Review
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Do stars govern our actions? Astrocyte involvement in rodent behavior

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Astrocytes have emerged as important partners of neurons in information processing. Important progress has been made in the past two decades in understanding the role of astrocytes in the generation of neuron–astrocyte network outputs resulting in behavior. We review evidence for astrocyte involvement across four different behavioral domains: cognition, emotion, motor, and sensory processing. Accumulating evidence from animal models has provided a wealth of data that largely supports a direct involvement of astrocytes on diverse aspects of behavior. The development of tools for selectively controlling the temporal and spatial properties of astrocyte activity will help to consolidate our knowledge of the mechanisms underlying this involvement.

The neuron–astrocyte interaction 25 years later

The ability to understand astrocyte activity by recording and quantifying calcium dynamics in astrocytes using imaging techniques has allowed the elucidation of the functional properties of astrocytes in the brain [1–3]. These seminal studies prompted neuroscientists to acknowledge the active features of astrocytes, and triggered their curiosity to understand the level of interaction that these cells would maintain with neurons under physiological conditions. Since these pioneering efforts, numerous studies have pointed out five main features of astrocytes: (i) they play relevant roles in brain homeostasis, contributing to the blood–brain barrier and maintaining extracellular levels of ions and neurotransmitters; (ii) they are in close physical contact with synapses and neurons, other glial cells, and vascular structures in the brain; (iii) they express several functional neurotransmitter receptors (e.g., those for glutamate, ATP, GABA, acetylcholine, or endocannabinoids) which allow them to sense surrounding neuronal activity; (iv) they show intracellular calcium-based excitability with complex temporal and spatial properties and may trigger paracrine signaling to neighboring astrocytes; (v) they are able to release neuro- and vasoactive substances, such as glutamate, D-serine, ATP, GABA, tumor necrosis factor α (TNF-α), prostaglandins, or peptides, in a process known as gliotransmission which can lead to the modulation of synaptic function, blood flow, and metabolism (see [4–13] for detailed reviews]. These features underlie the dynamic interaction between astrocytes and neurons that complements and modulates the communication between pre- and postsynaptic structures, a concept termed the tripartite synapse [12,14]. In the tripartite synapse, astrocytes respond to surrounding synaptic activity with calcium signaling, which in turn cause feedback regulation of neuronal activity and synaptic strength through gliotransmitter release. Regarding the mechanism of the tripartite synapse, it was recently proposed that astrocytes incorporate information shared inside and outside synapses, and process signals in a scaled manner, but within temporal and spatial dimensions that differ from those of neurons. This view unifies the hundreds of observations published in the past two decades of intensive research in this field [5].

While important progress has been made in defining the modulatory role of astrocytes in synaptic transmission, and in identifying the underlying cellular mechanisms, fundamental aspects of the consequences of the functional interaction of astrocytes and neurons on information processing at higher organization levels, in other words neural networks and animal behavior, have only more recently begun to be explored. A major challenge for studying the role of astrocytes in information processing and behavior stems from the suitability of available approaches to specifically target astrocyte function and reliably measure the consequences on network output *in vivo*. Therefore, the main goal of this review is to compile evidence of the importance of neuron–astrocyte network outputs on the behavior of animal models in which astrocytes have been targeted specifically by pharmacological and/or genetic manipulation. While excellent recent reviews have focused on specific related topics, such as the role of glia in cognitive function [15], astrocyte and neuron–astrocyte networks [10,16], the complexity of astrocyte activation and calcium signaling [17–20], purinergic signaling and behavior [21], molecular approaches for *in vivo* studies [22–25], and astrocyte contributions to pathophysiological processes [6,26–30], we aim here to review and discuss the different *in vivo* evidence that indicates the involvement of astrocytes in animal behavior.

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Animal models to study astrocyte function *in vivo*

The study of astrocyte function *in vivo* is a daunting task owing to the difficulty of dissecting out the astrocytic component from the output of the neuron–astrocyte network. Neuroscientists have tried to overcome this difficulty by taking advantage of specific pharmacological and/or genetic tools that either reduce or enhance astrocyte functions in animal models, mostly in rodents. Table 1 summarizes a list of the animal models that have been used to study astrocyte function *in vivo*. The strategies used to obtain the different models include pharmacological modulation, injection of viral vectors for genetic manipulation, and/or the generation of genetically modified animals. The need for increased specificity in the brain region, cell type, or mechanism targeted has triggered in recent years the use of more-refined transgenic models. This has been achieved by (i) choosing specific astrocytic promoters (e.g., aldehyde dehydrogenase 1L1, *Aldh1L1*) [31–33], and (ii) the expression of machinery that allows inducible changes in gene expression. The latter include the tetracycline-controlled transcriptional activation and Cre/*loxP* recombination systems which allow temporal control of modulation, thus avoiding simultaneous compensatory mechanisms [22,23,34,35]. Importantly, the use of the Cre/*loxP* system expressed from different astrocyte-specific promoters has revealed that recombination has regional particularities. Therefore, careful characterization of functional alterations and reporter expression should be performed to confirm the suitability of a model for testing [23,36]. Future development of more specific probes, for example the CRISPR/Cas system (that allows easier and efficient modification of endogenous genes [37]), employed in specific brain areas and targeting specific spatiotemporal aspects of astrocyte activity, will further our understanding.

Astrocytes influence the outputs generated by neuron–astrocyte networks

In accordance with many available animal models described in Table 1, the current literature includes a large number of studies aimed at evaluating the consequences of *in vivo* manipulation of astrocyte function on animal behavior (Table 2). Generally, in this work researchers have performed behavioral analysis that compares experimental with control groups to estimate the effect of manipulation of the astrocytic component (see Box 1 for details). Complementary electrophysiology or imaging has been added in some cases. In this section we separately focus on studies of cognition, emotion, motor, and sensory functions.

Cognition

Cognition encompasses multiple functions including learning, memory, attention, and reasoning. Despite decades of research linking cognitive processing and fast synaptic transmission between connected neurons, cognitive output seems to also require complex integration of neuron–astrocyte networks in the temporal and spatial domains [5,15]. For example, incoming signals to astrocytes through cannabinoid receptor type 1 (CB1R) activation were shown to mediate resilience to spatial memory impairment and hippocampal long-term depression caused by cannabinoids [38]. Moreover, the absence of gliotransmitter release, specifically in astrocytes, or blockade of adenosine A1 receptor, affected neuronal plasticity and cortical oscillations, and promoted resilience to the sleep pressure and spatial long-term memory impairments caused by sleep deprivation [39–42]. Accordingly, the same approach was also shown to avoid lipopolysaccharide-induced sleep pressure [43].

These results suggest that hippocampal astrocytes mediate negative outputs under adverse conditions. Indeed, the generation of interleukin 1 receptor (IL-1R)-expressing astrocytes in the hippocampus rescued the characteristic phenotype of IL-1R full knockout mice, namely severe memory disturbances in contextual fear conditioning and spatial reference memory (hippocampus-dependent), and *in vivo* long-term potentiation of hippocampal synapses, disclosing a role for astrocyte IL-1 signaling in this region [44].

