2000; Wang et al., 2003). However, it is unclear how calcineurin controls GABA$_A$R accumulation at the postsynaptic terminal. Through investigation of GABA$_A$R using QD-SPT, we found that Ca$^{2+}$ influx from NMDA receptors, which is equivalent to LTD-inducing

![FIGURE 4](image-url)  
Two types of intracellular Ca$^{2+}$ signaling pathways. “Ca$^{2+}$ release” from the intracellular Ca$^{2+}$ store in the endoplasmic reticulum (ER) and “Ca$^{2+}$ influx” from the extracellular space
stimulation, increases the lateral diffusion of GABA_{A}R through activation of calcineurin (Bannai et al., 2009) (Figure 6a). It has been also shown that the elevation of GABA_{A}R lateral diffusion caused by Ca^{2+} influx was due to the dephosphorylation of Serine-327 in the \gamma_2-subunit of GABA_{A}R by calcineurin, similar to that in GABAergic LTD (Fricke et al., 2019; Muir et al., 2010).
Previously, synaptic plasticity was considered to have been induced by modification of the number of surface GABA<sub>R</sub>. However, we also showed that the expression level and the total number of GABA<sub>R</sub> on the cell surface did not change before and after LTD-inducing stimulation (Bannai et al., 2009). This discovery indicates for the first time that "changes in lateral diffusion of the neurotransmitter receptor" is a new molecular mechanism underlying synaptic plasticity.

Surprisingly, "GABAergic long-term potentiation (LTP)" is another form of GABAergic synaptic plasticity, is also caused by the control of lateral diffusion of GABA<sub>R</sub> depending on Ca<sup>2+</sup> influx from the NMDA receptor (Petrini et al., 2014). When neurons receive LTP-inducing stimulus, the calcium calmodulin-dependent kinase II (CaMKII) that is activated by Ca<sup>2+</sup> influx from NMDA receptors translocate to the GABAergic synapse, and promotes exocytosis of GABA<sub>R</sub> (Marsden, Beattie, Friedenthal, & Carroll, 2007; Marsden, Shemes, Bayer, & Carroll, 2010). Simultaneously, CaMKII phosphorylates Serine-383 in the β3-subunit of GABA<sub>R</sub>. Phosphorylation of the β3-subunit of GABA<sub>R</sub> causes an increase in the accumulation of the scaffold protein gephyrin at the synapse. QD-SPT revealed that interaction between gephyrin and GABA<sub>R</sub> selectively suppresses the lateral diffusion of synaptic GABA<sub>R</sub> (Petrini et al., 2014). The increase in the level of GABA<sub>R</sub> presented on cell surface due to CaMKII-dependent exocytosis and the increase in the number of synaptic GABA<sub>R</sub> due to suppression of lateral diffusion in the synapse is therefore the molecular mechanism of GABAergic synaptic long-term potentiation (Figure 6b). It is notable that this NMDA receptor and CaMKII-dependent signaling pathway is also involved in the input-specific potentiation of GABAergic inhibition from somatostatin-expressing interneurons in cerebral cortex (Chiu et al., 2018).

