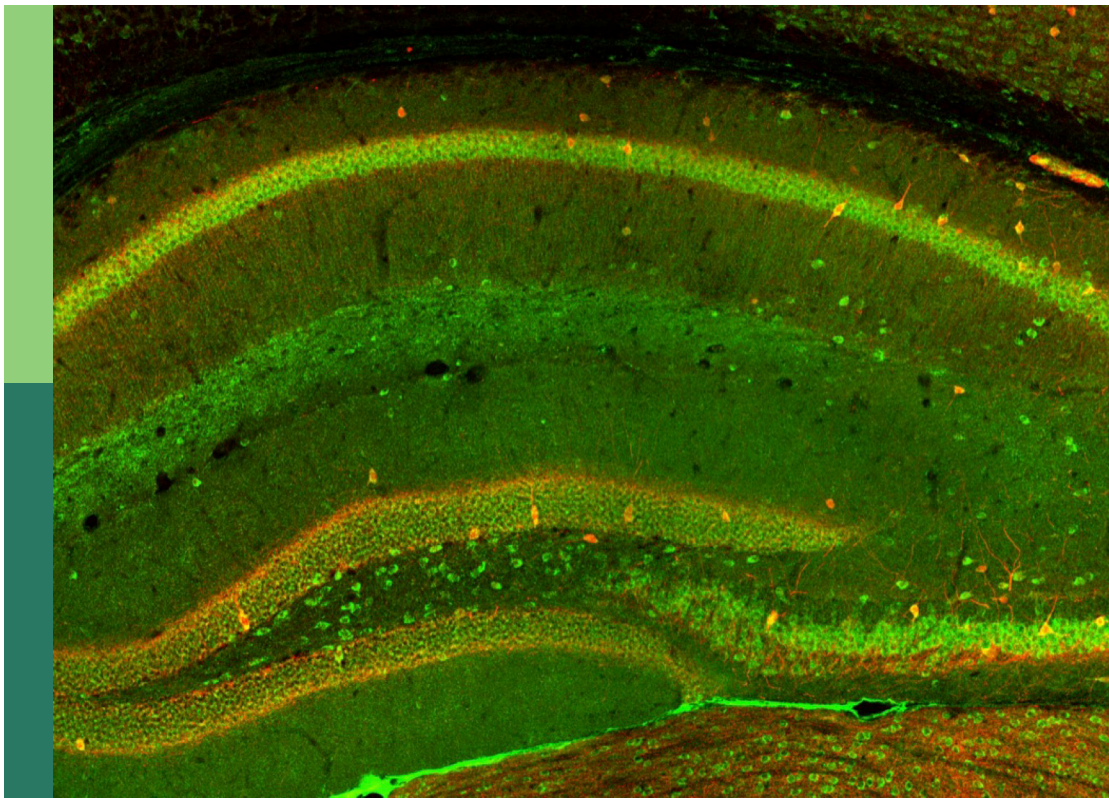
frontiers | Research Topics

Physiology and pathology of neuroglia

Edited by
Daniel Reyes-Haro, Alejandro López-Juárez
and Adrian Rodriguez-Contreras

Published in
Frontiers in Cellular Neuroscience



ISSN 1664-8714
ISBN 978-2-8325-3148-8
DOI 10.3389/978-2-8325-3148-8

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Physiology and pathology of neuroglia

Topic editors

Daniel Reyes-Haro — National Autonomous University of Mexico, Mexico

Alejandro López-Juárez — The University of Texas Rio Grande Valley, United States

Adrian Rodriguez-Contreras — Northwestern University, United States

Citation

Reyes-Haro, D., López-Juárez, A., Rodriguez-Contreras, A., eds. (2023). *Physiology and pathology of neuroglia*. Lausanne: Frontiers Media SA.

doi: 10.3389/978-2-8325-3148-8

Table of contents

frontiers | Frontiers in Cellular Neuroscience

TYPE Editorial
PUBLISHED 18 July 2023
DOI 10.3389/fncel.2023.1246885

OPEN ACCESS

EDITED AND REVIEWED BY
Marie-Ève Tremblay,
University of Victoria, Canada

*CORRESPONDENCE
Daniel Reyes-Haro
✉ dharo@unam.mx

RECEIVED 24 June 2023
ACCEPTED 07 July 2023
PUBLISHED 18 July 2023

CITATION
Reyes-Haro D, López-Juárez A and Rodríguez-Contreras A (2023) Editorial: Physiology and pathology of neuroglia. *Front. Cell. Neurosci.* 17:1246885. doi: 10.3389/fncel.2023.1246885

Editorial: Physiology and pathology of neuroglia

Daniel Reyes-Haro[1]*, Alejandro López-Juárez[2] and Adrián Rodríguez-Contreras[3]

[1]Universidad Nacional Autónoma de México, Instituto de Neurobiología - UNAM, Campus Juriquilla, Juriquilla, QRO, Mexico, [2]Department of Health and Biomedical Sciences, The University of Texas Rio Grande Valley, Brownsville, TX, United States, [3]The Roxelyn and Richard Pepper Department of Communication Sciences and Disorders, School of Communication, Northwestern University, Evanston, IL, United States

KEYWORDS

anorexia, Down syndrome, diabetes, Fetal Alcohol Syndrome, pain

Editorial on the Research Topic
Physiology and pathology of neuroglia

Neuroglia is the largest population of cells in the brain and participates in formation, maintenance, and modulation of synaptic circuits. This heterogenous group includes macroglia (astroglia and oligodendroglia) and microglia. Neurons and neuroglia form assemblies that potentiate the cognitive capability of the brain. In this topic, nine articles highlight structural and functional roles of neuroglia in brain physiology, which is fundamental to better understand the biology of neurodevelopmental and neurodegenerative diseases.

Our understanding of brain connectivity evolves rapidly and the cytoarchitecture of underexplored brain regions such as the cerebellum need to be revisited (De Zeeuw et al., 2021). Hence, Gómez-González et al. reviewed the organization of a peculiar cerebellar glial niche located at the subventricular zone of the fourth ventricle. In addition to neurons and ependymal cells, this region is rich in microglia as well as macroglia including astrocytes, Bergmann glia, and oligodendrocyte lineage cells. Further transcriptional and functional heterogeneity within these glial populations is discussed. Despite scarce to non-proliferative activity, this region shares similarity with adult neurogenic niches throughout the brain and various stimulating questions remain to be explored. Furthermore, glial organization is adapted to this highly vascularized niche and contribute to the glioneurovascular unit, a structural and functional element where glial cells respond to stimulation by coupling to increased sensory activity through development (Biesecker et al., 2016; Koehler, 2021). In a perspective contributed by Konecny et al., they hypothesize that augmented neuronal activity is associated with angiogenic factor production and creates an environment of intermittent hypoxia, promoting the expression of hypoxia-inducible transcription factors (HIFs) by the glioneurovascular unit. However, mechanisms triggering glioneurovascular coupling during early sensory neurodevelopment are unknown; therefore, further research using non-invasive approaches is proposed.

Neuron-glia coupling is physiologically tied to volume regulation. Ionic gradients permit neurons to communicate electrically, and glial cells help them to regulate volume. Astrocytes express a variety of cotransport systems and ion channels to maintain brain homeostasis through mobilization of osmolytes (Walch and Fiacco, 2022). Ochoa-de la Paz and Gulias-Cañizo identify glial cells as master regulators of the tripartite synapse volume, a property that gives them an important role in maintaining homeostasis. Dysfunction of volume regulation leads

to pathology in conditions such as edema, uremia, and diabetes, in which solute imbalances occur.

Neuron-glia communication is mediated by neurotransmitters including glutamate, the main excitatory neurotransmitter of the brain. This signaling occurs through ionotropic and metabotropic receptors expressed by neurons and glial cells. Particularly, astrocytes modulate neuronal activity through release of gliotransmitters like glutamate and D-serine (Reyes-Haro et al., 2010). Additionally, astrocytes express glutamate transporters to regulate the glutamatergic tone at the synaptic cleft and supply glutamine to neurons that convert it into glutamate or GABA to refill synaptic vesicles (Martínez-Lozada and Ortega, 2023). Dysfunction of the glutamate-glutamine shuttle results in excitotoxicity that has been linked to Alzheimer's and Huntington's diseases. Thus, Cuellar-Santoyo et al. summarize the astrocyte's contribution to glutamatergic neurotransmission in physiological and pathological conditions.

Astrocytes also respond to neuronal activity with calcium transients, a signaling mechanism that seems involved in pain and nociception (Prokhorenko and Smyth, 2023). Here, Higinio-Rodríguez et al. present an experimentally supported perspective in which coherent activity of astrocytes in pain-related brain areas plays critical roles in binding sensory, affective, and cognitive information, on a slow time scale. As astrocytes respond to noxious stimuli via calcium modulation likely independent of neuronal activation, this could represent the mechanism by which pain is created from nociception with the participation of astrocytes.

Glial cell responses in pathology involve inflammasomes, multi-protein intracellular signaling complexes which orchestrate inflammatory responses to a diverse range of pathogens and host-derived signals (Jewell et al., 2022). In their review article, Mata-Martínez et al., discuss aspects of the inflammatory process, focusing on accumulating evidence of multiprotein complexes that sense and respond in the context of inflammation. The authors argue that acute and chronic inflammation will engage a coordinated molecular response in various organs, involving glial cells in the brain. Interestingly, Down syndrome (DS) and Alzheimer's disease (AD) are characterized by chronic neuroinflammation, peripheral inflammation, astrogliosis, imbalanced excitatory/inhibitory neuronal function, and cognitive deficits in both humans and mouse models (Ahmed et al., 2022). Little is known about the causes of these pathologies, but patients with DS are suspected to be predisposed to developing AD late in life. García and Flores-Aguilar summarize data about glial cells in the context of DS-AD and inflammation.

Links for inflammation and glial cells could also exist in anorexia; food intake is reduced during acute and chronic inflammatory states in human and research models (Gautron and Layé, 2010). Reyes-Haro reviews features of physiological anorexia in research models in comparison with human pathological anorexia, emphasizing valid precautions when extrapolating results. Moreover, he discusses studies in murine models of anorexia in which glial cells putatively play central roles in classical hypothalamic mechanisms, as well as in systemic machineries including the prefrontal cortex. Specifically, the pro-inflammatory environment associated with microglia reactivity, the impact of astrocyte manipulation on food intake associated with purinergic gliotransmission, and the roles of Oligodendrocyte Precursor Cells (OPCs) mediating the anorexigenic action of leptin in mice, are presented.

Another pathology associated with glial cell dysfunction is alcohol exposure during pregnancy. Fetal Alcohol Syndrome (FAS) is a public health problem with a prevalence of 2–5% in the USA. FAS disturbs the structure and function of the brain, but the underlying mechanisms remain elusive (Holloway et al., 2023). Zheng et al., observed loss of the tubulin-binding cofactor B resulting in disorganized microtubules and shortening of astrocytic processes in a model of chronic alcohol exposure. Developmental pathological implications to consider include abnormal migration of neuronal precursors through aberrant radial glial processes and defective synaptic coverage by astrocytes.

Overall, articles in this topic cover diverse aspects of research on glial cells and serve as introductory information to several subfields of glial biology. Ideas presented encourage others to design studies to clarify the roles of physiological and pathological factors with potential use in therapeutic applications and engineering.

Author contributions

DR-H, AL-J, and AR-C wrote the original draft and edited the final version of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by CONAHCYT 319209 and UNAM-DGAPA-PAPIIT (IN209121) to DR-H. NU start-up funds to AR-C.

Acknowledgments

The editors of this topic are grateful to Bruce Ransom, Alfonso Araque, and Axel Nimmerjahn for participating and promoting the organization of the Symposium on Physiology and Pathology of Neuroglia, a biannual event that is becoming a referent in the field. We are grateful to Teresa Morales and Mauricio Díaz (INb-UNAM), Raúl Paredes and Aurea Orozco (ENES-Juriquilla), and Gerardo Piloni and Yolanda Chirino (Posgrado-UNAM, PAEP-UNAM) for their support. We also appreciate the support of the technical and administrative staff.

Conflict of interest

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

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher,

the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Ahmed, M. M., Wang, A. C., Elos, M., Chial, H. J., Sillau, S., Solano, D. A., et al. (2022). The innate immune system stimulating cytokine GM-CSF improves learning/memory and interneuron and astrocyte brain pathology in Dp16 Down syndrome mice and improves learning/memory in wild-type mice. *Neurobiol. Dis.* 15, 105694. doi: 10.1016/j.nbd.2022.105694

Biesecker, K. R., Srienc, A. I., Shimoda, A. M., Agarwal, A., Bergles, D. E., Kofuji, P., et al. (2016). Glial cell calcium signaling mediates capillary regulation of blood flow in the retina. *J. Neurosci.* 36, 9435–45. doi: 10.1523/JNEUROSCI.1782-16.2016

De Zeeuw, C. I., Lisberger, S. G., and Raymond, J. L. (2021). Diversity and dynamism in the cerebellum. *Nat. Neurosci.* 24, 160–167. doi: 10.1038/s41593-020-00754-9

Gautron, L., and Layé, S. (2010). Neurobiology of inflammation-associated anorexia. *Front. Neurosci.* 3, 59. doi: 10.3389/neuro.23.003.2009

Holloway, K. N., Douglas, J. C., Rafferty, T. M., Majewska, A. K., Kane, C. J. M., and Drew, P. D. (2023). Ethanol-induced cerebellar transcriptomic changes in a postnatal model of fetal alcohol spectrum disorders: Focus on disease onset. *Front. Neurosci.* 17, 1154637. doi: 10.3389/fnins.2023.1154637

Jewell, S., Herath, A. M., and Gordon, R. (2022). Inflammasome Activation in Parkinson's Disease. *J. Parkinsons Dis.* 12, S113–S128. doi: 10.3233/JPD-223338

Koehler, R. C. (2021). Regulation of the cerebral circulation during development. *Compr Physiol.* 11, 2371–2432. doi: 10.1002/cphy.c200028

Martínez-Lozada, Z., and Ortega, A. (2023). Milestone review: excitatory amino acid transporters - beyond their expected function. *J. Neurochem.* 165, 457–466. doi: 10.1111/jnc.15809

Prokhorenko, M. A., and Smyth, J. T. (2023). Astrocyte store-operated calcium entry is required for centrally mediated neuropathic pain. *bioRxiv [Preprint].* doi: 10.1101/2023.06.08.544231

Reyes-Haro, D., Müller, J., Boresch, M., Pivneva, T., Benedetti, B., Scheller, A., et al. (2010). Neuron-astrocyte interactions in the medial nucleus of the trapezoid body. *J. Gen. Physiol.* 135, 583–94. doi: 10.1085/jgp.200910354

Walch, E., and Fiacco, T. A. (2022). Honey, I shrunk the extracellular space: measurements and mechanisms of astrocyte swelling. *Glia.* 70, 2013–2031. doi: 10.1002/glia.24224

frontiers | Frontiers in Cellular Neuroscience

REVIEW
published: 16 June 2022
doi: 10.3389/fncel.2022.929529

Glial Cells and Brain Diseases: Inflammasomes as Relevant Pathological Entities

*Esperanza Mata-Martínez, Mauricio Díaz-Muñoz and Francisco G. Vázquez-Cuevas**

Laboratorio de Fisiología Celular, Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Juriquilla, Mexico

Inflammation mediated by the innate immune system is a physiopathological response to diverse detrimental circumstances such as microbe infections or tissular damage. The molecular events that underlie this response involve the assembly of multiprotein complexes known as inflammasomes. These assemblages are essentially formed by a stressor-sensing protein, an adapter protein and a non-apoptotic caspase (1 or 11). The coordinated aggregation of these components mediates the processing and release of pro-inflammatory interleukins (IL-β and IL-18) and cellular death by pyroptosis induction. The inflammatory response is essential for the defense of the organism; for example, it triggers tissue repair and the destruction of pathogen microbe infections. However, when inflammation is activated chronically, it promotes diverse pathologies in the lung, liver, brain and other organs. The nervous system is one of the main tissues where the inflammatory process has been characterized, and its implications in health and disease are starting to be understood. Thus, the regulation of inflammasomes in specific cellular types of the central nervous system needs to be thoroughly understood to innovate treatments for diverse pathologies. In this review, the presence and participation of inflammasomes in pathological conditions in different types of glial cells will be discussed.

Keywords: inflammasome, glia, astrocyte, microglia, oligodendrocyte, NLRP3, neuroinflammation

OPEN ACCESS

Edited by:
Adrian Rodriguez-Contreras, City College of New York (CUNY), United States

Reviewed by:
Tao Tao, Southern Medical University, China
Lai Wang, Henan University, China

***Correspondence:**
Francisco G. Vázquez-Cuevas
fvazquez@comunidad.unam.mx

Specialty section:
This article was submitted to Non-neuronal Cells, a section of the journal Frontiers in Cellular Neuroscience

Received: *27 April 2022*
Accepted: *27 May 2022*
Published: *16 June 2022*

Citation:
Mata-Martínez E, Díaz-Muñoz M and Vázquez-Cuevas FG (2022) Glial Cells and Brain Diseases: Inflammasomes as Relevant Pathological Entities. Front. Cell. Neurosci. 16:929529. doi: 10.3389/fncel.2022.929529

INTRODUCTION

In response to pathogens or damage, the innate immune system of multicellular organisms responds with an alarm signal known as inflammation. Detection of stressor agent hints, like pathogen-associated molecular patterns (PAMPs, e.g., lipopolysaccharide (LPS), flagellin or viral RNA) or damage-associated molecular patterns (DAMPs, e.g., ATP, cytochrome C, defensins, galectins or uric acid), triggers the assembly of cytosolic multimeric protein complexes named inflammasomes. Inflammasomes process and release IL-β and IL-18 and induce pyroptotic cell death, a kind of sophisticated apoptosis directly related to inflammation (Bryant and Fitzgerald, 2009).

Classification

First described around 20 years ago (Martiñon et al., 2002), inflammasomes constitute a complex sharing functional and structural characteristics and are appointed and classified in function of the sensor protein triggering their assembly. Thus, inflammasomes can belong to those containing nucleotide-binding oligomerization domains (NOD/NATCH) and leucine-rich domains (NLRs),

or those not containing the mentioned domains (no-NLRs) (Angosto-Bazarra et al., 2021; Zhang et al., 2021; **Figure 1**).

The NLR family includes NOD proteins, NOD-like receptor proteins (NLRPs) and NOD-like receptor C4 (NLRC4). Advances in NLR classification and nomenclature are available elsewhere (Mackenzie et al., 2008; Ting et al., 2008; Schroder and Tschopp, 2010). The NLR group is formed by 23 members in humans and 34 in mice; these proteins show characteristic motifs such as pyrin domain (PYD), a conserved central NATCH domain responsible for protein oligomerization, a carboxy terminal leucine-rich repeat (LRR) domain and a variable amino terminal region (reviewed in Walsh et al., 2014a) (**Figure 1**). NLRP1, NLRP2, NLRP3, NLRP4, NLRP6 and NLRP12 can participate in the formation of inflammasomes, being NLRP3 the best characterized (Rathinam et al., 2012). The importance of NLRP3 as a regulator of immune homeostasis is highlighted by the fact that mutations in the *NLRP3* gene cause autoimmune diseases such as the Muckle–Wells syndrome (also known as UDA syndrome) and the familial cold autoinflammatory syndrome (urticaria episodes triggered by exposure to cold) (Hoffman et al., 2001), collectively named cryopyrin-associated periodic syndromes (Broderick et al., 2015). Another relevant sensor protein is NLRC4 (originally named IPAF because it is related to APAF-1), which is characterized by a caspase recruitment domain (CARD), with high affinity for caspase-1, bound to NATCH and LRR domains (**Figure 1**); caspase-1 association promotes the autocatalytic activation of their protease activity (Poyet et al., 2001). NLRC4 is relevant in the context of microbial infection, as it can sense flagellin of *Salmonella typhimurium* (Amer et al., 2006) and is activated by a mechanism other than NLRP3, but in some circumstances both can be cooperative (Qu et al., 2016).