Given the above, it is interesting that the enhanced production of the gliotransmitter D-serine by overexpression of serine racemase in astrocytes did not alter memory processing in mice [45]. Moreover, under physiological conditions, the alternative blockade of astrocyte vesicular release in mice that produce the botulinum neurotoxin type B specifically in glutamate/aspartate transporter (GLAST)-positive cells did not trigger any alterations in spatial memory tests, although here the authors claimed that the recombination in the hippocampus was not sufficient ([46]; discussed in [35,36]). The level and extent of gene recombination, protein expression, and subcellular localization is a very sensitive issue that requires careful consideration when establishing conclusions from experiments using transgenic animals (see [5]). Indeed, in contrast to the results reported in [46], the expression of clostridial toxin (tetanus neurotoxin, TeNT) specifically in astrocytes to block astrocyte calcium-dependent vesicular release was shown recently to cause a decrease in cortical gamma power that correlated with a key impairment in long-term recognition memory [47]. In addition, the activation of Gs-protein-coupled receptor was shown to be crucial for working memory, but deleterious for memory consolidation [48,49]. Curiously, the increase or ablation of astrocyte Gq-protein-dependent calcium signaling failed to affect hippocampal neuronal activity, synaptic plasticity [50–52], or spatial memory [53]. However, numerous studies have demonstrated the involvement of astrocyte signaling in synaptic plasticity in different brain areas and through different regulatory mechanisms (Table 3). These contrasting results cannot be accounted for solely by the existence of multiple forms of synaptic plasticity generated by diverse mechanisms, and therefore highlight the importance of the experimental paradigms used to stimulate neurons and astrocytes (discussed in [5]). For example, Tanaka and colleagues found that mice in which astrocyte calcium-signaling was inhibited presented memory deficits in hippocampus-dependent long-term memory tasks [54], suggesting that astrocytes may interfere with memory processing by means of alternative calcium-dependent mechanisms. Supporting this idea, blockade of glycogen breakdown by astrocytes, inhibiting the lactate shuttle to neurons, severely impaired long-term and working memory in rats [55,56].

Table 1. Animal models used to study astrocyte function *in vivo*

Astrocyte function	Model	Mechanism used to alter function	References that use this model
Sensing/activation	GFAP-Cre CB1R$^{fl/fl}$	Lack of type-1 cannabinoid receptors (CB1R) in astrocytes	[38]
	GFAP-ChR2 (virus)	Optogenetic activation of channelrhodopsin 2 in GFAP^{+} astrocytes	[101]
	GFAP-MrgA1^{+}	Activation of transgenic astrocyte exogenous GPCR	[50,51,69,89]
	GFAP-hM3Dq	Chemogenetic control of astrocyte Gq-coupled signaling	[85]
	GFAP-hM3Dq × IP3R2 KO	Chemogenetic control of astrocyte Gq-coupled signaling independent of IP3	[85]
	AQP4 KO	Lack of the astrocyte AQP4 channel	[57,58]
	GLAST-Cre Gria1$^{fl/fl}$ × Gria4$^{fl/fl}$ (dKO)	Genetic deletion of AMPA subunits GluA1 and GluA4 in GLAST^{+} astrocytes	[88]
	GLAST KO	Lack of astrocyte GLAST	[92,98]
	Glt-1-eGFP BAC	eGFP expression in astrocytes	[104]
	Thy-1 GFP plus SR101 labeling	GFP expression in neurons and SR101 labeling of astrocytes	[105]
	NMRI mice plus SR101 labeling	SR101 labeling of astrocytes	[103]
	GFAP-Cre A2AR$^{fl/fl}$	Lack of A2AR in astrocytes	[48,49]
	GFAP-tTA/TetO-Rs1	Chemogenetic control of astrocyte Gs-coupled signaling	[49]
	IL-1R KO plus NPC transplantation	Ablation of IL-1 receptor expression and newly generated wild-type astrocytes	[44]
Calcium dynamics	IP3R2 KO	Lack of intracellular calcium signaling in astrocytes	[50,52,69, 100,105]
	IP3R2 KO	Lack of intracellular calcium signaling in astrocytes	[99]
	IP3R2 KO (C57BL/6)	Lack of intracellular calcium signaling in astrocytes; C57BL/6 background	[89]
	GFAP-IP3R2 cKO (C57BL/6)	Lack of intracellular calcium signaling in GFAP^{+} astrocytes; C57BL/6 background	[53,96]
	GLAST-Cre IP3R2$^{fl/fl}$	Lack of intracellular calcium signaling in GLAST^{+} astrocytes	[90]
	GLT1-IP3 'sponge'	Attenuation of intracellular calcium signaling in GLT-1^{+} astrocytes	[54]
	Mlc1-YC-Nano50- IP3R2 KO	Expression of the calcium indicator yellow Cameleon-Nano 50 in astrocytes lacking calcium signaling	[102]
	Mlc1-YC-Nano50	Expression of the calcium indicator yellow Cameleon-Nano 50 in astrocytes	[102]
	GLAST-CreER-R26-lsl-GCaMP3	Expression of the calcium indicator GCaMP3 in GLAST^{+} astrocytes	[87]
	Cx30-Cre-GCaMP3	Expression of the calcium indicator GCaMP3 in Cx30^{+} astrocytes	[97]
Gliotransmission	dnSNARE	Inducible impairment of SNARE-dependent exocytosis in GFAP^{+} astrocytes	[39–43,69,77, 78,84,91,106]
	GLAST-CreERT2-iBot	Inducible lack of SNARE-dependent exocytosis in GLAST^{+} cells	[46]
	GFAP-TeNT	Lack of SNARE-dependent exocytosis in GFAP^{+} astrocytes	[47]
	Lenti-BDNF	BDNF overexpression in astrocytes	[74]
	GFAP-Srr	Serine racemase overexpression in GFAP^{+} astrocytes	[45]
Neurotransmitter uptake	PDC	Lack of glutamate uptake through GLT-1	[80]
	DHK	Pharmacological blockade of the astrocyte glutamate transporter GLT-1	[59,79,80]
	Ceftriaxone	Pharmacological overexpression of the astrocyte glutamate transporter GLT-1	[60,81]
	Riluzole	Modulation of glutamate release; enhancement of glutamate uptake (also GLT-1 overexpression)	[73]
	GLAST-CreERT2 GLT1$^{fl/fl}$	Inducible deletion of GLT-1 in GLAST^{+} cells	[75]
Connection/adhesion to vicinal cells	mGFAP-Cre Cx43$^{fl/fl}$_Cx30$^{-/-}$ (dKO)	Lack of both connexins Cx43 and Cx30 in GFAP^{+} astrocytes	[61]
	hGFAP-Cre Cx43$^{fl/fl}$_Cx30$^{-/-}$ (dKO)	Lack of both connexins Cx43 and Cx30 in GFAP^{+} astrocytes	[107]
	Cx30$^{-/-}$	Lack of astrocyte connexin Cx30	[62,65,94]
	GFAP-Cre Cx43$^{fl/fl}$	Lack of connexin Cx43 in GFAP^{+} astrocytes	[70]
	hGFAP-Cre Cx43$^{fl/fl}$	Lack of connexin Cx43 in GFAP^{+} astrocytes	[63,86]
	S100B-Cx43	Lack of connexin Cx43 in S100B^{+} astrocytes	[93]
	TAT-Cx43L2	Pharmacological blockade of connexin 43	[64]
	GFAP-DNSynCAM1	Expression of a dominant-negative form of SynCAM1 in GFAP^{+} astrocytes	[71]
Astrocyte ablation/shutdown	L-aminoadipate	Pharmacological ablation of astrocytes	[66,72]
	Fluorocitrate	Pharmacological inhibition of astrocyte function	[76,90]
Human astrocytes	Human glial chimeric mice	Engraftment of human glial progenitor cells (GPCs) in immunodeficient mice	[67]
Metabolism	Lactate-shuttle impairment	Pharmacological blockade of the hippocampal lactate shuttle	[55,56]

Table 2. *In vivo* outputs of animal models of astrocyte modulation[a]

