Published in final edited form as:

Alcohol Clin Exp Res. 2016 September ; 40(9): 1802–1816. doi:10.1111/acer.13168.

Disentangling the role of astrocytes in alcohol use disorder

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Abstract

Several laboratories recently identified that astrocytes are critical regulators of addiction machinery. It is now known that astrocyte pathology is a common feature of ethanol exposure in both humans and animal models, as even brief ethanol exposure is sufficient to elicit long-lasting perturbations in astrocyte gene expression, activity, and proliferation. Astrocytes were also recently shown to modulate the motivational properties of ethanol and other strongly reinforcing stimuli. Given the role of astrocytes in regulating glutamate homeostasis, a crucial component of alcohol use disorder, astrocytes might be an important target for the development of next generation alcoholism treatments. This review will outline some of the more prominent features displayed by astrocytes, how these properties are influenced by acute and long term ethanol exposure, and future directions that may help to disentangle astrocytic from neuronal functions in the etiology of alcohol use disorder.

Keywords

Astrocyte; gliotransmission; glutamate; alcohol use disorder

Introduction

Alcohol use disorder (AUD) is a complex and chronically relapsing disorder that has a great impact on societies worldwide. Recent research has identified astrocytes as critical regulators of alcohol-intake, and ethanol-induced effects on astrocyte physiology appear to cause pronounced downstream effects on excitability, neurotransmission and neuronal health. This review begins by summarizing basic astrocyte functions prior to discussing the role of astrocytes in maintaining neuronal function during physiological conditions. The

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The authors have no competing or conflicting interests to declare.

objective of the second part of this review is to highlight the impact of acute or long-term ethanol exposure on astrocyte function, astrocyte-neuron crosstalk, and behavior. The last part of the review describes future research directions that could be pursued to more precisely define the role of astrocytes in the etiology of AUD. While we predominately focus on excitatory mechanisms, we briefly cover the role of astrocytes in modulating non-glutamatergic neurotransmission.

Astrocytes maintain extracellular homeostasis and support neurotransmission

Glial cells (astrocytes, oligodendrocytes, and microglia) represent the most abundant cell group in the central nervous system (Sherwood et al., 2006). The star-shaped astrocyte, often characterized by expression of the cytoskeletal protein Glial Fibrillary Acidic Protein (GFAP) or the astrocyte-specific marker ALDH1L1, is the most numerous cell type of the glia family (Figure 1) (Yang et al., 2011). Astrocytes give structural and metabolic support while maintaining homeostasis of the extracellular milieu through clearance of potassium and neurotransmitters. Astrocytes play critical roles in glutamatergic neurotransmission. Astrocytes can regulate excitability through the clearance of glutamate and glycine as well as by the release of gliotransmitters such as glutamate and D-serine (Figure 2) (Hansson et al., 2000, Malarkey and Parpura, 2008). Clearance of extracellular glutamate is primarily mediated via the astrocytic glutamate transporters GLAST/EAAT1 and GLT-1/EAAT2, while glycine is taken up through glycine transporters GlyT1 and GlyT2 (Zhou and Danbolt, 2013, Aroeira et al., 2014). Glutamate and glycine are co-transported with Na^+, which activates the Na^+/K^+-ATPase, thereby promoting astrocytic metabolism while simultaneously reducing neuronal excitability via clearing K^+ from the extracellular space (Kirischuk et al., 2015). Following astrocytic uptake, glutamate is converted to its non-excitable precursor glutamine by glutamine synthetase; glutamine is subsequently released to the extracellular space and taken up by neurons to be converted back into glutamate (Figure 2) (Yudkoff et al., 1993). This astrocyte-dependent glutamate-glutamine cycle is required to maintain active neurotransmission at excitatory terminals, and may also play a vital role in GABAergic neurotransmission since glutamate can be converted into GABA via glutamate decarboxylase (Kaczor et al., 2015, Tani et al., 2014). The importance of astrocytes in maintaining glutamatergic tone are highlighted in mice lacking the glutamate transporter GLAST. These mice show reduced synaptic plasticity and phenotypic abnormalities thought to model the negative and cognitive symptoms of schizophrenia (Karlsson et al., 2012, Karlsson et al., 2008, Karlsson et al., 2009).

The end feet of astrocytic processes encapsulate capillaries, allowing exchange of small molecules including ions, glucose, and amino acids between smaller blood vessels and the brain tissue. This anatomical arrangement allows astrocytes to provide biochemical, metabolic, and structural support to surrounding neurons. For example, the intercellular trafficking of glucose through astroglial networks is increased during energy consuming processes (Magistretti and Pellerin, 1999, Magistretti et al., 1999, Rouach et al., 2008). Astrocytic glycogen breakdown into lactate and subsequent transport of lactate into neurons is required for long-term memory formation (Suzuki et al., 2011), and astrocytes may also

support synaptic plasticity mechanisms through the release of thrombospondins and gliotransmitters (Crawford et al., 2012, Henneberger et al., 2010). Astrocytes can also modulate neurotransmission via specialized extracellular matrix components known as perineuronal nets that can be found near glial processes. Perineuronal nets are a form of molecular scaffold deposited at the interface between the astrocyte and neuronal synapse (Dzyubenko et al., 2016). Thus, astrocytes appear to play a key role in regulating the formation, maturation, stability, and maintenance of neuronal synapses (Slezak and Pfrieger, 2003). Malfunctioning of astrocytes may thus not only cause instability of neuronal networks, but also impinge upon the intrinsic ability of the central nervous system to appropriately adapt to external influences due to impaired synaptic plasticity.

Calcium signaling through the astrocytic network

Electrophysiological recordings and functional analyses have demonstrated that astrocytes are extensively interconnected through gap junction channels, built up by connexins (Giaume et al., 1991, Adermark and Lovinger, 2008). Several different connexins have been identified, but connexin-43 is the primary subtype interconnecting astrocytes. Because gap junction channels allow diffusion of small molecules, such as Ca^{2+} and inositol (1,4,5) trisphosphate (IP_3), these channels represent a direct pathway for bidirectional electrical coupling and metabolic signaling (Blomstrand et al., 1999a) while also promoting coordinated activation of neuronal networks and synaptic plasticity (Pannasch et al., 2011, Chever et al., 2014, Chever et al., 2016). Connexin-43 is highly expressed by astrocytes located at the blood-brain barrier (BBB) interface (Nagy and Rash, 2000) where they play critical roles in the clearance of glutamate and potassium. Gating of gap junction channels is influenced by neuronal activity as well as ion concentrations within the astrocyte, indirectly resulting in neuronal control of intercellular signal propagation through the astrocytic syncytium (Blomstrand et al., 1999b).

