Gliotransmitter Release from Astrocytes: Functional, Developmental, and Pathological Implications in the Brain

Kazuki Harada, Taichi Kamiya and Takashi Tsuboi*

Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan

Astrocytes comprise a large population of cells in the brain and are important partners to neighboring neurons, vascular cells, and other glial cells. Astrocytes not only form a scaffold for other cells, but also extend foot processes around the capillaries to maintain the blood–brain barrier. Thus, environmental chemicals that exist in the blood stream could have potentially harmful effects on the physiological function of astrocytes. Although astrocytes are not electrically excitable, they have been shown to function as active participants in the development of neural circuits and synaptic activity. Astrocytes respond to neurotransmitters and contribute to synaptic information processing by releasing chemical transmitters called “gliotransmitters.” State-of-the-art optical imaging techniques enable us to clarify how neurotransmitters elicit the release of various gliotransmitters, including glutamate, D-serine, and ATP. Moreover, recent studies have demonstrated that the disruption of gliotransmission results in neuronal dysfunction and abnormal behaviors in animal models. In this review, we focus on the latest technical approaches to clarify the molecular mechanisms of gliotransmitter exocytosis, and discuss the possibility that exposure to environmental chemicals could alter gliotransmission and cause neurodevelopmental disorders.

Keywords: astrocytes, exocytosis, glial cell, gliotransmitter, neurodevelopmental disorders, optical imaging, synaptic activity

INTRODUCTION

Astrocytes are the most abundant glial cells in the central nervous system (CNS) of mammals (Ventura and Harris, 1999). Based on electron microscopic analyses, astrocytes are located near to neurons and blood vessels (Figure 1A). Regarding vasculature, capillary endothelial cells are surrounded by pericytes and basal lamina, and astrocytes tightly wrap these microvascular structures (Abbott et al., 2006). Together with pericytes, astrocytes are an essential component of the blood–brain barrier (BBB), which selects and transports molecules from the bloodstream, and allows for the transfer of nutrients to neurons (Figure 1B). Regarding their relationship with neurons, astrocytic foot processes make close contact with pre- and post-synaptic areas, forming structures called “tripartite synapses” (Araque et al., 1999; Halassa et al., 2007). Indeed, in the hippocampus, 57% of synapses are associated with astrocytes (Ventura and Harris, 1999), suggesting that astrocytes might contribute to neural information processing in the CNS.
Despite their morphological characteristics described above, astrocytes have long been considered as mere metabolic supporters that nurture adjacent neurons (Halassa et al., 2007; Wang and Bordey, 2008; Cali et al., 2009). However, recent electrophysiology and optical imaging analyses have provided strong evidence that astrocytes respond to neurotransmitters and release chemical transmitters called “gliotransmitters” (Li et al., 2013). Gliotransmitters, including glutamate, D-serine, and ATP, bind to their respective receptors on neurons to modulate their firing frequency and/or synaptic transmission (Figure 1C; Halassa et al., 2007; Koizumi, 2010). In fact, the dysfunction of gliotransmitter release-related proteins (e.g., vesicular transporters and vesicle-associated membrane proteins) in astrocytes can cause serious brain disorders and abnormal behaviors (Rossi et al., 2011; Verkhratsky et al., 2014). At the same time, traumatic injury, stroke, or infection-induced astrogliosis (also known as reactive astrocytes). These reactive astrocytes produce and release neurotoxic levels of glutamate (Rossi et al., 2011; Verkhratsky et al., 2014). Astrocytes also contribute to proper development of the BBB by aligning endothelial cells and pericytes, transporting molecules selected from the bloodstream to neurons (Abbott et al., 2006), and providing a protective barrier against toxic substances (Pentreath and Slamon, 2000; Calabrese, 2008). Thus, chronic exposure to environmental chemicals, or inflammatory molecules from vasculature, may potentially affect the function of astrocytes and gliotransmitter release (Kim et al., 2014; Orellana et al., 2014; Avendano et al., 2015).

In this review, we present the latest methods that enable scientists to decipher the molecular mechanisms of gliotransmitter secretion. In particular, we focus on the vesicular exocytosis of gliotransmitters from astrocytes using optical microscopic imaging. We further discuss how genetic alterations, acute injuries, and chronically toxic conditions (including exposure to stress in utero) could impair gliotransmission and consequently lead to neuronal and behavioral disorders.