Model	Alteration/modulation	Approach	Phenotype	Refs
Cognition				
GFAP-Cre CB1R$^{fl/fl}$	Lack of type-1 cannabinoid receptors (CB1R) in astrocytes	Spatial working memory (MWM)	↑ Resilience to CB-induced spatial working memory impairment	[38]
		In vivo electrophysiology	↑ Resilience to CB-induced hippocampal LTD;	[38]
dnSNARE	Impairment of SNARE-dependent exocytosis in GFAP$^+$ astrocytes	Brain slice electrophysiology	↓ Hippocampal synaptic plasticity	[42]
		EEG/EMG *in vivo*	↑ Resilience to sleep deprivation (SD)-induced sleep pressure Modulation of cortical oscillations	[39,41]
		Novel object recognition	↑ Resilience to SD-induced long-term memory impairment	[41]
		Contextual fear conditioning	= Contextual fear memory	[41]
		Spatial object recognition	↑ Resilience to SD-induced long-term memory impairment	[40]
		EEG/EMG *in vivo*	↑ Resilience to LPS-induced sleep pressure	[43]
IL-1R KO plus NPC transplantation	Ablation of IL-1 receptor expression plus newly generated WT astrocytes	Fear-conditioning spatial reference memory (MWM)	↑ Rescue of IL-1R KO memory impairment	[44]
		In vivo electrophysiology	↑ Rescue of IL-1R KO LTP impairment	[44]
GFAP-Srr	Serine racemase overexpression in GFAP$^+$ astrocytes	Y-maze spontaneous alternation test	= Spatial working memory	[45]
		Spatial reference memory (MWM)	= Long-term memory	[45]
		Operant Learning	= Long-term memory	[45]
Glast-CreERT2-iBot	Lack of SNARE-dependent exocytosis in GLAST$^+$ cells	Novel object recognition	= Short-term recognition memory	[46]
		Spatial reference memory (MWM)	= Long-term spatial memory	[46]
GFAP-TeNT	Lack of SNARE-dependent exocytosis in GFAP$^+$ astrocytes	Novel object recognition	↓ Long-term recognition memory	[47]
		In vivo electrophysiology	↓ Cortical gamma power	[47]
GFAP-Cre A2AR$^{fl/fl}$	Lack of A2AR in astrocytes	Y-maze spontaneous alternation test	↓ Working memory; prevented by DHK and by Tat-Glur2$_{3y}$	[48]
		Radial arm maze	↓ Working memory	[48]
		Spatial reference memory (MWM)	↑ Long-term memory	[49]
		Contextual memory	↑ Long-term memory	[49]
GFAP-tTA-TetO-Rs1	Chemogenetic control of astrocyte Gs-coupled signaling	Spatial reference memory (MWM)	↓ Long-term memory	[49]
IP3R2 KO	Lack of intracellular calcium signaling in astrocytes	Brain slice electrophysiology	= Hippocampal spontaneous/evoked activity; = synaptic plasticity;	[50,52]
GFAP-MrgA1$^+$	Activation of transgenic astrocyte exogenous GPCR	Brain slice electrophysiology	= Hippocampal spontaneous/evoked activity = Synaptic plasticity	[50,51]
GFAP-IP3R2 cKO (C57BL/6)	Lack of intracellular calcium signaling in GFAP$^+$ astrocytes; C57BL/6 background;	Spatial reference memory and reversal learning (MWM)	= Long-term spatial memory = Reversal learning	[53]
GLT1-IP3 'sponge'	Attenuation of intracellular calcium signaling in GLT-1$^+$ astrocytes	Spatial reference memory (MWM) and fear conditioning	↓ Long-term memory	[54]
Lactate-shuttle impairment	Pharmacological blockade of the hippocampal lactate shuttle	Inhibitory avoidance test	↓ Long-term memory	[56]
		In vivo electrophysiology	↓ Hippocampal LTP	[56]
		Spontaneous alternation task	↓ Spatial working memory, reverted by lactate or glucose administration	[55]
AQP4 KO	Lack of the astrocyte AQP4 channel	Fear conditioning	↓ Long-term memory; rescue by increasing GLT-1 expression	[57]
		Fear conditioning	= Long-term memory	[58]
		Spatial object recognition	↓ Short-term memory	[58]

Table 2 (*Continued*)

Model	Alteration/modulation	Approach	Phenotype	Refs
DHK	Pharmacological blockade of the astrocyte glutamate transporter GLT-1	Spatial reference memory (MWM)	↓ Long-term memory	[59]
Ceftriaxone	m	Spatial reference Memory (MWM)	= Long-term memory	[60]
mGFAP-Cre $Cx43^{fl/fl}$ $Cx30^{-/-}$ (dKO)	Lack of both connexins Cx43 and Cx30 in $GFAP^+$ astrocytes	Spatial object recognition	↓ Spatial working memory	[61]
$Cx30^{-/-}$	Lack of the astrocyte connexin Cx30	Spatial object recognition	↓ Spatial memory	[62]
		Contextual fear conditioning	↓ Long-term memory	[65]
hGFAP-Cre $Cx43^{fl/fl}$	Lack of the connexin Cx43 in $GFAP^+$ astrocytes	Spatial reference memory (MWM)	= Long-term memory	[63]
TAT-Cx43L2	Pharmacological blockade of connexin 43	Fear conditioning	↓ Long-term memory, reverted by gliotransmitter administration	[64]
L-aminoadipate	Pharmacological ablation of astrocytes	Spatial working memory (MWM)	↓ Spatial working memory	[66]
		Attentional set-shifting	↓ Attention and behavioral flexibility	[66]
Human glial chimeric mice	Engraftment of human glial progenitor cells (GPCs) in immunodeficient mice	Fear conditioning	↑ Long-term memory	[67]
		Barnes maze	↑ Long-term memory	[67]
		Spatial object recognition	↑ Short-term recognition memory	[67]
Emotion				
Anxiety-like behavior				
GFAP-Srr	Serine racemase overexpression in $GFAP^+$ astrocytes	Novelty suppressed feeding	↓ Anxiety-like behavior	[45]
		Ultrasonic vocalizations	↓ Anxiety-like behavior	[45]
dnSNARE	Impairment of SNARE-dependent exocytosis in $GFAP^+$ astrocytes	Zero maze	= Anxiety-like behavior	[41]
GLAST-CreERT2-iBot	Lack of SNARE-dependent exocytosis in $GLAST^+$ cells	Open field	= Anxiety-like behavior	[46]
GFAP-Cre $A2AR^{fl/fl}$	Lack of A2AR in astrocytes	Open field	= Anxiety-like behavior	[49]
GFAP-tTA/TetO-Rs1	Chemogenetic control of astrocyte Gs-coupled signaling	Open field	= Anxiety-like behavior	[49]
		Elevated plus maze	= Anxiety-like behavior	[49]
IP3R2 KO	Lack of intracellular calcium signaling in astrocytes	Elevated plus maze	= Anxiety-like behavior	[69]
GLT1-IP3 'sponge'	Attenuation of intracellular calcium signaling in $GLT\text{-}1^+$ astrocytes	Open field	= Anxiety-like behavior	[54]
hGFAP-Cre $Cx43^{fl/fl}$	Lack of the connexin Cx43 in $GFAP^+$ astrocytes	Open field	↓ Anxiety-like behavior	[63]
		Elevated plus maze	= Anxiety-like behavior	[63]
GFAP-Cre $Cx43^{fl/fl}$	Lack of the connexin Cx43 in $GFAP^+$ astrocytes	Open field	↓ Anxiety-like behavior	[70]
$Cx30^{-/-}$	Lack of the astrocyte connexin Cx30	Open field	↑ Anxiety-like behavior	[62]
		Elevated plus maze	= Anxiety-like behavior	[62]
GFAP-DNSynCAM1	Expression of a dominant-negative form of SynCAM1 in $GFAP^+$ astrocytes	Home cage activities	↑ home-cage activity	[71]
		Elevated zero maze	↓ Anxiety-like behavior	[71]
		Acoustic startle	↓ Anxiety-like behavior	[71]
L-aminoadipate	Pharmacological ablation of astrocytes	Novelty suppressed feeding	↑ Anxiety-like behavior	[72]
		Active avoidance test	↑ Anxiety-like behavior	[72,73]
Lenti-BDNF	BDNF overexpression in astrocytes	Novelty suppressed feeding	↓ Anxiety-like behavior	[74]
		Elevated plus maze	↓ Anxiety-like behavior	[74]
GLAST CreERT2-$GLT1^{fl/fl}$ (cKO)	Inducible deletion of GLT-1 in $GLAST^+$ cells	Animal welfare	↑ Grooming behavior and tic-like head shakes; ↓ grooming behavior after memantine i.p.	[75]
		Elevated plus maze	= Anxiety-like behavior	[75]
		Light–dark box	= Anxiety-like behavior	[75]
		Open field	= Anxiety-like behavior	[75]
Depression-like behavior				
L-aminoadipate	Pharmacological ablation of astrocytes	Sucrose preference test	↑ Anhedonia	[72,73]
		Forced swim test	↑ Learned helplessness	[72]
Fluorocitrate	Pharmacological inhibition of astrocyte function	Conditioned avoidance test	↓ Antidepressant effect of imipramine	[76]
GFAP-Srr		Forced swim test	↓ Learned helplessness	[45]