In addition, the absent in melanoma 2 (AIM2)-like receptors (AIM2) are other well-characterized no-NLR inflammasome sensors expressed in the central nervous system (CNS) (Heinisch et al., 2021). AIM2 protein is an inflammasome component specialized in the detection of mislocated or foreign DNA (from viruses, bacteria or other parasites). It has been demonstrated that disruption of the nuclear envelope also induces its aggregation (Di Micco et al., 2016); when the AIM2 inflammasome is assembled, it promotes pyroptosis (Lugrin and Martiñon, 2018).

Structure

Inflammasomes are multiprotein complexes (∼700 kDa) constituted by three basic elements: (1) the previously described *cytosolic pattern recognition receptors*, (2) an *adapter protein* and (3) *an effector*, typically pro-caspase-1. For inflammasome aggregation, extracellular (i.e., ATP as a signaling molecule) or intracellular (i.e., uric acid, an adenosine catabolite) DAMPs trigger the activity of NLR; then NLR recruits the adapter protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), which contains PYD and CARD domains, to interact with the CARD domain of pro-caspase-1 (Bryant and Fitzgerald, 2009). The three components of the inflammasome unit can be self-assembled into filamentous structures with complex stoichiometries (Broderick et al., 2015).

Caspases are cysteinyl-aspartate proteases essential for inflammation, apoptosis and pyroptosis. In humans, the caspase family has 11 members. Caspases involved in inflammation are caspases 1, 4 and 5 in humans and 1, 11 and 12 in rodents (reviewed in Lamkanfi et al., 2007); recruitment of pro-caspase-1 induces its proteolytic processing to generate mature IL-β and IL-18 (Angosto-Bazarra et al., 2021; Zhang et al., 2021). Active caspase-1 also hydrolyzes Gasdermin D (GsdmD), the main protein related to pyroptosis execution, generating a fragment known as GsdmD N-terminal (Burdette et al., 2021), which forms pores in the plasma membrane that allow the entry of water toward the cytoplasm followed by an osmotic shock and cell outbreak, promoting the release of the cell content to the extracellular space including accumulated interleukins. In this context, caspase-1 activates other executer caspases to complete the death process by destroying nuclear DNA and cytoskeleton proteins (Bergsbaken et al., 2009; **Figure 2**).

Activation

Activation of the inflammatory process by DAMPs is a well-understood event when extracellular ATP (eATP) is the trigger (Adinolfi et al., 2018). When this nucleotide is accumulated in the extracellular space, it binds to the P2X7 receptor activating the depolarizing influx of Ca^{2+} and Na^{+} as well as the efflux of K^{+}; the ATP-induced reduction in cytoplasmic K^{+} leads to NLRP3 activation *via* NEK7 kinase, promoting NLRP3 inflammasome assembly (Pétrilli et al., 2007; He et al., 2016). P2X7 receptor activation also induces the formation of a pore with high conductance that allows the transit of molecules of ∼1 kDa; the stabilization of this *megapore* involves the participation of pannexin-1 (Pelegrin and Surprenant, 2006). Since pannexin-1 has been detected in association with the NLRP2/P2X7 receptor complex (Minkiewicz et al., 2013), it could be a player of P2X7 receptor-dependent inflammasome activation. Moreover, the increment in reactive oxygen species (ROS) by P2X7 receptor also favors inflammasome activation (Hung et al., 2013). In addition, protein-protein interaction between P2X7 receptor and the scaffold protein NLRP3 has been demonstrated (Franceschini et al., 2015), suggesting a cooperative mechanism between different cellular events elicited by P2X7 receptor to accomplish inflammasome activation.

It has also been described that the inflammasome activated by LPS mediates ATP/P2X7 receptor-dependent pyroptosis; thus, LPS administration induces caspase-11 activation, which cleaves pannexin-1 originally located in the plasma membrane. This proteolytic event induces a dramatic increment in the eATP triggering NLRP3 inflammasome activation, which guides the cell to pyroptosis. In agreement with the role played by the multiprotein complex of P2X7 receptor-dependent inflammasomes, LPS priming was unable to induce pyroptosis in *Panx1*$^{-/-}$, *P2X7*$^{-/-}$ or *Casp11*$^{-/-}$ mice (Yang et al., 2015).

Acute activation of inflammatory responses helps the injured tissue to restore homeostasis or overcome pathogen infections; however, ample evidence supports the notion that chronic inflammation is a harmful mechanism that contributes to the development of various nervous system pathologies such as Alzheimer's disease (AD; Heneka et al., 2013), stroke (Fann et al., 2013), depression (Iwata et al., 2013), autism

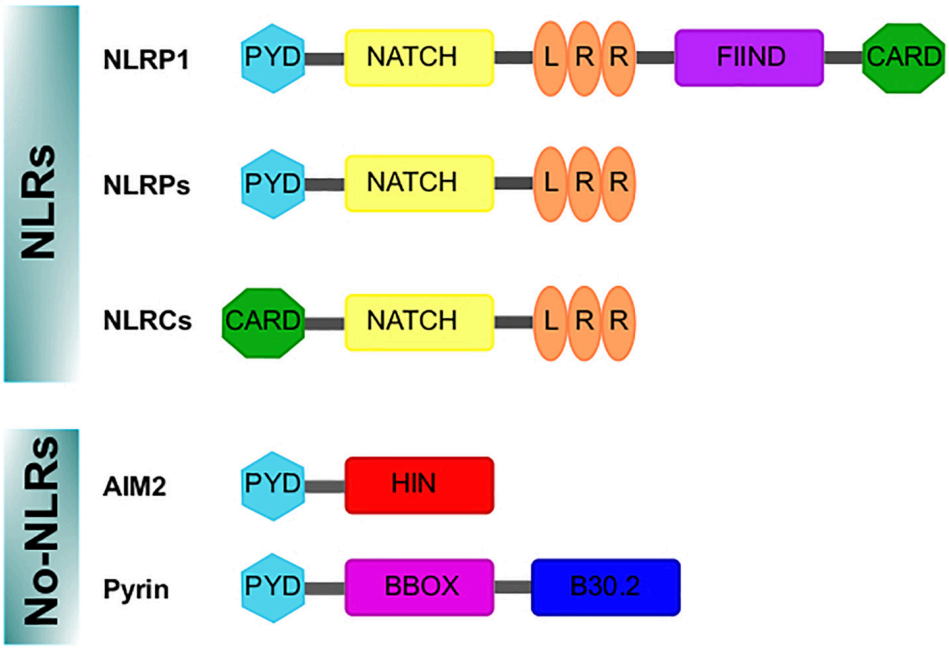


FIGURE 1 | Domains structure of the inflammasomes sensor proteins. Nod like receptor (NLRs) proteins have a nucleotide-binding and oligomerization domain (NACHT/NBD), a leucine-rich repeat (LRR) motifs, typically located in the center and carboxy terminus of the NLR proteins, respectively. The NACHT motif is usually flanked by an additional amino-terminal domain, either a caspase recruitment domain (CARD) or a pyrin domain (PYD). For NLRP1, a FIIND and CARD domain are in the C-terminal position after the LRR domain. In addition, the absent in melanoma 2 (AIM2)-like receptor and Pyrin are no-NLR inflammasome sensors. AIM2 is characterized by an amino-terminal PYD domain and one or two DNA-binding HIN domains. Pyrin, features a PYD domain, two B-boxes, and a C-terminal B30.2 domain.

(Saresella et al., 2016a), bipolar disorder (Kim et al., 2015a), sclerosis (Keane et al., 2018) and Parkinson's disease (PD; Wang et al., 2022). Expression and regulation of inflammasomes in glial cells in this pathological context will be discussed.

INFLAMMASOMES IN MICROGLIAL CELLS

Microglial cells are considered specialized resident macrophages of the brain, showing particular genetic expression patterns compared with circulating macrophages (Lavin et al., 2014) and self-renewal within the brain (Ajami et al., 2007). Microglial cells have diverse functions, such as clearing apoptotic cells by phagocytosis and supporting synaptogenesis by secreting neural factors during the learning process (Labzin et al., 2018).

In their resting state, microglial cells display a basal stellated morphology and establish contact with neurons, astrocytes and endothelial cells; but when an injury or a metabolic challenge occurs, they change their morphology to acquire an amoeboid shape with high migratory abilities to reach the damaged zone and secrete pro-inflammatory mediators that promote astrocytic activation and recruitment of peripheral immune cells (Colonna and Butovsky, 2017). Microglia can secrete proinflammatory cytokines like IL-1β, IL-6, tumor necrosis factor alpha (TNF-α), IL-23, and IL-18 in response to DAMPs, including PAMPs, endogenous alarmins, and misfolded proteins (Prinz et al., 2019). Moreover, they are equipped with a set of receptors, called the microglial sensome, which allow the recognition of invading pathogens, misfolded proteins, chemokines and cytokines, metabolites, inorganic substances, and changes in pH or extracellular matrix components (Hickman et al., 2013). The murine microglial sensome comprises purinergic receptors (P2X4, P2X7, P2Y12, P2Y13, and P2Y6), chemokine receptors (CCR5, CX3CR1, CXCR4, and CXCR2), Fc receptors (FCERer1G and FCGR3), interferon-induced transmembrane proteins (IFITM2, IFITM3, and IFITM6), Toll-like receptors (TLR2 and TLR7), and Siglecs (SiglecH and Siglec3/CD33) (Prinz et al., 2019).

Regarding inflammasome machinery, it has been reported that LPC-dependent inflammation specifically induces caspase-1 activity mediated by NLRP3 and NLRP4 inflammasomes in microglia (Scholz and Eder, 2017). Additionally, the expression of NLRP3 components (*NLRP3, Asc* and *casp-1*) was detected by polymerase chain reaction in primary cultures of microglial cells, suggesting that inflammasomes are functional in these cells. On another hand, treatment of microglial cells with DAMPs, such as ATP, nigericin or alum, induces IL-1β production, supporting the role played by microglial cells in inflammasome aggregation (Gustin et al., 2015).

Furthermore, cooperative actions of AIM2 and NLRP3 have been observed when microglial cells were primed by ligands derived from *Brucella abortus*; AIM2 senses *Brucella* DNA

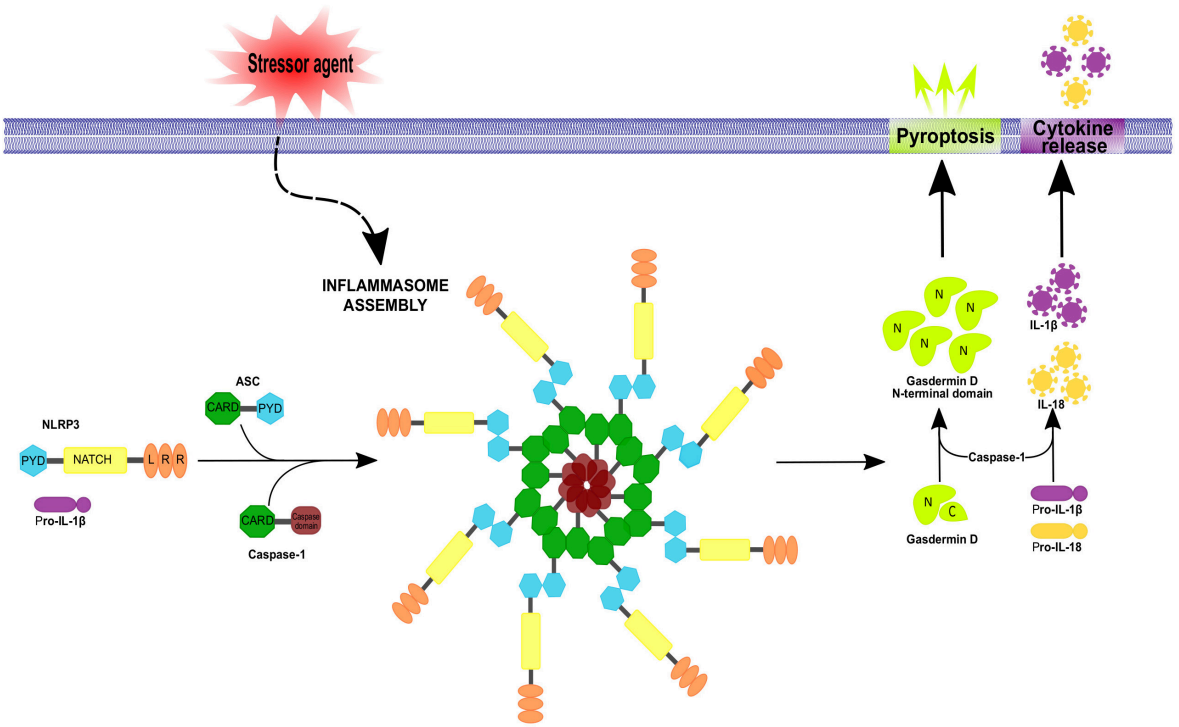


FIGURE 2 | Inflammasome assembly. Inflammasomes assemble in a stimulus-specific manner. Different stressor agents are able to induce inflammasome activation by NLRs or no-NLR inflammasome sensors proteins. Activation of the NLRP3 inflammasome involves ASC and procaspase-1 recruitment, resulting in ASC oligomerization into a macromolecular aggregate and subsequent activation of procaspase-1. Active caspase-1 then cleaves pro-IL-1β and pro-IL-18 to their mature forms IL-1 β and IL-18 which get secreted. In addition, caspase-1 can cleave gasdermin D, releasing its N-terminal fragment which translocate to the plasma membrane inducing pore formation and pyroptotic cell death. The apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) is an adaptor protein for many inflammasome complexes and is composed of CARD and PYD domains, the latter being necessary for homotypic interaction with a PYD-containing inflammasome sensor. Procaspase-1 features a CARD domain, in addition to its caspase domain, and homotypic CARD interactions result in direct or indirect (*via* ASC) recruitment of procaspase-1 to the inflammasome complex.

and NLRP3 inflammasomes detect ROS (Marim et al., 2017) contributing to an immunopathological state and generating a disease named neurobrucellosis.

Taken together, these observations indicate that modulation of the immune response by microglia is critical to maintaining homeostasis in the brain.

Inflammasome Activation in Microglial Cells

Aggregation and activation of inflammasomes is a general mechanism to face different types of cell injury; within the brain, it has been suggested that the immune response begins in microglial cells. A wide variety of stimuli that promote the activation of inflammasomes involve many pathogens and different classes of DAMPs.

When microglial cells face a pathogen, for instance *Streptococcus pneumoniae*, a microorganism whose infections produce meningitis, they respond by assembling inflammasome NLRP3, activating caspase-1, secreting IL-1β and IL-18 and inducing cell death by pyroptosis. In addition, the infection is associated with autophagy; nevertheless, the relationship with pyroptosis is not well understood (Kim et al., 2015b).

Likewise, when infection by HIV affects the CNS, a sustained NLRP3 inflammasome-dependent response leads to neurological damage specifically affecting microglial cells. By using microglia-derived cell lines, researchers observed that HIV promotes the synthesis of pro-IL-1β after 4 h post-infection and the release of this cytokine after 24 h; in agreement, brains of patients with AIDS showed high levels of IL-1β, IL-18 and caspase-1 (Walsh et al., 2014b). These data have been replicated in a model of feline immunodeficiency virus infection of cats, showing that IL-1β induction and microglial activation are associated with the occurrence of neurobehavioral deficits. Further, it was found that the HIV-1 transactivator of transcription (Tat) protein is instrumental for the upregulation of NLRP3 and ASC levels themselves and for the increase in caspase-1 cleavage and subsequent IL-1β release (Chivero et al., 2017). Similarly, the HIV-1 viral protein R (Vpr) can induce NLRP3-dependent caspase-1 cleavage and IL-1β release. Furthermore, treatment of Vpr transgenic animals with the caspase-1 inhibitor VX-765 improved neurobehavioral deficits (Mamik et al., 2017).

On the other hand, prions are misfolded endogenous proteins whose accumulation induces serious neurological disorders. One of the most common prion diseases in bovines and

goats is *scrapie*, characterized by uncontrolled movements, altered behavior and finally death. The prionic disease is mediated by inflammation, unleashed in response to the fragment of the protein PrP^{sc} (PrP^{sc} 106-126). It involves the activation of the NLPR3 inflammasome in microglial cells primed with LPS, producing the release of IL-1β (Shi et al., 2012). Additionally, stimulation of microglia with PrP fibrils was also shown to induce toxicity in neurons (Hafner-Bratkovič et al., 2012). Mechanistically, NLRP3 inflammasome activation was suggested to negatively regulate autophagy in microglia, thereby contributing to neurodegeneration (Lai et al., 2018). However, controversial results in mice have shown that the activity of NLRP3 inflammasome is not necessary for establishing *scrapie*; thus, the effect of intraventricular injection of PrP^{sc} 106-126 was identical in knockout mice for *NLRP3* and *pycard* (the gene coding for the adapter protein ASC) (Nuvolone et al., 2015). Further studies are required to clarify the specificity of inflammasome activity in response to prion activation.

Furthermore, a notable characteristic of neurodegenerative diseases is the prevalent high levels of proinflammatory cytokines, IL-1β, IL-16 and TNF-α (Heneka et al., 2014). It is considered that these substances have local origins and are mainly produced by microglial cells. Because these diseases are sterile-type damage, the main DAMPs involved in neurodegeneration are fibrillar and soluble β-amyloid, α-synuclein (α-syn), ATP, LPC and the high-mobility group box protein 1 (HMGB1) (Labzin et al., 2018).

There is evidence showing that microglial cells play a role in neurodegenerative diseases associated with neuroinflammation (**Figure 3**). In the following lines, we will describe the main results supporting this statement.

Alzheimer's Disease

Alzheimer's disease is a highly prevalent form of dementia characterized by the accumulation of extracellular amyloid beta (Aβ) plaques in the brain, insoluble filaments of tau —the microtubule-associated protein accumulated as neurofibrillary tangles in the brain— neuronal cell death, and neuroinflammation. It has been demonstrated that the concentration of IL-1β and IL-18 is increased in this disease as a consequence of NLRP3 inflammasome activation (Medeiros et al., 2011; Heneka et al., 2013; Saresella et al., 2016b; Awad et al., 2017; La Rosa et al., 2019).

