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·Research Highlight·

Connexin 30 controls the extension of astrocytic processes into the synaptic cleft through an unconventional non-channel function

Jerome Clasadonte, Philip G. Haydon
Department of Neuroscience, Tufts University School of Medicine, Boston, MA 02111, USA
Corresponding author: Jerome Clasadonte. E-mail: jerome.clasadonte@tufts.edu

Neurons and glial cells, particularly astrocytes, are the two main cell populations in the central nervous system. While it is established that brain functions primarily rely on neuronal activity, an active contribution of astrocytes to information processing is only starting to be considered. There is growing evidence that astrocytes, as part of the tripartite synapse, participate in this challenge by receiving and integrating neuronal signals and, in turn, by sending signals that target neurons[1]. The involvement of astrocytes in information processing has mainly been studied at the level of the single astrocyte, often missing the role of astrocyte networks in this process. These networks result from the extensive intercellular communication between astrocytes through connexins, the proteins forming gap junction channels[2]. Pioneering research from Rouach and colleagues has demonstrated the role of astrocyte networks mediated by the two main astroglial connexins 43 (Cx43) and 30 (Cx30) in metabolic support[3] and clearance of extracellular potassium during synaptic activity[4]. While these critical functions have been shown to be mainly dependent on the channel properties of connexins, Pannasch and colleagues have now demonstrated that astroglial Cx30, through a non-channel function, regulates synaptic transmission and memory by changing astrocyte morphology and controlling the insertion of astrocytic processes into synaptic clefts[5].

In their study using acute hippocampal slices, Pannasch and colleagues found that selective inactivation of the Cx30 gene in the astrocytes of mice ($Cx30^{-/-}$ mice) decreased basal evoked excitatory synaptic transmission at the CA1 Schaffer collateral pathway without affecting inhibitory synaptic transmission. This reduction in excitatory transmission was specific to α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors. The size of the AMPA receptor current evoked in CA1 pyramidal neurons with local application of AMPA was similar in $Cx30^{-/-}$ and wild-type $Cx30^{+/+}$ mice, suggesting no change in the membrane AMPA receptor density. However, the amplitude of miniature excitatory postsynaptic currents was reduced in $Cx30^{-/-}$ mice, indicating that this decreased excitatory synaptic transmission was a consequence of a reduction in synaptic glutamate levels, rather than to postsynaptic effects. Remarkably, this reduction in excitatory synaptic transmission had functional consequences on hippocampal plasticity and contextual learning. Indeed, $Cx30^{-/-}$ mice displayed striking reduction in long-term potentiation (LTP) and contextual fear memory 24 h after conditioning.

Because glutamate is primarily cleared by astrocytes through glutamate transporters (GLTs)[6], Panasch and colleagues next hypothesized that the reduced synaptic glutamate levels in $Cx30^{-/-}$ mice could be due to increased astroglial glutamate uptake. In agreement with this possibility, the GLT current evoked in $Cx30^{-/-}$ astrocytes by Schaffer collateral stimulation in hippocampal slices was doubled compared to $Cx30^{+/+}$ astrocytes, which is indicative of enhanced glutamate transport in $Cx30^{-/-}$ mice. Then, by reducing the astroglial GLT current in $Cx30^{-/-}$ mice to wild-type levels by pharmacological inhibition of GLT1, the authors were able to restore normal glutamate synaptic concentrations, basal excitatory synaptic transmission, and LTP. Thus, the selective lack of Cx30 in astrocytes enhanced glutamate clearance and consequently attenuated glutamate synaptic transmission.

Intriguingly, the authors demonstrated that this

increased astroglial glutamate clearance in Cx30$^{-/-}$ mice was not due to either changes in the GLT expression level or alteration in the channel function of Cx30 that involves gap junction or hemichannel activity. Connexins also display non-channel functions through their intracellular carboxy-terminal (C-terminal) domain in cell growth, differentiation, and tumorigenicity by interacting with a plethora of connexin-associated proteins including cytoskeletal elements, enzymes (kinases and phosphatases), adhesion molecules, and signaling molecules[7]. Consequently, Pannasch and colleagues tested the hypothesis that this domain could contribute to the mechanism by which Cx30 regulates synaptic transmission. Stereotaxic lentiviral injection of full-length Cx30 targeting hippocampal astrocytes in the stratum radiatum of Cx30$^{-/-}$ mice restored normal glutamate synaptic transmission, while the C-terminally truncated form of Cx30 (C30ΔCter) did not, suggesting that non-channel functions of Cx30 involving its C-terminal domain mediate the regulation of excitatory synaptic transmission.