A functional astrocytic network may thus be essential for precise information transfer between neurons, and reduced function of astrocyte connexins 30 and 43 has been associated with severe psychiatric conditions such as increased risk of suicide (Ernst et al., 2011, Nevin, 2012). Connexins, and the closely related pannexins, can also form hemichannels, which are gap junction channel-like pores that open to the extracellular space instead of directly coupling neighboring cells (Iglesias et al., 2009). Hemichannels can release gliotransmitters such as adenosine, glutamate, and D-serine, and these channels are tightly regulated during physiological conditions. However, dysregulation of hemichannel properties may occur in response to injury or inflammatory factors, resulting in relatively nonselective opening of large membrane pores (Montero and Orellana, 2015, Orellana and Stehberg, 2014, Kim et al., 2016). It should be additionally noted that gliotransmitters can also be released through the two-pore domain potassium channel TREK-1, and the Ca^{2+}-activated anion channel Best1; these channels can differentially influence neighboring neurons due to discrete channel distribution in distinct astrocytic subdomains (Woo et al., 2012).

Although astrocytes do not generate action potentials, they show intrinsic cellular excitability based on intracellular Ca^{2+} transients (Figure 3). These Ca^{2+} variations may

arise spontaneously or as a consequence of different stimuli including depolarization of neighboring neurons and/or neurotransmitter binding. Astrocytic Ca^{2+} transients produce a nonlinear input–output response that may tune neuronal activity via gliotransmitter release and changes in metabolic support (Navarrete et al., 2012, Schummers et al., 2008, Takata et al., 2011). Transient astrocytic Ca^{2+} signals are primarily mediated through Gαq-coupled G-protein receptor-mediated activation of phospholipase C (PLC) and subsequent formation of IP_3, which leads to release of Ca^{2+} from internal stores and, to a lesser extent, Ca^{2+} influx through voltage-dependent and in-dependent Ca^{2+} channels (Verkhratsky and Kettenmann, 1996) (Figure 3). The amplitude and intracellular propagation of Ca^{2+} signals are partially modulated by activity of different synaptic inputs, but astrocytes also display cell-specific intrinsic properties that regulate excitability (Perea and Araque, 2005). Elevated levels of intracellular IP_3 or $[Ca^{2+}]_i$ induce the release of gliotransmitters, and neuronal activity has been shown to mediate gliotransmitter-release in several brain areas (Nedergaard, 1994, Wang et al., 2013, Zorec et al., 2012), including in human brain preparations (Navarrete et al., 2013). In addition to calcium signaling, neuronal activity may also evoke astrocytic sodium transients that can spread through the astrocytic syncytium (Rose and Chatton, 2015). Increased $[Na^+]_i$ can influence the driving force for GABA and glutamate uptake, as well as increase the release of gliotransmitters, thereby modulating the spatio-temporal profiles of transmitter levels in the extracellular space (Kirischuk et al., 2015). Gliotransmitters act on pre-, post-, and extrasynaptic targets, thereby producing a complex reciprocal communication between astrocytes and neurons. Interfering with astrocytic Ca^{2+} homeostasis, or IP_3 signaling, disrupts gliotransmitter-release, and perturbs synaptic transmission and plasticity, supporting the hypothesis that astrocytes play crucial roles in activity-dependent adaptations of neural circuits (Henneberger et al., 2010, Takata et al., 2011).

Astrocyte-neuron crosstalk occurs in a pathway specific manner

A single astrocyte can make contact with thousands of synapses. This enables the astrocyte to serve as an active processing bridge for synaptic interaction and crosstalk between synapses that have no direct neuronal connectivity (Bushong et al., 2002, Covelo and Araque, 2015) (Figure 4). Moreover, astrocytes can interact with neurons at multiple levels including through direct communication at the synapse (Perea et al., 2014). Subtypes of astrocytes can form direct synaptic contacts with axons (Bergles et al., 2010), while others ensheathe pre- and postsynaptic terminals, thereby constituting an integral element of the neuronal synapse (Rossi, 2015, Halassa et al., 2007). Astrocyte process motility often correlates with movement in associated dendritic protrusions, and this structural relationship undergoes activity-dependent changes with consequences on synaptic regulation and metaplasticity (Perez-Alvarez et al., 2014). At the site of synaptic contact on stratum radiatum, hippocampal, and cerebellar parallel fiber neurons, astrocyte processes can preferentially appose postsynaptic spines. This structural arrangement is hypothesized to favor fast presynaptic autoreceptor feedback while preserving specificity of the postsynaptic input (Lehre and Rusakov, 2002).

Astrocytes have been shown to discriminate activity of synaptic terminals belonging to specific neural circuits (Perea and Araque, 2005). This study found that astrocytes

specifically respond to activation of discrete axon pathways that release different neurotransmitters, and that astrocytes distinguish between synapses belonging to different axon pathways that use the same neurotransmitter. In addition, because of intrinsic cellular properties, the astrocytic Ca^{2+} signal is bidirectionally modulated through interaction of different synaptic inputs. Together, these data indicate that astrocytes can integrate synaptic information in a pathway-specific manner (Perea and Araque, 2005). Thus, despite anatomically apposed neurons participating in dissimilar networks, the neighboring astrocytes can help coordinate activity of neurons within the same network (Martin et al., 2015, Perea and Araque, 2005)).

