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TOPICAL REVIEW

A TRP among the astrocytes

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Abstract TRP channels were first identified as membrane proteins mediating phototransduction in fruit flies. Astrocytes were initially referred to as the silent elements of the nervous system. At the time these discoveries were made, few would have suspected TRP channels and astrocytes could contribute significantly to our understanding of brain signalling. Recent findings, however, put TRP channels and astrocytes in the spotlight, describe their ability to modulate the activity of specific sets of synapses, and raise some interesting questions. What makes astrocytes capable of exerting cell-specific effects on interneuronal signals? How do different synapses respond to changes in astrocytic function and in the local micro-structure of the neuropil? Can astrocytes be considered good candidate targets for therapeutic intervention to treat neurological diseases? Here I discuss the recent developments on TRP channels and astrocytes that have made us aware of the many structural and functional features of synapses that still need to be discovered and that could lead a new avant-garde in decoding the cellular and molecular basis of brain (dys)function.

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TRP channels in a nutshell

The major organic compounds present in spices like chili pepper, mint, mustard oil, cinnamon and garlic (i.e. capsaicin, menthol, allyl isothiocyanate, cinnamaldehyde, and allicin, respectively) were first identified 70–150 years ago, but only recently the molecular mechanisms that allow them to act on particular molecular targets, the transient receptor potential (TRP) channels, have started to be elucidated. By sequence homology, in mammals, 28 different TRP channels have been identified and grouped in six subfamilies (TRPA, TRPC, TRPM, TRPML, TRPP, TRPV) (Wu *et al.* 2010). Each TRP channel assembles as a tetramer of six transmembrane domain subunits, and shows non-selective cation permeability and weak voltage sensitivity (Clapham, 2003). TRP channels are particularly abundant in epithelial cells and nerve endings in the skin and mouth, but are also present in a variety of peripheral tissues and brain regions like the hypothalamus, substantia nigra, locus coeruleus, amygdala, cortex, hippocampus and cerebellum (Mori *et al.* 1998). In the brain, TRP channels are activated by a wide range of stimuli including changes in temperature, pressure and inflammatory agents, and mediate diverse functions including thermoregulation, inflammatory hyperalgesia (Bautista *et al.* 2006) and mechanosensation (Kwan *et al.* 2006).

TRPA channels

Among all TRP channels, TRPA channels have a peculiar structural signature, characterized by the presence of at least 14 modular protein–protein interaction motifs

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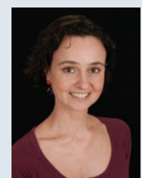

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(the ankyrin repeats) in their cytoplasmic N-terminal domain (Story *et al.* 2003) (Fig. 1*A* and *B*), which are suggested to act as gating springs for mechanotransduction (Sotomayor *et al.* 2005) and to be involved in trafficking the channels from intracellular organelles to the cell surface (Nilius *et al.* 2011). TRPA1 channels, the only members of the mammalian TRPA subfamily known so far, are activated directly by allyl isothiocyanate and cinnamaldehyde, and indirectly via G protein-coupled signalling cascades triggered by growth factors and proinflammatory peptides such as bradykinin. The jury is still out and with no prospect of immediate agreement on whether TRPA1 channels also play a major role in cold sensation (Story *et al.* 2003; Caspani & Heppenstall, 2009) (Fig. 1*C*), but while this is being evaluated, new important features of TRPA1 channels have emerged.

Shigetomi *et al.* (2011) recently reported that, in the hippocampus, spontaneous openings of TRPA1 channels in astrocytes shape fast inhibitory synaptic transmission in GABAergic interneurons (INs), but not in pyramidal cells (PCs). By monitoring the fluorescent signal of a genetically encoded Ca^{2+} indicator expressed in astrocytes (Lck-GCaMP3), the authors showed that spontaneous openings of TRPA1 channels mediate spatially confined ($\sim 5\ \mu$m) and slow-decaying ($\sim$4 s) increments in cytosolic Ca^{2+} (up to 0.5 μM) (Shigetomi *et al.* 2010*a*,*b*). Blocking TRPA1 channels prevented these events and led to a reduction in the astrocytic