MOLECULAR MECHANISMS UNDERLYING THE RELEASE OF GLIOTRANSMITTERS

There have been two major methodological breakthroughs that have allowed for profound understanding of astrocytic activities including gliotransmission: calcium imaging and advanced optical microscopy (Li et al., 2013). The initial discovery made by using chemical calcium indicators was that astrocytes exhibit increased intracellular calcium concentration ([Ca\(^{2+}\)]), which spreads to adjacent astrocytes. This phenomenon is called Ca\(^{2+}\) waves (Cornell-Bell et al., 1990; Charles et al., 1991; Rusakov et al., 2014). Genetically encoded calcium indicators have enabled more detailed analysis of astrocyte functions (Shigetomi et al., 2013).

Two-photon microscopy enabled scientists to observe fluorescence with superior penetration depth. Thus, studies on astrocytes have been expanded to experiments using brain slices and in vivo models (Nimmerjahn et al., 2004; Nishida and Okabe, 2007). Moreover, thanks to total internal reflection fluorescence microscopy, which can visualize fluorescent molecule behaviors beneath the plasma membrane, the interaction between [Ca\(^{2+}\)]; elevation and subsequent vesicular trafficking became precisely clarified (Bezzi et al., 2004; Shigetomi et al., 2012; Oya et al., 2013).

Because of these experimental advancements, accumulating evidence suggests the paradigm that: (1) inositol 1,4,5-trisphosphate-mediated Ca\(^{2+}\) release from endoplasmic
reticulum causes $[\text{Ca}^{2+}]_i$ increases in astrocytes in response to the activity of adjacent astrocytes and neurons; (2) elicited $[\text{Ca}^{2+}]_i$ elevation induces release of gliotransmitters (Halassa et al., 2007; Oya et al., 2013; Khakh and McCarthy, 2015). Although the exact mechanisms of gliotransmission are unclear, recent studies have partially revealed the release mechanisms of glutamate, D-serine, and ATP in astrocytes (Figure 2; Gucek et al., 2012; Li et al., 2013).

**GLUTAMATE**

Although, glutamate is well-known as a neurotransmitter, it also acts as a gliotransmitter. Application of bradykinin to cultured astrocytes induces glutamate release and influences adjacent neurons through N-methyl-D-aspartate (NMDA) receptors (Parpura et al., 1994). In contrast, application of clostridium, tetanus, and botulinum neurotoxins, which differentially cleave the exocytosis-regulating soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, reduces $\text{Ca}^{2+}$-dependent glutamate release. These findings suggest that the SNARE proteins, including vesicle-associated membrane protein-2 (VAMP2), syntaxin-1, and synaptosome-associated protein-23, mediate $\text{Ca}^{2+}$-dependent glutamate release (Montana et al., 2006; Parpura and Zorec, 2010).

The uptake of cytoplasmic glutamate into exocytotic vesicles is mediated by vesicular glutamate transporters (VGLUTs), which are driven by a proton gradient produced by vacuolar-type H$^+$ ATPases (V-ATPases; Takamori et al., 2000; Gucek et al., 2012). Inhibition of V-ATPases blocks $\text{Ca}^{2+}$-dependent glutamate release (Parpura and Zorec, 2010). Furthermore,
VGLUT1 and 2 are colocalized with synaptic-like vesicles (Bezzi et al., 2004), suggesting that glutamate is packaged into synaptic-like vesicles and released from astrocytes in a Ca^{2+}-dependent manner.

Meanwhile, other release mechanisms have been identified: (1) reverse operation of plasma membrane glutamate transporters (Longuemaire and Swanson, 1995); (2) cell swelling-induced anion transporter opening (Kimelberg et al., 1990); (3) release via P2X7 receptors (Duan et al., 2003); (4) gap junction channels (i.e., hemichannels) on the cell surface of astrocytes (Ye et al., 2003). However, it is not clear how often and to what extent astrocytes employ these different mechanisms. Further studies will be needed to clarify whether there are specific release mechanisms that operate under particular conditions.

**D-SERINE**

The discovery of D-serine as a gliotransmitter was remarkable because it was long thought that mammalian tissues only produced L-isomers of amino acids (Oliet and Mothet, 2006; Henneberger et al., 2012). D-serine is thought to be produced from L-serine by serine racemase (de Miranda et al., 2002). In cultured astrocytes, application of glutamate enhanced Ca^{2+}-dependent secretion of D-serine via the activation of a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors (AMPA/KARs) and metabotropic glutamate receptors (Mothet et al., 2005). Correspondingly, agonists for AMPA/KARs and metabotropic glutamate receptors were found to increase [Ca^{2+}], as well as subsequent secretion of D-serine, which is reduced by inhibition of these receptors. Furthermore, tetanus neurotoxins and V-ATPase inhibitors suppress agonist-evoked secretion of D-serine, and VAMP2/3 and VGLUT2-containing vesicles that are colocalized with D-serine. These results suggest that D-serine is stored in the synaptic-like vesicles and released from the vesicles in a Ca^{2+}-dependent manner (Martineau et al., 2013).