Modulation of astrocyte number by ethanol

Astrocytes are a major target of ethanol in the human brain (de la Monte and Kril, 2014, Butterworth, 1995). Astrocyte adaptations are commonly measured by immunoreactivity of their major structural filament GFAP (Sofroniew and Vinters, 2010). For example, density (cells per region volume) of GFAP-expressing ($GFAP^+$) astrocytes is lower in the prelimbic prefrontal cortex of ethanol-naive rats bred for high ethanol preference over water (Miguel-Hidalgo, 2005). Moreover, a reduction in cell number has been reported for several glial cell types in animal models of Fetal Alcohol Syndrome (FAS) (Wilhelm and Guizzetti, 2015). In contrast, increased $GFAP^+$ astrocyte number is observed throughout the rat cerebral cortex following repeated gavage of a high ethanol dose or ethanol-containing diet (Udomuksorn et al., 2011, Dalcik et al., 2009). Astrocyte density also increases in the prelimbic cortex of ethanol-preferring rats during ethanol abstinence (Miguel-Hidalgo, 2006). Importantly, in standard outbred rats, $GFAP^+$ astrocyte density changes are dependent on time, region, and method of ethanol administration (Bull et al., 2015). For example, after 3 weeks abstinence, $GFAP^+$ astrocyte density decreases in prelimbic and orbitofrontal prefrontal cortex of rats that had continuous access to ethanol in the home cage, whereas anterior cingulate and orbitofrontal cortex astrocyte density decreases in rats abstinent from operant ethanol self-administration. No change in astrocyte density was seen in the infralimbic cortex or after intermittent access to ethanol in the home cage. Lower astrocyte density is also seen in the dorsolateral and orbitofrontal prefrontal cortex (Miguel-Hidalgo et al., 2002, Miguel-Hidalgo et al., 2006) and hippocampus (Korbo, 1999) of human alcoholics. Thus, astrocyte number is sensitive to ethanol in a region and exposure-dependent manner, and the importance of these changes is only beginning to be unraveled. For example, nucleus accumbens astrocytes also display time-, region-, and treatment-dependent changes in astrocyte density that can correlate with ethanol-seeking behavior (Bull et al., 2014). More specifically, $GFAP^+$ astrocyte density in the nucleus accumbens shell increases after either 24 hours or 3 weeks abstinence from intermittent access to ethanol in the home cage. In the nucleus accumbens core, astrocyte density changes were only seen after 3 weeks abstinence, and this increase positively correlated with the motivation to resume ethanol self-administration after abstinence (Bull et al., 2014). Interestingly, astrocyte density increases in nucleus accumbens core after ethanol self-administration, yet decreases after sucrose self-administration (Bull et al., 2014). Ethanol might thus exert long-lasting and widespread perturbations in neurotransmission given these region-specific changes in astrocyte number during protracted abstinence. It should be noted, however, that reactive astrocytes are

characterized by upregulated GFAP, and the increase in $GFAP^+$ astrocyte number could in this respect also reflect a long-lasting inflammatory response to ethanol (Ben Haim et al., 2015). Thus, while it is currently unknown if changes in astrocyte density reflect general inflammatory processes or alterations in innate immunity, it is clear that astrocyte density can be related to the motivation to resume ethanol self-administration after abstinence.

Ethanol induced inflammation and glia

Ethanol intoxication is associated with oxidative stress, pro-inflammatory mediators and cytokine production, which may contribute to neuronal injury (He and Crews, 2008). Unlike neurons, microglia and to a lesser extent astrocytes mediate innate immunity, and both microglia and astrocytes express Toll-like receptors (TLRs). The TLRs are activated by a wide variety of microbial components and elicit innate immune responses (Kawai and Akira, 2007, Beutler et al., 2006). Distinct inflammatory signaling appears to occur in response to ethanol intoxication, causing the secretion of pro-inflammatory molecules from glial cells, predominately the cytokines interleukin-1 β (IL-1β) and tumor necrosis factor α (TNF-α) as well as chemokines (Blanco et al., 2004) (Figure 5). This is followed by release of inflammatory mediators such as cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS) via activation of nuclear factor kappa B (NF-κB), mitogen-activated protein kinase, and activator protein 1 (Akira and Takeda, 2004). The TLRs, which belong to the IL-1 receptor family, appear to be a key receptor mediating this signaling cascade. TLRs promote secretion of pro-inflammatory molecules in response to ethanol in a biphasic manner (Blanco et al., 2004, Blanco et al., 2005), and some of the acute effects of ethanol on GABAergic signaling are mediated by TLR4 (Bajo et al., 2014). Moreover, chronic ethanol consumption has been shown to impair proteolytic pathways, and the immune response mediated by TLR4 receptors (Pla et al., 2016). Since inflammatory processes mediated through TLRs also have been implicated in the development of neurological disorders such as Alzheimer's disease (Walter et al., 2007), TLR4 recruitment in response to ethanol abuse could play a key role in ethanol-mediated neurodegeneration (Floreani et al., 2010). Further supporting a role for immune signaling in AUD are data from adolescent TLR4 knockout mice, which in contrast to wild type littermates, do not escalate ethanol preference over time (Montesinos et al., 2016). These mice also appear protected from ethanol-induced elevation of BDNF and FosB in mPFC, which might play a role in epigenetic transformation (Montesinos et al., 2016). Taken together, ethanol appears to enhance glial inflammatory signaling, which in turn may further promote the escalation of ethanol intake and subsequent neurodegeneration. At the same time, ethanol may also increase in the release of glutathione from astrocytes, which protects neurons from oxidative stress and apoptosis (Rathinam et al., 2006). Importantly, these processes have the potential for therapeutic intervention. For example, the dietary supplement N-acetylcysteine is essentially a prodrug that reduces oxidative stress by facilitating astrocytic glutathione production. In short, N-acetylcysteine is taken up by the astrocytic cysteine-glutamate antiporter prior to enzymatic conversion into glutathione. Thus, N-acetylcysteine increases extracellular glutamate while reducing oxidative stress. Increasing extracellular glutamate with N-acetylcyesteine has been shown to reduce relapse-like behavior for a number of substances (Baker et al., 2003, Bowers et al., 2016, Ramirez-Nino et al., 2013, Reissner et al., 2015). However, N-acetylcysteine failed to

block cue-primed reinstatement of ethanol-seeking behavior in rats (Weiland et al., 2015). Interestingly, N-acetylcysteine restores GLT-1 expression following ethanol exposure (Roberts-Wolfe and Kalivas, 2015). Thus, N-acetylcysteine, and other reagents such as ceftriaxone that restore GLT-1, may exert a therapeutic effect for AUD via a number of mechanisms (Ferreira Seiva et al., 2009, Roberts-Wolfe and Kalivas, 2015).