Interestingly, Cx30 has been shown to interact with cytoskeletal proteins that regulate cell adhesion and motility[8]. This, taken together with the fact that the glial coverage of synapses can control glutamate clearance[9] prompted the authors to investigate whether Cx30 could regulate astrocyte morphology and thereby glutamate synaptic levels. Histological studies indicated important morphological changes in astrocytes from Cx30$^{-/-}$ mice. Indeed, immunostaining for the glial marker, glial fibrillary acidic protein, revealed a larger domain area, elongated processes, and enhanced ramification in astrocytes from these mice. These morphological changes were blunted when Cx30$^{-/-}$ mice received intrahippocampal injections of lentiviral vector expressing full-length Cx30 but not when they were injected with C30ΔCter, suggesting that Cx30 controls astrocyte morphology through its C-terminal domain. Accordingly, Pannasch and colleagues demonstrated that migration and cell adhesion to the extracellular matrix was inhibited in HeLa cells transfected with full-length Cx30 but not with C30ΔCter. In addition, detailed ultrastructural analysis in Cx30$^{-/-}$ mice using electron microscopy uncovered an increase in the size and number of fine distal processes of astrocytes in close proximity to postsynaptic densities as well as a greater number of synaptic clefts contacted by astrocytes.

To investigate whether this enhanced astroglial coverage of synapses could potentiate glutamate clearance, the authors developed a mathematical model in which a synapse expressing AMPA receptors was surrounded by astroglial processes with membrane protrusions expressing GLTs. This model revealed that the invasion of a synapse by an astrocytic protrusion of 150 nm reduced synaptic AMPA receptor currents by 50% while it doubled the astrocytic GLT currents. Furthermore, the model predicted that inhibiting GLTs or increasing their density on wild-type astrocytes lacking protrusions would have no effect on synaptic AMPA receptor currents, confirming that the extension of astrocytic processes into synaptic clefts is required to enhance glutamate clearance and consequently reduce excitatory synaptic transmission.

Altogether, this is an elegant study establishing astroglial Cx30 as a regulator of astroglial invasion of synapses to control the efficacy of glutamate clearance and thereby, glutamatergic synaptic transmission and contextual memory (Fig. 1). The authors unexpectedly uncovered a non-channel function of astroglial Cx30, involving its C-terminal domain, in the control of astrocyte morphology. Although the role of the C-terminal tail of connexins in the regulation of cell morphology has been described, mainly in the context of cell adhesion, migration, and proliferation during disease and cancer development[7], this study is the first to demonstrate its role in a physiological process. However, how the C-terminus of connexins control cell behavior remains elusive[7]. It would be interesting to determine the precise molecular mechanisms by which the C-terminal tail of Cx30 controls the development of membrane protrusions in astrocytic processes. The physiological relevance of the astrocyte plasticity identified here is not well understood, but the authors suggest that it might contribute to the maturation of efficient synaptic strength during the postnatal development of the brain and basic cognitive functions in the adult brain by controlling the access of astrocytic processes to synaptic glutamate. It is noteworthy that similar changes in the glial coverage of synapses were previously observed in the hypothalamus, contributing to the regulation of several key processes such as lactation[9], osmoregulation[10], reproductive function[11] and more recently, feeding behavior[12]. Since astroglial