Table 2 (*Continued*)

Model	Alteration/modulation	Approach	Phenotype	Refs
	Serine racemase overexpression in GFAP$^+$ astrocytes			
GFAP-MrgA1$^+$	Activation of transgenic astrocyte exogenous GPCR	Forced swim test	↓ Learned helplessness	[69]
dnSNARE	Impairment of SNARE-dependent exocytosis in GFAP$^+$ astrocytes	Forced swim test	↑ Learned helplessness	[69]
		Forced swim test	= Learned Helplessness; absent after sleep deprivation	[77]
		Tail suspension test	= Learned Helplessness; absent after sleep deprivation	[77]
		Conditioned place preference	↓ Drug-seeking behavior	[78]
IP3R2 KO	Lack of intracellular calcium signaling in astrocytes	Sucrose preference test	↑ Anhedonia	[69]
		Forced swim test	↑ Learned helplessness	[69]
		Coat score	↑ Depressive behavior	[69]
DHK	Pharmacological blockade of the astrocyte glutamate transporter GLT-1	Intracranial self-stimulation	↑ Depressive behavior	[59,79]
		Place conditioning	= Aversive state	[59]
		Sucrose consumption test	↑ Depressive behavior	[79]
PDC	Lack of glutamate uptake through GLT-1	Social interaction test	↑ Depressive behavior	[80]
Ceftriaxone	Pharmacological overexpression of the astrocyte glutamate transporter GLT-1	Forced swim test	↓ Learned helplessness	[81]
		Tail suspension test	↓ Learned helplessness	[81]
GLAST-CreERT2 GLT1$^{fl/fl}$	Inducible deletion of GLT-1 in GLAST$^+$ cells	Social interaction test	= Depressive behavior	[75]
		Three-chamber social interaction test	= Depressive behavior	[75]
Motor activity and coordination				
dnSNARE	Impairment of SNARE-dependent exocytosis in GFAP$^+$ astrocytes	Novel object recognition training	= Exploratory/locomotor behavior	[41]
		Rotarod	= Motor coordination	[41]
		Open field	= Exploratory/locomotor behavior	[69,77,84]
GLAST-CreERT2-iBot	Lack of SNARE-dependent exocytosis in GLAST$^+$ cells	Open field	= Exploratory/locomotor behavior	[46]
		Wheel-running activity	= Motor activity	[46]
GFAP-Srr	Serine racemase overexpression in GFAP$^+$ astrocytes	Open field	= Exploratory behavior	[45]
IP3R2 KO	Lack of intracellular calcium signaling in astrocytes	Open field	= Exploratory/locomotor behavior	[69]
GLT1-IP3 'sponge'	Attenuation of intracellular calcium signaling in GLT-1$^+$ astrocytes	Open field	= Exploratory behavior	[54]
GFAP-MrgA1$^+$	Activation of transgenic astrocyte exogenous GPCR	Open field	= Exploratory/locomotor behavior	[69]
		Electrocorticogram	Astrocyte–cortical upstate synchrony	[89]
GFAP-hM3Dq × IP3R2 KO	Chemogenetic control of astrocyte Gq-coupled signaling independent of IP3	Open field	= Exploratory behavior	[85]
		Rotarod	↓ Motor coordination	[85]
		Righting reflex analysis	↓ Motor coordination	[85]
GFAP-DNSynCAM1	Expression of a dominant-negative form of SynCAM1 in GFAP$^+$ astrocytes	Open field	↑ Exploratory behavior, attenuated by amphetamine	[71]
		Pre-pulse inhibition (PPI)	= Sensorimotor behavior	[71]
hGFAP-Cre Cx43$^{fl/fl}$	Lack of the connexin Cx43 in GFAP$^+$ astrocytes	Open field	↑ Exploratory behavior	[63,86]
		Rotarod	= Motor coordination	[63]
Cx30$^{-/-}$	Lack of the astrocyte connexin Cx30	Open field	= Exploratory behavior	[62]
		Rotarod	= Motor coordination	[62]
GLAST-CreER-R26-lsl-GCaMP3	Expression of the calcium indicator GCaMP3 in GLAST$^+$ astrocytes	Locomotion behavioral paradigm	Astrocyte synchronization with locomotion	[87]
GLAST-Cre Gria1$^{fl/fl}$ × Gria4$^{fl/fl}$ (dKO)	Genetic deletion of AMPA subunits GluA1 and GluA4 in GLAST$^+$ astrocytes	Eyeblink conditioning	↓ Motor coordination	[88]
		Erasmus ladder	↓ Motor coordination	[88]
IP3R2 KO (C57BL/6)	Lack of intracellular calcium signaling in astrocytes; C57BL/6 background;	Electrocorticogram	↓ Astrocyte–cortical upstate synchrony	[89]

Table 2 (*Continued*)

Model	Alteration/modulation	Approach	Phenotype	Refs
GLAST Cre IP3R2$^{fl/fl}$	Lack of intracellular calcium signaling in GLAST^{+} astrocytes	Forelimb reaching task	↓ Motor-skill learning	[90]
GFAP-tTA/TetO-Rs1	Chemogenetic control of astrocyte Gs-coupled signaling	Rotarod	= Motor coordination	[49]
dnSNARE	Impairment of SNARE-dependent exocytosis in GFAP^{+} astrocytes	Rung walk	↑ Resilience to stroke-induced impairment	[91]
		Adhesive dot removal	↑ Resilience to stroke-induced impairment	[91]
Fluorocitrate	Pharmacological inhibition of astrocyte function	Forelimb reaching task	↓ Motor-skill learning, rescued by D-serine i.p.	[90]
GLAST KO	Lack of the astrocyte GLAST	Rotarod	↓ Motor coordination	[92]
mGFAP-Cre Cx43$^{fl/fl}$ Cx30$^{-/-}$ (dKO)	Lack of both connexins Cx43 and Cx30 in GFAP^{+} astrocytes	Rotarod	↓ Motor coordination	[61]
S100B-Cx43	Lack of the connexin Cx43 in S100B^{+} astrocytes	Rotarod	= Motor coordination	[93]
		Eyeblink conditioning	= Motor coordination	[93]
Sensory processing				
Hearing				
Cx30$^{-/-}$	Lack of the astrocyte connexin Cx30	Audiometry tests	Severe constitutive hearing impairment	[94]
Smell				
GLAST KO	Lack of the astrocyte GLAST	Odor stimulation plus *in vivo* electrophysiology	Altered olfactory responses	[98]
Cx30-Cre-GCaMP3	Expression of the calcium indicator GCaMP3 in Cx30^{+} astrocytes	Odor stimulation plus *in vivo* two photon	Astrocyte–neurovascular coupling	[97]
Tactile: whisker plus tail pinch				
IP3R2 KO	Lack of intracellular calcium signaling in astrocytes	Whisker stimulation plus *in vivo* electrophysiology	↓ Somatosensory plasticity, rescued by D-serine administration;	[99]
		Tail stimulation plus *in vivo* electrophysiology	↓ Cholinergic-dependent hippocampal plasticity	[100]
GFAP-Cre Cx43$^{fl/fl}$	Lack of the connexin Cx43 in GFAP^{+} astrocytes	Whisker stimulation plus *in vivo* electrophysiology	↓ Cortical plasticity	[70]
		Slit experiment	↓ Whisker-dependent perception	[70]
		Jump-stand	= Sensory discrimination learning	[70]
Glt-1-eGFP BAC	eGFP expression in astrocytes	Whisker/startle stimulation plus *in vivo* electrophysiology	↑ Fast cortical astrocyte responses	[104]
Thy-1-GFP plus SR101 labeling	GFP expression in neurons and SR101 labeling of astrocytes	Whisker stimulation plus *in vivo* imaging	↑ Astrocyte process motility	[105]
Vision				
GFAP-IP3R2 cKO (C57BL/6)	Lack of intracellular calcium signaling in GFAP^{+} astrocytes; C57BL/6 background;	Visual stimulation plus *in vivo* electrophysiology	↓ NB-induced potentiation of visual responses	[96]
GFAP-ChR2 (virus)	Optogenetic activation of channelrhodopsin 2 in GFAP^{+} astrocytes	Visual and optogenetic stimulation plus *in vivo* electrophysiology	↑ Excitatory and inhibitory synaptic transmission	[101]
Mlc1-YC-Nano50	Expression of the calcium indicator yellow Cameleon-Nano 50 in astrocytes	Tail stimulation plus two photon imaging	↑ Astrocyte calcium response to sensory stimulation	[102]
Mlc1-YC-Nano50-IP3R2 KO	Expression of the calcium indicator yellow Cameleon-Nano 50 in astrocytes lacking calcium signaling	Tail stimulation plus two-photon imaging	Absent astrocyte calcium response to sensory stimulation	[102]
NMRI mice plus SR101 labeling	SR101 labeling of astrocytes	Electrical stimulation plus *in vivo* laser Doppler flowmetry/ electrophysiology	Astrocyte–neurovascular coupling	[103]
Nociception				
dnSNARE	Impairment of SNARE-dependent exocytosis in GFAP^{+} astrocytes	Von Frey fiber test	↓ Baseline nociception; = neuropathic nociception	[106]