Selective effects of ethanol on astrocytic calcium signaling

Increased $[Ca^{2+}]_i$ is both necessary and sufficient for most forms of astrocytic gliotransmitter release (Parpura et al., 1994). Thus, it is significant that physiologically relevant ethanol concentrations increase astrocytic $[Ca^{2+}]_i$ in a manner that parallels release of several gliotransmitters including glutamate, glutamine, and taurine (Salazar et al., 2008, Kimelberg et al., 1990, Kimelberg et al., 1993, Allen et al., 2002, Fonseca et al., 2001). Although ethanol does not enhance overall IP_3 formation (Simonsson et al., 1989), ethanol can induce intracellular calcium transients in a subset of astrocytes (Allansson et al., 2001). In addition, ethanol pre-treatment appears to affect calcium responses in a neurotransmitter-dependent manner, where ethanol stimulates serotonin induced inositol metabolism, while suppressing muscarinic receptor-mediated calcium responses (Catlin et al., 2000, Simonsson et al., 1989). Ethanol might thereby indirectly shift the balance between different signaling pathways. Importantly, ethanol acutely suppresses gap junction permeability in defined brain regions, and may thus further impinge upon signal processing in a circuit specific manner (Adermark and Lovinger, 2006, Adermark et al., 2004). Additionally, immunoreactive puncta of the gap junction protein connexin-43 is reduced in post mortem samples from subjects with AUD, supporting a role for altered connectivity or hemichannel-based communication in the pathophysiology of alcoholism (Miguel-Hidalgo et al., 2014). Thus, it is interesting that subpopulations of astrocytes are known to have sexually dimorphic roles (Collado et al., 1995, Mong and McCarthy, 2002), which can include regulation of the gap junction protein connexin 43 (Gulinello and Etgen, 2005), and other gene expression changes in response to ethanol that are hypothesized to underlie increased vulnerability to ethanol-mediated neurotoxicity in females (Wilhelm et al., 2015). Ethanol can also modulate the biophysics of other ion channels expressed in astrocytes that are related to the connexins. For example, unlike the connexins, pannexin-containing hemichannels are opened by both ethanol and increased $[Ca^{2+}]_i$ (Locovei et al., 2006, Scemes et al., 2007, Dahl and Harris, 2008). Thus, ethanol can modulate the ability of astrocytes to communicate by regulating both calcium transients and channel permeability.

Ethanol affects astrocytes in a manner that can disrupt the balance between excitation and inhibition

Alteration of glutamatergic neurotransmission is a hallmark of alcohol dependence, and ethanol can cause glutamatergic dysfunction by impinging upon important astrocytic functions (Smith, 1997, Ayers-Ringler et al., 2016, Dahchour et al., 2000, Ding et al., 2013, Mulholland et al., 2009). As noted earlier, a primary function of astrocytes is glutamate clearance via the glial excitatory amino acid transporters GLAST/EAAT1 and GLT-1/EAAT2. Because greater than 70% of glial processes immunolabeled for GLAST or GLT-1

were found to ensheathe dendritic spines or excitatory synapses (Cholet et al., 2002), it is important to note that ethanol can modulate expression of these transporters. For example, both GLAST and GLT-1 expression increased in organotypic cortical cultures chronically treated with ethanol (Zink et al., 2004). This upregulation may explain why glutamate uptake is increased following chronic treatment of organotypic cortical cultures with ethanol (Smith, 1997). Upregulation of GLT-1, but not GLAST, has also been found in the rat nucleus accumbens following voluntary ethanol consumption (Alhaddad et al., 2014, Das et al., 2015, Sari et al., 2016). Importantly, modulating expression and/or function of glial excitatory amino acid transporters affects AUD-associated phenotypes. For example, mice with a homozygous GLAST deletion do not exhibit a conditioned place preference for ethanol, and these mice also show reduced voluntary ethanol intake compared to wild type littermates (Karlsson et al., 2012). A similar effect was seen after inhibition of glial excitatory amino acid transporters via intracerebral ventricular infusion of the GLT-1 blocker dihydrokainic acid (Smith et al., 2014). On the other hand, mice with a genetic deletion of the circadian clock gene Per2, show reduced GLAST expression, elevated extracellular levels of glutamate in the nucleus accumbens, and increased ethanol intake (Spanagel et al., 2005). However, interpretation of this finding is complicated by the fact that, when compared to wild type mice, GLAST levels were only suppressed at one specific time point over the day.

Glutamate uptake can also be regulated by other transmitters that shape ethanol-responsive behaviors, such as the gliotransmitter adenosine. Adenosine and purinergic signaling regulate numerous vital biological processes in the central nervous system, such as circadian rhythmicity, sleep, and energy homeostasis (Lindberg et al., 2015). Ethanol increases extracellular adenosine levels, and purinergic receptors have been proposed as a novel target for the development of therapeutics to treat AUD (Franklin et al., 2014). Activation of the adenosine A1 receptor (Wu et al., 2011) or the astrocytic equilibrative nucleoside transporter 1 (ENT1) (Wu et al., 2010) decreases GLT-1 mRNA expression and glutamate uptake (Figure 6). At the same time, acute ethanol inhibits adenosine uptake via ENT1 (Nam et al., 2012), and knockdown of ENT1 expression has been shown to prevent ethanol-induced elevation of GLT-1 (Wu et al., 2010). The acute increase in adenosine seen following ethanol exposure partly mediates the ataxic and hypnotic effects of acute ethanol, but adenosine is also associated with cellular tolerance and dependence (Diamond et al., 1991), and insomnia can develop as adenosine levels decrease following chronic ethanol (Ruby et al., 2014). Importantly, adenosine also exerts a complex effect on GABAergic neurotransmission by enhancing astrocyte GABA uptake via adenosine A2A receptors, while inhibiting uptake via A1 receptors (Cristovao-Ferreira et al., 2013). In addition, purinergic receptor activation may lead to disruption of the Na^+ gradient, thereby suppressing high-affinity uptake of both GABA and glutamate (Barros-Barbosa et al., 2015) (Figure 6).