The CNS resident microglia population (Sarlus and Heneka, 2017; Shi and Holtzman, 2018) primarily drives

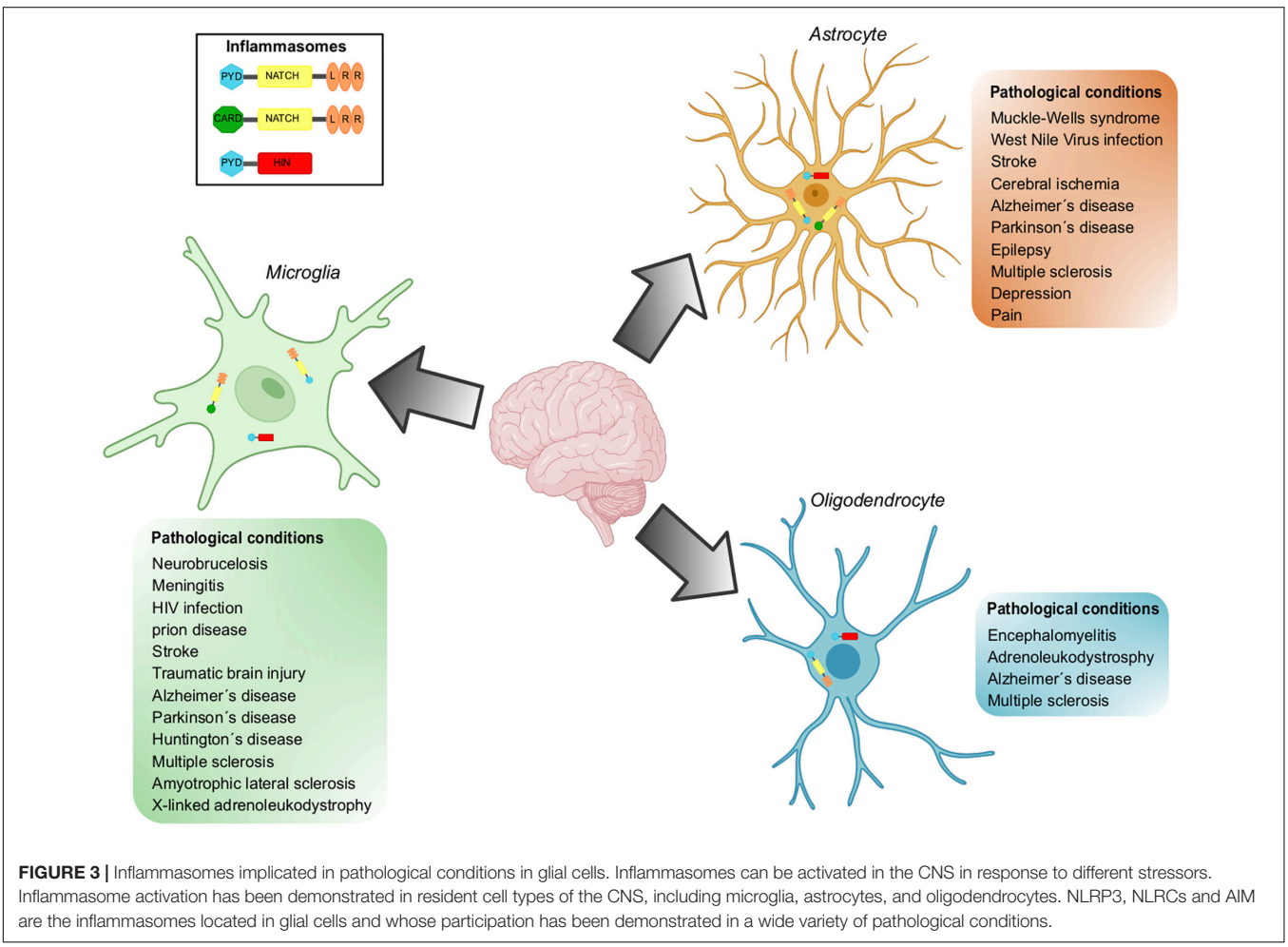


FIGURE 3 | Inflammasomes implicated in pathological conditions in glial cells. Inflammasomes can be activated in the CNS in response to different stressors. Inflammasome activation has been demonstrated in resident cell types of the CNS, including microglia, astrocytes, and oligodendrocytes. NLRP3, NLRCs and AIM are the inflammasomes located in glial cells and whose participation has been demonstrated in a wide variety of pathological conditions.

neuroinflammation in AD. Although it is recognized that microglia may exert benign and reparative activities in AD through the phagocytic removal of Aβ deposits, the accumulation of Aβ may also prime microglial cells and promote their activation to produce inflammatory mediators. Moreover, upon Aβ accumulation, microglial cells may become progressively impaired in their ability to phagocytize Aβ plaques (Sarlus and Heneka, 2017; Shi and Holtzman, 2018).

Besides the elevated expression of IL-1β, the presence of Aβ plaques in microglia of AD patients has been reported (Griffin et al., 1989; Simard et al., 2006). On the other hand, *in vitro* studies have shown that fibrillar Aβ activates the NLRP3 inflammasome when phagocytized by microglia, leading to the activation of caspase-1 and the release of IL-1β (Halle et al., 2008).

Furthermore, indirect inhibition of NLRP3 inflammasome activation by clinically approved fenamate, as well as non-steroidal anti-inflammatory drugs that target cyclooxygenase enzymes and volume-regulated anion channels (VRAC), suppressed microglia-mediated neuroinflammation and memory loss in 3 × TgAD mice (Daniels et al., 2016). Collectively, these findings suggest that misfolded Aβ activates the microglial NLRP3 inflammasome, which triggers the release of pro-inflammatory factors that perpetrate a chronic neuroinflammatory environment and promote AD pathology.

Although the focus has been on the function of NLRP3 in AD, the role of other inflammasomes has also been characterized in the context of AD. It has been reported that AIM2 inflammasome increases Aβ deposition, microglia activation, and cytokine production, but it does not affect behavior or memory in transgenic 5xFAD mice (Wu et al., 2017).

Multiple Sclerosis

This pathology is an autoimmune demyelinating disease of the CNS characterized by immune cell infiltration from the periphery into the CNS as well as by the activation of the microglia and astrocytes, which together promote neuroinflammation and neurodegeneration (Baecher-Allan et al., 2018). Most studies on the involvement of inflammasomes in multiple sclerosis (MS) have focused on the peripheral immune response that is shaped by lymphocytes and macrophages that enter the CNS during this pathology. However, direct genetic evidence points to the relevance of inflammasome signaling in microglia and border-associated macrophages during experimental autoimmune encephalomyelitis (EAE), a widely used rodent model of MS (Ransohoff, 2012; Voet et al., 2018). Also, it has been shown that the anti-inflammatory protein A20 negatively regulates NLRP3 inflammasome activation (Vande Walle et al., 2014), and the deletion of A20 in microglia and CNS macrophages exacerbates EAE in mice due to NLRP3 hyperactivation, resulting in increased IL-1β secretion and CNS inflammation (Voet et al., 2018).

CNS-intrinsic inflammasome activation was further reported in another study that showed caspase-1 and GsdmD mediated pyroptosis in microglia, as well as in myelin-forming oligodendrocytes (ODCs) in the CNS of MS patients and EAE mice (McKenzie et al., 2018).

Furthermore, the elevated expression of P2X7 receptor, a purinergic receptor that detects and amplifies the release of ATP and, therefore, the activation of NLRP3 inflammasome, was shown in spinal cords of MS patients (Yiangou et al., 2006; Matute, 2007). Taken together, these results seem to suggest that endogenous metabolic danger signals, ATP, and uric acid are likely involved in the activation of the NLRP3 inflammasome pathway observed in MS.

Parkinson's Disease

Parkinson's disease is a progressive neurodegenerative disorder mostly characterized by the depletion of dopaminergic neurons in the substantia nigra and the accumulation of cytoplasmic inclusions of fibrillar α-syn, also called Lewy bodies (Petrucci et al., 2014). Different intracellular mechanisms allow the release of α-syn outside of the cell (Lee, 2008), but the common endpoint of α-syn accumulation is the activation of astrocytes and microglia to produce IL-1β (Lee, 2008; Béraud and Maguire-Zeiss, 2012). Notably, this phenomenon also facilitates the recruitment of immune cells from the periphery into the CNS (Harms et al., 2017).

Most of the evidence linking PD to inflammasome signaling comes from *in vitro* studies, but the importance of inflammasomes for the disease is not completely understood. α-Syn was shown to trigger activation of the NLRP3 inflammasome in human monocytes and BV2 microglial cells (Codolo et al., 2013; Gustot et al., 2015; Zhou et al., 2016), but not in primary microglia (Gustin et al., 2015).

Additionally, mutations in Parkin, PARK2, PARK6, and PINK1 have been identified in patients with autosomal recessive early-onset PD, and microglia and macrophages from PARK2 and PINK1 knockout mice and patients with PARK2 mutations have been shown to display an exacerbated NLRP3 inflammasome response, possibly due to impaired expression of the anti-inflammatory protein A20 that negatively regulates NLRP3 inflammasome activation (Mouton-Liger et al., 2018).

Although several studies have shown that α-syn can elicit the activation of inflammasomes in monocyte and microglial cell lines and in PD animal models, the possible role of NLRP3 in patients with a diagnosis of PD still needs to be clarified.

Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by the selective loss of motor neurons in the motor cortex, the brainstem, and the spinal cord, which leads to muscle weakness and wasting (Taylor et al., 2016).

Neuroinflammation has an important role in the pathogenesis of ALS, as demonstrated by lymphocyte and macrophage infiltration in the CNS, microglial activation, and the presence of reactive astrocytes in the same anatomical sites where motor neuron injuries are observed. Some studies have suggested that deregulated and excessive inflammasome activation contributes to the neuroinflammation observed in this disease (McCombe and Henderson, 2011; Lall and Baloh, 2017).

Furthermore, data obtained from G93A-*SOD1* transgenic mice, the most common animal model for ALS, showed the

activation of caspase-1 and IL-1β in the microglia by ALS-linked mutant SOD1 and demonstrated that caspase-1 or IL-1β gene knockout or the use of recombinant IL-1Ra resulted in a reduction of inflammation. Notably, augmented caspase-1 and IL-1β production appeared to be NLRP3-independent in this model, suggesting the possible involvement of other inflammasome complexes (Meissner et al., 2010; Lehmann et al., 2018). However, other analyses performed in the *SOD1* transgenic mice showed an upregulation of NLRP3 and ASC in the anterior dorsal thalamic nucleus of G93A mice (Debye et al., 2018) and the transactive response DNA-binding protein-43 (TDP-43) in the microglia (Zhao et al., 2015). Additionally, the authors noticed the microglial expression of ASC but not that of NLRP3, suggesting that other inflammasome sensor molecules may play a role in microglia-driven neuroinflammation in ALS.

On the other hand, clinical studies using an IL-1 receptor antagonist in ALS patients have not shown a significant reduction in disease progression (Maier et al., 2015), suggesting that inflammasome activation does not play a major role in ALS, or that the pathology is driven by IL-18 or by DAMPs *via* pyroptosis.

Huntington's Disease

Huntington's Disease (HD) is an autosomal dominant progressive neurodegenerative disease caused by the expansion of a trinucleotide CAG repeat in the 5′ coding region of the Huntingtin gene, leading to the expression of an abnormal protein that gradually damages cells in the brain (Caron et al., 2018). Caspase-1 activation can be detected in the brains of HD patients and in mouse models of HD, whereas caspase-1 inhibition was shown to slow down disease progression in the R6/2 mouse model of HD (Ona et al., 1999). Mechanistically, caspase-1 was shown to cleave mutant and wild-type Huntingtin *in vitro* (Wellington et al., 1998) and *in vivo* (Ona et al., 1999), potentially contributing to the neurodegeneration seen in HD. Similarly, treatment with the tetracycline derivative minocycline delayed disease progression by inhibition of caspase-1 and caspase-3 expression (Chen et al., 2000).

X-Linked Adrenoleukodystrophy

This genetic disease that affects the nervous system and the adrenal cortex is caused by a loss-of-function mutation of the gene encoding for the ATP-binding cassette subfamily D member 1 (ABCD1). ABCD1 is involved in the transport of very-long-chain fatty acids from the cytosol to peroxisomes, a critical step for their oxidation and disposal. In the brain, dysfunctional ABCD1 causes axonopathy of the spinal cord and inflammatory demyelination (Kemp et al., 2016). 25-Hydrocholesterol (25-HC) is a downstream product of cholesterol 25-hydroxylase, and this enzyme is increased in X-linked adrenoleukodystrophy (X-ALD) patient-derived induced pluripotent stem cells. Indeed, 25-HC activates microglia and increases cerebral IL-1β levels when injected into the murine corpus callosum (Jang et al., 2016). In LPS-primed mouse microglia, 25-HC causes NLRP3- dependent caspase-1 cleavage, leading to the release of IL-1β. This activation also requires 25-HC-induced K^+ efflux and mitochondrial ROS production. Additionally, NLRP3 deficiency or treatment with IL-1 receptor antagonist protein (IL-1ra) decreased microglial activation upon cerebral 25-HC injection and prevented ODC cell death (Jang et al., 2016).

Other Maladies

Neuroinflammation plays a crucial pathological role in stroke, and IL-1β has been identified as a key cytokine in this pathology. So far, four distinct inflammasomes have been implicated in stroke: NLRP1, NLRP3, NLRC4, and AIM2 (Barrington et al., 2017). NLRC4- and AIM2-deficient mice were shown to have significantly smaller infarct volumes compared to wild-type mice subjected to middle cerebral artery occlusion, which was associated with strongly reduced microglia cell activation and leukocyte recruitment to the infarct site (Denes et al., 2015).

In patients with traumatic brain injury (TBI), higher levels of inflammasome markers in their cerebrospinal fluid have been detected, including ASC, caspase-1, NLRP1, and NLRP3 (Adamczak et al., 2012; Wallisch et al., 2017). Nevertheless, NLRPβ and ASC knockout mice did not show any improvement in motor recovery or lesion volume in conditions of TBI, compared to control mice, although their levels of IL-1β were reduced (Brickler et al., 2016). NLRP3 expression localizing to neurons, microglia, and astrocytes was also detected in experimental TBI (Liu et al., 2013), and NLRP3-deficient mice as well as pharmacological blockade of NLRP3 activation were shown to improve recovery from TBI (Irrera et al., 2017); demonstrating that inflammasome activation plays an important role in this pathological condition.

Regarding spinal cord injury (SCI), it has been demonstrated that this condition induced higher levels of NLRP1, ASC, caspase-1, IL-1β, and IL-18 in mice, and therapeutic neutralization of ASC was proposed to result in tissue sparing and functional improvement due to reduced inflammasome activation in neurons (de Rivero Vaccari et al., 2008). Besides, in SCI, enhanced NLRP3 expression has been demonstrated predominantly in neurons, but also in microglia and astrocytes (Zendedel et al., 2016).

Given that microglial cells are essential for the regulation of inflammasome activities in the brain, further research into the regulation and control of these responses is required for treating diverse pathologies and developing a deep understanding of cellular damage in the brain.

INFLAMMASOMES AND ASTROCYTES

Some years ago, the expression and aggregation of inflammasomes in astrocytes was a controversial topic, since it had been reported that inflammasomes were exclusively expressed in microglial cells (Gustin et al., 2015). However, more contemporaneous and overwhelming experimental evidence supports that astrocytes also express functional inflammasomes. A search in PubMed with the terms "inflammasome" and "astrocyte" resulted in 260 references (March 2022); 84% of these references (219 reports) have been published in the last 4 years, since 2018.

It is now clear that astrocytes have the capacity to assemble different types of inflammasomes and play relevant

pro-inflammatory roles in neurological diseases such as depression, epilepsy, PD, AD, pain, ischemic damage, and others (Nourbakhsh et al., 2021). In a complementary context, some examples of how isolated astrocytes are able to react to known DAMPs, such as HMGB1 and ATP, are the reports by Minkiewicz et al. (2013), Yao et al. (2019), and Wang et al. (2020). In the first, HMGB1 promoted NLRP3 inflammasome formation *via* NF-κB nuclear translocation in a signaling pathway sensitive to PPARγ activation; in the second, the LPS-induced inflammation response was dependent on endoplasmic reticulum stress activation; in the third, extracellular ATP induced the aggregation of NLRP2 through the activation of purinergic P2X7 receptor; this effect was sensible to the specific antagonist reactive blue. In addition, ATP actions required the association of P2X7 receptor with Pannexin-1, as revealed by the inhibitory action of probenecid over pre-incubated astrocytes, which blocked the ATP-dependent activation of pro-caspase-1. Interestingly, silencing NLRP2 expression reduced the innate immune response mediated by ATP.

Isolated astrocytes are also able to react to the presence of ethanol, a compound that generates a pro-inflammatory state associated with an intense upregulation of ROCK2 (a serine-threonine kinase), and enhanced oxidative stress (Li et al., 2020).

It was also demonstrated in mice sensitized with LPS and primed with LPC that both canonical and non-canonical inflammasomes, namely NLRP3 and NLRC4, are assembled in microglia and astrocytes, and in consequence produce microgliosis and astrogliosis (Freeman et al., 2017). NLRC4 was initially characterized by its interaction with pro-caspase-1 through the CARD domain present in both proteins (Geddes et al., 2001; Poyet et al., 2001). In astrocytes, the activation of NLRC4 involved ASC, caspase-1, Ca^{2+} mobilization and K^{+} efflux (Freeman et al., 2017).

Numerous reports have demonstrated an outstanding role of the inflammatory process within astrocytes in different experimental models of neurological maladies (**Figure 3**) as well as neurological damage related to pharmacological treatments.

Muckle-Wells Syndrome

It has also been shown that human astrocytes have the capacity to express NLRP2, a cytosolic inflammasome that is associated with ASC and caspase-1, inhibiting the activation of the transcriptional factor NF-κB (Bruey et al., 2004). A variant of this protein complex has been related to the Muckle–Wells syndrome (Fontalba et al., 2007), a rare genetic disease characterized by pro-inflammatory episodes of fever and rashes.

Multiple Sclerosis

Multiple sclerosis is a chronic disease characterized by demyelination and axonal damage in the nervous system. It is accompanied with severe cognitive deficits and eventually becomes a disabling condition. Using the model of autoimmune encephalomyelitis, an animal model of multiple sclerosis, Hou et al. (2020) reported the generalized activation of the NLRP3 inflammasome that was associated with the alteration of the astrocyte phenotype. This group demonstrated that the experimental MS converted astrocytes to the neurotoxic phenotype A1, an action that was prevented by an IL-18 antagonist, which is related to the NLRP3 effect *via* the NF-κB pathway.

Alzheimer's Disease

Neurodegeneration associated with diverse forms of AD, including transgenic experimental models, is the subject of intense analytical scrutiny given the pressing health issue that this illness has become for modern societies. It is well accepted that neuroinflammation is one of the pathological events associated with AD, and that astrocytes are one cellular type with the capacity to participate in this phenomenon (Bandyopadhyay, 2021). In this context, efforts have been made to ameliorate neuroinflammation in AD to find a therapeutic strategy that improves the outcome of the patients. For example, it has been proposed that glucagon-like peptide-1 analogs could mitigate astrocytic NLRP2 activation in 5xFAD transgenic mice, improving cognitive dysfunction *in vivo* and protecting astrocytes *in vitro* (Zhang et al., 2022a); in APP/PS1 mice, AVE0991 —an angiotensin-(1-7) analog— was effective in inhibiting astrocyte-mediated neuroinflammation *via* the SNHG14/miR-223-3p/NLRP3 pathway (Duan et al., 2021). Treatment with Aβ to astrocytes promotes NLRP3 inflammasome activation. Ebrahimi et al. (2018) and Hong et al. (2019) reported neuroprotective actions of α1-antitrypsin and progesterone, mitigating the inflammatory process by downregulating the expression of NLRP3 in the first study and enhancing the autophagy-lysosomal pathway in the second.

Parkinson's Disease

Parkinson's disease is now recognized as a neurodegenerative entity that involves much more than a dopamine deficit. In this context, it has also been postulated that astrocytic inflammation plays a role in the onset of this pathology (Miyazaki and Asanuma, 2020). In the experimental model of PD by MPTP treatment, it was shown in mice that the neuroinflammation process was associated with upregulation of the NLRP3 inflammasome and glia maturation factor. This factor was coincident with the presence of α-syn in an astrocyte population. The authors demonstrated that molecular ablation of the glia maturation factor significantly reduced the presence of NLRP3 in astrocytes (Javed et al., 2020). Again, with mice treated with MPTO, Zhu et al. (2018) reported a protective role for dopamine D2 receptor agonist inhibiting astrocytic NLRP3 inflammasome activation *via*β-arrestin 2. It was shown in primary mouse astrocytes and in the U373 cell line that the tumor necrosis factor-like weak inducer of apoptosis (TWEAK) treatment enhanced the activation of the Stat3/NLRC4 inflammasome signaling axis with the participation of PKCδ. TWEAK has been involved in PD-like neuropathology (Samidurai et al., 2020). Given that Parkinsonian patients experience olfactory deficits, Zhang et al. (2020a) treated mice with rotenone to simulate olfactory disturbances in PD. These authors reported a role of the mitochondrial fission factor Drp1 in the promotion of pro-oxidant responses that favored NF-κB nuclear translocation and subsequent NLRP3 inflammasome activation.

Viral Infections

Astrocytes also express AIM2 inflammasome, and its assembly is efficiently induced by double-stranded DNA, suggesting a role of AIM2 in detecting DNA viruses. In response to mimetic DNA, AIM2 activation induced the synthesis of IL-6 and INF-γ (Cox et al., 2015). It was reported by Soung et al. (2022), that after an infection by West Nile Virus, the recovery of the resultant encephalitis was associated with continued astrocyte inflammasome-mediated production of IL-1β, which was maintained by hippocampal astrogenesis *via* IL-1R1 signaling in neural stem cells.