Table 2 (*Continued*)

Model	Alteration/modulation	Approach	Phenotype	Refs
hGFAP-Cre $Cx43^{fl/fl}$ $Cx30^{-/-}$ (dKO)	Lack of both connexins Cx43 and Cx30 in $GFAP^+$ astrocytes	Von Frey fiber test	↓ SCI-triggered neuropathic pain	[107]
		Plantar test	↓ SCI-triggered neuropathic pain	[107]
Cx30 KO	Lack of the astrocyte connexin Cx30	Von Frey fiber test	= SCI-triggered neuropathic pain	[107]
		Plantar test	= SCI-triggered neuropathic pain	[107]
GLAST-CreERT2 $GLT1^{fl/fl}$	Inducible deletion of GLT-1 in $GLAST^+$ cells	Hot plate test	= Nociception	[75]
GFAP-tTA/TetO-Rs1	Chemogenetic control of astrocyte Gs-coupled signaling	Hot plate test	= Nociception	[49]
Human glial chimeric mice	Engraftment of human glial progenitor cells (GPCs) in immunodeficient mice	Von Frey fiber test	= Nociception	[67]
		Plantar test	= Nociception	[67]

[a]↑, increased; ↓, decreased; =, no change.

Recent evidence also suggests a role for aquaporin-4 channels in the modulation of network outputs. Mice lacking this channel were shown to display differently spatial or fear memory impairments, which may be justified by specific regional correlates [57,58]. In particular, the decrease in long-term fear memory correlated with a marked decrease in the expression of glutamate transporter GLT-1 in the amygdala, and was reverted by ceftriaxone administration that triggered GLT-1 expression in this region [57]. Interestingly, pharmacological blockade of GLT-1 was shown to induce by itself a decrease in spatial memory [59], whereas the administration of ceftriaxone in basal conditions did not alter spatial memory performance [60]. Together, these data suggest that continuous glutamate buffering is essential for neuron–astrocyte network activity.

Astrocyte coupling was also suggested as an alternative mechanism of cognitive modulation. Astrocyte circuits built up through gap junctions based on connexins Cx30 (gap junction protein β6, Gjb6) and Cx43 (gap junction protein α1, Gja1) enable rapid intercellular exchange of ions, metabolites, and neuroactive substances between astrocytes, thus allowing selective spatial communication. Mice lacking both astrocyte connexins [glial fibrillary acidic protein (GFAP)-Cre $Cx43^{fl/fl}$ $Cx30^{-/-}$ double knockout] have impaired spatial memory [61], a pattern also observed for the single knockout $Cx30^{-/-}$ mice [62], but not for the GFAP-Cre $Cx43^{fl/fl}$ mice [63]. More recently, specific antagonism of Cx43 was shown to trigger a long-term fear memory impairment that was rescued by gliotransmitter replacement [64]. Accordingly, $Cx30^{-/-}$ mice were shown to display severe contextual fear conditioning memory impairment [65], confirming a crucial role of astrocyte coupling based on Cx30 for neuron–astrocyte network function.

While the deletion of specific astrocyte mechanisms seems to impair aspects of cognitive processing, it also seems to be true that astrocytes as a whole are crucial for network function. The specific ablation of astrocytes in the prefrontal cortex of rats is by itself responsible for a key impairment of reversal learning and working memory, tasks that are highly dependent on this brain region [66]. Accordingly, mice engrafted with human glia (about 20× larger and more complex than those of mice) in the hippocampus display enhanced learning abilities in tasks dependent on this region [67].

It is clear that interfering with astrocytes or astrocytic pathways causes consequences at the level of neuronal communication (measured by electrophysiological readouts), translated ultimately into behavioral outputs.

Emotion

Emotions are complex states of feeling that have been described as discrete and consistent responses to internal or external events. Emotional states result in physical and psychological changes that influence our behavior [68].

Regarding anxiety-like behavior, the increased availability of astrocyte D-serine leads to a less-anxious phenotype [45]. However, exocytotic release [41,46], Gs-protein-coupled receptor activation [49], and astrocyte IP3 signaling [54,69] do not seem to interfere with the anxious state. Interestingly, a lack of physical communication through Cx43 [63,70] or synaptic adhesion molecule SynCAM1/CADM1 [71] appears to induce a less-anxious state. On the other hand, the ablation of Cx30 induces anxiety-like behavior [62]. Of note, the antagonistic effects displayed by mice lacking Cx43 or Cx30 were visible in the open-field arena, but were not confirmed in the elevated plus maze, a gold-standard test for anxiety-like behavior. Therefore, this effect needs further clarification using additional paradigms to assess anxiety. The pharmacological ablation of astrocytes in the prefrontal cortex was shown to induce anxious behavior *per se* [72,73], and brain-derived neurotrophic factor (BDNF) overexpression in hippocampal astrocytes produced an anxiolytic-like effect [74]. However, blockade of astrocyte glutamate uptake did not alter the anxiety phenotype, but triggered obsessive/compulsive-like behavior [75].

Together these data suggest that astrocytes are important for the anxiety balance and that astrocyte activation may represent a possible therapeutic target for the treatment of anxiety.

Box 1. Behavioral tests used to analyze astrocyte involvement in brain function

Ultimately the outputs of the neuron–astrocyte network are translated in the form of behavior. We present in Table I an overview of the behavior tests used by researchers to analyze the impact of astrocytes on network function in the main behavioral dimensions.