As above, analysis of GABA<sub>R</sub> lateral diffusion through QD-SPT showed that "Ca<sup>2+</sup> signal" and "glutamate" are common signaling molecules involved in all of: (a) GABA<sub>R</sub> accumulation in the synapse under the normal state (Figure 4), (b) GABAergic synaptic long-term depression (Figure 6a) and (c) long-term potentiation (Figure 6b). Interestingly, glutamate, an excitatory neurotransmitter, controls the inhibitory synaptic transmission efficiency. How can three different types of target molecules, i.e., PKC, Calcineurin, and CaMKII, be selectively activated using the same signaling molecules such as Ca<sup>2+</sup> and glutamate? One possible mechanism that produces the specificity of the downstream signal molecule is thought to be the extracellular glutamate concentration. Under normal conditions, low concentration glutamate released from the surrounding excitatory synapses and glial cells is expected to preferentially activate mGluRs, which have relatively high binding affinity with glutamate, resulting in dominance of the IP<sub>3</sub>R and PKC signaling pathways. On the contrary, synaptic plasticity that involves release of large amounts of glutamate from the excitatory synapse elevates the extracellular concentration of glutamate significantly and activates the NMDA receptors. Despite Ca<sup>2+</sup> influx from the same NMDA receptor, the LTD stimulation reportedly migrates calcineurin to the inhibitory synapse, whereas LTP stimulation specifically migrates the CaMKII to the inhibitory synapse (Marsden et al., 2010). A model has been proposed where the difference in the level of Ca<sup>2+</sup> entering from the NMDA receptors induces different types of synaptic plasticity (Petrini & Barberis, 2014). It should be noted that Ca<sup>2+</sup> can activate not only phosphatase/kinase but also other proteins. For example, calpain is directly activated by Ca<sup>2+</sup> and control gephyrin clustering (Tyagarajan et al., 2011). In addition, cAMP which produced with adenylyl cyclase whose activity is controlled by Ca<sup>2+</sup> also control the diffusion of inhibitory neurotransmitters (Niwa et al., 2019). Therefore, localization of Ca<sup>2+</sup> signal itself is another important factor to be taken into consideration. Indeed, recent studies have discussed a possible relationship between compartmentalization of glutamate-induced Ca<sup>2+</sup> signals and the specification of synapses that causes synaptic plasticity (Chiu et al., 2018). With all the combinations of these factors, i.e. Ca<sup>2+</sup> signals and diverse enzymes, spatially and temporally complex regulation of GABAergic synapse via Ca<sup>2+</sup> is processed. It will be a challenge to elucidate the detailed mechanism for GABA<sub>R</sub> dynamics by local stimulation and compartmentalization of Ca<sup>2+</sup> signals.

### 6 | DECODING OF Ca<sup>2+</sup> SIGNALS

Analysis of GABA<sub>R</sub> dynamics highlighted amazing aspects of neurons wherein the same signaling molecule, Ca<sup>2+</sup>, can have a completely different effect on synaptic regulation depending on where it comes from. In neurons, Ca<sup>2+</sup> release and Ca<sup>2+</sup> influx also work in opposite directions to determine the direction of extension for the growth cone of axons, causing attraction and repulsion, respectively (Tojima, Hines, Henley, & Kamiguchi, 2011). Therefore, elucidating the source of the Ca<sup>2+</sup> signal and analyzing the precise time and space patterns of the Ca<sup>2+</sup> signal is an important challenge in the field of neuroscience.

To achieve this objective, we recently created a genetically encoded Ca<sup>2+</sup> indicator that preferentially reports Ca<sup>2+</sup> release from the ER (Niwa et al., 2016). By targeting GCaMP6f to the outer membrane of the ER, we succeeded in reporting the moment of Ca<sup>2+</sup> release from the ER at a higher spatiotemporal resolution than the conventional cytosolic GCaMP6f. This outer ER-targeted-GCaMP6f (OER-GCaMP6f) was available for in vivo Ca<sup>2+</sup> imaging of C. elegans. It was also found that the plasma membrane-targeted genetically encoded Ca<sup>2+</sup> indicator Lck-GCaMP6f that detects Ca<sup>2+</sup> influx with good sensitivity (Shigetomi, Kracun, Sofroniew, & Khakh, 2010), shows a different pattern of Ca<sup>2+</sup> signal from that of OER-GCaMP6f. This result suggests that a Ca<sup>2+</sup> signal can be diverse within a single cell. Furthermore, the aforementioned results indicate that the combination of the "ER-targeting sensor" and "cell membrane-targeting sensor" makes it possible to estimate the source of the Ca<sup>2+</sup> signal (Bannai, 2018). The Ca<sup>2+</sup> signal is a secondary messenger that induces diverse biological outputs in the nervous system as well as in various cells. Ca<sup>2+</sup> imaging which elucidates the source of Ca<sup>2+</sup> signal is expected to contribute to decode Ca<sup>2+</sup> signals in various cells in the future.
7 | CONCLUSION

In this review, we have demonstrated that “GABA\textsubscript{A}R diffusion dynamics” is a new element that can control inhibitory neurotransmission. In recent years, the relationship between the abnormal diffusion dynamics of plasma membrane molecules including neurotransmitter receptors in Alzheimer’s disease and other neurodegenerative diseases has been demonstrated (Bannai, 2018; Shrivastava, Aperia, Melki, & Triller, 2017). Elucidating the molecular mechanism that controls the lateral diffusion of membrane molecules may provide a new therapeutic strategy for cranial nerve diseases.