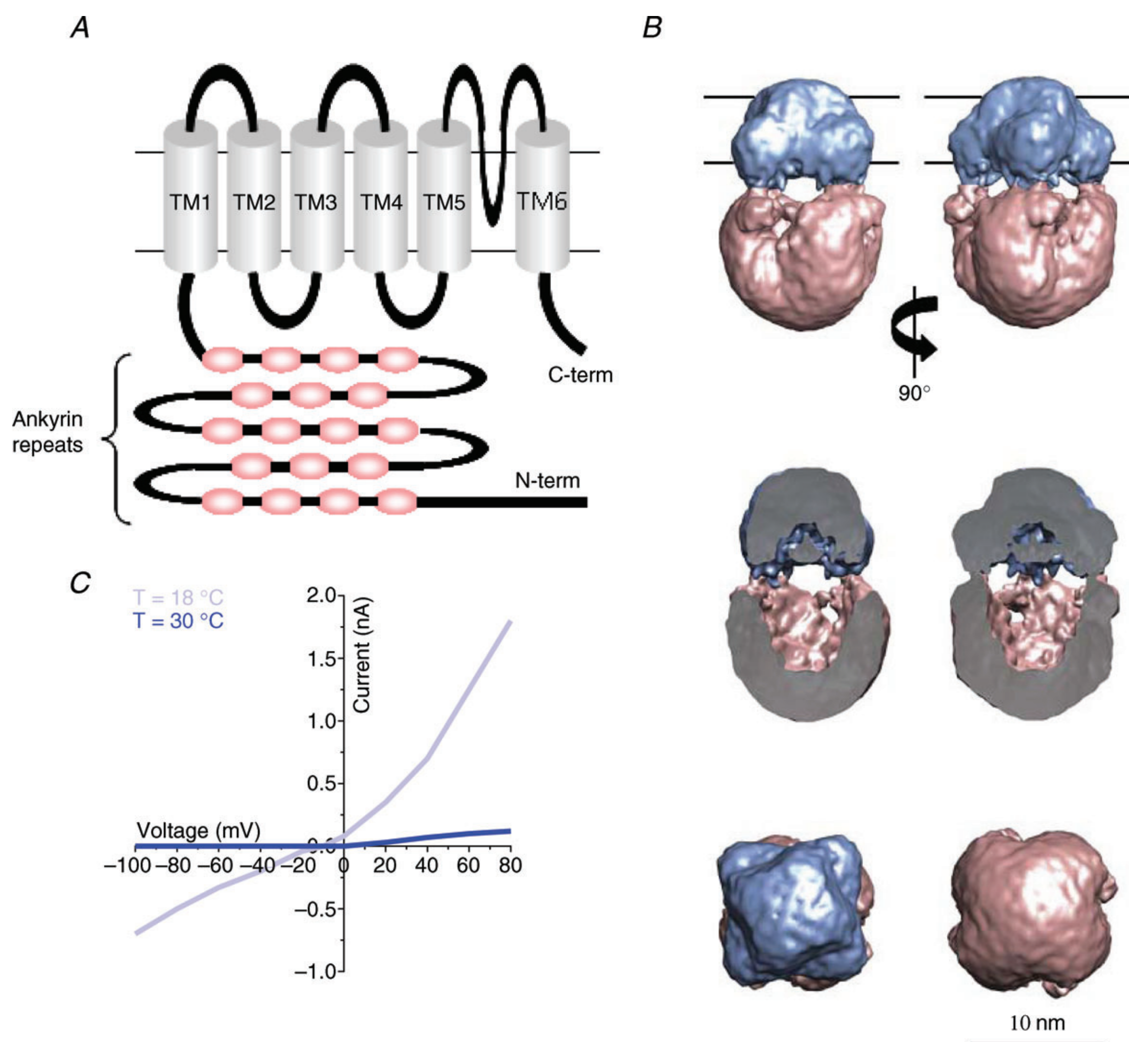

Figure 1. Architecture and functional properties of TRPA1 channels
A, TRPA1 channels are composed of four identical subunits organized as the one schematized here. Each subunit contains six transmembrane domains (TM1–6), 14 intracellular ankyrin repeats, and a long intracellular N-terminal domain. *B*, side, cut-away, top and bottom views of a TRPA1 channel according to electron microscopy reconstructions. Modified with permission from Cvetkov *et al.* (2011); © 2011 The American Society for Biochemistry and Molecular Biology. *C*, the current–voltage relation for TRPA1 channels shows a weak outward rectification at 30°C. The inward and outward currents are both increased by lowering the temperature to 18°C. Modified with from Story *et al.* (2003) with permission from Elsevier (© 2003).