Epilepsy

Seizures and epilepsy are another neuropathology that has been related to inflammatory responses. In mice treated with kainic acid, they presented an epileptic outcome that was associated with NLRP3 activation; the inflammasome promoted an epileptic crisis by enhancing the expression of astrocytic adenosine kinase. The mechanism of this action was further explored using astrocytes in culture, and it was demonstrated that adenosine kinase upregulation depended on the CREB/REST/SP1 signaling pathway (Zhang et al., 2022b).

Cerebral Ischemia and Stroke

Both NLRP2 and NLRP3 have been shown to participate in the proinflammatory events related to experimentally induced cerebral ischemia after cerebral ischemia-reperfusion injury and acrolein-induced astrocytic inflammation in brain affected by ischemic stroke. Cheon et al. (2018) demonstrated that cerebral ischemia activated NLRP2 inflammasome in a set of events that were dependent on the apoptosis signal-regulating kinase 1 (ASK1), a finding that was confirmed in an astrocyte cell line that was subjected to oxygen-glucose deprivation and reperfusion injury. A study using the model of middle cerebral artery occlusion as well as TNA2 astrocytes (astrocytic cell line containing the oncogenic early region of SV40) exposed to oxygen-glucose deprivation showed that gastrodin (GAS) was able to inhibit NLRP3 and NLRC4 expression (Sui et al., 2019). Liu et al. (2020) reported the suppression of NLRP3 inflammasome in astrocyte treatment with adiponectin in a model of cerebral ischemia-reperfusion injury. The protective adiponectin action was mediated by the AMPK/GSK-3β signaling pathway. In another report, it was shown that acrolein, a neurotoxin produced by pro-oxidant reactions in tissues affected by ischemic stroke, promoted NF-κB and NLRP3 inflammasome activation. This action was mediated by the induction of ADAM10 and the participation of p38 MAPK (Park et al., 2020).

Pain

It has been reported that pain-related clinical situations and experimental models are associated with inflammatory response activation in astrocytes (Tan et al., 2021). Both NLRP2 and NLRP3 have been identified in spinal cord astrocytes during in situations of mechanical pain hypersensitivity in the complete Freund adjuvant-induced persistent pain model with IL-1β as effector and by activating the sphingosine-1-phosphate receptor (Doyle et al., 2019; Ducza et al., 2021). It has been shown that the inflammatory activation in spinal cord astrocytes, and the associated pain, can be alleviated by treatment with the stilbenoid resveratrol (Fan et al., 2021), and the molecular reduction of heat shock protein family A member 8 (HSPA8) (Mi et al., 2021). In a different model, Carranza-Aguilar et al. (2022), reported that daily treatment with morphine and fentanyl differentially induced cell-specific activation of NLRP3 inflammasome and pyroptosis in the dorsal raphe nucleus through TLR4 receptors in astrocytes and opioid receptors in neurons, indicating that neuroinflammation is involved in opioid-induced analgesia and fentanyl-induced hyperalgesia.

Depression

Depression is one of the most complex mood disorders. It is usually a long-term disabling condition that has an enormous repercussion in the health system. In both clinical and experimental model observations, it has been recognized that inflammatory events play an important role in this neuropathological condition, including alterations presented by astrocytes (Kim et al., 2018). By using sleep deprivation as an inducer of the depressive condition, one study showed that NLRP3 activation in astrocytes reduced BDNF levels, whereas combined treatment with fluoxetine and leptin ameliorated the depression induced by sleep deprivation (Li et al., 2019). Depressive mice were shown to present astrocytic loss that was promoted by pyroptotic cellular death. The pyroptotic process was triggered by the activation of the NLRP3/caspase-1/GSDMD pathway (Li et al., 2021). Also, in mice with depression induced by chronic mild stress, it was reported that kynurenine, a tryptophan metabolite, acted as a pro-inflammatory factor in astrocytes. In this model, hippocampal astrocytes expressed NLRP2 inflammasome *via* NF-κB nuclear translocation (Zhang et al., 2020b). In mice with molecular ablation of neuroligin3, it was shown that this factor is needed for NLRP3 inflammasome activation in a chronic unpredictable mild stress model. Hence, suppression of neuroligin3 down-regulated NLRP3 and the ASC protein, preventing the loss of astrocytic cells during depressive episodes (Li et al., 2018).

Astrocytic Inflammation in Non-pathological Conditions

Inflammasomes in astrocytes are relevant not only in pathological entities, but also as part of several physiological and protective responses. For example, NLRP3 deficiency in mice is associated with hippocampal dysfunction as well as anxiety-like behavior (Komleva et al., 2021). Absence of NLRP3 renders astrocytes highly permissive to *Trypanosoma cruzi* replication in the context of Chagas disease (Pacheco et al., 2019). Mice treated with LPS in their hippocampus responded by enhancing the astrocytic production of neopterin, a biomarker for immune system activation; neopterin inhibited inflammasome activation in pre-conditioned human astrocytes (de Paula Martins et al., 2018).

Although microglia were once considered the only cell type related to immunity in the brain, it is now clear that inflammasome aggregation also occurs in astrocytes. This fact

reveals a new paradigm in the immune responses of the CNS with implications for the functional concepts of cell regulation in the brain and for the development of new therapeutic strategies against neurodegenerative diseases.

INFLAMMASOMES AND OLIGODENDROCYTES

Oligodendrocytes are specialized glial cells in the CNS whose main tasks include the production of myelin sheath to wrap and electrically insulate neuronal axons, provide metabolic support to axons and participate in neuroplasticity processes; their lineage in the CNS includes immature precursors (OPCs) and mature ODCs (Baumann and Pham-Dinh, 2001; Zhou et al., 2021).

Seminal work suggested a contribution of inflammasome-mediated responses to demyelinating pathologies whose main target are ODCs. Thus, in a demyelination model induced by cuprizone feeding in mice, the expression level of *Nlrp3* transcript incremented dramatically (> 100 fold); in agreement, in *Nlrp3* knockout mice, cuprizone-induced inflammation, demyelination and ODC death were significantly delayed (Jha et al., 2010), suggesting localized activity of Nlrp3 inflammasome in ODCs. In EAE, an animal model of MS, a role was also demonstrated for the adaptor protein PYCARD/ASC and caspase-1, but the sensor protein remains elusive (Shaw et al., 2010).

On the other hand, in childhood cerebral adrenoleukodystrophy (CC-ALD), the most severe form of X-ALD, characterized by severe demyelination, upregulation of *CH25H* transcript (coding for cholesterol 25-hydroxilase) was found. The product of this enzymatic activity, 25-hydroxycholesterol (25-HC), resulted a potent inductor of neuroinflammation in CC-ALD. CH25H mRNA was specifically incremented in OPCs differentiated from pluripotent stem cells (iPSCs) isolated from CC-ALD patients. In agreement, injection of 25-HC into the corpus callosum induced microglia recruitment and IL-β production, which conduced to apoptotic ODC death. These effects were significantly attenuated in Nlrp3-deficient mice, suggesting an inflammatory response dependent on NLRP3 inflammasome in ODCs (Jang et al., 2016).

Distribution of NLRP1/NALP1 and NLRP3/NALP3 in several tissues of the body was explored using self-designed monoclonal antibodies, and it was detected that NLRP1 inflammasome is expressed in ODCs (Kummer et al., 2007). Despite these approaches, inflammasome activity in ODCs was only recently recognized.

In a study aimed at investigating if inflammasomes are activated in MS, a neurological disease characterized by loss of neuron-covering myelin, it was observed that in homogenates from postmortem brain samples of MS patients, transcripts from I-1β, IL-18, CASP1, GsdmD, NLRP3, NLRP1 and AIM2 incremented in comparison with control samples; in the same biopsies, it was documented by immunofluorescence that GsdmD co-localized with glutathione S-transferase-π^+ (GST-π^+), a marker of mature ODCs, indicating specific inflammasome activity in these glial cells (McKenzie et al., 2018). Furthermore, in mature ODCs *in vitro*, it was shown that in response to a challenge with TNF-α, the expression level of CASP1 and GsdmD transcripts and their respective proteins incremented. These changes were concomitant with cell death-related morphological changes, such as a condensed nucleus and short and few cellular processes in a caspase-1 dependent way, indicating the induction of pyroptosis (McKenzie et al., 2018).

Demyelination is one of the main alterations of the white matter observed in AD (Desai et al., 2009; Zhan et al., 2014). Studies on mature ODCs from AD patients and AD transgenic mice demonstrated that NLRP3 inflammasome is active in ODCs and participates in pathologic demyelination through a pathway triggered by hyperactivated Drp1, a mitochondrial GTPase involved in organelle division (Smirnova et al., 2001); this factor inhibits hexokinase 1 (HK1), which in turn activates NLRP3 inflammasome. This metabolic stress and chronic inflammation conduce to demyelination and tissular degeneration of white matter and contribute to the cognitive impairment of AD (Zhang et al., 2020c).

The exposed evidence supports the notion that deleterious neuroinflammation observed in pathologies where demyelination is characteristic, is substantially supported by inflammasome activity (**Figure 3**).

CONCLUDING REMARKS

Inflammasomes are multiprotein machineries mediating maturation and secretion of IL-β and IL-18 in response to various sterile and infectious stimuli. Inflammasomes are functional in microglial cells and, surprisingly, in astrocytes and ODCs as well, and they mediate responses to specific stressors. However, chronic activation or mutations in inflammasomes lead to a variety of diseases (**Figure 3**). Obtaining detailed information about affected cell populations, molecular identity and function is an important opportunity for the development of innovative therapies.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was supported by Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT), Universidad Nacional Autónoma de México, grants IN202620 and IN202121. EM-M is a postdoctoral fellow funding by DGAPA-UNAM.

ACKNOWLEDGMENTS

We are grateful to Jéssica González Norris for expert proofreading.

REFERENCES

Adamczak, S., Dale, G., de Rivero Vaccari, J. P., Bullock, M. R., Dietrich, W. D., and Keane, R. W. (2012). Inflammasome proteins in cerebrospinal fluid of brain-injured patients as biomarkers of functional outcome: clinical article. *J. Neurosurg.* 117, 1119–1125. doi: 10.3171/2012.9.JNS12815

Adinolfi, E., Giuliani, A. L., De Marchi, E., Pegoraro, A., Orioli, E., and Di Virgilio, F. (2018). The P2X7 receptor: a main player in inflammation. *Biochem. Pharmacol.* 151, 234–244. doi: 10.1016/j.bcp.2017.12.021

Ajami, B., Bennett, J. L., Krieger, C., Tetzlaff, W., and Rossi, F. M. (2007). Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat. Neurosci.* 10, 1538–1543. doi: 10.1038/nn2014

Amer, A., Franchi, L., Kanneganti, T. D., Body-Malapel, M., Ozören, N., Brady, G., et al. (2006). Regulation of *Legionella* phagosome maturation and infection through flagellin and host Ipaf. *J. Biol. Chem.* 281, 35217–35223. doi: 10.1074/jbc.M604933200

Angosto-Bazarra, D., Molina-López, C., Peñín-Franch, A., Hurtado-Navarro, L., and Pelegrín, P. (2021). Techniques to study inflammasome activation and inhibition by small molecules. *Molecules (Basel, Switzerland)* 26:1704. doi: 10.3390/molecules26061704

Awad, F., Assrawi, E., Jumeau, C., Georgin-Lavialle, S., Cobret, L., Duquesnoy, P., et al. (2017). Impact of human monocyte and macrophage polarization on NLR expression and NLRP3 inflammasome activation. *PLoS One* 12:e0175336. doi: 10.1371/journal.pone.0175336

Baecher-Allan, C., Kaskow, B. J., and Weiner, H. W. (2018). Multiple sclerosis: mechanisms and immunotherapy. *Neuron* 97, 742–768. doi: 10.1016/j.neuron.2018.01.021

Bandyopadhyay, S. (2021). Role of neuron and glia in Alzheimer's disease and associated vascular dysfunction. *Front. Aging Neurosci.* 13:653334. doi: 10.3389/fnagi.2021.653334

Barrington, J., Lemarchand, E., and Allan, S. M. (2017). A brain in flame; do inflammasomes and pyroptosis influence stroke pathology? *Brain Pathol.* 27, 205–212. doi: 10.1111/bpa.12476

Baumann, N., and Pham-Dinh, D. (2001). Biology of oligodendrocyte and myelin in the mammalian central nervous system. *Physiol. Rev.* 81, 871–927. doi: 10.1152/physrev.2001.81.2.871

Béraud, D., and Maguire-Zeiss, K. A. (2012). Misfolded α-synuclein and toll-like receptors: therapeutic targets for Parkinson's disease. *Parkinsonism Relat. Disord.* 18, S17–S20. doi: 10.1016/S1353-8020(11)70008-6

Bergsbaken, T., Fink, S. L., and Cookson, B. T. (2009). Pyroptosis: host cell death and inflammation. *Nat. Rev. Microbiol.* 7, 99–109. doi: 10.1038/nrmicro2070

Brickler, T., Gresham, K., Meza, A., Coutermarsh-Ott, S., Williams, T. M., Rothschild, D. E., et al. (2016). Nonessential role for the NLRP1 inflammasome complex in a murine model of traumatic brain injury. *Mediators Inflamm.* 2016:6373506. doi: 10.1155/2016/6373506

Broderick, L., De Nardo, D., Franklin, B. S., Hoffman, H. M., and Latz, E. (2015). The inflammasomes and autoinflammatory syndromes. *Ann. Rev. Pathol.* 10, 395–424. doi: 10.1146/annurev-pathol-012414-040431

Bruey, J. M., Bruey-Sedano, N., Newman, R., Chandler, S., Stehlik, C., and Reed, J. C. (2004). PAN1/NALP2/PYPAF2, an inducible inflammatory mediator that regulates NF-kappaB and caspase-1 activation in macrophages. *J. Biol. Chem.* 279, 51897–51907. doi: 10.1074/jbc.M406741200

Bryant, C., and Fitzgerald, K. A. (2009). Molecular mechanisms involved in inflammasome activation. *Trends Cell. Biol.* 19, 455–464. doi: 10.1016/j.tcb.2009.06.002

Burdette, B. E., Esparza, A. N., Zhu, H., and Wang, S. (2021). Gasdermin D in pyroptosis. *Acta Pharm. Sin. B.* 11, 2768–2782. doi: 10.1016/j.apsb.2021.02.006

Caron, N. S., Dorsey, E. R., and Hayden, M. R. (2018). Therapeutic approaches to Huntington disease: from the bench to the clinic. *Nat. Rev. Drug Discov.* 17, 729–750. doi: 10.1038/nrd.2018.133

Carranza-Aguilar, C. J., Hernández-Mendoza, A., Mejias-Aponte, C., Rice, K. C., Morales, M., González-Espinosa, C., et al. (2022). Morphine and fentanyl repeated administration induces different levels of NLRP3-dependent pyroptosis in the dorsal raphe nucleus of male rats via cell-specific activation of TLR4 and opioid receptors. *Cell. Mol. Neurobiol.* 42, 677–694. doi: 10.1007/s10571-020-00957-5

Chen, M., Ona, V. O., Li, M., Ferrante, R. J., Fink, K. B., Zhu, S., et al. (2000). Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nat. Med.* 6, 797–801. doi: 10.1038/77528

Cheon, S. Y., Kim, E. J., Kim, S. Y., Kim, J. M., Kam, E. H., Park, J. K., et al. (2018). apoptosis signal-regulating kinase 1 silencing on astroglial inflammasomes in an experimental model of ischemic stroke. *Neuroscience* 390, 218–230. doi: 10.1016/j.neuroscience.2018.08.020

Chivero, E. T., Guo, M. L., Periyasamy, P., Liao, K., Callen, S. E., and Buch, S. (2017). HIV-1 tat primes and activates microglial NLRP3 inflammasome-mediated neuroinflammation. *J. Neurosci.* 37, 3599–3609. doi: 10.1523/JNEUROSCI.3045-16.2017

Codolo, G., Plotegher, N., Pozzobon, T., Brucale, M., Tessari, I., Bubacco, L., et al. (2013). Triggering of inflammasome by aggregated α-synuclein, an inflammatory response in synucleinopathies. *PLoS One* 8:e55375. doi: 10.1371/journal.pone.0055375

Colonna, M., and Butovsky, O. (2017). Microglia Function in the central nervous system during health and neurodegeneration. *Annu. Rev. Immunol.* 35, 441–468. doi: 10.1146/annurev-immunol-051116-052358

Cox, D. J., Field, R. H., Williams, D. G., Baran, M., Bowie, A. G., Cunningham, C., et al. (2015). DNA sensors are expressed in astrocytes and microglia in vitro and are upregulated during gliosis in neurodegenerative disease. *Glia* 63, 812–825. doi: 10.1002/glia.22786

Daniels, M. J., Rivers-Auty, J., Schilling, T., Spencer, N. G., Watremez, W., Fasolino, V., et al. (2016). Fenamate NSAIDs inhibit the NLRP3 inflammasome and protect against Alzheimer's disease in rodent models. *Nat. Commun.* 7:12504. doi: 10.1038/ncomms12504

de Paula Martins, R., Ghisoni, K., Lim, C. K., Aguiar, A. S. Jr., Guillemin, G. J., and Latini, A. (2018). Neopterin preconditioning prevents inflammasome activation in mammalian astrocytes. *Free Radic. Biol. Med.* 115, 371–382. doi: 10.1016/j.freeradbiomed.2017.11.022

de Rivero Vaccari, J. P., Lotocki, G., Marcillo, A. E., Dietrich, W. D., and Keane, R. W. (2008). A molecular platform in neurons regulates inflammation after spinal cord injury. *J. Neurosci.* 28, 3404–3414. doi: 10.1523/JNEUROSCI.0157-08.2008

Debye, B., Schmülling, L., Zhou, L., Rune, G., Beyer, C., and Johann, S. (2018). Neurodegeneration and NLRP3 inflammasome expression in the anterior thalamus of SOD1(G93A) ALS mice. *Brain Pathol.* 28, 14–27. doi: 10.1111/bpa.12467

Denes, A., Coutts, G., Lénárt, N., Cruickshank, S. M., Pelegrin, P., Skinner, J., et al. (2015). AIM2 and NLRC4 inflammasomes contribute with ASC to acute brain injury independently of NLRP3. *Proc. Natl. Acad. Sci. U.S.A.* 112, 4050–4055. doi: 10.1073/pnas.1419090112

Desai, M. K., Sudol, K. L., Janelsins, M. C., Mastrangelo, M. A., Frazer, M. E., and Bowers, W. J. (2009). Triple-transgenic Alzheimer's disease mice exhibit region-specific abnormalities in brain myelination patterns prior to appearance of amyloid and tau pathology. *Glia* 57, 54–65. doi: 10.1002/glia.20734

Di Micco, A., Frera, G., Lugrin, J., Jamilloux, Y., Hsu, E. T., Tardivel, A., et al. (2016). AIM2 inflammasome is activated by pharmacological disruption of nuclear envelope integrity. *Proc. Natl. Acad. Sci. U.S.A.* 113, E4671–E4680. doi: 10.1073/pnas.1602419113

Doyle, T. M., Chen, Z., Durante, M., and Salvemini, D. (2019). Activation of sphingosine-1-phosphate receptor 1 in the spinal cord produces mechanohypersensitivity through the activation of inflammasome and Il-1β pathway. *J. Pain.* 20, 956–964. doi: 10.1016/j.jpain.2019.02.007

Duan, R., Wang, S. Y., Wei, B., Deng, Y., Fu, X. X., Gong, P. Y., et al. (2021). Angiotensin-(1-7) analogue AVE0991 modulates astrocyte-mediated neuroinflammation via lncRNA SNHG14/miR-223-3p/NLRP3 pathway and offers neuroprotection in a transgenic mouse model of Alzheimer's disease. *J. Inflamm. Res.* 14, 7007–7019. doi: 10.2147/JIR.S343575

Ducza, L., Szücs, P., Hegedűs, K., Bakk, E., Gajtkó, A., Wéber, I., et al. (2021). NLRP2 is overexpressed in spinal astrocytes at the peak of mechanical pain sensitivity during complete freund adjuvant-induced persistent pain. *Int. J. Mol. Sci.* 22:11408. doi: 10.3390/ijms222111408