Table I. Behavioral tests to analyze astrocyte involvement in brain function

Test	Task	Function tested	Refs
Cognition			
Morris water maze	Learn to escape to a hidden platform in a pool guided by spatial cues.	Spatial reference memory; spatial working memory; cued learning; spatial reversal learning	[38,44–46,54,58–60,63,66]
Radial arm maze	Explore the arms that contain a reward.	Spatial working memory; reference memory	[48]
Barnes maze	Locate a hidden escape box on an open platform.	Spatial reference memory; spatial working memory	[67]
Attentional set-shifting task	Select a baited bowl by its odor or texture for food reward.	Non-spatial working memory: attentional set-shifting and reversal learning functions	[66]
Novel object recognition	Explore a familiar and a novel object in an arena.	Working memory; 'pure' recognition memory test	[41,46,47,62]
Object placement	Explore two identical objects in the training day, recognize relocated object on test day.	Spatial learning and memory	[58]
Jump-stand sensory-discrimination learning	Stretch across a gap to palpate textured surfaces with vibrissae.	Exploratory and discriminative behavior; sensory learning	[70]
T-maze	Explore a T-maze apparatus and enter the goal arm.	Spatial working memory	[38]
Spontaneous alternation task	Explore the maze freely during 5 min.	Spatial working memory	[45,47,48,55]
Spatial object recognition task	Explore two different objects in an arena.	Spatial working memory	[40,61,67]
Fear-conditioning paradigm	Recognize that a novel environment is associated with exposure to an aversive stimulus.	Fear/emotion-based learning and memory	[44,47,54,57,58,64,65,67,71]
Inhibitory avoidance	Explore a small starting compartment or a small platform and receive a single footshock after entering a larger compartment or stepping down from the platform.	Fear-motivated instrumental learning	[56]
Emotion			
Elevated plus maze	Explore an apparatus with four arms (two open and two closed).	Anxiety-like behavior	[38,54,57,63,69,74,75]
Elevated zero maze	Explore an apparatus with two open and two enclosed elevated arms that form a zero or circle.	Anxiety-like behavior	[71]
Open field	Explore an open and brightly illuminated square arena.	Exploratory behavior and general activity	[45,46,54,57,62,63,69–71,75,85,86,93]
Light-dark box	Explore an open field arena divided into two areas: dark and light.	Exploratory behavior and anxiety-like behavior	[75]
Slit experiment	Explore an apparatus that is divided into two areas (dark and light) by a baffle with holes of different sizes.	Whisker-related exploratory behavior	[70]
Social interaction task (Crawley's)	Interact with familiar VS novel mouse in a three-chamber arena.	Social interaction	[67,75]
Social interaction test	Evaluate the interaction between two animals in a box.	Social interaction	[75,80]
Home-cage activity tests	Monitor animals in their home cage environment.	Basic motor and sensory function	[54,71]
Graded anxiety test	Explore an adapted elevated-plus maze apparatus.	High-discriminative anxiety test	[62]
Sucrose preference test	Evaluate the preference for sucrose solution or plain water during a determined period.	Anhedonia/depression	[69,72]
Sucrose intake	Intake of sucrose from two sucrose drinking bottles.	Anhedonia/depression	[79]
Novelty suppressed feeding test	Reach a pellet placed in the center of the arena.	Anxiety-like behavior	[45,72,74,81]
Forced swim test	Swim in cylinders filled with water where there is no possible escape.	Depression-like behavior	[45,69,72,81]
Tail suspension test	Struggle to face upward and climb to a solid surface where there is no possible escape.	Depression-like behavior	[74,81]
Splash test	Measure the latency until the animal starts grooming the dorsal coat sprayed with 10% sucrose.	Depression-like and apathetic behavior	[74]
Two-way active avoidance test	Explore a box where the animals are exposed to stimulus and shocks.	Anxiety-like behavior	[72]
Two-way conditioned avoidance test	Explore an apparatus where animals are exposed to a conditioned stimulus tone followed by electric shocks.	Anxiety-like behavior	[76]

Table I (*Continued*)

Test	Task	Function tested	Refs
Prepulse inhibition of startle reflex	Exposure to a low intensity stimulus (prepulse) followed by a test stimulus of a higher intensity.	Anxiety-like behavior	[71]
Acoustic startle	Exposure to different acoustic stimuli elicits a body startle response.	Anxiety-like behavior	[71]
Coat score assay	Classify the coat state of the animal in seven different parts of the body.	Measure of stress-induced behavioral effects	[69]
Grooming and tic-like behavior	Record the time spent in grooming and the tic-like movements in a novel and familiar environment.	Grooming behavior	[75]
Conditioned place-preference paradigm	Explore two identical chambers, designated as drug-paired compartment and as vehicle-paired compartment.	Reward/addiction	[59,78]
Operant drug/food self-administration	Press the lever with reward delivery.	Reward/addiction	[45,78]
Ultrasonic vocalization test	Record and quantify ultrasonic vocalizations in different frequencies.	Anxiety-like behavior; motivation; social interaction	[45]
Motor activity and coordination			
Locomotor activity	Explore a maze/home cage.	Motor function	[38,59,78]
Motor balance	Evaluate the ability to stay on a bar fixed horizontally (max for 10 s).	Motor balance	[38]
Rotarod	Try to stay on the rotating cylinder without falling.	Balance, grip strength, and motor coordination	[61–63,71, 85,92,93]
Balance beam assay	Traverse a graded series of narrow beams to reach an enclosed safety platform.	Motor coordination and balance	[61]
Actimetry	Evaluate the activity of animals (movement, immobility, wheel-running activity) during light/dark cycles.	Motor function and coordination	[46,72]
Loss of righting reflex	Right itself when placed on its back in a V-shaped trough after administration of a drug.	Sedative/hypnotic state	[85]
Rung walk	Walk along a horizontal ladder with randomly spaced rungs.	Motor function	[91]
Locomotor behavioral paradigm	Walk in a treadmill (freely movable/controlled by motor) with the head immobilized.	Motor function	[87]
Erasmus ladder	Run on a horizontal ladder composed of sensitive rungs with pressure sensors.	Motor coordination	[88]
Forelimb reaching task	Learn to reach through the slit with the preferred forelimb and grasp to retrieve a pellet.	Motor-skill learning	[90]
Eyeblink conditioning	Exposure to an auditory or visual stimulus (the conditioned stimulus), paired with an eyeblink-eliciting unconditioned stimulus.	Motor learning	[88,93]
Sensory processing			
Footshock sensitivity test	Respond to electrical shocks (intensity ranging between 0.05 and 1 mA).	Nociception	[54,57]
Adhesive dot removal	Detect and remove pieces of adhesive paper from their wrists.	Sensorimotor function	[79,91]
Von Frey fiber test	Stimulate the hind limb by Von Frey monofilaments until the animal removes its paw or maximal force is reached.	Mechanical nociception	[67,106,107]
Plantar test	Stimulate the hindpaw under a controlled heat source.	Thermal nociception	[67,107]
Hot plate test	Place the footpad in contact with a heated surface.	Thermal nociception	[49,75]

Regarding effects of astrocyte modulation on mood, astrocyte ablation induced learned helplessness and anhedonia, widely accepted hallmarks of depressive-like behavior in rats [72,73], and the anti-depressant effect of imipramine was absent in a model of depression induced by blocking astrocyte metabolism [76]. Accordingly, the enhanced availability of astrocyte D-serine led to a reduced depressive phenotype [45]. Moreover, it was shown that specific activation of astrocytes through the Mas-related gene A1 receptor (MrgA1R) seems to follow the tendency observed for the anxiety state in this model and trigger a less-depressive phenotype [69]. Both effects seem to be mediated by astrocyte mechanisms because gliotransmission and inositol 1,4,5-trisphosphate receptor type 2 (IP3R2)-dependent signaling were shown to be crucial for both mood hallmarks [69] (although these data could not be confirmed in a different laboratory using two different tests to assess learned helplessness [77]). To clarify the role of gliotransmission, these effects should be carefully confirmed.