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intracellular [Ca^{2+}] from ∼100 nM (Kuchibhotla *et al.* 2009) to possibly ∼35 nM (a level of cytosolic Ca^{2+} similar to that measured with 13 mM intracellular BAPTA). This reduction in astrocytic baseline intracellular [Ca^{2+}] levels was associated with clathrin-dependent internalization of the GABA transporter GAT3 in astrocytes and with increased amplitude of tonic inhibitory currents and reduced amplitude of GABAergic miniature inhibitory postsynaptic currents (mIPSCs) in INs.

This story provided valuable insights into possible novel roles of TRPA1 channels in a cell type where the expression and function of TRP channels (Pizzo *et al.* 2001; Song *et al.* 2005; Shibasaki *et al.* 2007), and in particular of TRPA channels (Golovina, 2005), had not been clearly resolved. Additionally, it drew attention to a number of potential mechanisms that, unexpectedly, could allow astrocytes to finely tune the activity of specific sets of synapses, rather than non-specifically modulating the activity of large groups of cells, as generally thought. Is this proposed synapse specificity due to the fact that astrocytic processes at IN–IN/IN–PC synapses have distinct structural and functional features or do IN–IN/IN–PC synapses have different sets of molecules that allow them to respond differently to similar changes in astrocytic function?

To answer this question, it is useful to review the currently available information on: (1) heterogeneous subcellular distribution, trafficking and cell regulation of GABA transporters in astrocytes; (2) differences in astrocytic coverage and membrane composition at IN–IN/IN–PC synapses; and (3) differences in (extra)synaptic $GABA_A$ receptor ($GABA_A$-R) composition at IN–IN/IN–PC synapses.

GABA transporters: differences in expression and regulation

GAT3 and GAT1 are the two major types of GABA transporters in the brain: GAT3 is more abundant than GAT1 in astrocytes, whereas GAT1 prevails in neuronal inhibitory terminals (but is also present in astrocytes). In the developing brain, the subcellular distribution of GAT3 and GAT1 in astrocytes differs (Vitellaro-Zuccarello *et al.* 2003). During the first two postnatal weeks, when GABA is depolarizing (Ben-Ari *et al.* 1989; Rivera *et al.* 1999) and when the large extracellular space fraction of the neuropil favours extrasynaptic GABA diffusion, GAT3 is expressed evenly in distal, proximal and perisomatic astrocytic regions, while GAT1 is targeted to distal astrocytic processes close to synaptic contacts (Vitellaro-Zuccarello *et al.* 2003; Beenhakker & Huguenard, 2010). By the end of the third postnatal week, when GABA has become hyperpolarizing and when the structure of the neuropil resembles more closely that of the adult brain, GAT3 acquires its mature pattern of expression, and both GAT1 and GAT3 become confined to distal astrocytic processes and intermediate filaments adjacent to active synapses (Vitellaro-Zuccarello *et al.* 2003). This suggests that GAT3 undergoes more profound regulatory processes than GAT1. Biochemical studies have shown that surface expression of GAT1 in neurons can be regulated by extracellular [GABA] (Gadea & Lopez-Colome, 2001), tyrosine phosphorylation (Law *et al.* 2000) and G protein-coupled (Corey *et al.* 1994; Beckman *et al.* 1999), syntaxin 1A-mediated (Beckman *et al.* 1998) phosphorylation by protein kinase C, but it is not known whether similar modifications also target GAT3. More importantly, there is really no indication that one transporter may have different binding/transport efficiency than the other one at particular sets of synapses. Likewise, even though broad regional differences in the expression level of GAT1/3 have been observed (Engel *et al.* 1998), any direct evidence supporting different expression of these transporters at different sets of synapses is currently missing.