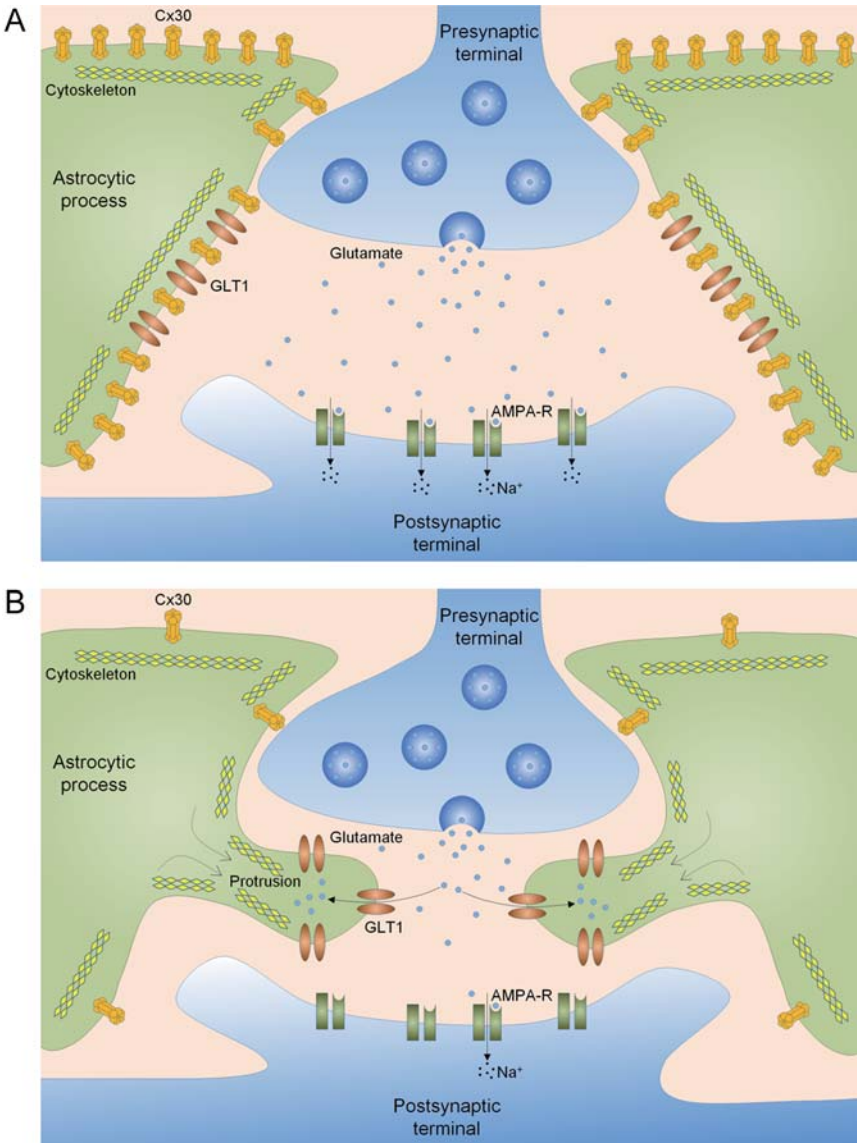

Fig. 1. Schematic depicting the mechanism by which astroglial Cx30 regulates the activity of AMPA receptors at the hippocampal synapse. A: The neurotransmitter glutamate released by the presynaptic terminal *via* exocytosis activates AMPA receptors (AMPA-Rs) in the postsynaptic terminal, causing excitatory synaptic currents generated by the influx of sodium (Na^+). Under steady-state conditions, astrocytic processes containing glutamate transporter 1 (GLT1) are held back from the synaptic cleft, which limits glutamate clearance and allows efficient activation of postsynaptic AMPA-Rs. B: Downregulation of astroglial Cx30, whose intracellular carboxy-terminal domain interacts with regulatory and structural elements of the cytoskeleton (e.g. actin filaments and tubulin), promotes the extension of astrocytic processes into the synaptic cleft (protrusion). Consequently, GLT1 becomes sufficiently close to the synaptic cleft to lower synaptic glutamate levels and reduces the activity of postsynaptic AMPA-Rs and the magnitude of excitatory synaptic transmission.

Cx30 is highly expressed in the hypothalamus[13], it would be of great interest to investigate the potential implications of their channel-independent functions on neuron-glia interactions in this area.