Ebrahimi, T., Rust, M., Kaiser, S. N., Slowik, A., Beyer, C., Koczulla, A. R., et al. (2018). α1-antitrypsin mitigates NLRP3-inflammasome activation in amyloid β1-42-stimulated murine astrocytes. *J. Neuroinflamm.* 15:282. doi: 10.1186/s12974-018-1319-x

Fan, R., Zhang, Y., Botchway, B., and Liu, X. (2021). Resveratrol can attenuate astrocyte activation to treat spinal cord injury by inhibiting inflammatory responses. *Mol. Neurobiol.* 58, 5799–5813. doi: 10.1007/s12035-021-02509-4

Fann, D. Y., Lee, S. Y., Manzanero, S., Chunduri, P., Sobey, C. G., and Arumugam, T. V. (2013). Pathogenesis of acute stroke and the role of inflammasomes. *Ageing Res. Rev.* 12, 941–966. doi: 10.1016/j.arr.2013.09.004

Fontalba, A., Gutierrez, O., and Fernandez-Luna, J. L. (2007). NLRP2, an inhibitor of the NF-kappaB pathway, is transcriptionally activated by NF-kappaB and exhibits a nonfunctional allelic variant. *J. Immunol.* 179, 8519–8524. doi: 10.4049/jimmunol.179.12.8519

Franceschini, A., Capece, M., Chiozzi, P., Falzoni, S., Sanz, J. M., Sarti, A. C., et al. (2015). The P2X7 receptor directly interacts with the NLRP3 inflammasome scaffold protein. *FASEB J.* 29, 2450–2461. doi: 10.1096/fj.14-268714

Freeman, L., Guo, H., David, C. N., Brickey, W. J., Jha, S., and Ting, J. P. (2017). NLR members NLRC4 and NLRP3 mediate sterile inflammasome activation in microglia and astrocytes. *J. Exp. Med.* 214, 1351–1370. doi: 10.1084/jem.20150237

Geddes, B. J., Wang, L., Huang, W. J., Lavellee, M., Manji, G. A., Brown, M., et al. (2001). Human CARD12 is a novel CED4/Apaf-1 family member that induces apoptosis. *Biochem. Biophys. Res. Comm.* 284, 77–82. doi: 10.1006/bbrc.2001.4928

Griffin, W. S., Stanley, L. C., Ling, C., White, L., MacLeod, V., Perrot, L. J., et al. (1989). Brain interleukin 1 and S-100 immunoreactivity are elevated in down syndrome and Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* 86, 7611–7615. doi: 10.1073/pnas.86.19.7611

Gustin, A., Kirchmeyer, M., Koncina, E., Felten, P., Losciuto, S., Heurtaux, T., et al. (2015). NLRP3 Inflammasome is expressed and functional in mouse brain microglia but not in astrocytes. *PLoS One* 10:e0130624. doi: 10.1371/journal.pone.0130624

Gustot, A., Gallea, J. I., Sarroukh, R., Celej, M. S., Ruysschaert, J. M., and Raussens, V. (2015). Amyloid fibrils are the molecular trigger of inflammation in Parkinson's disease. *Biochem. J.* 471, 323–333. doi: 10.1042/BJ20150617

Hafner-Bratkovič, I., Benčina, M., Fitzgerald, K. A., Golenbock, D., and Jerala, R. (2012). NLRP3 inflammasome activation in macrophage cell lines by prion protein fibrils as the source of IL-1β and neuronal toxicity. *Cell. Mol. Life Sci.* 69, 4215–4228. doi: 10.1007/s00018-012-1140-0

Halle, A., Hornung, V., Petzold, G. C., Stewart, C. R., Monks, B. G., Reinheckel, T., et al. (2008). The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. *Nat. Immunol.* 9, 857–865. doi: 10.1038/ni.1636

Harms, A. S., Delic, V., Thome, A. D., Bryant, N., Liu, Z., Chandra, S., et al. (2017). α-Synuclein fibrils recruit peripheral immune cells in the rat brain prior to neurodegeneration. *Acta Neuropathol. Commun.* 5:85. doi: 10.1186/s40478-017-0494-9

He, Y., Zeng, M. Y., Yang, D., Motro, B., and Núñez, G. (2016). NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux. *Nature* 530, 354–357. doi: 10.1038/nature16959

Heinisch, O., Zeyen, T., Goldmann, T., Prinz, M., Huber, M., Jung, J., et al. (2021). Erythropoietin abrogates post-ischemic activation of the NLRP3, NLRC4, and AIM2 inflammasomes in microglia/macrophages in a TAK1-dependent manner. *Transl. Stroke Res.* 13, 462–482. doi: 10.1007/s12975-021-00948-8

Heneka, M. T., Kummer, M. P., and Latz, E. (2014). Innate immune activation in neurodegenerative disease. *Nat. Rev. Immunol.* 14, 463–477. doi: 10.1038/nri3705

Heneka, M. T., Kummer, M. P., Stutz, A., Delekate, A., Schwartz, S., Vieira-Saecker, A., et al. (2013). NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature* 493, 674–678. doi: 10.1038/nature11729

Hickman, S. E., Kingery, N. D., Ohsumi, T. K., Borowsky, M. L., Wang, L. C., Means, T. K., et al. (2013). The microglial sensome revealed by direct RNA sequencing. *Nat. Neurosci.* 16, 1896–1905. doi: 10.1038/nn.3554

Hoffman, H. M., Mueller, J. L., Broide, D. H., Wanderer, A. A., and Kolodner, R. D. (2001). Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and muckle-wells syndrome. *Nat. Gen.* 29, 301–305. doi: 10.1038/ng756

Hong, Y., Liu, Y., Yu, D., Wang, M., and Hou, Y. (2019). The neuroprotection of progesterone against Aβ-induced NLRP3-Caspase-1 inflammasome activation via enhancing autophagy in astrocytes. *Int. Immunopharmacol.* 74:105669. doi: 10.1016/j.intimp.2019.05.054

Hou, B., Zhang, Y., Liang, P., He, Y., Peng, B., Liu, W., et al. (2020). Inhibition of the NLRP3-inflammasome prevents cognitive deficits in experimental autoimmune encephalomyelitis mice via the alteration of astrocyte phenotype. *Cell Death Dis.* 11:377. doi: 10.1038/s41419-020-2565-2

Hung, S. C., Choi, C. H., Said-Sadier, N., Johnson, L., Atanasova, K. R., Sellami, H., et al. (2013). P2X4 assembles with P2X7 and pannexin-1 in gingival epithelial cells and modulates ATP-induced reactive oxygen species production and inflammasome activation. *PLoS One* 8:e70210. doi: 10.1371/journal.pone.0070210

Irrera, N., Pizzino, G., Calò, M., Pallio, G., Mannino, F., Famà, F., et al. (2017). Lack of the Nlrp3 inflammasome improves mice recovery following traumatic brain injury. *Front. Pharmacol.* 8:459. doi: 10.3389/fphar.2017.00459

Iwata, M., Ota, K. T., and Duman, R. S. (2013). The inflammasome: pathways linking psychological stress, depression, and systemic illnesses. *Brain, Behav. Immun.* 31, 105–114. doi: 10.1016/j.bbi.2012.12.008

Jang, J., Park, S., Jin Hur, H., Cho, H. J., Hwang, I., Pyo Kang, Y., et al. (2016). 25-hydroxycholesterol contributes to cerebral inflammation of X-linked adrenoleukodystrophy through activation of the NLRP3 inflammasome. *Nat. Commun.* 7:13129. doi: 10.1038/ncomms13129

Javed, H., Thangavel, R., Selvakumar, G. P., Dubova, I., Schwartz, N., Ahmed, M. E., et al. (2020). NLRP3 inflammasome and glia maturation factor coordinately regulate neuroinflammation and neuronal loss in MPTP mouse model of Parkinson's disease. *Int. Immunopharmacol.* 83:106441. doi: 10.1016/j.intimp.2020.106441

Jha, S., Srivastava, S. Y., Brickey, W. J., Iocca, H., Toews, A., Morrison, J. P., et al. (2010). The inflammasome sensor, NLRP3, regulates CNS inflammation and demyelination via caspase-1 and interleukin-18. *J. Neurosci.* 30, 15811-15820. doi: 10.1523/JNEUROSCI.4088-10.2010

Keane, R. W., Dietrich, W. D., and de Rivero Vaccari, J. P. (2018). Inflammasome proteins as biomarkers of multiple sclerosis. *Front. Neurol.* 9:135. doi: 10.3389/fneur.2018.00135

Kemp, S., Huffnagel, I. C., Linthorst, G. E., Wanders, R. J., and Engelen, M. (2016). Adrenoleukodystrophy — neuroendocrine pathogenesis and redefinition of natural history. *Nat. Rev. Endocrinol.* 12, 606–615. doi: 10.1038/nrendo.2016.90

Kim, H. K., Chen, W., and Andreazza, A. C. (2015a). The potential role of the Nlrp3 inflammasome as a link between mitochondrial complex I dysfunction and inflammation in bipolar disorder. *Neural Plast.* 2015:408136. doi: 10.1155/2015/408136

Kim, J. Y., Paton, J. C., Briles, D. E., Rhee, D. K., and Pyo, S. (2015b). Streptococcus pneumoniae induces pyroptosis through the regulation of autophagy in murine microglia. *Oncotarget* 6, 44161–44178. doi: 10.18632/oncotarget.6592

Kim, R., Healey, K. L., Sepulveda-Orengo, M. T., and Reissner, K. J. (2018). Astroglial correlates of neuropsychiatric disease: From astrocytopathy to astrogliosis. *Prog. Neuropsychopharmacol. Biol. Psychiatry.* 87, 126–146. doi: 10.1016/j.pnpbp.2017.10.002

Komleva, Y. K., Lopatina, O. L., Gorina, I. V., Shuvaev, A. N., Chernykh, A., Potapenko, I. V., et al. (2021). NLRP3 deficiency-induced hippocampal dysfunction and anxiety-like behavior in mice. *Brain Res.* 1752:147220. doi: 10.1016/j.brainres.2020.147220

Kummer, J. A., Broekhuizen, R., Everett, H., Agostini, L., Kuijk, L., Martinon, F., et al. (2007). Inflammasome components NALP 1 and 3 show distinct but separate expression profiles in human tissues suggesting a site-specific role in the inflammatory response. *J. Histochem. Cytochem.* 55, 443–452. doi: 10.1369/jhc.6A7101.2006

La Rosa, F., Saresella, M., Marventano, I., Piancone, F., Ripamonti, E., Al-Daghri, N., et al. (2019). Stavudine reduces NLRP3 inflammasome activation and modulates amyloid-β autophagy. *J. Alzheimers Dis.* 72, 401–412. doi: 10.3233/JAD-181259

Labzin, L. I., Heneka, M. T., and Latz, E. (2018). Innate immunity and neurodegeneration. *Annu. Rev. Med.* 69, 437–449. doi: 10.1146/annurev-med-050715-104343

Lai, M., Yao, H., Shah, S., Wu, W., Wang, D., Zhao, Y., et al. (2018). The NLRP3-Caspase 1 inflammasome negatively regulates autophagy via TLR4-TRIF in prion peptide-infected microglia. *Front. Aging Neurosci.* 10:116. doi: 10.3389/fnagi.2018.00116

Lall, D., and Baloh, R. H. (2017). Microglia and C9orf72 in neuroinflammation and ALS and frontotemporal dementia. *J. Clin. Invest.* 127, 3250–3258. doi: 10.1172/JCI90607

Lamkanfi, M., Kanneganti, T. D., Franchi, L., and Núñez, G. (2007). Caspase-1 inflammasomes in infection and inflammation. *J. Leukoc. Biol.* 82, 220–225. doi: 10.1189/jlb.1206756

Lavin, Y., Winter, D., Blecher-Gonen, R., David, E., Keren-Shaul, H., Merad, M., et al. (2014). Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* 159, 1312–1326. doi: 10.1016/j.cell.2014.11.018

Lee, S. J. (2008). Origins and effects of extracellular alpha-synuclein: implications in Parkinson's disease. *J. Mol. Neurosci.* 34, 17–22. doi: 10.1007/s12031-007-0012-9

Lehmann, S., Esch, E., Hartmann, P., Goswami, A., Nikolin, S., Weis, J., et al. (2018). Expression profile of pattern recognition receptors in skeletal muscle

of SOD1(G93A) amyotrophic lateral sclerosis (ALS) mice and sporadic ALS patients. *Neuropathol. Appl. Neurobiol.* 44, 606–627. doi: 10.1111/nan.12483

Li, S., Sun, Y., Song, M., Song, Y., Fang, Y., Zhang, Q., et al. (2021). NLRP3/caspase-1/GSDMD-mediated pyroptosis exerts a crucial role in astrocyte pathological injury in mouse model of depression. *JCI Insight* 6:e146852. doi: 10.1172/jci.insight.146852

Li, X., Liang, S., Li, Z., Li, S., Xia, M., Verkhratsky, A., et al. (2019). Leptin increases expression of 5-HT2B receptors in astrocytes thus enhancing action of fluoxetine on the depressive behavior induced by sleep deprivation. *Front. Psychiatry* 9:734. doi: 10.3389/fpsyt.2018.00734

Li, X., Tong, J., Liu, J., and Wang, Y. (2020). Downregulation of ROCK2 attenuates alcohol-induced inflammation and oxidative stress in astrocytes. *Int. J. Neurosci.* 1–10. doi: 10.1080/00207454.2020.1825421 [Epub ahead of print].

Li, Z. Q., Yan, Z. Y., Lan, F. J., Dong, Y. Q., and Xiong, Y. (2018). Suppression of NLRP3 inflammasome attenuates stress-induced depression-like behavior in NLGN3-deficient mice. *Biochem. Biophys. Res. Commun.* 501, 933–940. doi: 10.1016/j.bbrc.2018.05.085

Liu, H., Wu, X., Luo, J., Zhao, L., Li, X., Guo, H., et al. (2020). Adiponectin peptide alleviates oxidative stress and NLRP3 inflammasome activation after cerebral ischemia-reperfusion injury by regulating AMPK/GSK-3β. *Exp. Neurol.* 329:113302. doi: 10.1016/j.expneurol.2020.113302

Liu, H. D., Li, W., Chen, Z. R., Hu, Y. C., Zhang, D. D., Shen, W., et al. (2013). Expression of the NLRP3 inflammasome in cerebral cortex after traumatic brain injury in a rat model. *Neurochem. Res.* 38, 2072–2083. doi: 10.1007/s11064-013-1115-z

Lugrin, J., and Martiñon, F. (2018). The AIM2 inflammasome: sensor of pathogens and cellular perturbations. *Immunol. Rev.* 281, 99–114. doi: 10.1111/imr.12618

Mackenzie, A., Maltais, L. J., Nunez, G., Ogura, Y., Otten, L. A., Philpott, D., et al. (2008). The NLR gene family: a standard nomenclature. *Immunity* 28, 285–287.

Maier, A., Deigendesch, N., Müller, K., Weishaupt, J. H., Krannich, A., Röhle, R., et al. (2015). Interleukin-1 antagonist anakinra in amyotrophic lateral sclerosis–a pilot study. *PLoS One* 10:e0139684. doi: 10.1371/journal.pone.0139684

Mamik, M. K., Hui, E., Branton, W. G., McKenzie, B. A., Chisholm, J., Cohen, E. A., et al. (2017). HIV-1 Viral protein R activates NLRP3 inflammasome in microglia: implications for HIV-1 associated neuroinflammation. *J. Neuroimmune. Pharmacol.* 12, 233–248. doi: 10.1007/s11481-016-9708-3

Marim, F. M., Franco, M., Gomes, M., Miraglia, M. C., Giambartolomei, G. H., and Oliveira, S. C. (2017). The role of NLRP3 and AIM2 in inflammasome activation during *Brucella abortus* infection. *Semin. Immunopathol.* 39, 215–223. doi: 10.1007/s00281-016-0581-1

Martiñon, F., Burns, K., and Tschopp, J. (2002). The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol. Cell* 10, 417–426. doi: 10.1016/s1097-2765(02)00599-3

Matute, C. (2007). Interaction between glutamate signalling and immune attack in damaging oligodendrocytes. *Neuron Glia Biol.* 3, 281–285. doi: 10.1017/S1740925X08000033

McCombe, P. A., and Henderson, R. D. (2011). The role of immune and inflammatory mechanisms in ALS. *Curr. Mol. Med.* 11, 246–254. doi: 10.2174/156652411795243450

McKenzie, B. A., Mamik, M. K., Saito, L. B., Boghozian, R., Monaco, M. C., Major, E. O., et al. (2018). Caspase-1 inhibition prevents glial inflammasome activation and pyroptosis in models of multiple sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 115, E6065–E6074. doi: 10.1073/pnas.1722041115

Medeiros, R., Baglietto-Vargas, D., and LaFerla, F. M. (2011). The role of tau in Alzheimer's disease and related disorders. *CNS Neurosci Ther.* 17, 514–524. doi: 10.1111/j.1755-5949.2010.00177.x

Meissner, F., Molawi, K., and Zychlinsky, A. (2010). Mutant superoxide dismutase 1- induced IL-β accelerates ALS pathogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 107, 10346–13050. doi: 10.1073/pnas.1002396107

Mi, J., Yang, Y., Yao, H., Huan, Z., Xu, C., Ren, Z., et al. (2021). Inhibition of heat shock protein family A member 8 attenuates spinal cord ischemia-reperfusion injury via astrocyte NF-κB/NLRP3 inflammasome pathway : HSPA8 inhibition protects spinal ischemia-reperfusion injury. *J. Neuroinflamm.* 18:170. doi: 10.1186/s12974-021-02220-0

Minkiewicz, J., de Rivero Vaccari, J. P., and Keane, R. W. (2013). Human astrocytes express a novel NLRP2 inflammasome. *Glia* 61, 1113–1121. doi: 10.1002/glia.22499

Miyazaki, I., and Asanuma, M. (2020). Neuron-astrocyte interactions in Parkinson's disease. *Cells* 9:2623. doi: 10.3390/cells9122623

Mouton-Liger, F., Rosazza, T., Sepulveda-Diaz, J., Ieang, A., Hassoun, S. M., Claire, E., et al. (2018). Parkin deficiency modulates NLRP3 inflammasome activation by attenuating an A20-dependent negative feedback loop. *Glia* 66, 1736–1751. doi: 10.1002/glia.23337

Nourbakhsh, F., Read, M. I., Barreto, G. E., and Sahebkar, A. (2021). Astrocytes and inflammasome: a possible crosstalk in neurological diseases. *Curr. Med. Chem.* 28, 4972–4994. doi: 10.2174/0929867328666210301105422

Nuvolone, M., Sorce, S., Schwarz, P., and Aguzzi, A. (2015). Prion pathogenesis in the absence of NLRP3/ASC inflammasomes. *PLoS One* 10:e0117208. doi: 10.1371/journal.pone.0117208

Ona, V. O., Li, M., Vonsattel, J. P., Andrews, L. J., Khan, S. Q., Chung, W. M., et al. (1999). Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature* 399, 263–267. doi: 10.1038/20446

Pacheco, A. L., Vicentini, G., Matteucci, K. C., Ribeiro, R. R., Weinlich, R., and Bortoluci, K. R. (2019). The impairment in the NLRP3-induced NO secretion renders astrocytes highly permissive to T. cruzi replication. *J. Leukoc. Biol.* 106, 201–207. doi: 10.1002/JLB.4AB1118-416RR

Park, J. H., Choi, J. Y., Jo, C., and Koh, Y. H. (2020). Involvement of ADAM10 in acrolein-induced astrocytic inflammation. *Toxicol. Lett.* 318, 44–49. doi: 10.1016/j.toxlet.2019.10.005

Pelegrin, P., and Surprenant, A. (2006). Pannexin-1 mediates large pore formation and interleukin-1beta release by the ATP-gated P2X7 receptor. *EMBO J.* 25, 5071–5082. doi: 10.1038/sj.emboj.7601378