Besides changes in glutamatergic neurotransmission, ethanol also has pronounced effects on GABAergic neurotransmission. In vivo electrophysiology has shown that ethanol acutely suppresses GABAergic neuron firing frequency in the nucleus accumbens (Burkhardt and Adermark, 2014), and that voluntary ethanol intake leads to a long-lasting decrease in GABAergic neurotransmission in the striatum (Adermark et al., 2013, Wilcox et al., 2014). In the ventral tegmental area, ethanol exerts subregion-specific effects on GABAergic

neuron activity (Guan et al., 2012). Research aimed at elucidating the role of astrocytes in ethanol-induced modulation of GABAergic neurotransmission is sparse. But, astrocytes express GABA receptors, and astrocytes may also release GABA, which could be sensitive to ethanol exposure (Lee et al., 2011, Yoon et al., 2014, Itzhak et al., 1993). In fact, the metabolic inhibitor fluorocitrate, which is preferably taken up by astrocytes, has been shown to enhance glutamatergic neurotransmission in the supraoptic nucleus by reducing the release of taurine from astrocytes (Choe et al., 2012). In addition, unpublished observations from our laboratory suggest that fluorocitrate increases excitation in the striatum by decreasing the firing frequency of inhibitory neurons, indicating that astrocytes also exert a powerful control over GABAergic neurotransmission. These data may be significant given that microinjection of fluorocitrate into the rat prefrontal cortex increased ethanol preference (Miguel-Hidalgo et al., 2009). Taken together, astrocytes appear to modulate the conditioned positive reinforcing effects of ethanol via complex regulation of uptake and release of a variety of neurotransmitters (Lee et al., 2013, Ruby et al., 2014) (Figure 6).

Ethanol-mediated cell swelling in astrocytes

Extrasynaptic volume transmission critically depends on the morphological and functional features of astrocytes, which can swell and shrink during physiological and pathological activity as a result of changes in osmolarity, or intensified neuronal activity (Fuxe et al., 2012). Changes in astrocytic cell volume may not only affect extrasynaptic homeostasis, but also size and geometry of the extracellular space, which can alter diffusion of neuroactive substances (Hansson and Ronnback, 2003, Vargova and Sykova, 2014). Glutamate has been shown to increase astrocytic cell volume in a complex manner that depends on the transport of K^+, Na^+ and Ca^{2+} over astrocytic cell membranes (Figure 7) (Bender et al., 1998, Schneider et al., 1992). Acute administration of ethanol can also induce astrocyte swelling in a manner that is sensitive to inhibition of the $Na^+/K^+/2Cl^-$-co-transporter or the Na^+/K^+-ATPase, suggesting that similar mechanisms might apply for both glutamate- and ethanol-induced swelling (Adermark et al., 2011c, Allansson et al., 2001, Aschner et al., 2001, Vargova and Sykova, 2014). Interestingly, connexin-43 containing gap junction channels appear to regulate astrocyte cell volume, and ethanol induced suppression of gap junction permeability also involves changes in sodium homeostasis (Chever et al., 2014). Maintaining intracellular sodium homeostasis might thus be crucial for sustained astrocytic function during intense neuronal activity and during ethanol exposure.

Rapid swelling and regulatory volume decrease is facilitated by astrocytic water channels containing aquaporin-4 (Figure 7) (Kuppers et al., 2008, Risher et al., 2009). Aquaporin-4 has been linked to reward-related motivational processes including alcohol consumption (Lee et al., 2013) and extracellular dopamine concentration. Specifically, the expression of aquaporin-4 correlates with dopamine levels in the nucleus accumbens (Kuppers et al., 2008), and inhibition of cell swelling suppresses ethanol induced dopamine release (Adermark et al., 2011a). Another important osmoregulator is the gliotransmitter taurine, which can be released from astrocytes during cell swelling and subsequently activate ligand gated ion channels such as NMDA, glycine, or $GABA_A$ receptors (Adermark et al., 2011b, Ericson et al., 2011, Ericson et al., 2006). In fact, this release of taurine appears to be vital for ethanol-induced dopamine release in the nucleus accumbens of rat (Ericson et al., 2011).

Although better known for its role in modulating glutamatergic neurotransmission, acamprosate (Campral®), is a homotaurine that can increase nucleus accumbens dopamine by glycine receptor activation (Chau et al., 2010a, Chau et al., 2010b). Thus, the effectiveness of acamprosate in treating AUD may stem from both glutamatergic and dopaminergic mechanisms. Interestingly, pharmacological inhibition of either the dopamine D1 or D2 receptor facilitates gap junction permeability and astrocyte calcium excitability (Bosson et al., 2015), indicating that dopamine, by itself, may further promote taurine release. Thus, astrocytes appear to play a key role in supporting the dopamine elevating property of ethanol, which is believed to be important for the conditioned reinforcing properties of commonly abused substances.

Disentangling the role of astrocytes in alcohol use disorder

Because neurons and astrocytes express an overlapping array of receptors and channels, current approaches do not facilitate disentangling the role of astrocytes in mediating complex phenomena with traditional pharmacological approaches. However, through the use of optogenetic and chemogenetic approaches, unexpected roles of astrocytes have been recently uncovered. For example, mimicking pH-evoked Ca^{2+} responses, by optogenetic stimulation of astrocytes expressing channelrhodopsin-2, has been shown to trigger robust respiratory responses *in vivo* via an ATP-dependent mechanism (Gourine et al., 2010). An alternative approach to define the role of specific cell populations and circuits in complex phenomena are G-protein coupled receptors that have been modified such that they are activated by an otherwise physiologically inert ligand (Designer Receptors Exclusively Activated by Synthetic Ligand; DREADD) (Armbruster et al., 2007). Responding for intracranial self-stimulation (ICSS) has been shown to be facilitated by activation of astrocytes expressing DREADDs coupled to $G\alpha q$, revealing that astrocytes in the nucleus accumbens can regulate ethanol-seeking behavior (Bull et al., 2014). Specifically, $G\alpha q$-DREADD activation allows astrocyte-selective increases in $[Ca^{2+}]_i$, which is both necessary and sufficient for gliotransmitter release from astrocytes. Stimulation of nucleus accumbens core astrocytes in this manner decreases the motivation of abstinent rats to resume ethanol self-administration. Intriguingly, because $G\alpha q$-DREADD-mediated astrocyte stimulation left-shifted the frequency-effect curve for intracranial self-stimulation, astrocyte stimulation may have reduced the motivation to self-administer ethanol by facilitating reward-associated phenomena (Bull et al., 2014). Subsequently, stimulation of nucleus accumbens astrocytes via Gq-DREADD activation was found to increase extracellular glutamate and reduce the reinstatement of cue-induced cocaine-seeking behavior (Scofield et al., 2015). Thus, selective astrocyte stimulation could be used to abate behaviors associated with drug abuse and these approaches will likely be facilitated by the identification of glia specific genes (Nwaobi et al., 2016) that could be exploited for therapeutic benefit.