Astrocytic coverage of inhibitory synapses

Previous quantification of astrocytic coverage at excitatory synapses relied on serial section electron microscopy (EM) reconstructions (Ventura & Harris, 1999), but a systematic analysis of astrocytic coverage at inhibitory synapses is not currently available. Getting this information with traditional serial EM reconstructions is feasible, but the technique encounters some issues when one wants to go more into detail and resolve the anatomy on synapses onto specific target cells. It is not an intrinsic limitation of the technique, but is rather due to the fact that the technique is generally used to reconstruct tiny volumes of the neuropil. Identifying synapses onto particular target cells might need the reconstruction of larger volumes and ideally of entire cells. The recent advances in EM-based reconstruction techniques now allow reconstructing larger volumes of tissue (Denk & Horstmann, 2004; Micheva & Smith, 2007; Knott *et al.* 2008). This, combined with the use of cell- (Livet *et al.* 2007) and molecule-specific labelling strategies, may now provide an unprecedented opportunity to understand whether there is any unsuspected heterogeneity in the geometry of different sets of synaptic contacts and in the structure and membrane protein composition of astrocytic processes around them.

Extrasynaptic $GABA_A$ receptors in different cell types

The effects of varying astrocytic GABA transporter expression, function or localization are ultimately determined by the biophysical properties of the $GABA_A$-Rs that are being activated. $GABA_A$-Rs containing the δ

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subunit (δGABA$_A$-Rs) are preferentially expressed peri- and extrasynaptically and are abundant in hippocampal (Wei *et al.* 2003) and cerebellar (Nusser *et al.* 1995) glutamatergic granule cells, not in glutamatergic and GABAergic cells of the hippocampus proper (Wei *et al.* 2003). Accordingly, tonic inhibitory signals, thought to be mediated primarily by extrasynaptic GABA$_A$-Rs, largely rely on activation of δGABA$_A$-Rs in hippocampal (Glykys *et al.* 2008) and cerebellar granule cells (Hamann *et al.* 2002), and on activation of γ_2 and α_5 subunit containing GABA$_{A-}$-Rs in hippocampal stratum radiatum INs (Semyanov *et al.* 2003) and CA1-PCs (Caraiscos *et al.* 2004; Scimemi *et al.* 2005), respectively. A distinguishing feature of γ subunit-containing GABA$_A$-Rs (γGABA$_A$-Rs) and δGABA$_A$-Rs is their steady-state affinity for GABA (higher for δGABA$_A$ than γGABA$_A$-Rs; Saxena & Macdonald, 1996). δGABA$_A$-Rs are sensitive to small changes in extracellular [GABA], but can have a high level of occupancy/desensitization in physiological [GABA] (Scimemi *et al.* 2005; Santhakumar *et al.* 2006; Bright *et al.* 2011; Brickley & Mody, 2012). In contrast, γGABA$_A$-Rs are better detectors of large changes in extracellular [GABA]. An apparently cell-specific effect of astrocytes, like the one reported by (Shigetomi *et al.* 2011), may therefore be consistent with a relatively higher abundance of extrasynaptic γGABA$_A$-Rs in INs. The reason for focusing on extrasynaptic GABA$_A$-Rs is that the subunit composition of synaptic GABA$_A$-Rs in IN/PCs is fairly similar (i.e. γGABA$_A$-Rs associated with α_1, α_2 or α_3 subunits; Farrant & Nusser, 2005). Because synaptic GABA$_A$-Rs are much closer to their presynaptic counterpart than to neighbouring astrocytic processes, they are also likely to be relatively insensitive to subtle functional/structural modifications in nearby astrocytic processes.

Can astrocytes be valuable therapeutical targets?