Pétrilli, V., Papin, S., Dostert, C., Mayor, A., Martinon, F., and Tschopp, J. (2007). Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. *Cell Death Differ.* 14, 1583–1589. doi: 10.1038/sj.cdd.4402195

Petrucci, S., Consoli, F., and Valente, E. M. (2014). Parkinson disease genetics: a "continuum" from mendelian to multifactorial inheritance. *Curr. Mol. Med.* 14, 1079–1088. doi: 10.2174/1566524014666141010155509

Poyet, J. L., Srinivasula, S. M., Tnani, M., Razmara, M., Fernandes-Alnemri, T., and Alnemri, E. S. (2001). Identification of Ipaf, a human caspase-1-activating protein related to Apaf-1. *J. Biol. Chem.* 276, 28309–28313. doi: 10.1074/jbc.C100250200

Prinz, M., Jung, S., and Priller, J. (2019). Microglia biology: one century of evolving concepts. *Cell* 179, 292–311. doi: 10.1016/j.cell.2019.08.053

Qu, Y., Misaghi, S., Newton, K., Maltzman, A., Izrael-Tomasevic, A., Arnott, D., et al. (2016). NLRP3 recruitment by NLRC4 during *Salmonella* infection. *J Exp. Med.* 213, 877–885. doi: 10.1084/jem.20132234

Ransohoff, R. M. (2012). Animal models of multiple sclerosis: the good, the bad and the bottom line. *Nat. Neurosci.* 15, 1074–1077. doi: 10.1038/nn.3168

Rathinam, V. A., Vanaja, S. K., and Fitzgerald, K. A. (2012). Regulation of inflammasome signaling. *Nat. Immunol.* 13, 333–342. doi: 10.1038/ni.2237

Samidurai, M., Tarale, P., Janarthanam, C., Estrada, C. G., Gordon, R., Zenitsky, G., et al. (2020). Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) enhances activation of STAT3/NLRC4 inflammasome signaling axis through PKCδ in astrocytes: implications for Parkinson's disease. *Cells* 9:1831. doi: 10.3390/cells9081831

Saresella, M., Piancone, F., Marventano, I., Zoppis, M., Hernis, A., Zanette, M., et al. (2016a). Multiple inflammasome complexes are activated in autistic spectrum disorders. *Brain Behav. Immun.* 57, 125–133. doi: 10.1016/j.bbi.2016.03.009

Saresella, M., La Rosa, F., Piancone, F., Zoppis, M., Marventano, I., Calabrese, E., et al. (2016b). The NLRP3 and NLRP1 inflammasomes are activated in Alzheimer' disease. *Mol. Neurodegener.* 11:23. doi: 10.1186/s13024-016-0088-1

Sarlus, H., and Heneka, M. T. (2017). Microglia in Alzheimer's disease. *J.Clin. Invest.* 127, 3240–3249. doi: 10.1172/JCI90606

Scholz, H., and Eder, C. (2017). Lysophosphatidylcholine activates caspase-1 in microglia via a novel pathway involving two inflammasomes. *J. Neuroimmunol.* 310, 107–110. doi: 10.1016/j.jneuroim.2017.07.004

Schroder, K., and Tschopp, J. (2010). The inflammasomes. *Cell* 140, 821–832. doi: 10.1016/j.cell.2010.01.040

Shaw, P. J., Lukens, J. R., Burns, S., Chi, H., McGargill, M. A., and Kanneganti, T. D. (2010). Cutting edge: critical role for PYCARD/ASC in the development of experimental toimmune encephalomyelitis. *J. Immunol.* 184, 4610–4614. doi: 10.4049/jimmunol.1000217

Shi, F., Yang, L., Kouadir, M., Yang, Y., Wang, J., Zhou, X., et al. (2012). The NALP3 inflammasome is involved in neurotoxic prion peptide-induced microglial activation. *J. Neuroinflamm.* 9:73. doi: 10.1186/1742-2094-9-73

Shi, Y., and Holtzman, D. M. (2018). Interplay between innate immunity and Alzheimer disease: APOE and TREM2 in the spotlight. *Nat. Rev. Immunol.* 18, 759–772. doi: 10.1038/s41577-018-0051-1

Simard, A. R., Soulet, D., Gowing, G., Julien, J. P., and Rivest, S. (2006). Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron* 49, 489–502. doi: 10.1016/j.neuron.2006.01.022

Smirnova, E., Griparic, L., Shurland, D. L., and van der Bliek, A. M. (2001). Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. *Mol. Biol. Cell* 12, 2245–2256. doi: 10.1091/mbc.12.8.2245

Soung, A. L., Davé, V. A., Garber, C., Tycksen, E. D., Vollmer, L. L., and Klein, R. S. (2022). IL-1 reprogramming of adult neural stem cells limits neurocognitive recovery after viral encephalitis by maintaining a proinflammatory state. *Brain Behav. Immun.* 99, 383–396. doi: 10.1016/j.bbi.2021.10.010

Sui, Y., Bian, L., Ai, Q., Yao, Y., Yu, M., Gao, H., et al. (2019). Gastrodin inhibits inflammasome through the STAT3 signal pathways in TNA2 astrocytes and reactive astrocytes in experimentally induced cerebral ischemia in rats. *Neuromol. Med.* 21, 275–286. doi: 10.1007/s12017-019-08544-8

Tan, P. H., Ji, J., Yeh, C. C., and Ji, R. R. (2021). Interferons in pain and infections: emerging roles in neuro-immune and neuro-glial interactions. *Front. Immunol.* 12:783725. doi: 10.3389/fimmu.2021.783725

Taylor, J. P., Brown, R. H. Jr., and Cleveland, D. W. (2016). Decoding ALS: from genes to mechanism. *Nature* 539, 197–206. doi: 10.1038/nature20413

Ting, J. P., Lovering, R. C., Alnemri, E. S., Bertin, J., Boss, J. M., Davis, B. K., et al. (2008). The NLR gene family: a standard nomenclature. *Immunity* 28, 285–287. doi: 10.1016/j.immuni.2008.02.005

Vande Walle, L., Van Opdenbosch, N., Jacques, P., Fossoul, A., Verheugen, E., Vogel, P., et al. (2014). Negative regulation of the NLRP3 inflammasome by A20 protects against arthritis. *Nature* 512, 69–73. doi: 10.1038/nature13322

Voet, S., Mc Guire, C., Hagemeyer, N., Martens, A., Schroeder, A., Wieghofer, P., et al. (2018). A20 critically controls microglia activation and inhibits inflammasome-dependent neuroinflammation. *Nat. Commun.* 9:2036. doi: 10.1038/s41467-018-04376-5

Wallisch, J. S., Simon, D. W., Bayır, H., Bell, M. J., Kochanek, P. M., and Clark, R. (2017). Cerebrospinal fluid NLRP3 is increased after severe traumatic brain injury in infants and children. *Neurocrit. Care* 27, 44–50. doi: 10.1007/s12028-017-0378-7

Walsh, J. G., Muruve, D. A., and Power, C. (2014a). Inflammasomes in the CNS. *Nat. Rev. Neurosci.* 15, 84–97. doi: 10.1038/nrn3638

Walsh, J. G., Reinke, S. N., Mamik, M. K., McKenzie, B. A., Maingat, F., Branton, W. G., et al. (2014b). Rapid inflammasome activation in microglia contributes to brain disease in HIV/AIDS. *Retrovirology* 11, 35. doi: 10.1186/1742-4690-11-35

Wang, J., Zhang, X. N., Fang, J. N., Hua, F. F., Han, J. Y., Yuan, Z. Q., et al. (2022). The mechanism behind activation of the Nod-like receptor family protein 3 inflammasome in Parkinson's disease. *Neural Regen. Res.* 17, 898–904. doi: 10.4103/1673-5374.323077

Wang, J., Zheng, B., Yang, S., Tang, X., Wang, J., and Wei, D. (2020). The protective effects of phoenixin-14 against lipopolysaccharide-induced inflammation and inflammasome activation in astrocytes. *Inflamm. Res.* 69, 779–787. doi: 10.1007/s00011-020-01355-9

Wellington, C. L., Ellerby, L. M., Hackam, A. S., Margolis, R. L., Trifiro, M. A., Singaraja, R., et al. (1998). Caspase cleavage of gene products associated with triplet expansion disorders generates truncated fragments containing the polyglutamine tract. *J. Biol. Chem.* 273, 9158–9167. doi: 10.1074/jbc.273.15.9158

Wu, P. J., Hung, Y. F., Liu, H. Y., and Hsueh, Y. P. (2017). Deletion of the inflammasome sensor Aim2 mitigates Aβ deposition and microglial activation but increases inflammatory cytokine expression in an alzheimer disease mouse model. *Neuroimmunomodulation* 24, 29–39. doi: 10.1159/000477092

Yang, D., He, Y., Muñoz-Planillo, R., Liu, Q., and Núñez, G. (2015). Caspase-11 requires the pannexin-1 channel and the purinergic P2X7 pore to mediate pyroptosis and endotoxic shock. *Immunity* 43, 923–932. doi: 10.1016/j.immuni.2015.10.009

Yao, X., Jiang, Q., Ding, W., Yue, P., Wang, J., Zhao, K., et al. (2019). Interleukin 4 inhibits high mobility group box-1 protein-mediated NLRP3 inflammasome formation by activating peroxisome proliferator-activated receptor-γ in astrocytes. *Biochem. Biophys. Res. Commun.* 509, 624–631. doi: 10.1016/j.bbrc.2018.11.145

Yiangou, Y., Facer, P., Durrenberger, P., Chessell, I. P., Naylor, A., Bountra, C., et al. (2006). COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. *BMC Neurol.* 6:12. doi: 10.1186/1471-2377-6-12

Zendedel, A., Johann, S., Mehrabi, S., Joghataei, M. T., Hassanzadeh, G., Kipp, M., et al. (2016). Activation and regulation of NLRP3 inflammasome by intrathecal application of SDF-1a in a spinal cord injury model. *Mol. Neurobiol.* 53, 3063–3075. doi: 10.1007/s12035-015-9203-5

Zhan, X., Jickling, G. C., Ander, B. P., Liu, D., Stamova, B., Cox, C., et al. (2014). Myelin injury and degraded myelin vesicles in Alzheimer's disease. *Curr. Alzheimer Res.* 11, 232–238. doi: 10.2174/1567205011666140131120922

Zhang, M., Wu, Y., Gao, R., Chen, X., Chen, R., and Chen, Z. (2022a). Glucagon-like peptide-1 analogs mitigate neuroinflammation in Alzheimer's disease by suppressing NLRP2 activation in astrocytes. *Mol. Cell. Endocrinol.* 542, 111529. doi: 10.1016/j.mce.2021.111529

Zhang, H., Yu, S., Xia, L., Peng, X., Wang, S., and Yao, B. (2022b). NLRP3 inflammasome activation enhances ADK expression to accelerate epilepsy in mice. *Neurochem. Res.* 47, 713–722. doi: 10.1007/s11064-021-03479-8

Zhang, W. J., Chen, S. J., Zhou, S. C., Wu, S. Z., and Wang, H. (2021). Inflammasomes and fibrosis. *Front. Immunol.* 12:643149.

Zhang, X., Huang, W., Shao, Q., Yang, Y., Xu, Z., Chen, J., et al. (2020a). Drp1, a potential therapeutic target for Parkinson's disease, is involved in olfactory bulb pathological alteration in the rotenone-induced rat model. *Toxicol. Lett.* 325, 1–13. doi: 10.1016/j.toxlet.2020.02.009

Zhang, Q., Sun, Y., He, Z., Xu, Y., Li, X., Ding, J., et al. (2020b). Kynurenine regulates NLRP2 inflammasome in astrocytes and its implications in depression. *Brain Behav. Immun.* 88, 471–481. doi: 10.1016/j.bbi.2020.04.016

Zhang, X., Wang, R., Hu, D., Sun, X., Fujioka, H., Lundberg, K., et al. (2020c). Oligodendroglial glycolytic stress triggers inflammasome activation and neuropathology in Alzheimer's disease. *Sci. Adv.* 6:eabb8680. doi: 10.1126/sciadv.abb8680

Zhao, W., Beers, D. R., Bell, S., Wang, J., Wen, S., Baloh, R. H., et al. (2015). TDP-43 activates microglia through NF-κB and NLRP3 inflammasome. *Exp. Neurol.* 273, 24–35. doi: 10.1016/j.expneurol.2015.07.019

Zhou, B., Zhu, Z., Ransom, B. R., and Tong, X. (2021). Oligodendrocyte lineage cells and depression. *Mol. Psychiatry* 26, 103–117. doi: 10.1038/s41380-020-00930-0

Zhou, Y., Lu, M., Du, R. H., Qiao, C., Jiang, C. Y., Zhang, K. Z., et al. (2016). MicroRNA-7 targets Nod-like receptor protein 3 inflammasome to modulate neuroinflammation in the pathogenesis of Parkinson's disease. *Mol. Neurodegener.* 11:28. doi: 10.1186/s13024-016-0094-3

Zhu, J., Hu, Z., Han, X., Wang, D., Jiang, Q., Ding, J., et al. (2018). Dopamine D2 receptor restricts astrocytic NLRP3 inflammasome activation via enhancing the interaction of β-arrestin2 and NLRP3. *Cell Death Differ.* 25, 2037–2049. doi: 10.1038/s41418-018-0127-2

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

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Cellular Neuroscience

TYPE Review
PUBLISHED 25 July 2022
DOI 10.3389/fncel.2022.955550

OPEN ACCESS

EDITED BY
Alejandro López-Juárez,
The University of Texas Rio Grande Valley, United States

REVIEWED BY
Tatiana Olivares,
Universidad Autónoma de Baja California, Mexico
Esther López-Bayghen,
Instituto Politécnico Nacional de México (CINVESTAV), Mexico

*CORRESPONDENCE
Ataúlfo Martínez-Torres
ataulfo@unam.mx

SPECIALTY SECTION
This article was submitted to Non-Neuronal Cells, a section of the journal Frontiers in Cellular Neuroscience

RECEIVED 28 May 2022
ACCEPTED 06 July 2022
PUBLISHED 25 July 2022

CITATION
Gómez-González GB, Becerra-González M, Martínez-Mendoza ML, Rodríguez-Arzate CA and Martínez-Torres A (2022) Organization of the ventricular zone of the cerebellum.
Front. Cell. Neurosci. 16:955550.
doi: 10.3389/fncel.2022.955550

Organization of the ventricular zone of the cerebellum

Gabriela B. Gómez-González, Marymar Becerra-González, Marianne Lizeth Martínez-Mendoza, Cynthia Alejandra Rodríguez-Arzate and Ataúlfo Martínez-Torres*

Laboratorio de Neurobiología Molecular y Celular, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Juriquilla, Mexico

The roof of the fourth ventricle (4V) is located on the ventral part of the cerebellum, a region with abundant vascularization and cell heterogeneity that includes tanycyte-like cells that define a peculiar glial niche known as ventromedial cord. This cord is composed of a group of biciliated cells that run along the midline, contacting the ventricular lumen and the subventricular zone. Although the complex morphology of the glial cells composing the cord resembles to tanycytes, cells which are known for its proliferative capacity, scarce or non-proliferative activity has been evidenced in this area. The subventricular zone of the cerebellum includes astrocytes, oligodendrocytes, and neurons whose function has not been extensively studied. This review describes to some extent the phenotypic, morphological, and functional characteristics of the cells that integrate the roof of the 4V, primarily from rodent brains.

KEYWORDS

astrocytes, Bergmann glia, choroid plexus, ependymal glial cells, fourth ventricle

Introduction

The presence of neurogenic niches throughout the ventricular system has stimulated intense research aiming to understand the organization and role of cell groups of glial origin that contact the cerebrospinal fluid and gate relevant information toward and from the brain parenchyma. The roof of the fourth ventricle (4V) is formed by three cerebellar lobules (I, II, and X). Diverse studies have shown little or non-proliferative activity in this area; however, it has been highlighted the presence of heterogeneous cell types expressing GABA-Aρ receptors in the ependymal glial cells (Reyes-Haro et al., 2013; Pétriz et al., 2014), these receptors gate a Cl^- channel and desensitize very little upon activation and are insensitive to bicuculline and baclofen. About 60% of the ependymal glial cells respond to GABA by generating Cl^- currents insensitive to pentobarbital; these findings supported the view that this area should be formed by a diverse population of cell phenotypes. The cellular diversity was highlighted when an inspection of cellular electrophysiological profiles was conducted in the subventricular zone (SVZ) (Reyes-Haro et al., 2013; González-González et al., 2017); those studies revealed the presence, of astrocytes, oligodendrocytes, neurons and other groups of cells at the ventricular and subventricular cerebellar zones, that remain to be fully described and studied.

The cells that form the wall of the fourth ventricles are crucial for neurogenesis and gliogenesis in early development. During embryonic mouse development, the ventricular zone of the cerebellum is a niche of cells that gives rise to all the neurons of the cerebellum. Individual progenitors are multipotent and produce sequentially different cell types, first to the deep cerebellar nuclei, then the Purkinje neurons and finally to the rest of cerebellar neurons. Also, in the ventricular zone a set of multipotent cortical progenitors is found, these cells produce sequentially cortical neurons, however, lose their potential to produce deep nuclei when longitudinal cell dispersion in the cerebellum becomes limited (Altman and Bayer, 1997; Mathis and Nicolas, 2003). In the mouse embryo (embryonic day 12–14), radial glia fascicles appear at the site of midline fusion at the roof of the fourth ventricle, also radial fascicles appear at the site of midline fusion of the metencephalic plate, radial glia fibers become concentrated within the zone that bounds the germinal trigone which is the site of origin of the external granular layer (Edwards et al., 1990). The early origins of radial glial cells of the cerebellum can be traced to cells of the primitive bipolar cells in ventricular germinal zones. This review focuses on the cell diversity that form the roof of the 4V.

Diversity of glial cells

The anatomical and functional complexity of the periventricular zone of the cerebellum is derived from the presence of different neuronal types, but also from the heterogeneity of glial cells (Buffo and Rossi, 2013; González-González et al., 2017). According to location, morphology and physiology, the following types of glial cells can be identified in the roof of the 4V: (a) astrocytes, (b) Bergmann glia (BG), (c) nerve-glial antigen two cells (NG2-glia), (d) microglia, and (e) ependymal glial cells (EGCs). The microglia originate from the mesoderm, while the rest share the same ectodermal embryonic origin.

Astrocytes

Astrocytes, also known as astroglia, are the most abundant and diverse population of glial cells in the central nervous system (CNS). This term was popularized by Santiago Ramón y Cajal who also developed the gold sublimate-method which labeled GFAP (glial fibrillary acidic protein), with this technique it was demonstrated that astrocytes originated from radial glia (Ramóny Cajal, 1916; García-Marín et al., 2007). Astrocytes develop in the embryonic stage and in the late postnatal period (Miale and Sidman, 1961). There are two sites of proliferation in the embryonic ventricular neuroepithelium, (1) the region anterior to the rhombic lip, and (2) the region between the isthmus and the superior cerebellar peduncle (Altman and Bayer, 1987). It is believed that cerebellar glia may be generated by progenitors inhabiting the cerebellar parenchyma (Altman and Bayer, 1987); clonal studies in mouse, showed that astrocytes from the vermis arise from gliogenic radial glia that reaches the ventricular zone in early stages, and at postnatal stages, progenitors from the Purkinje cell layer originate BG and velated astrocytes located in the granular layer (Cerrato et al., 2018).

In general, astrocytes possess multiple primary processes that originate from the soma. In addition, this cell type has intermediate filaments that integrate the cytoskeleton, formed by proteins that define this cell type, including the GFAP and vimentin (Verkhratsky and Butt, 2007). Astrocytes have been classified in protoplasmic and fibrous, according to differences in their morphology and location. Protoplasmic astrocytes are found in the gray matter and exhibit multiple finely branched and complex processes (of $\sim$50 μm long, in rodents). Fibrous astrocytes are found in the white matter and exhibit processes that are longer (300 μm long, in rodents) and less complex compared to protoplasmic astrocytes (Verkhratsky and Butt, 2007).