Conclusion and future directions

Here, we reviewed a rapidly expanding literature uncovering the critical roles that astrocytes play in modulating neurotransmission with relevance to AUD. Ethanol is well known to interrupt blood brain barrier integrity and to impart long-lasting perturbations in neuronal function. More recently, increasing attention is being given to the ability of glial cells, in

particular astrocytes, to actively respond to and modulate neuronal communication and ultimately impact behavior. We now know that astrocyte number is altered following ethanol administration and that density of the astrocyte population positively correlates with the relapse-like motivation to self-administer ethanol. While the precise mechanisms underlying this relationship remain unknown, it may stem from altered diffusion or uptake of neuroactive substances or by altered bidirectional communication between astrocytes and neurons. Moreover, we know that several AUD-associated phenotypes can be influenced by manipulating astrocyte function including ethanol preference, self-administration, and conditioned reinforcement. Because astrocytes play major roles in regulating extracellular glutamate and ethanol can regulate many forms of astrocytic glutamate uptake, release, and metabolism, astrocytes likely exert many of their effects on AUD-associated phenotypes via glutamate. AUD-associated phenotypes are also critically regulated via the astrocytic role in modulating inflammation and neurotrophic factors. Moreover, an elegant series of reports established that the ethanol-mediated release of the astrocytic osmoregulator taurine regulates ethanol-mediated increases in nucleus accumbens dopamine. Thus, akin to the role of astrocytes in responding to and modulating neurotransmission, astrocytes respond to ethanol and mediate many ethanol effects at the molecular, cellular, and behavioral levels.

A focus of future astrocyte addiction research will undoubtedly center around capitalizing upon therapeutic potential. Given that astrocytes and neurons express a similar complement of receptors and channels as neurons, targeting astrocytes with traditional pharmaceutics is unlikely, but capitalizing on astrocyte-specific gene expression (Nwaobi et al., 2016) will likely facilitate these efforts. Studies outlined above indicate that AUD-like behaviors can be reduced by employing innovative approaches that selectively manipulate astrocytes. Indeed, novel strategies using engineered receptors activated by synthetic ligands (DREADDS) or by light (optogenetics) have significantly contributed to a new era of brain research that allows for precise experimental manipulation of cellular and circuitry activity, and increasingly these techniques are being applied to non-neuronal cells.

Future research directions will likely include novel approaches that identify and target astrocyte subtypes, as it is not uncommon for astrocytes to exhibit functional heterogeneity even within brain region (Adermark and Lovinger, 2008, Isokawa and McKhann, 2005). Identifying these subpopulations will probably involve imaging using a combination of genetically encoded calcium or voltage sensors such as glial specific gcAMP or ArcLight-like imaging (Jackson and Robinson, 2015, Lee et al., 2011), via either implanted fiber optics or cranial windows, in awake behaving animals. Other novel approaches may interface with brain energetics via modulating neurovascular coupling (Robinson and Jackson, 2016), or cell-type-specific gene silencing (Merienne et al., 2015).

In conclusion, it is clear that astrocytes, quiescent cells that until fairly recently were relegated to performing purely homeostatic functions, are actively shaping the pathophysiology of ethanol abuse and relapse. Precisely defining the molecular milieu of these astrocytes will lead to targeted and highly efficacious therapeutics to combat AUD.

Acknowledgments

This work was supported by Stiftelsen Psykiatriska Forskningsfonden (LA), the Swedish Medical Research Council (LA), the Swedish Brain foundation (LA), the Alcohol Research Council of the Swedish Alcohol Retailing Monopoly (LA), Åke Wibergs stiftelse (LA), ABMRF/The foundation for Alcohol Research (MSB), and NIH/NIAAA P50 AA022537 (MSB).

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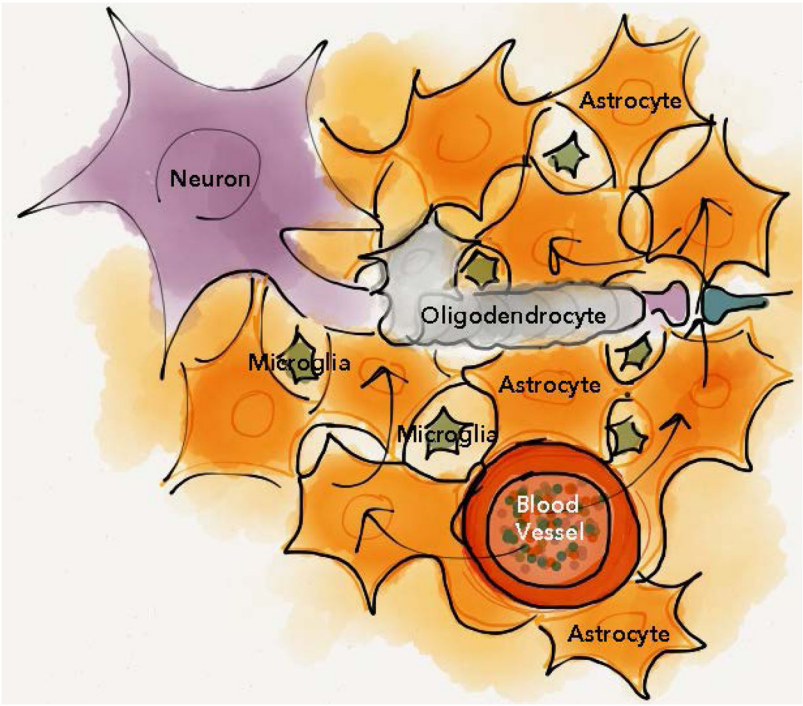

Figure 1. Glial cells provide metabolic support while buffering the extracellular milieu

The glia cell family is pivotal for neuronal function, and astrocytes are the most numerous glial cells in mammalian brain. By enseathing blood vessels, astrocytes can serve as a conduit for delivery of energetic metabolites to distal neurons during energy consuming processes in addition to buffering excess central nervous system ions and small molecules. In the mature brain, oligodendrocytes increase conduction velocity of larger nerve fibers by enseathing axons between nodes of Ranvier. Microglia, and to a limited extent astrocytes, provide innate immune responses. Arrows represents the flow of nutrients that is taken up by astrocytes from the blood vessels, and transported to surrounding cells.