The inducible dominant-negative SNARE (dnSNARE; SNARE from 'soluble NSF attachment protein receptor') model allowed the demonstration that adenosine derived from astrocyte ATP mediates the learned helplessness caused by sleep deprivation [77], and that gliotransmission is necessary for reinstatement of drug-seeking behaviors

Table 3. Involvement of astrocyte signaling in synaptic plasticity

Brain area	Stimuli	Gliotransmitter	Refs
Hippocampus	Endogenous	ATP	[42]
	Calcium uncaging	Glutamate	[109]
	Depolarization	Glutamate	[110]
	Endogenous	D-serine	[111]
	Glutamate	ATP	[112]
	Acetylcholine	Glutamate	[100]
	Cannabinoids	Glutamate	[38]
	Endocannabinoids	Glutamate	[113]
Cortex	Acetylcholine	D-serine	[99]
	Endocannabinoids	Glutamate	[114]
Supraoptic nucleus	Endogenous	D-serine	[115]

by cocaine or associated cues [78]. Accordingly, pharmacological blockade of astrocyte glutamate buffering by blockade of the astrocyte GLT-1 glutamate transporter triggered depressive-like behavior in the form of increase of intracranial self-stimulation, latency to drink sucrose solution [59,79], or decrease in social interaction [80]. Moreover, the chronic administration of ceftriaxone, that leads to the overexpression of GLT-1, was shown to produce a reduction in learned helplessness [81]; however, its deletion in GLAST$^+$ cells did not affect depressive behavior [75]. These data provide some hints that support a link between astrocyte function and the modulation of depressive behavior, but further work will be needed to clarify the extent of modulation and the mechanisms involved.

Motor activity and coordination

Assessment of motor performance is of great relevance when evaluating behavior in animal models, and each novel model should be carefully characterized for this dimension. Motor activity is obviously important for research on sensorimotor function; however, it might have broader behavioral implications (e.g., if an animal with normal learning capabilities has motor deficits it will have difficulties to reach a platform in the Morris water maze, leading to false results). Therefore, behavioral experiments intended to assess other dimensions (e.g., cognition or emotion) should always be interpreted with combined assessment of sensorimotor function. Furthermore, many paradigms used to test behavior in rodent models are based in the observation that rodents have a high exploratory drive. In fact, the analysis of spontaneous exploratory behavior is of great importance because it provides a simple and direct measure of motor function and emotional state, and alterations in exploratory behavior may condition the analysis of additional phenotypic parameters (e.g., cognitive tasks) [82,83,119].

Exploratory activity was shown to be unaltered in mice in which gliotransmitter release [41,45,46,69,77,84] or intracellular calcium elevations are impaired [54,69]. The activation of Gq-protein-coupled MrgA1 receptors expressed specifically in astrocytes also failed to interfere with exploratory behavior [69]. However, mice expressing alternative Gq-protein-coupled hM3Dq in astrocytes displayed alteration of the exploratory pattern in a longer protocol, an effect that was independent of inositol trisphosphate (IP3) signaling [85]. Curiously, astrocytic coupling seems to interfere differentially with exploratory behavior. The conditional ablation of Cx43 or of adhesion molecule SynCAM1 in astrocytes seems to enhance exploration [63,71,86], while the full ablation of Cx30 does not has an impact on the horizontal exploratory drive [62]. These data might suggest that astrocyte mechanisms do not interfere with general exploratory behavior, but investigations in tests of longer duration are required.

Motor function was shown to induce activation of astrocyte networks in multiple brain regions through concerted norepinephrine signaling [87], suggesting a role for astrocytes in the integration of motor information. In addition to adrenergic input, glutamatergic signaling through AMPA receptor activation in the cerebellum was shown to support fine motor coordination [88]. By contrast, astrocyte Gq-protein-coupled receptor activation led to impairment of motor coordination that was independent of the IP3R2 [85], although it was also shown to cause IP3R2-dependent astrocyte calcium increase and consequent Purkinje cell activation [89]. Accordingly, IP3R2-dependent astrocyte calcium was also shown to enhance motor skill learning [90]. Curiously, activation of Gs-protein-coupled receptors in astrocytes did not interfere with motor coordination [49].

Regarding the impact of gliotransmitter release on motor function, its conditional blockade does not seem to interfere with motor coordination [41,46], but offered resilience to stroke-triggered motor impairment [91]. Interestingly, D-serine administration can rescue fluorocitrate-induced inactivation of astrocyte function in a motor skill-learning task [90]. The absence of GLAST leads to impaired motor coordination [92], probably due to cerebellar excitotoxicity. In addition, astrocyte coupling through Cx43, but not through Cx30, was shown to be specifically important for correct motor coordination [61–63]. Curiously, the effect of Cx43 ablation in GFAP$^+$ cells was not observed in mice that lack CX43 in S100β$^+$ cerebellar astrocytes and Bergmann glia [93], and this may support the hypothesis that astrocytes expressing different markers also perform different functions.

Sensory processing

Complementary to motor performance, sensory function is of great importance for appropriate evaluation of behavior. A sensory impairment might bias interpretation of experimental results (e.g., if an animal with normal learning capabilities has visual deficits it will face a difficulty in following external cues to reach a platform in the Morris water maze, leading to false results). Hence, sensory abilities should be assessed for all animal models [82,83,119].

Regarding the basic sensory functions, Cx30 knockout mice were reported to have a severe hearing impairment [94]. In addition, IP3R2 knockout mice generated on a Black Swiss background may display retinal degeneration, leading to performance deficits in cognitive tests [95], which may be reverted (for instance, by backcrossing the line with C57BL/6 mice [53,89,96]). Although blockade of exocytosis in astrocytes altered the function of Müller cells in the retina, the visual abilities of these mice remained unaffected [46]. Regarding the role of astrocytes in odor

processing, it has been shown that astrocytes respond with fast calcium elevations to odor stimulation [97], and that the lack of astrocyte transporter GLAST led to misprocessing of odor inputs that was ascribed to alterations in gamma oscillations [98].

Sensory stimulation was shown to potentiate cortical responses when paired with electrical stimulation in the nucleus basalis of Meynert (NBM), the primary cholinergic input to the cerebral cortex. This effect was absent in mice lacking astrocyte calcium signaling, although it was rescued by D-serine administration in these animals [99]. Accordingly, cortical plasticity triggered by whisker stimulation alone was abolished by deleting Cx43, which translated to a severe impairment of whisker-dependent tactile function [70]. Similarly, astrocyte calcium signaling was shown to be crucial for cholinergic dependent plasticity in the hippocampus [100] and for NBM-induced potentiation of visual responses [96]. These results indicate an important role of astrocytes in the processing of cholinergic input in corticolimbic circuits. More recently, an elegant study in which optogenetic tools were used to specifically activate $GFAP^+$ astrocytes demonstrated that this activation is responsible for both excitatory and inhibitory synaptic transmission in the primary visual cortex [101]. In addition, different studies have reported that sensory stimulation triggers fast calcium transients in astrocytes [102], coupled with hemodynamic responses in the same brain region [103]. These fast signals were also observed in the locus coeruleus after whisker air-puff startle stimulation; this has implications for the effects of adrenergic projections to cortical areas [104]. Finally, similar sensory stimulation triggered astrocyte process motility and calcium signals, which correlated with enhanced synaptic plasticity [105].

Lastly, astrocytes seem to interfere with pain processing because impairment of astrocyte exocytosis in the dnSNARE animals reduced baseline mechanical nociception, although nociception during neuropathic pain was unaltered [106], and astrocyte-specific deletion of Cx43 prevented the development of chronic neuropathic pain following spinal cord injury [107]. These results suggest a clear involvement of astrocytes in nociceptive processing by brain networks, but the involvement appears to be very specific because Gs-coupled protein activation of astrocytes, blockade of glutamate uptake, or engraftment of human astrocytes failed to interfere with nociception [49,67,75].