The cerebellar cortex is formed by two main types of astroglia: Bergmann glia, specialized cells located in the Purkinje cell layer (PCL) and velate astrocytes, which are more common in the granular layer (Farmer and Murai, 2017). Velate astrocytes have a small soma and relatively short processes, where they partially surround the glomeruli and slip between granular cells which synapse with mossy fibers, separating the glomeruli into distinct entities. The velate astrocytes also gather around blood vessels in form of a plaque around the basal lamina forming part of the blood-brain barrier (Landis and Reese, 1982). Velate astrocytes form a syncytial network through gap junctions with nine neighboring cells, giving rise to a highly elaborated and functional coordinated structure in the cerebellum (Kiyoshi et al., 2018).

In the SVZ of the mouse cerebellum, a region delimited dorsally by BG end feet and ventrally by the ependymal cells, the astrocytes are abundant and express GABA-A receptors (Reyes-Haro et al., 2013). A study conducted by Pétriz et al. (2014) showed that GFAP$^+$ cells of the granular layer of the cerebellum express GABAρ subunits during early postnatal development. Electrophysiological studies revealed that astrocytes express functional GABA-A receptors, whereas evoked currents were inhibited by bicuculline and TPMPA, indicating the presence of GABAρ subunits. In that study, three populations of GABA-A receptors were identified in astrocytes: classic GABA-A receptors, bicuculline insensitive GABAρ, and GABA-A-GABAρ hybrids. The presence of GABAρ subunits independently or in combination, confers complexity to GABAergic signaling in the area. Also, the modulation of GABAρ dynamics can be a novel extra-synaptic transmission mechanism that regulates GABAergic control of GFAP$^+$ cells of the SVZ of the cerebellum in early development (Pétriz et al., 2014).

In recent experiments in which carmustine was administered prenatally, a reduction in the morphology and complexity of astrocytic cells of the 4V of the cerebellum was observed (Rodríguez-Arzate et al., 2021). In a transcriptomic analysis using the well-established hEGP-eGFP reporter mouse line (Nolte et al., 2001), differences were found between protoplasmic cortical astrocytes and cerebellar astrocytes (mainly Bergmann glia). It seems that a specific transcriptional program associated to the Zic/Irx families of transcription factors play a central role in cerebellar astrocytes (Welle et al., 2021).

Bergmann glia

These cells originate from the radial glia and are a landmark of cerebellum. They are found from early stages of development around embryonic day 15 in mouse, playing an essential role during neurogenesis and cell migration since they form a structural scaffold for cellular migration of granular cells during postnatal development as well as in the dendritic growth of PCs (Bellamy, 2006). In the mouse adult brain, the cell bodies of BGs are ∼15 μm diameter and is settled in the Purkinje cell layer, the somas surround these neurons in a proportion of 8 to 1. The BG exhibits multiple processes, with numerous lateral protrusions named microdomains, these processes go through the molecular layer forming a conical terminal foot (Rakic, 1972, 2003). The plane of the processes of BG is organized as a palisade, oriented in parallel to the longitudinal axis of the folia and perpendicular to the plane formed by the dendritic trees of the Purkinje cells (Buffo and Rossi, 2013).

The BG surrounds and covers the dendritic trees of Purkinje neurons, wrapping between 2000 and 6000 synapses (Kettenmann and Ransom, 2005). The BG microdomains respond to synaptic activity by changing Ca^{2+} concentration, which eventually propagates from the processes toward different regions of the cell (Bellamy, 2006). Also, the microdomains are highly dynamic, displaying motility patterns similar to neuronal spines, since they have the capacity to form new processes that grow and retract laterally (Lippman et al., 2008, 2010).

The lateral protuberances of BG processes present glutamate receptors of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) subtype, composed exclusively by the GluA1/GluA4 subunits. A study conducted by Saab et al. (2012), demonstrated that BG is essential for synaptic integration and cerebellar output signal and a double knock out of AMPA receptors of the BG leads to deficiencies and alterations of fine motor coordination (Saab et al., 2012).

While the BG located in the dorsal cerebellar lobes (lobule III-IX) extend their processes to the pia establishing the *glia limitans* (Yamada et al., 2000), the BG located in the ventral lobules (I, II and X) at the roof of the 4V, projects their processes toward the SVZ, where the end feet limit the upper part of a structure named the subventricular cellular cluster (SVCC) (González-González et al., 2017). The SVCC, extends rostral-caudally along the lobules I and X, and is formed by a diversity of cell types, camera lucida drawing of three sample cells are shown in Figure 1. These cells express GFAP, nestin or both (González-González et al., 2017).

NG2 glial cells

The nerve-glial antigen two cells (NG2-glia) are also known as oligodendrocyte precursor cells (OPC), NG2 progenitor cells, synantocytes or polydendrocytes (Butt et al., 2002; Nishiyama et al., 2002; Dimou and Gallo, 2015). During the development these cells have different origins (ventral and dorsal ganglionic eminence, SVZ, cerebral cortex), are widespread in the CNS and present diverse morphology (Nishiyama et al., 2002, 2014).

NG2 glial cells are considered the fourth type of glial cells in the central nervous system, these are immature cells with high proliferative capacity and widely distributed throughout the gray and white matter in the postnatal and adult brain, where they make 5–8 of the glial cells population (Dawson et al., 2003; Clarke et al., 2012; Lomoio et al., 2012). NG2-glia can be identified by the expression of several molecular markers, such as platelet-derived growth factor receptor (PDGFRα), Olig2, proteolipid promoter (Plp) (Chung et al., 2013) and Sox 10 (Trotter et al., 2010); besides NG2, which is permanently expressed after birth (Rivers et al., 2008).

In the mouse adult cerebellum, the NG2-glia has a stellate shape and is present in the white and gray matter (Bouslama-Oueghlani et al., 2005; Lomoio et al., 2012; Chung et al., 2013). The general distribution of the NG2-glia in the cerebellum was described in a study conducted by Lomoio et al. (2012) in rats, that showed that NG2 glia at the posterior cerebellar lobules (VI–VIII) is evenly and densely spread in all the cell layers; however, in the anterior and ventral lobes (I–VI and IX–X lobules) most of these cells are in the molecular layer, while in the white matter and the internal granular cell layer (IGL) their presence is sporadic, and decreases with age. NG2-glia from the molecular layer exhibits numerous processes that extend between the dendrites of the Purkinje neurons (Palay and Chan-Palay, 1974; Levine and Card, 1987; Lin et al., 2005), while the NG2-glia from the white matter is highly branched with thin processes and no cell-coupling (Labrada-Moncada et al., 2020).

One of the major roles of the NG2-glia is the generation of myelinating and non-myelinating oligodendrocytes (OL) during postnatal development (P12-P23 in rodents), crucial in the repairment of the myelin loosed under demyelinating pathologies (Watanabe et al., 2002; Nishiyama et al., 2014, 2021), either at the spinal cord or brain (Sherafat et al., 2021). Nevertheless, the fate of NG2-glia as precursor cells has been further investigated *in vivo* using different inducible transgenic mice lines, unveiling that secondary to the OL generation,

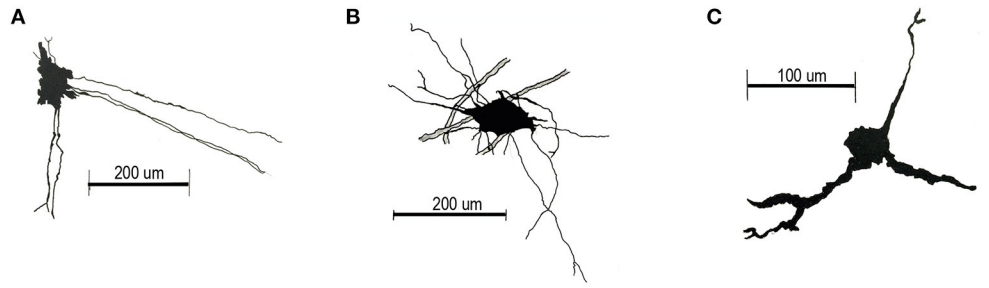


FIGURE 1
Camera lucida drawings of main cell types of the SVCC. **(A)** Cells located in the lateral region of the SVCC have an elongated soma (diameter 32.506 ± 0.808 μm), long processes that project toward the middle region and processes that extend ventrally. **(B)** Star-shaped soma cells (diameter 36.957 ± 0.774 μm) with a long lateral process and one that projects ventrally. **(C)** Small cells (diameter 17.728 ± 2.223 μm), with two processes projecting ventrally and a short lateral process.

NG2-glia cells give rise to astrocytes before birth, and this capacity decreases along time (Zhu et al., 2008; Trotter et al., 2010; Nishiyama et al., 2014; Huang et al., 2018). In the adult life, NG2-glia derived astrocytes are found in the gray matter from the forebrain (mainly at ventral cortex, striatum; Zhu et al., 2008) and cerebellum; where the Olig2/Plp positive NG2-glia exhibit a gliogenic fate and generates Bergmann glia cells (postnatal day 7 in mice), however upon hypoxic-ischemic injury the cerebellar oligodendroglial lineage increases while Bergmann cells and astrocytes remain constant (Chung et al., 2013). Evidence strongly suggests the proliferative capacity of NG2-glia cells in response to injury by giving rise to astrocytes, microglia (Sellers et al., 2009) and Schwann cells (Zawadzka et al., 2010). However, despite discussions, most of the evidence support that NG2-glia cells do not generate neurons (Nishiyama et al., 2014; Huang et al., 2018; Sánchez-González et al., 2020).

The multipotential capacity of the NG2-glia is linked to its functional properties that suggest these cells are involved in the proper function of different brain circuits (Akay et al., 2021; Zhang et al., 2021), for instance it has been reported that in the cerebellum these cells express AMPA receptors permeable to Ca^{2+} that sense through synaptic junctions the input from climbing fibers (Lin et al., 2005), and the NG2-glia from white matter expresses functional GABA-A receptors (Labrada-Moncada et al., 2020). On the other hand, cell fate of NG2-glia cells can be modulated by local cues, such as glutamate signaling in the cerebellum (Chung et al., 2013).

Microglia

Microglia, also known as Hortega cells, who described them in 1932 (Del Rio, 1932), are a population of immune cells that reside in the CNS within the parenchyma associated to the capillary vasculature (Prinz and Mildner, 2011). In the adult murine nervous system, the microglial population is regularly organized as an independent mosaic of cell layers (Lawson et al., 1990). Depending on the region analyzed microglia represents 5–12% of the total number of glial cells (Lawson et al., 1990), showing higher densities in gray matter than in white matter (Mittelbronn et al., 2001). Microglia presents diversity in its morphology, which is mostly arborized with radial orientation; other microglial cells are elongated and mainly oriented to the white matter while others are amoeboid in the circumventricular organs (Lawson et al., 1990).

These cells play a role as the first line of defense in the nervous system (Prinz and Priller, 2014), participating in phagocytosis, acute responses to injury, release of inflammatory modulators and cytokines, and are important for normal brain development and synaptic plasticity (Prinz and Priller, 2014). In the absence of injury or disease, microglia is in a "resting" state constantly "monitoring" its microenvironment (Gómez-Nicola and Perry, 2015), in this state microglia maintains its soma in a fixed position while its multiple mobile processes allow to explore its surroundings while maintaining a mosaic distribution and organization (Dávalos et al., 2005; Nimmerjahn et al., 2005; Wake et al., 2009). Depending on the specific signals of the modulators of microglial activation after injury detection (Hanisch and Kettenmann, 2007; Prinz and Priller, 2014), the microglia transforms into a "reactive" state, in which the motility of their processes allows its migration to the site of injury (Dávalos et al., 2005; Hanisch and Kettenmann, 2007) generating multiple phenotypes of reactive microglia, which present phenotypes similar to the polarization states of M1 and M2 macrophages (Wang et al., 2013; Prinz and Priller, 2014). Reactive microglia have also been shown to release reactive oxygen species, guanosine triphosphate (GTP), ATP, inflammatory cytokines (IL-1β and IL-6), and tumor necrosis factor (TNF-α) (Pascual et al., 2012; Zhan et al., 2014; Prinz and Priller, 2017).

Unlike the microglia located in the brain cortex, the cerebellar microglia display certain unique characteristics. A

study carried out by Tay et al. (2017) showed that microglia isolated from the cerebellum of rodents have a higher expression of immune-related genes and are renewed in a shorter time compared to microglia from other regions of the brain. In the cerebellum, microglia are scarce, and their branches are less complex, having only two main processes extending from the soma. Cerebellar microglia show different morphological and dynamic profiles in the molecular layer, Microglia from Purkinje and granular cell layers show soma motility and a dynamic interaction with Purkinje neurons (Stowell et al., 2017). Yamamoto et al. (2019) showed the interaction between microglia and cerebellar neurons through TNF-α signaling, which induced long-term potentiation and thus modulation of motor behavior.

Ependymal glial cells

The ependyma is a specialized epithelial tissue derived from the radial glia (Spassky et al., 2005), hence these are glial cells with a cuboid shape and multi-ciliated simple columnar form lining the ventricles and interconnected by either gap junction or zonula adherens (Bruni and Anderson, 1987; Oda and Nakanishi, 1987; Telano and Baker, 2021). A network of modified ependymal cells known as the choroid plexus (see following section) produces 70–80% of the cerebrospinal fluid (CSF). EGCs are covered by cilia and microvilli on its apical side, permitting CSF circulation and absorption. Damage to the ependyma may result in low volume of CSF which is also caused by age-related atrophy of ependymal cells (Oda and Nakanishi, 1987; Telano and Baker, 2021; Javed et al., 2022).

Other functions of the EGCs include roles in barrier formation, transport of ions, molecules and water between the CSF and the parenchyma (Albors et al., 2022). The EGCs surround clusters of fenestrated capillaries that permit the filtration of plasma (Telano and Baker, 2021). EGCs are a key component of the subventricular zone stem cell niche, such as in the spinal cord, where some of the EGC lining the central canal are in contact with neural stem cells (Albors et al., 2022). The transcriptomic profile of EGCs is quite diverse in the ventricular system, but all express the gene FoxJ1, which is critical for the production of cilia (Zeisel et al., 2018; Albors et al., 2022), however at the moment there is not a comprehensive transcriptome of ependymal cells of the 4V to help to understand the morphological diversity in this area that includes tanycyte-like cells and biciliated cells in the midline of the cerebellum (González-González et al., 2017).

The surface of the cells that form the wall of the roof of the 4V has been observed under the scanning electron microscope (Oda and Nakanishi, 1987; Alvarez-Morujo et al., 1992). The anterior section is divided in territories rich in cilia, other territories show only a few cilia while others are covered by microvilli but not cilia (Alvarez-Morujo et al., 1992). In addition, numerous spherical bulbs of unknown function face the lumen (Alvarez-Morujo et al., 1992; González-González et al., 2017). Transmission electron microscopy showed that cilia exhibit a 9 + 2 microtubule pattern, and some cilia have a "dilated end", possibly related with a receptor like function (Alvarez-Morujo et al., 1992; González-González et al., 2017). Along the midline of the ventricle a formation named the ventromedial cord includes biciliated cells intermingled with EGCs. These cells express GFAP, vimentin and nestin and respond to hypoxic conditions by transiently regulating the expression of GFAP (González-González et al., 2017; Becerra-González et al., 2020).

In the EGCs, GFAP expression is related to the state of differentiation during ontogeny and is an indicator of different levels of functional maturity, nevertheless, in adulthood a variation of GFAP expression correlates with diverse functional states. During the postnatal life, EGC's decrease the expression of GFAP but rebounds in the mature brain. Interestingly, EGC's are mitotically active in the adult brain and therefore are in risk of developing tumors (Leonhardt et al., 1987).

In the cerebellum, EGCs express GABA-A receptors with different characteristics from those expressed in neurons, this includes the presence of the GABA-Aρ receptors that confer resistance to bicuculline and insensitivity to pentobarbital (Reyes-Haro et al., 2013). Under the scanning electron microscope ependymal EGCs of the roof of the 4V show diverse features: (1) cells with numerous microvilli and cilia, (2) cells with numerous microvilli and few cilia, located next to the choroid plexus, and (3) cells with sparse microvilli and one or a few cilia (Oda and Nakanishi, 1987; González-González et al., 2017). Within the later, there are some biciliated cells that correspond to a group of EGCs compacted and ordered medially in antero-posterior direction in the roof of the 4V. These cells co-express vimentin and nestin (a marker commonly used to detect precursor cells) that form the ventromedial cord (VMC) which is present since embryonic development and through adulthood (González-González et al., 2017). Some insights about the functionality of these tanycyte-like cells that form the VMC have been revealed by using a model of hypoxic preconditioning in mice, in which expression of GFAP showed a transient reduction upon induction of hypoxic pre-conditioning. In addition, expression of nestin was upregulated in the whole cerebellum but incorporation of BrdU was not detected in the EGCs of the area. As the VMC EGCs responded to this mild stimulation (hypoxic preconditioning), it is suggested that VMC GFAP-positive cells may relay chemical information from the CSF to underlying neural circuits such as the ones extending along the floor of the 4V (Mirzadeh et al., 2017; Becerra-González et al., 2020). It is clear that the diversity of EGCs of the cerebellum and the entire CNS points out to specializations of their functional role, and a map of the distribution of every phenotype will certainly help to understand their selective role according to their distribution along the surface of the 4V.

Choroid plexus

Four choroid plexuses reside inside the ventricular system of the brain: one in each of the two lateral ventricles, one in the third ventricle, and one in the 4V. Their most important function is the production of the cerebrospinal fluid (CSF). The CSF is formed at a rate of ∼4 ml/min per gram of choroid plexus tissue, in the rat, which is ∼10 times higher than the rate of the blood supply to the brain parenchyma (Keep and Jones, 1990). The total volume of CSF in the entire human CNS amounts about 150 ml, and ∼500–600 ml is produced in 24 h; thus, the CSF is replaced three to four times per day (Cserr, 1971).

The choroid plexus of the 4V is T-shaped, extended toward the lateral angles of the rhomboid shaped roof and its vertical limb extends caudally. It is composed by a single layer of cuboidal epithelial cells, with numerous club-shaped microvilli and occasional short cilia at the ventricular surface. Choroid cells are connected by tight and gap junctions that form a *zonula adherens* (Oda and Nakanishi, 1987).

Epithelial cells are the predominant cell-type in the choroid plexus (Keep et al., 1986; Keep and Jones, 1990), at the caudal end of the 4V the epithelial cells surround 1–4 cell clusters composed of 15–20 cells that form structures named "choroid bodies" whose function is not known yet (Levine and Saltzman, 2003). Transcriptomic studies have revealed the expression of different coding and non-coding regulatory mRNAs in the choroid plexus. A comparative transcriptomic analysis of the choroid plexus from the lateral and fourth ventricles (from mice, macaque, and human) revealed specialized domains of secretory cells that predict that cells from both regions contribute differently to cell-signaling to the CSF (Lun et al., 2015). One interesting signaling molecule produced by the choroid plexus of the 4V is the bone morphogenic protein or BMP, which negatively modulates neuron differentiation of rhombic lip-derived cells, that gives rise to cerebellar granular neurons (Krizhanovsky and Ben-Arie, 2006). A non-coding RNA expressed in the choroid plexus of the 4V is the microRNA (miRNA) 449. miR449 is expressed in the epithelial cells of the choroid plexus in the 4V in developing and adult mouse (Redshaw et al., 2009) and is known to regulate ciliogenesis by inhibiting the Notch pathway. If the molecular circuit downstream of miR449 is perturbed it may lead to ciliopathies (Marcet et al., 2011).

Due to the inaccessibility of the plexus choroid cells within the 4V, experimental analysis of their electrophysiological properties has been very limited. Whole-cell patch-clamp of choroid plexus epithelial cells maintained in culture have shown a lower mean capacitance than those from the 3V (55 vs. 61 pF) and express several conductances that include a delayed-rectifying potassium channel, an inwardly rectifying chloride channel and a volume sensitive anion channel, which are very similar to those of the lateral ventricle (Kibble et al., 1996; Speake and Brown, 2004). Epithelial cells of the choroid plexus present ion-transport proteins that mediate the transcellular movement of molecules from the blood to the CSF and maintenance of ion concentrations is important for a continuous regulation of cell volume of these cells that are immersed in the CSF. Control of cell volume of choroid plexus epithelial cells of the 4V depend on a Na^+-K^+ exchanger and a Cl^--HCO_3^- exchanger that confer the capacity to respond to variations in extracellular osmolality (Hughes et al., 2010).