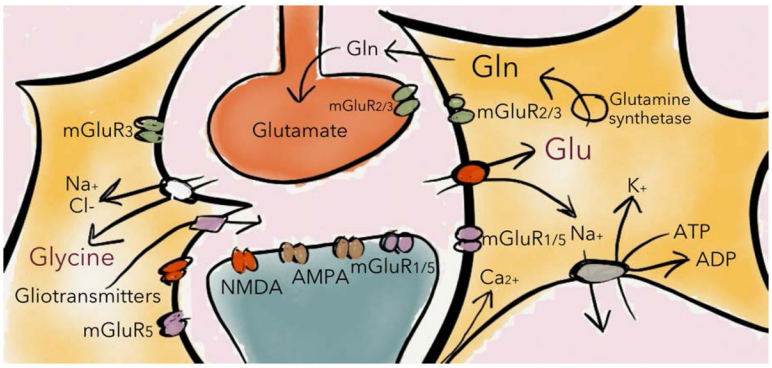

Figure 2. Astrocytes control excitatory neurotransmission

Astrocytes regulate glutamatergic signaling on multiple levels. Not only are astrocytes primarily responsible for clearing glutamate in the brain, they also take up glycine, which is a co-agonist for the NMDA receptor. In addition, astrocytes release gliotransmitters such as D-serine and glutamate, which could further influence excitatory transmission especially at ionotropic NMDA receptors. See text for further details. Glu=glutamate, Gln=glutamine, mGluR=metabotropic glutamate receptors, AMPA/NMDA=ionotropic glutamate receptors, ADP=adenosine diphosphate.

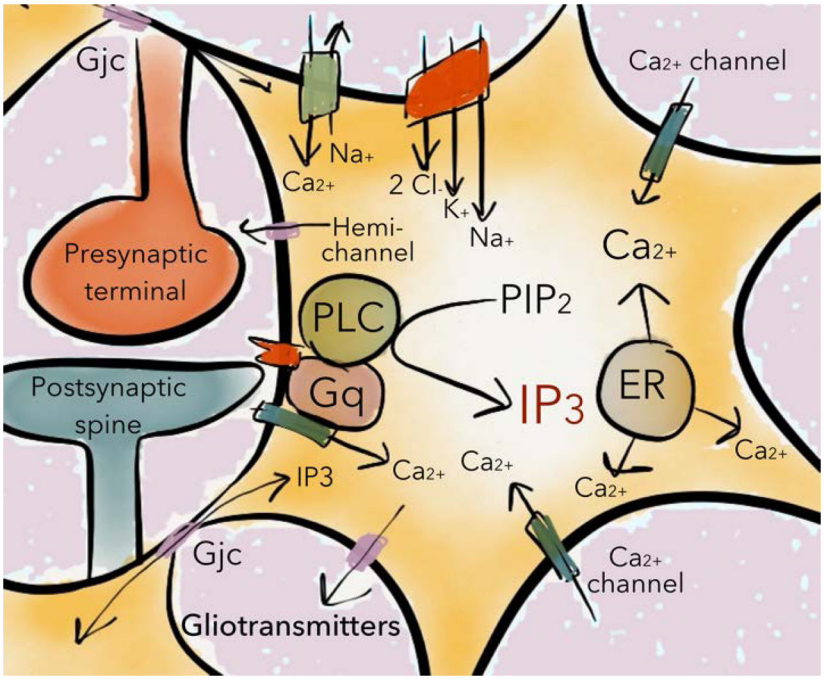

Figure 3. Receptor activated calcium signaling in astrocytes

Astrocytic calcium transients are primarily mediated through the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol (1,4,5) trisphosphate (IP3) following activation of G-protein coupled receptors. IP3 promotes the release of Ca2+ predominately from intracellular pools (endoplasmatic reticulum; ER), into the cytoplasm, and influx of calcium through calcium channels primarily activated by changes in membrane potential. Following formation/release of IP3 or Ca2+, these signaling molecules may spread to surrounding astrocytes through gap junction channels (Gjc) and lead to coordinated release of gliotransmitters.

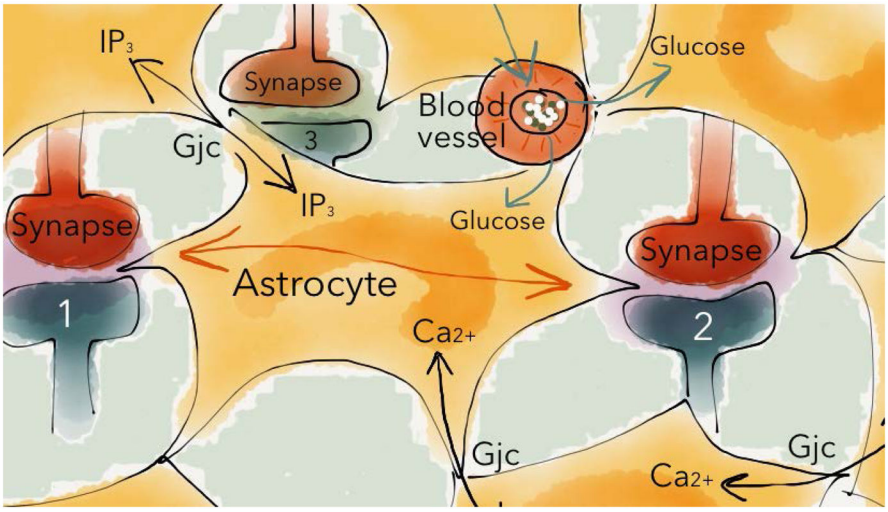

Figure 4. Pathway specific integration of synaptic information

Astrocytes are important information processors in the neural network, and astrocytes may support and regulate neurotransmission in a pathway specific manner (for instance interacting with synapse 1 and 2 but not 3). Neuronal activity evokes complex intrinsic changes in intracellular Ca2+ that may spread through the astrocytic network, and provoke the release of gliotransmitters at distal synapses. One astrocyte may be in contact with thousands of synapses, enabling synaptic interaction without direct neuronal connectivity, as shown between synapse 1 and 2. See text for further details. Gjc=gap junction coupling.