In addition to understanding the physiological role of this glial morphological reorganization involving non-channel functions of Cx30, it is important to determine the contribution of this process to potential pathophysiologic

processes and/or how the reorganization itself might be altered in such states. For instance, is this process normal, decreased, or enhanced in various brain disorders? Do changes in the expression level of astroglial Cx30 observed during epilepsy[14] or in the brains of patients with major depression[15] or of suicide completers[16] impact the glial coverage of synapses and thereby contribute to the etiology of these brain disorders? Even as the exciting findings of Pannasch and colleagues have reinforced our emerging understanding of the working brain as a constant interaction between neurons and glial cells, they also raise many intriguing and important questions for the field.

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REFERENCES

[1] Araque A, Carmignoto G, Haydon PG, Oliet SH, Robitaille R, Volterra A. Gliotransmitters travel in time and space. Neuron 2014, 81: 728–739.

[2] Pannasch U, Rouach N. Emerging role for astroglial networks in information processing: from synapse to behavior. Trends Neurosci 2013, 36: 405–417.

[3] Rouach N, Koulakoff A, Abudara V, Willecke K, Giaume C. Astroglial metabolic networks sustain hippocampal synaptic transmission. Science 2008, 322: 1551–1555.

[4] Pannasch U, Vargová L, Reingruber J, Ezan P, Holcman D, Giaume C, *et al*. Astroglial networks scale synaptic activity and plasticity. Proc Natl Acad Sci U S A 2011, 108: 8467–8472.

[5] Pannasch U, Freche D, Dallérac G, Ghézali G, Escartin C, Ezan P, *et al*. Connexin 30 sets synaptic strength by controlling astroglial synapse invasion. Nat Neurosci 2014, 17: 549–558.

[6] Tzingounis AV, Wadiche JI. Glutamate transporters: confining runaway excitation by shaping synaptic transmission. Nat Rev Neurosci 2007, 8: 935–947.

[7] Zhou JZ, Jiang JX. Gap junction and hemichannel-independent actions of connexins on cell and tissue functions--an update. FEBS Lett 2014, 588: 1186–1192.

[8] Qu C, Gardner P, Schrijver I. The role of the cytoskeleton in the formation of gap junctions by Connexin 30. Exp Cell Res 2009, 315: 1683–1692.

[9] Oliet SH, Piet R, Poulain DA. Control of glutamate clearance and synaptic efficacy by glial coverage of neurons. Science 2001, 292: 923–926.

[10] Gordon GR, Baimoukhametova DV, Hewitt SA, Rajapaksha WR, Fisher TE, Bains JS. Norepinephrine triggers release of glial ATP to increase postsynaptic efficacy. Nat Neurosci 2005, 8: 1078–1086.

[11] Clasadonte J, Sharif A, Baroncini M, Prevot V. Gliotransmission by prostaglandin e(2): a prerequisite for GnRH neuronal function? Front Endocrinol (Lausanne) 2011, 2: 91.

[12] Kim JG, Suyama S, Koch M, Jin S, Argente-Arizon P, Argente J, *et al*. Leptin signaling in astrocytes regulates hypothalamic neuronal circuits and feeding. Nat Neurosci 2014, 17: 908–910.

[13] Allard C, Carneiro L, Grall S, Cline BH, Fioramonti X, Chrétien C, *et al*. Hypothalamic astroglial connexins are required for brain glucose sensing-induced insulin secretion. J Cereb Blood Flow Metab 2014, 34: 339–346.

[14] Condorelli DF, Mudò G, Trovato-Salinaro A, Mirone MB, Amato G, Belluardo N. Connexin-30 mRNA is up-regulated in astrocytes and expressed in apoptotic neuronal cells of rat brain following kainate-induced seizures. Mol Cell Neurosci 2002, 21: 94–113.

[15] Bernard R, Kerman IA, Thompson RC, Jones EG, Bunney WE, Barchas JD, *et al*. Altered expression of glutamate signaling, growth factor, and glia genes in the locus coeruleus of patients with major depression. Mol Psychiatry 2011, 16: 634–646.

[16] Ernst C, Nagy C, Kim S, Yang JP, Deng X, Hellstrom IC, *et al*. Dysfunction of astrocyte connexins 30 and 43 in dorsal lateral prefrontal cortex of suicide completers. Biol Psychiatry 2011, 70: 312–319.