ACKNOWLEDGEMENTS

This paper was written through subsidies provided by PRESTO-JST (JPMJPR15F8); MEXT (JP17H05710, JP18H05414), JSPS (JP16K07316) and the Takeda Science Foundation.

ORCID

Hiroko Bannai https://orcid.org/0000-0002-0951-488X
Fumihiro Niwa https://orcid.org/0000-0002-0206-1902
Shigeo Sakuragi https://orcid.org/0000-0003-0214-3681
Katsuhiko Mikoshiba https://orcid.org/0000-0002-3487-6970

REFERENCES

Al Awabdh, S., Gupta-Agarwal, S., Sheehan, D. F., Muir, J., Norkett, R., Twelvetrees, A. E., ... Kittler, J. T. (2016). Neuronal activity mediated regulation of glutamate transporter GLUT-1 surface diffusion in rat astrocytes in dissociated and slice cultures. Glia, 64, 1252–1264.

Bannai, H. (2018). Molecular membrane dynamics: Insights into synaptic function and neuropathological disease. Neuroscience Research, 129, 47–56.

Bannai, H., Inoue, T., Hirose, M., Niwa, F., & Mikoshiba, K. (2020). Synaptic Function and Neuropathological Disease Revealed by Quantum Dot-Single-Particle Tracking. In N. Yamamoto, & Y. Okada (Eds.), Single molecule microscopy in neurobiology, neuromethods (Vol. 154, pp.131-155). New York, NY: Springer Nature.

Bannai, H., Levi, S., Schweizer, C., Dahan, M., & Triller, A. (2006). Imaging the lateral diffusion of membrane molecules with quantum dots. Nature Protocols, 1, 2628–2634.

Bannai, H., Levi, S., Schweizer, C., Inoue, T., Launey, T., Racine, V., ... Triller, A. (2009). Activity-dependent tuning of inhibitory neurotransmission based on GABA\textsubscript{A}R diffusion dynamics. Neuron, 62, 670–682.

Bannai, H., Niwa, F., Sherwood, M. W., Shrivastava, A. N., Arizono, M., Miyamoto, A., ... Mikoshiba, K. (2015). Bidirectional control of synaptic GABA\textsubscript{A}R clustering by glutamate and calcium. Cell Reports, 13, 2768–2780.

Biermann, B., Sokoll, S., Klueva, J., Missler, M., Wiegent, J. S., Sibarita, J. B., & Heine, M. (2014). Imaging of molecular surface dynamics in brain slices using single-particle tracking. Nature Communications, 5, 3024.

Bogdanov, Y., Michels, G., Armstrong-Gold, C., Haydon, P. G., Lindstrom, J., Pangalos, M., & Moss, S. J. (2006). Synaptic GABA\textsubscript{A} receptors are directly recruited from their extrasynaptic counterparts. EMBO Journal, 25, 4381–4389.

Chiu, C. Q., Martenson, J. S., Yamazaki, M., Natsume, R., Sakimura, K., Tomita, S., ... Higley, M. J. (2018). Input-Specific NMDAR-dependent potentiation of dendritic GABAergic inhibition. Neuron, 97, 368–377, e3.

Choquet, D. (2018). Linking nanoscale dynamics of AMPA receptor organization to plasticity of excitatory synapses and learning. Journal of Neuroscience, 38, 9318–9329.

Choquet, D., & Triller, A. (2013). The dynamic synapse. Neuron, 80, 691–703.

Dahan, M., Lévi, S., Luccardini, C., Rostaing, P., Riveau, B., & Triller, A. (2003). Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking. Science, 302, 442–445.