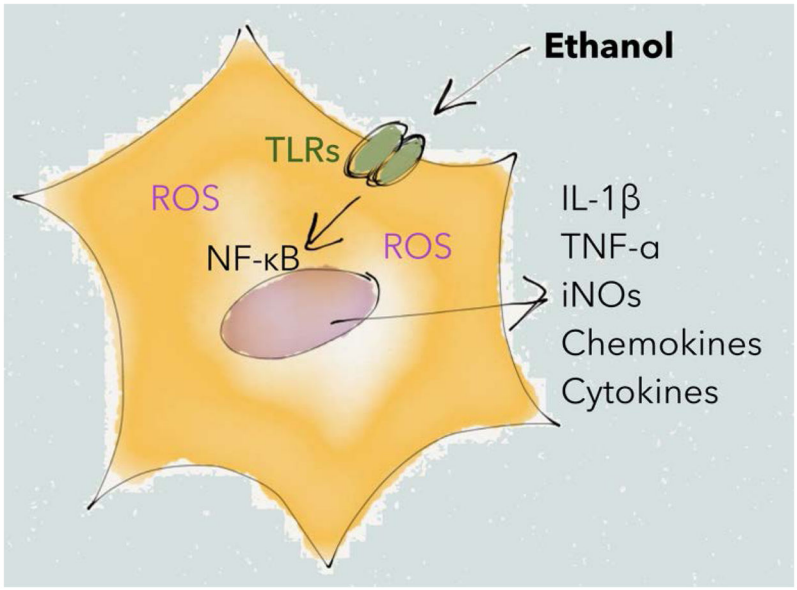

Figure 5. Immune signaling during ethanol exposure

Simplified schematic showing how ethanol exposure can promote signaling via Toll like receptors (TLRs), leading to activation of nuclear factor kappa B (NF-κB), secretion of pro-inflammatory cytokines and chemokines, and elevated levels of Reactive Oxygen Species (ROS). See text for further details.

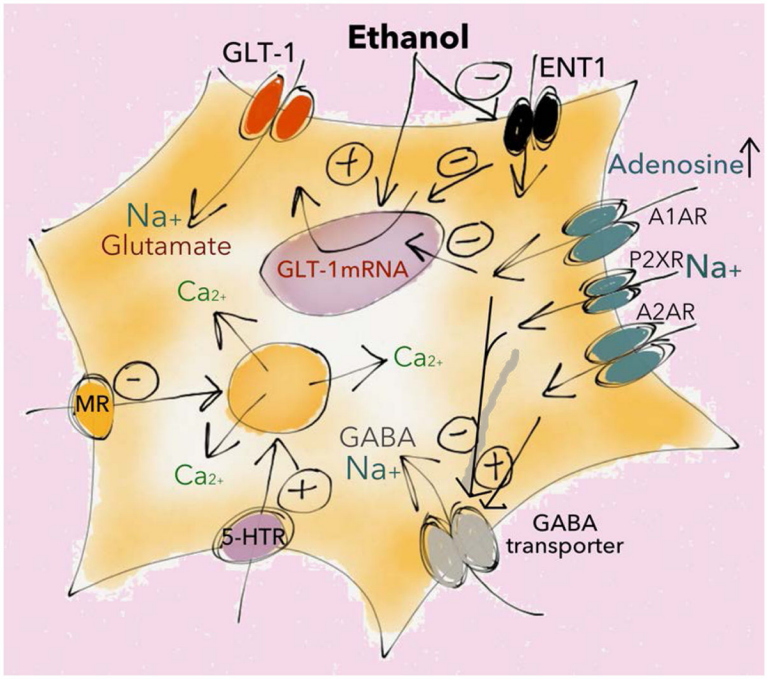

Figure 6. Ethanol-mediated disruption of the balance between excitation and inhibition

Ethanol can perturb excitatory balance by altering astrocyte calcium signaling and the clearance of neurotransmitters. Ethanol modulates receptor-mediated responses in a pathway specific manner, and may directly increase Ca^{2+} signaling in a subset of astrocytes. Ethanol also suppresses adenosine-uptake via the astrocytic equilibrative nucleoside transporter 1 (ENT1), leading to increased levels of adenosine and activation of purinergic receptors (A1AR, A2AR, P2XR) resulting in indirect modulation of glutamate and GABA clearence. MR=muscarinic receptor, 5-HTR=serotonin receptor. See text for further details.

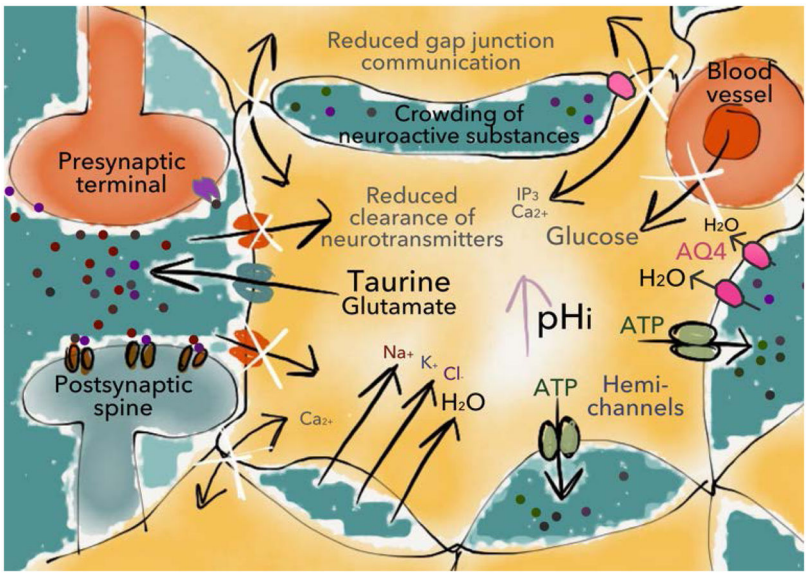

Figure 7. Ethanol-induced cell swelling

A subset of astrocytes responds to ethanol exposure with an increase in cell volume, which can lead to crowding of molecules in the extracellular space, changes in volume transmission and possibly neurotoxicity. In addition, ethanol-induced cell swelling may lead to impaired communication through gap junction channels, reduced clearance of neuroactive substances and decreased glucose transport (illustrated with white x-marks) (Abdul Muneer et al., 2011), thereby indirectly affecting neurotransmission. Importantly, cell swelling can also promote the release of taurine, which appears to play a key role in the dopamine-elevating properties of ethanol. See text for further details. AQ4=aquaporin-4.