**ATP**

Although ATP is the primary energy currency of the cells, ATP can also act as a signaling molecule through purinergic receptors. A recent study showed that culture medium from cultured astrocytes exhibiting Ca^{2+} waves contained more ATP than control culture medium. Interestingly, the addition of collected culture medium to astrocytes induced Ca^{2+} waves that were inhibited by purinergic receptor antagonists (Guthrie et al., 1999). Thus, the ATP released from astrocytes induces Ca^{2+} waves, which astrocytes use to communicate with each other. However, the ATP release mechanisms still remain controversial; several lines of investigation have suggested various putative models for ATP release from astrocytes (Koizumi, 2010).

Connexin 43 (Cx43) assembles into a hemichannel which constitutes gap junctions in astrocytes, and exchanges signaling molecules, including Ca^{2+} and inositol 1,4,5-trisphosphate, between adjacent astrocytes (Orellana and Stehberg, 2014). Bioluminescence imaging of ATP combined with single channel recording showed that Cx43 hemichannels in rat glia C6 cells and CA1 hippocampal astrocytes are permeable to ATP (Kang et al., 2008). Consistent with this finding, glutamate evoked [Ca^{2+}], increase and ATP release in astrocytes of hippocampal slices, which were inhibited by application of a hemichannel blocker and in Cx43/Cx30 knockout mice (Torres et al., 2012), suggesting that ATP is released extracellularly through Cx43 hemichannels.

However, some studies have shown the involvement of secretory lysosomes in ATP release from astrocytes. In fact, primary cultured astrocytes express a secretory lysosome marker called vesicle-associated membrane protein-7 (also called T1-VAMP), and T1-VAMP-positive secretory lysosomes contain ATP which is Ca^{2+}-dependently released (Verderio et al., 2012). In an experiment using primary cultured astrocytes and C6 cells, vesicular nucleotide transporter (VNUT)-positive lysosomes were labeled with fluorescent ATP, and application of VNUT inhibitor reduced the number of fluorescent ATP-containing vesicles. Observation by total internal reflection fluorescence microscopy revealed exocytotic events of secretory lysosomes in the cells following the application of a calcium ionophore, ATP, and glutamate. Thus, ATP is stored in lysosomes and released from lysosomes in a Ca^{2+}-dependent manner (Oya et al., 2013).

**CONTRIBUTION OF GLIOTRANSMITTER RELEASE TO DEVELOPMENT AND DISEASE**

Release of gliotransmitters regulates synaptic transmission between neurons and the extracellular environment in the brain. It is known that glutamate and D-serine excite synaptic transmission. However, whether ATP potentiates or inhibits synaptic transmission is still under debate because adenosine, a metabolite synthesized from ATP, usually inhibits synaptic activity via adenosine A1 receptors (Koizumi, 2010; Nam et al., 2012; Delekate et al., 2014). It is therefore reasonable to speculate that imbalance in the release of these gliotransmitters could result in altered neuronal activity. Various pathological conditions, including CNS diseases, traumatic brain injuries, developmental disorders, and prenatal exposure to deleterious molecules have been reported to be closely associated with impairment of gliotransmission.

**CNS DISEASES**

Many CNS diseases are attributed to hyperactivity of neurons or unregulated neuronal cell death. Although such conditions have long been the focus of “neurocentric” studies, recent progress in the study of astrocytic gliotransmission has provided accumulating evidence for the contribution of astrocytes (Rossi et al., 2011; Verkhratsky et al., 2014).

Epilepsy is one of the most common CNS diseases, and is characterized by sudden and frequent seizures resulting from excessive firings by neurons (Wetherington et al., 2008). In slices from epilepsy model mice, astrocytic glutamate release was found to cause abnormal and prolonged depolarization in
neurons (Tian et al., 2005). Furthermore, tumor necrosis factor-α (TNFα) and prostaglandins (PGs), released from astrocytes under traumatic events, can reactivate their calcium signaling, and can cause increased glutamate release (Bezzi et al., 1998, 2001; Domercq et al., 2006).