Davenport, E. C., Pendolino, V., Kontou, G., Mcgee, T. P., Sheehan, D. F., Lopez-Domenegh, C., ... Kittle, T. J. (2017). An essential role for the tetrasmus LHFLPL4 in the cell-type-specific targeting and clustering of synaptic GABA\textsubscript{A} receptors. Cell Reports, 21, 70–83.

De Luca, E., Rivasenga, T., Petrini, E. M., Polenghi, A., Nieus, T., Guazzi, S., & Barberis, A. (2017). Inter-synaptic lateral diffusion of GABA\textsubscript{A} receptors shapes inhibitory synaptic currents. Neuron, 95, 63–69, e5.

Fagioliini, M., Fritschy, J. M., Low, K., Mohler, H., Rudolph, U., & Hensch, T. K. (2004). Specific GABA(A) circuits for visual cortical plasticity. Science, 303, 1681–1683.

Farrant, M., & Nusser, Z. (2005). Variations on an inhibitory theme: Phasic and tonic activation of GABA(A) receptors. Nature Reviews Neuroscience, 6, 215–229.

Fricke, S., Metzendorf, K., Ohm, M., Haak, S., Heine, M., Korte, M., & Zagrebelsky, M. (2019). Fast regulation of GABA\textsubscript{A}R diffusion dynamics by Nogo-A signaling. Cell Reports, 29, 671–684, e6.

Groc, L., Heine, M., Cognet, L., Brickley, K., Stephenson, F. A., Lounis, B., & Choquet, D. (2004). Differential activity-dependent regulation of the lateral mobilities of AMPA and NMDA receptors. Nature Neuroscience, 7, 695–696.

Groc, L., Lafourcade, M., Heine, M., Renner, M., Racine, V., Sibarita, J. B., ... Cognet, L. (2007). Surface trafficking of neurotransmitter receptor: Comparison between single-molecule/quantum dot strategies. Journal of Neuroscience, 27, 12433–12437. https://doi.org/10.1523/JNEUROSCI.3349-07.2007

Han, W., Li, J., Pelkey, K. A., Pandey, S., Chen, X., Wang, Y. X., ... Lu, W. (2019). Shisa7 is a GABA\textsubscript{A} receptor auxiliary subunit controlling benzodiazepine actions. Science, 366, 246–250.

Hausrat, T. J., Muhia, M., Gerrow, K., Hirdes, W., Tsukita, S., ... Kneussel, M. (2015). Radixin regulates synaptic GABA\textsubscript{A} receptor density and is essential for reversal learning and short-term memory. Nature Communications, 6, 6872.

Heine, M., Groc, L., Frischknecht, R., Beique, J. C., Lounis, B., Rumbaugh, G., ... Choquet, D. (2008). Surface mobility of postsynaptic AMPARs tunes synaptic transmission. Science, 320, 201-205.

Jacob, T. C., Moss, S. J., & Jurd, R. (2008). GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. Nature Reviews Neuroscience, 9, 331–343.

Kasuga, Y., Swiny, J. D., Roberts, J. D. B., Dalezios, Y., Fukazawa, Y., Sieghart, W., ... Somogyi, P. (2010). Quantitative localisation of synaptic and extrasynaptic GABA(A) receptor subunits on hippocampal pyramidal cells by freeze-fracture replica immunolabelling. European Journal of Neuroscience, 32, 1868–1888.

Leri, M., Bemporad, F., Oropeza-Nunez, R., Canale, C., Calamai, M., Nosi, D., ... Bucciantini, M. (2016). Molecular insights into cell toxicity of a novel familial amyloidogenic variant of beta2-microglobulin. Journal of Cellular and Molecular Medicine, 20, 1443–1456.

Liu, S. L., Wang, Z. G., Xie, H. Y., Liu, A. A., Lamb, D. C., & Pang, D. W. (2020). Single-virus tracking: From imaging methodologies to virological applications. Chemical Reviews, 120, 1936–1979.