The ability to selectively dampen the inhibitory tone of INs (or maybe of specific sets of INs) with fairly gross manipulations of astrocytes could provide a remarkable tool to alter the responsiveness of these cells to excitatory inputs and change the balance of inhibition and excitation in the brain (Clarke & Attwell, 2011). However, the types of INs in the brain and the functions they serve are so diverse (Somogyi & Klausberger, 2005) that it is hard to get a good intuition of how changing astrocytic function can ultimately regulate the behaviour of the whole brain, in health and disease. A role of astrocytes in regulating the onset and amplification of epileptic seizures has been previously proposed, possibly due to synchronization of the activity of large neuronal networks via release of glutamate and other modulators of excitatory neurotransmission by astrocytic syncytia (Fellin & Haydon, 2005; Tian *et al.* 2005). Conversely, in pathological conditions associated with glutamate excitotoxicity and neurological damage, astrocytes have been proposed to be good candidate therapeutical targets due to their ability to rapidly remove glutamate from the extracellular space (Bergles & Jahr, 1997; Danbolt, 2001). In an extensive screen of 1040 FDA-approved drugs and nutritionals, Rothstein *et al.* 2005 showed that a number of β-lactam antibiotics that enhance the expression of the glutamate transporter GLT1 in astrocytes also delay the onset of neuronal loss in animal models of amyotrophic lateral sclerosis (Rothstein *et al.* 2005) and improve the survival of neurons and rats after stroke (Lipski *et al.* 2007; Thone-Reineke *et al.* 2008). However, for most neurodegenerative diseases, the potential therapeutic relevance of astrocytes has been related only to their ability to act as scavengers for reactive oxygen species which accumulate in the brain of patients affected by Parkison's and Alzheimer's disease. Despite the fact that multiple lines of evidence suggest the existence of correlations between changes in the physiology of astrocytes and the onset or progression of various disease states, the development of therapeutical approaches specifically aimed at regulating astrocytic function has not been extensively pursued yet. One of the main reasons is that many membrane proteins or signalling cascades present in astrocytes are also functional in neurons, so targeting selectively one or the other cell type, despite its great potential for future clinical applications, has so far remained a challenging task. Of course this trend may change dramatically if specific astrocytic molecules regulating synaptic function are being identified.

An integrated view of astrocytes and neurons

Astrocytes and neurons have often been studied as separate entities, and for a long time the only form of activity that astrocytes were thought to be able of generating were occasional (0.001–0.002 Hz) and slow-decaying (5–160 s) Ca^{2+} waves in cell bodies and thick primary processes (Hirase *et al.* 2004). Recent high-resolution imaging experiments suggest that smaller astrocytic processes generate much more frequent and fast Ca^{2+} signals (Di Castro *et al.* 2011). These fine processes are presumably closer to synaptic contacts, and here the Ca^{2+} signals have been suggested to regulate the probability of transmitter release at excitatory synaptic terminals (Di Castro *et al.* 2011). Other studies also indicate that the functional interactions between astrocytes and neurons may be subject to substantial remodelling (Theodosis *et al.* 2008), for example during development (Tashiro & Kawai, 2007), exposure to enriched environments (Jones *et al.* 1996), hormonal fluctuations (Panatier *et al.* 2006) and, over shorter periods of time, after induction of synaptic plasticity (Wenzel *et al.* 1991; see also Agulhon *et al.* 2010; Henneberger *et al.* 2010). These structural changes

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are likely to have important functional consequences on synaptic function, possibly regulating the ability of synapses to process information independently of each other, and of changing their strength in response to modulation by various neurotransmitter receptor agonists (Min & Nevian, 2012) and co-agonists that astrocytes release (Panatier *et al.* 2006).

The currently emerging view, therefore, is that astrocytes are far from being as silent and static as initially thought (Kuffler *et al.* 1966; Ransom & Goldring, 1973), but may have dynamic, subtle and diverse interactions with neurons. This requires to move from a descriptive to a more mechanistic understanding of astrocytic signalling.

Conclusions

A rapidly emerging body of work suggests that astrocytes might be able to exert synapse-specific effects and have a much finer control of brain function than previously thought. The time may have come to leave behind the long-standing contentions about astrocytes doing nothing or everything in the brain: the challenge now is to define the molecular mechanisms that underlie any dynamic interaction between neurons and astrocytes and how these can be programmed to modulate the activity of specific sets of synapses in particular disease states.

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