Reactive astrocytes are also involved in the pathogenesis of other neuronal disorders. In a mouse model of Huntington’s disease, cultured astrocytes exhibited hyperactivated Ca\(^{2+}\)-dependent glutamate release. This activity was owing to increased expression of pyruvate carboxylase (Lee et al., 2013), or reduced expression of glutamate transporter-1 and K\(_{ir}\)4.1 K\(^+\) channels, which are key regulators for the clearance of extracellular glutamate and maintenance of membrane potentials, respectively (Behrens et al., 2002; Tong et al., 2014). In addition to overpotentiating neuronal activity, excessive accumulation of extracellular glutamate causes cytotoxicity. For instance, mice with genetic deletion in glutamate transporter-1 exhibit reduced glutamate clearance, and consequently display abnormal cell death in motor neurons, reminiscent of amyotrophic lateral sclerosis (Staats and Van Den Bosch, 2009).

In Alzheimer’s disease (AD) mouse models, reactive astrocytes are detected near β-amyloid plaques (Nagele et al., 2004). Although chronic rise in [Ca\(^{2+}\)]\(_i\) is a well-known phenomenon in reactive astrocytes in AD, its underlying mechanisms remain unclear. A recent study demonstrated that purinergic signaling through Cx43 hemichannels and P2Y1 receptors mediated the hyperactivity of astrocytes in AD (Delekate et al., 2014). Consistent with this finding, upregulation of Cx43 hemichannels was observed in an AD mouse model (Mei et al., 2010), and AD patients displayed higher levels of ATP in brain regions surrounding β-amyloid plaques (Mecheri et al., 1997; Mandal et al., 2012).

Gliotransmitter release from astrocytes is also required for correct development of neuronal circuits. In particular, glial-neuronal communication through NMDA receptors is an essential process for proper dendritic morphogenesis and establishment of synaptic connections (Rabacchi et al., 1992; Sin et al., 2002; Espinosa et al., 2009). Although NMDA receptors are activated by both glutamate and D-serine, recent discoveries suggest that D-serine plays an important role in dendritic development and long-term potentiation (Henneberger et al., 2010; Devito et al., 2011; Balu and Coyle, 2012; Diniz et al., 2012). Mice with a deletion of serine racemase showed reduced levels of brain D-serine and brain-derived neurotrophic factor, and loss of glutamatergic neurotransmission, and consequently had less complex dendrites (Morita et al., 2007; Balu and Coyle, 2012). Because NMDA receptor malfunction has been considered to be responsible for schizophrenia, deficiency in D-serine secretion from astrocytes can be a potent schizophrenia risk factor (Van Horn et al., 2013). Indeed, association studies of schizophrenia patients revealed several mutations in genes for serine racemase, as well as D-amino acid oxidase and its interacting protein G72 (Boks et al., 2007; Morita et al., 2007; Müller et al., 2011; Caldinelli et al., 2013).

INJURY AND INFECTION

Acute brain insults, caused by ischemia or infection, affect neuronal circuitry through direct inflammatory responses in neurons and through signals from glial cells (Vesce et al., 2007; Cali et al., 2009). Astrocytes under acute inflammatory conditions undergo reactive astrogliosis similarly to those in CNS diseases, albeit with differences in gene expression and cell structure (Khakh and Sofroniew, 2015). Upon injury or ischemia, damaged neurons, endothelial cells and glial cells are known to release considerable amounts of ATP (Cook and McCleskey, 2002; Wang et al., 2004; Davalos et al., 2005; Nedergaard et al., 2010). Increased levels of extracellular ATP activate purinergic receptors on astrocytes, particularly P2Y1 (Domercq et al., 2006), thereby inducing [Ca\(^{2+}\)]\(_i\) elevation and release of glutamate, as well as ATP (Domercq et al., 2006; Nedergaard et al., 2010). Furthermore, inflammatory molecules including TNFα, interleukin-1β, and PGs, are profoundly engaged in these responses. Not only the activated microglia converge to the site of injury and secrete cytokines; astrocytes themselves synthesize TNFα and PGs (Domercq et al., 2006; Santello et al., 2011). TNFα and PGs either interact with certain processes in the stimulus-secretion coupling machinery within astrocytes (Domercq et al., 2006; Santello et al., 2011), or bind to TNFα and PGs receptors on astrocytes after secretion (Bezzi et al., 2001; Vesce et al., 2007).