Conceptual challenges and future perspectives

The evidence obtained thus far points to important roles played by astrocytes in network computation of behavioral outputs in the four main behavioral dimensions discussed in this review (summarized in Figure 1). It is clear that the

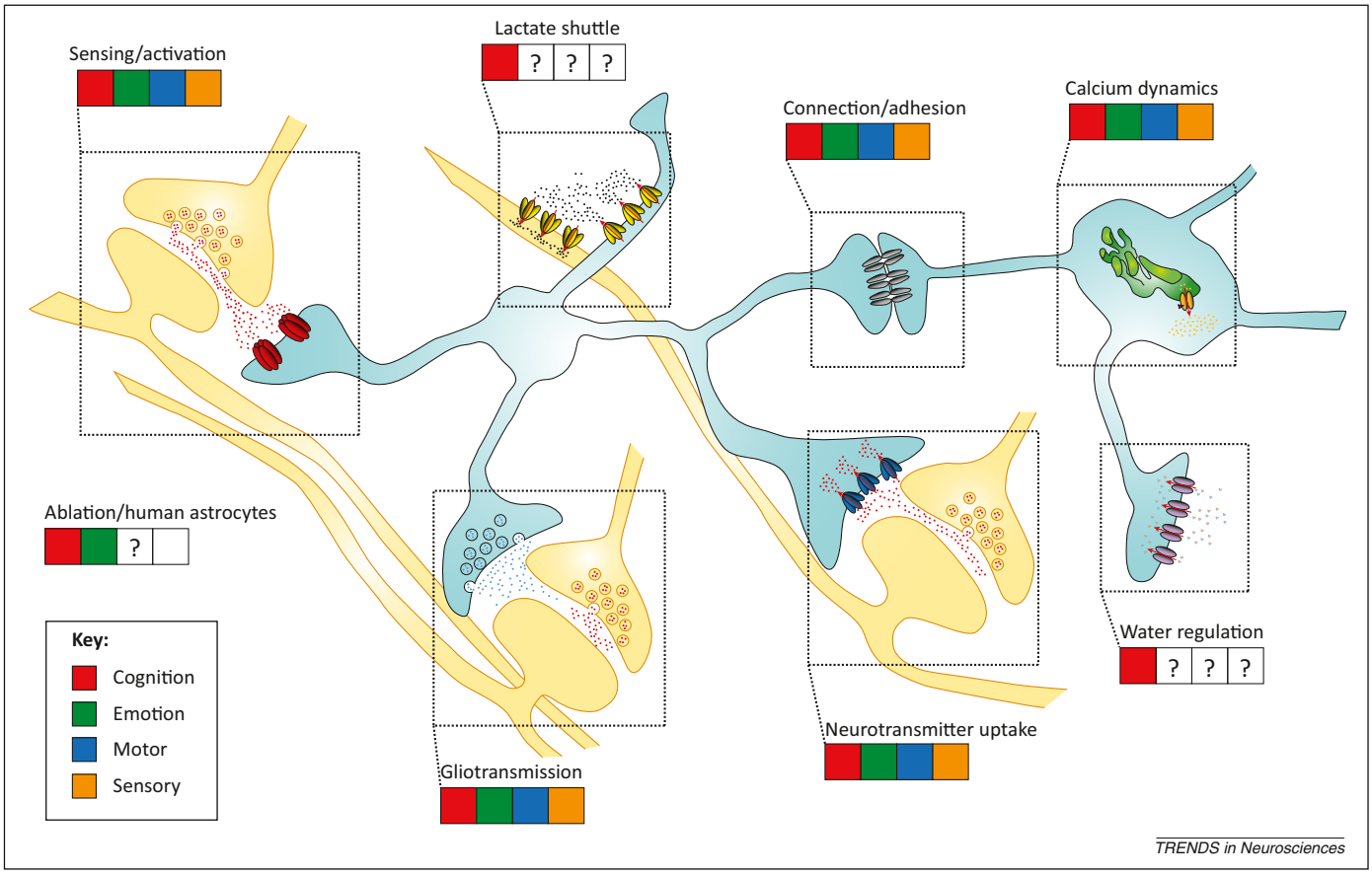

Figure 1. Behavioral dimensions affected by different astrocytic functions. The scheme represents the behavioral dimensions affected after modulation of each astrocytic function. Each frame with four squares represents the behavioral dimensions, from left to right: cognition (red); emotion (green); motor activity and coordination (blue); sensory processing (orange). A colored square indicates that at least one study has implicated astrocytes in that particular behavioral dimension. White squares refer to dimensions not affected by astrocyte modulation, while question marks identify dimensions that are yet to be assessed. Figure elements: neurons (yellow); astrocytes (blue). Depicted structures: G-protein-coupled receptors (red); lactate transporters (yellow); glutamate transporters (blue); aquaporin channels (purple); connexins/adhesion molecules (grey); inositol trisphosphate (IP3) receptors (orange). Molecules: neurotransmitters (red dots); gliotransmitters (blue dots); lactate (black dots); water (red/blue dots); IP3 (orange). Arrows (red) indicate the direction of the net molecule movement.

dialog between astrocytes and neurons is crucial for correct performance of the neuron–astrocyte network in all the behavioral domains analyzed. One should note, however, that although we know now that a given astrocyte function is important for network performance, these observations are still very general because the functions studied include general astrocyte mechanisms (e.g., exocytosis or calcium dynamics), and usually across extensive brain areas (e.g., in whole-brain receptor knockouts). It is also noteworthy that, for each function, the results differed both across different models and even within the same model. These discrepancies must be explained by further studies (Box 2).

The establishment of the contribution of astrocytes to behavior faces important experimental and conceptual challenges that derive from the relatively initial stages of our knowledge on the specific properties of astrocyte physiology and astrocyte–neuron communication. For example, astrocytes are emerging as a more heterogeneous cell type than was previously thought [108,120]. Regional heterogeneity as well as cellular subtype specificity within brain areas need to be considered when modulating astrocytes to evaluate their consequences on animal behavior (Box 2). Moreover, astrocyte control of behavior will include long-range spatial and temporal integration of information, and this adds an extra layer of complexity, putatively providing the network with increased degrees of freedom to perform a given task [5].

In summary, accumulating evidence indicates a direct involvement of astrocyte function in animal behavior, based on the key properties of astrocyte function in regulating neuronal and synaptic function through the control of brain homeostasis, neuronal metabolism, and gliotransmission. It is our belief that future research should be guided by innovation in the design of more-accurate models and techniques and, most importantly, by the adequate selection of the best option for *in vivo* analysis taking into account the functional heterogeneity of astrocytes.

Box 2. Outstanding questions

How might model diversity affect my results?

- The first concern is related to the inherent divergence of available models. For instance, there are at least five different IP3R2 KO strains. The generation of different strains arises from the consensual need for a consistent genetic background and cellular specificity. However, researchers should take this compilation into account and try to focus on common strains for better integration of the data obtained.
- The second concern is that new (and perhaps also existing) models should be fully validated. The molecular mechanisms underlying the genetic manipulation are crucial for the interpretation of data. For example, the use of Cre/loxP recombination or tetracycline-inducible mice should be characterized in detail because they may lead to unexpected outcomes, such as lack of recombination, regional specificities, or even unspecific transgene expression [36,116], that will require further clarification and confirmation for each model in each laboratory [49].

Which aspects shall we take into consideration when interpreting the data available so far and in preparing future in vivo *studies?*

- Phenotypes of animal models of astrocyte dysfunction should be carefully characterized because impairments in basic processing such as motor or sensory functions may lead to false results by, for instance, affecting the performance of cognitive tasks.
- Behavioral testing should be addressed and analyzed very carefully, preferably using standard protocols to permit better comparison between different studies, and different behavioral paradigms to assess a similar function (e.g., the Morris water maze and the radial arm maze to assess spatial reference memory).
- The analysis of brain outputs should include complementary techniques such as behavioral analysis, *in vivo* electrophysiology, and imaging to obtain valuable and consistent information; the approaches used should evolve to address (i) the temporal and spatial resolution characteristic of astrocyte somata and fine processes [117,118], and (ii) the direct impact of astrocytes on calcium oscillations or electrophysiological signals rather than an indirect neuronal readout.
- Classically, different tasks rely on specific networks (e.g., spatial memory relies on the dorsal hippocampus) whose neurons display characteristic anatomical and functional features; similarly, the apparent molecular, cellular, and functional heterogeneity presented by astrocytes [108,120] may lead to an unexpected diversity of effects and needs careful interpretation.

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