Liu, S. L., Zhang, Z. L., Sun, E. Z., Peng, J., Xie, M., Tian, Z. Q., ... Pang, D. W. (2011). Visualizing the endocytic and exocytic processes. Biotechnology, 32, 7616–7624.
Shigetomi, E., Kracun, S., Sofroniew, M. V., & Khakh, B. S. (2010). A genetically targeted optical sensor to monitor calcium signals in astrocyte processes. *Nature Neuroscience*, 13, 759–766.

Shrivastava, A. N., Aperia, A., Meilke, R., & Triller, A. (2017). Physiopathologic mechanisms involved in neurodegeneration: misfolded protein-plasma membrane interactions. *Neuron*, 95, 33–50. https://doi.org/10.1016/j.neuron.2017.05.026

Smith, K. R., Davenport, E. C., Wei, J., Li, X., Pathania, M., Vaccaro, V., ... Kittler, J. T. (2014). GIT1 and betaPIX are essential for GABA(A) receptor synaptic stability and inhibitory neurotransmission. *Cell Reports*, 9, 298–310.

Swift, J. L., & Cram, D. T. (2008). Nanoparticles as fluorescent labels: Is size all that matters? *Biophysical Journal*, 95, 865–876.

Tajima, T., Hines, J. H., Henley, J. R., & Kamiguchi, H. (2011). Second messengers and membrane trafficking direct and organize growth cone steering. *Nature Reviews Neuroscience*, 12, 191–203.

Tyagarajan, S. K., Ghosh, H., Yevenes, G. E., Nikonenko, I., Ebeling, C., Schwerdel, C., ... Fritschy, J. M. (2011). Regulation of GABAergic synaptic formation and plasticity by GSK3beta-dependent phosphorylation of gephyrin. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 379–384.

Uezu, A., Kanak, D. J., Bradshaw, T. W., Soderblom, E. J., Catavero, C. M., Burette, A. C., ... Soderling, S. H. (2016). Identification of an elaborate complex mediating postsynaptic activation. *Science*, 353, 1123–1129.

Varela, J. A., Dupuis, J. P., Etchepare, L., Espana, A., Cognet, L., & Groc, L. (2016). Targeting neurotransmitter receptors with nanoparticles in vivo allows single-molecule tracking in acute brain slices. *Nature Communications*, 7, 10947.

Wang, J., Liu, S., Haditsch, U., Tu, W., Cochrane, K., Ahmadian, G., ... Lu, Y. M. (2003). Interaction of calcineurin and type-A GABA receptor gamma 2 subunits produces long-term depression at CA1 inhibitory synapses. *Journal of Neuroscience*, 23, 826–836.

Wang, J. H., & Stelzer, A. (1996). Shared calcium signaling pathways in the induction of long-term potentiation and synaptic disinhibition in CA1 pyramidal cell dendrites. *Journal of Neurophysiology*, 75, 1687–1702. https://doi.org/10.1152/jn.1996.75.4.1687

Wen, X., Van Hook, M. J., Grassmeyer, J. J., Wiesman, A. I., Rich, G. M., Cork, K. M., & Thoreson, W. B. (2018). Endocytosis sustains release at photoreceptor ribbon synapses by restoring fusion competence. *Journal of General Physiology*, 150, 591–611.

Yamasaki, T., Hoyos-Ramirez, E., Martenson, J. S., Morimoto-Tomita, M., & Tomita, S. (2017). GARLH family proteins stabilize GABA_A receptors at synapses. *Neuron*, 93, 1138–1152, e6.

Yan, D., & Tomita, S. (2012). Defined criteria for auxiliary subunits of glutamate receptors. *Journal of Physiology*, 590, 21–31.

Zhang, Q., Li, Y., & Tsiern, R. W. (2009). The dynamic control of kiss-and-run and vesicular reuse probed with single nanoparticles. *Science*, 323, 1448–1453.

**How to cite this article:** Bannai H, Niwa F, Sakuragi S, Mikoshiba K. Inhibitory synaptic transmission tuned by Ca²⁺ and glutamate through the control of GABA AR lateral diffusion dynamics. *Develop Growth Differ*. 2020;62:398–406. https://doi.org/10.1111/dgd.12667