CHRONIC AND PRENATAL EXPOSURE TO CHEMICALS

Increasing evidence shows significant correlations between environmentally deleterious chemicals and the risk of neurodevelopmental disorders (Feng et al., 1990; Leonardsson and Ny, 1997). Previous studies have focused on the effects of toxic substances on neurons, but recently it was suggested that astrocytes are also involved in the pathogenesis of those conditions.

Owing to their close connections with microvascular units via BBB, astrocytes tend to be chronically exposed to noxious molecules in circulation. Probably because of their interactions with environmental toxins, astrocytes possess more resilient and adaptive machinery against toxic molecules compared with neurons (Pentreath and Slamon, 2000; Calabrese, 2008). These protective systems include the glutathione system, superoxide dismutase, and hemeoxygenase (Dwyer et al., 1995; Huang and Philbert, 1995; Blauwegeers et al., 1996; Pentreath and Slamon, 2000). Nevertheless, excessive passage of harmful substances across the BBB seriously affects astrocyte homeostasis and functionality.

The toxicological effects of heavy metals (e.g., mercury, zinc, manganese, and aluminum) on neurons and glial cells have been studied for decades (Calabrese, 2008; De Keyser et al., 2008). However, it is unclear how these metals affect gliotransmitter release. Some studies have shown that lead and manganese induce cytotoxic cell death by impairing glutamate uptake in astrocytes (Normandin and Hazell, 2002; Struzynska et al., 2005). However, pathological effects on gliotransmission by lifestyle-associated factors, such as smoking, drinking, and insufficient
sleep, are becoming the focus of growing interest. Because nicotinic acetylcholine receptors are expressed on astrocytes, they exhibit nicotine sensitivity and $[\text{Ca}^{2+}]_i$ increase (Oikawa et al., 2005; Delbro et al., 2009). Ethanol causes reactive oxygen species production, and $[\text{Ca}^{2+}]_i$ increase and glutamate secretion from astrocytes (Salazar et al., 2008). Astrocytes exposed to ethanol also exhibit alterations in Golgi complex morphology, secretory vesicle biogenesis, and expression levels of Rab GTPases and motor proteins (Tomas et al., 2005), which may be an additional factor for the dysfunction of brain development caused by ethanol.

Because adenosine plays a critical role in the control of sleep-wakefulness (Thakkar et al., 2003), and chronic alcoholism is frequently accompanied by sleep disorders (Brower, 2001), changes in sleep pattern may also induce alteration in gliotransmitter release. Interestingly, hypothalamic astrocytes from rats following sleep deprivation exhibited different proteome profiles, and the expression of VAMP2, which is an essential protein for vesicular exocytosis (Kim et al., 2014), was significantly increased. These findings suggest a strong association between alcohol intake, sleep disorders, and astrocytic gliotransmission.

Additionally, certain ambient ultrafine particles, which are defined as particulate substances with a diameter less than 100 nm, are emerging as another toxic substance that may deleteriously affect brain function (Block and Calderón-Garcidueñas, 2009; Loane et al., 2013). In a recent study, ultrafine carbon black, a surrogate for ultrafine particles, was shown to induce the release of glutamate and ATP from astrocytes by activating Cx43 and pannexin-1 hemichannels (Wei et al., 2014).

Recent epidemiological and experimental studies have demonstrated that children born from mothers who are exposed to infections or are addicted to alcohol or drugs have a higher risk of neuronal disorders and abnormal behaviors (Jacobsen et al., 2006; Stringari et al., 2008; Boks, 2010; Broese et al., 2015). However, the effects of these agents on astrocytes still remain largely unknown. Some studies have shown that prenatal exposure to lipopolysaccharides or nicotine together with postnatal high-fat/cholesterol diet result in enhancement of Cx43 hemichannel activity, and consequently increases the release of glutamate and ATP (Orellana et al., 2014; Avendano et al., 2015).

**CONCLUSIONS**

Over several decades, researchers have attempted to understand the properties and pathologies of the CNS by focusing solely on neurons; however, recent improvements in molecular and cellular imaging techniques are increasingly indicating that this neurocentric approach needs to be revised. In addition to neurons, glial cells including astrocytes are important elements for brain functions. Astrocytes are located in close morphological and functional relationships with blood vessels and neurons, and various genetic or environmental factors are implicated in gliotransmission impairment. Considering these characteristics of astrocytes, further studies will provide new insight on the significance of gliotransmitter release for fetal neurodevelopment. Thus, new therapies can be developed to overcome environmental chemical-induced neurodevelopmental disorders.

**AUTHOR CONTRIBUTIONS**

KH, TK, and TT wrote the paper.

**FUNDING**

This work was funded in part by a research grant from The Grant of National Center for Child Health and Development, Tokyo, Japan (grant numbers 27-9), and by a Grant-in-Aid for Science Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

**ACKNOWLEDGMENTS**

The authors thank Dr. Fumihiko Maekawa, Dr. Kazuaki Nakamura, and Dr Manami Oya for their helpful discussion and comments.

**REFERENCES**

Abbott, N. J., Rönnback, L., and Häusson, E. (2006). Astrocyte-endothelial interactions at the blood-brain barrier. Nat. Rev. Neurosci. 7, 41–53. doi: 10.1038/nrn1824

Araque, A., Parpura, V., Sanzgiri, R. P., and Haydon, P. G. (1999). Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci. 22, 208–215. doi: 10.1016/S0166-2236(98)01349-6

Avendano, B. C., Montero, T. D., Chavez, C. E., Von Bernhardi, R., and Orellana, J. A. (2015). Prenatal exposure to inflammatory conditions increases Cx43 and Panx1 unopposed channel opening and activation of astrocytes in the offspring effect on neuronal survival. Glia. 63, 2058–2072. doi: 10.1002/glia.22877

Balu, D. T., and Coyle, J. T. (2012). Neuronal D-serine regulates dendritic architecture in the somatosensory cortex. Neurosci. Lett. 517, 77–81. doi: 10.1016/j.neulet.2012.04.020

Behrens, P. P., Franz, P., Woodman, B., Lindenberg, K. S., and Landwehrmeyer, G. B. (2002). Impaired glutamate transport and glutamate-glutamine cycling: downstream effects of the Huntington mutation. Brain 125, 1908–1922. doi: 10.1093/brain/awf180

Betti, P., Carmignoto, G., Pasti, L., Vesce, S., Rossi, D., Rizzini, B. L. et al. (1998). Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. Nature 391, 281–285. doi: 10.1038/34651

Betti, P., Demercq, M., Brambilla, L., Galli, R., Schols, D., De Clercq, E., et al. (2001). CXCR4-activated astrocyte glutamate release via TNFα: amplification by microglia triggers neurotoxicity. Nat. Neurosci. 4, 702–710. doi: 10.1038/nn4910

Betti, P., Gundersen, V., Galbete, J. L., Seifert, G., Steinhäusser, C., Pilati, E., et al. (2004). Astrocytes contain a vesicular compartment that is competent for regulated exocytosis of glutamate. Nat. Neurosci. 7, 613–620. doi: 10.1038/nn1246

Blaauwgeers, H. G., Vianney De Jong, J. M., Verspaget, H. W., Van Den Berg, F. M., and Troost, D. (1996). Enhanced superoxide dismutase-2 immunoreactivity of astrocytes and occasional neurons in amyotrophic lateral sclerosis. J. Neurol. Sci. 140, 21–29. doi: 10.1016/0022-510X(96)00110-4
Loane, C., Pilinis, C., Lekkas, T. D., and Politis, M. (2013). Ambient particulate matter and its potential neurological consequences. Rev. Neurosci. 24, 323–335. doi: 10.1515/revneuro-2013-0001

Longuemare, M. C., and Swanson, R. A. (1995). Excitatory amino acid release from astrocytes during energy failure by reversal of sodium-dependent uptake. J. Neurosci. Res. 40, 379–386. doi: 10.1002/jnr.49040312

Mandal, P. K., Akolkar, H., and Tripathi, M. (2012). Mapping of hippocampal pH and neurochemicals from in vivo multi-voxel 31P study in healthy normal young male/female, mild cognitive impairment, and Alzheimer’s disease. J. Alzheimers. Dis. 31(Suppl. 3), S75–S86. doi: 10.3233/JAD-2012-120166

Martineau, M., Shi, T., Puyal, J., Kasnoff, A. M., Dulong, J., Gasnier, B., Mei, X., Ezan, P., Giaume, C., and Koulakoff, A. (2010). Astroglial connexin 30.2 is involved in amino acid-induced glutamate-transporter secretion. J. Biol. Chem. 285, 4531–4532. doi: 10.1074/jbc.M109.104277

Orellana, J. A., Busso, D., Ramirez, G., Campos, M., Rigotti, A., Eugenio, J., et al. (2014). Prenatal nicotine exposure enhances Ca3x and Panx1 unopposed channel activity in brain cells of adult offspring mice fed a high-fat/cholesterol diet. Front. Cell Neurosci. 8:403. doi: 10.3389/fncel.2014.00403

Orellana, J. A., and Stehberg, J. (2014). Hemichannels: new roles in astroglial function. Front. Physiol. 5:193. doi: 10.3389/fphys.2014.00193

Oya, M., Kitaguchi, T., Pias, R., Reimann, F., Gribble, F., and Tsuibo, T. (2013). The G protein-coupled receptor family C group 6 subtype A (GPRC6A) receptor is involved in amino acid-induced-glucagon-like-peptide-1 secretion from GLUTag cells. J. Biol. Chem. 288, 4531–4532. doi: 10.1074/jbc.288.5.4531

Parkpura, V., Basarsky, T. A., Liu, F., Jeftinija, K., Jeftinija, S., and Hayden, P. G. (1994). Glutamate-mediated astrocyte-neuron signalling. Nature 369, 744–747. doi: 10.1038/369744a0

Parpura, V., and Zorec, R. (2010). Gliotransmission: excitocytic release from astrocytes. Brain Res. Rev. 63, 83–92. doi: 10.1016/j.brainresrev.2009.11.008

Pentreath, V. W., and Slamon, N. D. (2000). Astrocyte phenotype and prevention against oxidative damage in neurotoxicity. Hum. Exp. Toxicol. 19, 641–649. doi: 10.1191/09663620076221595

Rabacchi, S., Bailly, Y., Delhaye-Bouchaud, N., and Mariani, J. (1992). Involvement of the N-methyl-D-aspartate (NMDA) receptor in synapse elimination during cerebellar development. Science 256, 1823–1825. doi: 10.1126/science.1352066

Rossi, D., Martorana, F., and Brambilla, L. (2011). Implications of gliotransmission for the pharmacotherapy of CNS disorders. CNS Drugs 25, 641–658. doi: 10.2165/11593909-000000000-00000

Rusakov, D. A., Bard, L., Stewart, M. G., and Henneberger, C. (2014). Diversity of astroglial functions alludes to subcellular specialisation. Trends Neurosci. 37, 228–242. doi: 10.1016/j.tins.2014.02.008

Salazar, M., Pareinte, J. A., Salido, G. M., and González, A. (2008). Ethanol induces glutamate secretion by Ca2+ mobilization and ROS generation in rat hippocampal astrocytes. Neurochem. Int. 52, 1061–1067. doi: 10.1016/j.neuint.2007.11.001

Santello, M., Bezzi, P., and Volterra, A. (2011). TNFalpha controls glutamatergic gliotransmission in the hippocampal dentate gyrus. Neuron 69, 988–1001. doi: 10.1016/j.neuron.2011.02.003

Shigetomi, E., Bushong, E. A., Haustein, M. D., Tong, X. P., Jackson-Weaver, O., Kracun, S., et al. (2013). Imaging calcium microdomains within entire astrocyte territories and endfeet with GCaMPs expressed using adeno-associated viruses. J. Gen. Physiol. 141, 633–647. doi: 10.1085/jgp.201210949

Shigetomi, E., Tong, X., Kwan, K. Y., Corey, D. P., and Khakh, B. S. (2012). TRPA1 channels regulate astrocyte resting calcium and inhibitory synapse efficacy through GAT-3. Nat. Neurosci. 15, 70–80. doi: 10.1038/nn.3000

Sin, W. C., Haas, K., Ruthazer, E. S., and Cline, H. T. (2002). Dendrite growth increased by visual activity requires NMDA receptor and Rho GTPases. Nature 419, 475–480. doi: 10.1038/nature00987

Staats, K. A., and Van Den Bosch, L. (2009). Astrocytes in amyotrophic lateral sclerosis: direct effects on motor neuron survival. J. Biol. Phys. 35, 337–346. doi: 10.1016/j.jbph.2009.03.004

Stringari, J., Nunes, A. K., Franco, J. L., Bohrer, D., Garcia, S. C., Dafre, A. L. L., et al. (2008). Prenatal methylmercury exposure hampers glutathione antioxidant system ontogenesis and causes long-lasting oxidative stress in the mouse brain. Toxicol. Appl. Pharmacol. 227, 147–154. doi: 10.1016/j.taap.2007.10.010

Struzynska, L., Chalimoniuk, M., and Sulkowski, G. (2005). The role of astroglia in Pb-exposed adult rat brain with respect to glutamate toxicity. Toxicology 212, 185–194. doi: 10.1016/j.tox.2005.04.013

Takamori, S., Rhee, J. S., Rosenmund, C., and Jahn, R. (2000). Identification of a vesicular glutamate transporter that defines a glutamatergic phenotype in neurons. Nature 407, 189–194. doi: 10.1038/35025070

Thakkar, M. M., Winston, S., and McCarley, R. W. (2003). A1 receptor and adenosinergic homeostatic regulation of sleep-wakefulness: effects of antisense to the A1 receptor in the cholinergic basal forebrain. J. Neurosci. 23, 4278–4287. doi: 10.1002/jn.20020000001

Tomás, M., Marín, P., Megías, L., Egea, G., and Renau-Piqueras, J. (2005). Ethanol induces glutamate secretion by Ca2+ mobilization and ROS generation in rat hippocampal astrocytes. J. Neurosci. Res. 81, U3413–U3605. doi: 10.1002/jnr.20356

Tian, G. F., Azmi, H., Takano, T., Xu, Q., Peng, W., Lin, J., et al. (2005). An excitatory neurotransmitter for astrocytes. J. Neurosci. 25, 641–658. doi: 10.1523/JNEUROSCI.4466-06.2007

Torres, A., Wang, F. S., Xu, Q. W., Fujita, T., Dobrowolski, R., Willecke, K., et al. (2012). Extracellular Ca2+ acts as a mediator of communication from neurons to glia. Sci. Signal. 5, ra8. doi: 10.1126/scisignal.2002160

Nedergaard, M., Rodríguez, J. J., and Verkhratsky, A. (2010). Gliarial calcium channels: from release to function. Rev. Neurosci. 21, 31–45. doi: 10.1523/JNEUROSCI.1044-10.2010

Oliet, S. H., and Mothet, J. P. (2006). Molecular determinants of D-serine-mediated gliotransmission: from release to function. Glia 54, 726–737. doi: 10.1002/glia.20356
Van Horn, M. R., Sild, M., and Ruthazer, E. S. (2013). D-serine as a gliotransmitter and its roles in brain development and disease. Front. Cell. Neurosci. 7:39. doi: 10.3389/fncel.2013.00039
Ventura, R., and Harris, K. M. (1999). Three-dimensional relationships between hippocampal synapses and astrocytes. J. Neurosci. 19, 6897–6906.
Verderio, C., Cagnoli, C., Bergami, M., Francolini, M., Schenk, U., Colombo, A., et al. (2012). TI-VAMP/VAMP7 is the SNARE of secretory lysosomes contributing to ATP secretion from astrocytes. Biol. Cell 104, 213–228. doi: 10.1111/boc.201100070
Verkhratsky, A., Parpura, V., Pekna, M., Pekny, M., and Sofroniew, M. (2014). Glia in the pathogenesis of neurodegenerative diseases. Biochem. Soc. Trans. 42, 1291–1301. doi: 10.1042/BST20140107
Vesce, S., Rossi, D., Brambilla, L., and Volterra, A. (2007). Glutamate release from astrocytes in physiological conditions and in neurodegenerative disorders characterized by neuroinflammation. Int. Rev. Neurobiol. 82, 57–71. doi: 10.1016/S0074-7742(07)82003-4
Wang, D. D., and Bordey, A. (2008). The astrocyte odyssey. Prog. Neurobiol. 86, 342–367. doi: 10.1016/j.pneurobio.2008.09.015
Wang, X., Arcuino, G., Takano, T., Lin, J., Peng, W. G., Wan, P., et al. (2004). P2X7 receptor inhibition improves recovery after spinal cord injury. Nat. Med. 10, 821–827. doi: 10.1038/nm.1082
Wei, H., Deng, F., Chen, Y., Qin, Y., Hao, Y., and Guo, X. (2014). Ultrafine carbon black induces glutamate and ATP release by activating connexin and pannexin hemichannels in cultured astrocytes. Toxicology 323, 32–41. doi: 10.1016/j.tox.2014.06.005
Wetherington, J., Serrano, G., and Dingledine, R. (2008). Astrocytes in the epileptic brain. Neuron 58, 168–178. doi: 10.1016/j.neuron.2008.04.002
Ye, Z. C., Wyeth, M. S., Baltan-Tekkok, S., and Ransom, B. R. (2003). Functional hemichannels in astrocytes: a novel mechanism of glutamate release. J. Neurosci. 23, 3588–3596.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2016 Harada, Kamiya and Tsuboi. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.