The temporal coordination of internal biological processes is crucial to mammals. A complex network of cell independent oscillators is found in different tissues and diverse studies indicate that the suprachiasmatic nucleus of the hypothalamus is the master circadian clock (Cheng and Cheng, 2021; Lu and Kim, 2021). Expression of critical clock genes and their products has been detected in the cells of the choroid plexus, which have a circadian oscillator modulated by estrogens (Quintela et al., 2013, 2018; Yamaguchi et al., 2020) and in explants of choroid plexus maintained *in vitro*, including from the 4V, the circadian rhythmicity remained and even exceeded that of the suprachiasmatic nucleus (Myung et al., 2018). Thus, the choroid plexus seems to affect the master clock in the suprachiasmatic nucleus most probably by biochemical signaling through the cerebrospinal fluid (Quintela et al., 2021).

Novel brain regenerative strategies apply transplantation of cells to induce functional rescue of brain lesions caused by trauma or neurodegenerative diseases. Cell therapy appears to increase neurotrophic factors levels and reduce neuroinflammation and several studies have demonstrated the potential of choroid plexus cells to reduce neurological deficits and promote neuroregeneration. For example, cells from the choroid plexus of the 3V and 4V were grafted in the 4V of rats with ischemic brain injury. This strategy reduced neurological deficits and infraction volume, it also reduced the number of apoptotic and inflammatory cells (Matsumoto et al., 2010). Furthermore, choroid plexus cells from the 3V and 4V promoted axonal extension and tissue repair in an experimental model of spinal cord lesion, in which axonal extension of the injured tissue was observed after transplantation (Kanekiyo et al., 2016), grafted cells also gave rise to astrocytes in lesioned spinal cord mice (Kitada et al., 2001) and induced nerve regeneration in the dorsal funiculus of rat spinal cord by supporting a massive growth of axons (Ide et al., 2001). All these effects of the grafted follicular cells may be associated to the expression and release of different neurotrophic factors that affect the local environment of the recipient tissue, although some neurogenic activity is not fully discarded in choroid plexus of the 4V (Itokazu et al., 2006).

Vascular organization

The vertebral arteries enter the cranium trough the *foramen magnum* to supply the cerebellum and the brainstem with blood,

carrying oxygen, nutrients and hormones obtained from the cerebrospinal fluid (Kulik et al., 2008; Cipolla, 2009; Fogwe and Mesfin, 2018), maintaining homeostasis. Three arteries supply the cerebellum: (1) the *superior cerebellar artery*, branching from the lateral portion of the *basilar artery* from which, (2) the *anterior inferior cerebellar artery* also branches, and (3) the *posterior inferior cerebellar artery* branching from the vertebral arteries, inferior to their junction with the basilar artery.

Vascularization occurs by angiogenesis as in the rest of the CNS, the cerebellar angioarchitecture of mouse starts its differentiation at embryonic day 9.5, from a blood vessel that arrives from the perineural vascular network. Vascular sprouts grow radially into the hindbrain parenchyma and by embryonic day 10.5 they are able to change their growth directionality to fuse with other sprouts and form a highly stereotyped vascular network named the *subventricular vascular plexus* with a honeycomb shape (Cipolla, 2009; Fogwe and Mesfin, 2018).

In the mature brain, two types of vessels are evident: (1) *pial vessels,* either free in the subarachnoid space surrounded by cerebrospinal fluid or on the surface of the brain contacting the pia-arachnoid and the glia-limitans; and (2) *intraparenchymal vessels*. Pial vessels (intracranial vessels) are predominantly: arteries, arterioles, veins, venules and a few capillaries (Holash et al., 1990). Intraparenchymal vessels are rich in capillaries with a low permeability rate and express γ-glutamyl transpeptidase, alkaline phosphatase and enzymes involved in catecholamine metabolism. While the vascular volume of the parenchymal vessels in the cerebrum and cerebellum remains almost identical, the volume of the vasculature corresponding to the pia is 10–60% higher in the cerebellum, where pial vessels are <6% of the total cerebral vasculature and >30% of the total cerebellar vasculature (Holash et al., 1990). The blood brain barrier (BBB) formed by dynamic endothelial cells that continuously develop tight junctions to limiting cross of molecules, is absent in the vasculature near the ventricular system and in the choroid plexus, where fenestration allows exchange of nutrients (Tata et al., 2015). It is not clear if BBB possesses specific properties to maintain the homeostasis of local neural circuits; however, vascularized areas next to the 4V present widespread passive permeability (Daneman and Prat, 2015).

In the adult life, neuropathologies such as ischemia as well as mild to moderate reduction of oxygen tension (hypoxic pre-conditioning) promote angiogenesis. Moreover, hypoxic pre-conditioning enhances angiogenesis and confers neuroprotective effects due to the generation of new blood vessels which in turn provide oxygen and nutrients to the brain (Li et al., 2013; Hoffmann et al., 2015). Studies in our laboratory demonstrated that hypoxic pre-conditioning induces the widening of the vasculature on the surface of the roof of the 4V. This response may be associated to the metabolic modifications in the environment that led to the reconfiguration of the local glial morphology, which includes BG, astrocytes and microglia. Images of the blood vessels from the 4V, before and after hypoxic preconditioning are shown in Figure 2. In this experiment GFAP-eGFP mice (Nolte et al., 2001) were perfused with the fluorescent lipophilic membrane dye DiI for labeling the vasculature and then fixed with paraformaldehyde. The cerebellum was removed, and the ventricular surface exposed for evidencing the vasculature. After 4 days of hypoxic conditioning, the vessels were considerable dilated; this was an expected effect related to mild hypoxia, in which robust vascular remodeling occurs.

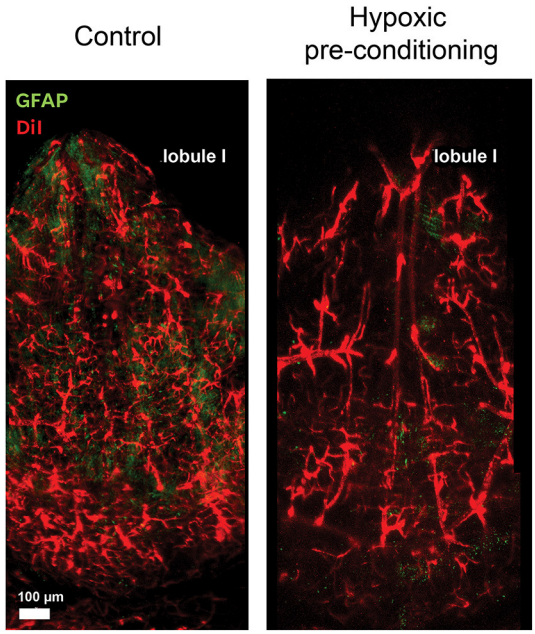


FIGURE 2
Blood vessels respond to hypoxic preconditioning. The surface of the roof of the 4V is profusely irrigated by blood vessels and arteries. These confocal microscope images show the vessels stained with DiI and the surface of the lobule I exposed by the whole mount preparation, before and four days after hypoxic pre-conditioning, where vessel dilation can be observed.

The neural component

We are usually familiarized with the concept that the cerebellum has a uniform set of layers with homogeneous cytoarchitectural organization along the lobules. This is mostly true, but peculiarities emerge when carefully analyzing the surface in contact with the 4V. Each zone of the cerebellar periventricular area has a specific neuronal population and therefore functions that contribute to the CNS homeostasis.

Subventricular zone

Axons from the descending section of the fastigial nucleus (FN) cross contralaterally along the roof of the ventricle mainly projecting to the vestibular system (Walberg et al., 1962;

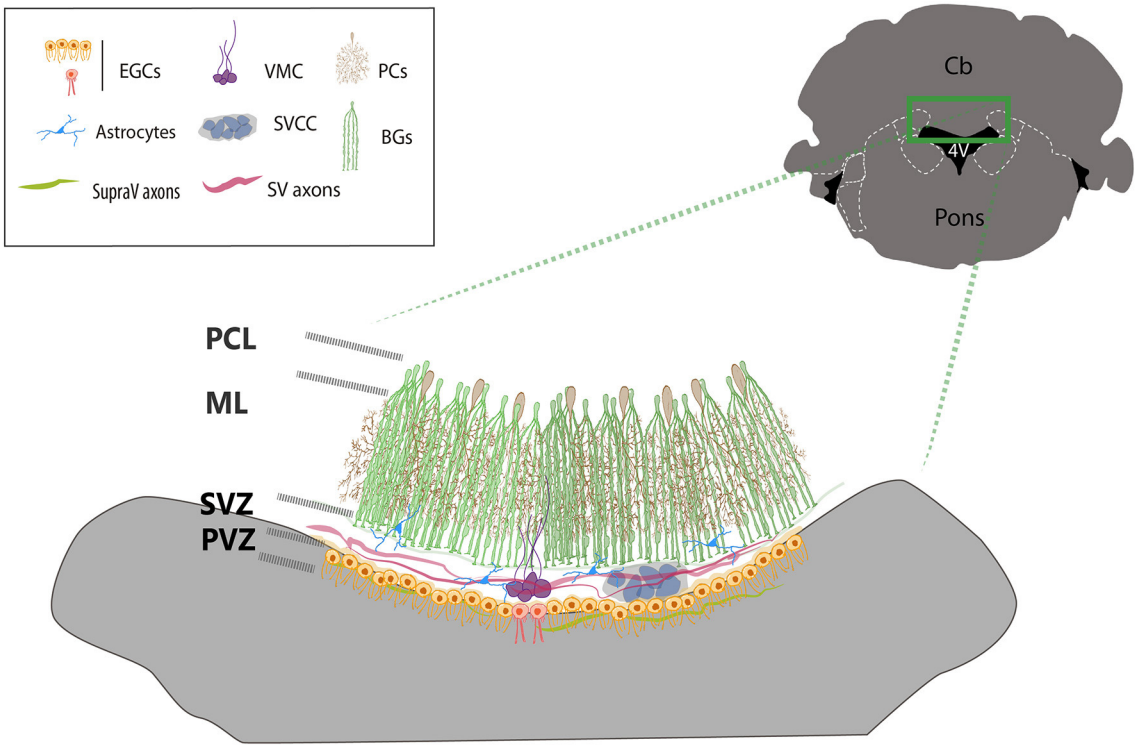


FIGURE 3
Cellular organization of the ventricular zone of the cerebellum. The drawing summarizes the main cell types found in the roof of the 4V. BG cells (green) surrounds and covers the dendritic trees of Purkinje neurons (brown). The end-feet of Bergmann glia borders the dorsal side of the SVZ, where the subventricular cellular cluster is located (SVCC, gray blue) which is ventrally bordered by multiciliated ependymal glial cells (orange cells). The ventromedial cord (VMC, purple), is integrated by local subventricular neurons and glial cells and contacted by local astrocytes (blue). Ependymal biciliated cells (red) are in the midline at the periventricular layer-zone (PVZ), facing the lumen of the ventricle. Two sets of axons transverse contralaterally the roof of the 4V, subventricular axons (SV axons, red) from the fastigial nucleus and supraventricular axons (SupraV axons, green) form the *vellum medularis* that project from the IV cranial nerve.

Voogd, 1967; Voogd and Glickstein, 1998; Gómez-González and Martínez-Torres, 2021). These axons are generally formed by neurons with a dense myelin sheath, and show diverse neurochemical identity either glutamatergic, glycinergic or GABAergic (Robinson et al., 1993; Uusisaari et al., 2007; Bagnall et al., 2009). Interestingly, in a study conducted by Gómez-González and Martínez-Torres (2021), it was demonstrated that the medial portion of the FN located between the cerebellar lobules I-II, form an inter-fastigial direct pathway composed mostly by GABAergic axons, adding complexity to this circuit. Functionally, the rostral region of the FN is associated with regulation of ocular movements (initiation, consistence, and accuracy of saccades), in addition its stimulation increases the systemic blood pressure (Nisimaru and Kawaguchi, 1984; Robinson et al., 1993; Takahashi et al., 2014).

The dorsal portion FN gives rise to the *locus coeruleus*, that is formed by A4 neurons (Grzanna and Mottiver, 1980), which are localized in the lateral region of the subventricular zone of the 4V. These dopamine β hydroxylase positive neurons, with spindle-shaped soma (Grzanna and Mottiver, 1980), run longitudinally and extend their dendrites through the ependymal cells toward the ventricular lumen (Demirjian et al., 1976).

Periventricular zone

A neuronal network called the cerebrospinal fluid-contacting neurons (CFCN) reaches the lumen of the ventricle with their axon or dendrites, forming neurohormonal nerve terminals and sensory cilia (Vigh-Teichmann and Vigh, 1983; Vígh et al., 2004); the long single-cilia has a 9 × 2+2 structure. These neurons are $GABA^{+}$ in small mammals (mouse and rats), and $AChE^{+}$ in fish (Vigh-Teichmann and Vigh, 1983). The function of these neurons has not been elucidated, nevertheless are associated with the sensing of CSF pressure, flow and composition (Vígh et al., 2004), maintaining the homeostasis of the whole brain by non-synaptic transmission, since the neurohormonal terminals release bioactive substances to the CSF.

Supraventricular zone

This zone includes the *vellum medullaris* and choroid plexus. The *vellum medullaris* consists of a sheet of tissue attached to the inferior colliculus and the cerebral vermis (Louvi et al., 2003). The anterior section of the vellum is located at the anterior section of the vermis and continues to the roof throughout the rostral region of the 4V, linking both cerebellar peduncles (Berry et al., 1995). It is a reminiscent zone that derives from the isthmus during embryonic development (Louvi et al., 2003). The anterior section of the vellum presents myelinated and non-myelinated axons (Berry et al., 1995) that arise from the fourth cranial nerve nucleus and project contralaterally to the IVth nerve rootlets rising to the trochlear nerve (Sliney et al., 1981; McConnell et al., 1984; Berry et al., 1995). Finally, this nerve enters to the ocular orbit by passing through the superior orbital fissure to innervate the superior oblique muscle (Brazis, 2014).

Conclusions

This brief analysis of the organization and function of the cells that form the roof of the 4V suggests that the area drastically differs from the rest of the lobules of the cerebellum. The microenvironment generated by the CSF, EGCs, choroid plexus, and blood vessels put forward an argument in favor of the peculiarities of the zone (summarized in Figure 3). There are major discrepancies yet to be resolved; for example, the diversification of functions of the EGCs regarding to their responses to GABA, cilia organization and distribution, the functional organization of axons crossing the roof of the 4V, the role of local non-myelinated neurons, the functional significance of the morphological varieties of glia and microglia among other stimulating questions worth to be explored with new experimental strategies.

Author contributions

AM-T conceived and designed the entire review. All authors wrote, reviewed, edited, and read and approved the manuscript.

Funding

This review was supported by Grants from CONACYT (A1S7659) and PAPIIT-DGAPA IN204520 to AM-T.

Acknowledgments

The authors acknowledge support of programs from UNAM: PAPIIT-DGAPA, Programa de Intercambio Académico-CIC, Programa de Doctorado en Ciencias Biomédicas-UNAM. The authors thank LCC Jessica González Norris for proofreading the English version of this review.

Conflict of interest

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

Publisher's note

References

Akay, L. A., Effenberger, A. H., and Tsai, L. H. (2021). Cell of all trades: oligodendrocyte precursor cells in synaptic, vascular, and immune function. *Genes Dev.* 35, 180–198. doi: 10.1101/gad.344218.120

Albors, A. R., Singer, G. A., May, A. P., Ponting, C. P., and Storey, K. G. (2022). Ependymal cell maturation is heterogeneous and ongoing in the mouse spinal cord and dynamically regulated in response to injury. *bioRxiv [Preprint]*. doi: 10.1101/2022.03.07.483249

Altman, J., and Bayer, S. A. (1987). Development of the precerebellar nuclei in the rat: I. The precerebellar neuroepithelium of the rhombencephalon. *J. Comp. Neurol.* 4, 477–489. doi: 10.1002/cne.902570402

Altman, J., and Bayer, S. A. (1997). *Development of the Cerebellar System in Relation to Its Evolution, Structure and Functions.* Boca Raton, FL: CRC Press.

Alvarez-Morujo, A. J., Toranzo, D., Blázquez, J. L., Peláez, B., Sánchez, A., Pastor, F. E., et al. (1992). The ependymal surface of the fourth ventricle of the rat: a combined scanning and transmission electron microscopic study. *Histol. Histopathol.* 7, 259–266.

Bagnall, M. W., Zingg, B., Sakatos, A., Moghadam, S. H., Zeilhofer, H. U., and du Lac, S. (2009). Glycinergic projection neurons of the cerebellum. *J. Neurosci.* 29, 10104–10110. doi: 10.1523/JNEUROSCI.2087-09.2009

Becerra-González, M., Varman Durairaj, R., Ostos Valverde, A., Gualda, E. J., Loza-Alvarez, P., Portillo Martínez, W., et al. (2020). Response to hypoxic preconditioning of glial cells from the roof of the fourth ventricle. *Neuroscience* 439, 211–229. doi: 10.1016/j.neuroscience.2019.09.015

Bellamy, T. C. (2006). Interactions between Purkinje neurons and Bergmann glia. *Cerebellum* 5, 116–126. doi: 10.1080/14734220600724569

Berry, M., Ibrahim, M., Carlile, J., Ruge, F., Duncan, A., and Butt, A. M. (1995). Axon-glial relationships in the anterior medullary velum of the adult rat. *J. Neurocytol.* 24, 965–983. doi: 10.1007/BF01215646

Bouslama-Oueghlani, L., Wehrlé, R., Sotelo, C., and Dusart, I. (2005). Heterogeneity of NG2-expressing cells in the newborn mouse cerebellum. *Dev. Biol.* 2, 409–421. doi: 10.1016/j.ydbio.2005.07.003

Brazis, P. W. (2014). "Trochlear nerve (cranial nerve IV)," in *Neuroscience and Biobehavioral Psychology, 2nd Edn*, eds M. J. Aminoff and R. B. Daroff (London: Academic Press Elsevier), 534–535.

Bruni, J. E., and Anderson, W. A. (1987). Ependyma of the rat fourth ventricle and central canal: response to injury. *Acta Anat.* 128, 265–273. doi: 10.1159/000146352

Buffo, A., and Rossi, F. (2013). Origin, lineage and function of the cerebellar glia. *Prog. Neurobiol.* 109, 42–63. doi: 10.1016/j.pneurobio.2013.08.001

Butt, A. M., Kiff, J., Hubbard, P., and Berry, M. (2002). Synantocytes: new functions for novel NG2 expressing glia. *J. Neurocytol.* 31, 551–565. doi: 10.1023/A:1025751900356

Cerrato, V., Parmigiani, E., Figueres-Oñate, M., Betizeau, M., Aprato, J., Nanavaty, I., et al. (2018). Multiple origins and modularity in the spatiotemporal emergence of cerebellar astrocyte heterogeneity. *PLoS Biol.* 16, 2005513. doi: 10.1371/journal.pbio.2005513

Cheng, A. H., and Cheng, H. M. (2021). Genesis of the master circadian pacemaker in mice. *Front. Neurosci.* 15, 659974. doi: 10.3389/fnins.2021.659974

Chung, S. H., Guo, F., Jiang, P., Pleasure, D. E., and Deng, W. (2013). Olig2/Plp-positive progenitor cells give rise to Bergmann glia in the cerebellum. *Cell Death Dis.* 4, e546. doi: 10.1038/cddis.2013.74

Cipolla, M. J. (2009). *The Cerebral Circulation. 1st Edn*. San Rafael, CA: Morgan and Claypool Life Sciences, 70.

Clarke, L. E., Young, K. M., Hamilton, N. B., Li, H., Richardson, W. D., and Attwell, D. (2012). Properties and fate of oligodendrocyte progenitor cells in the corpus callosum, motor cortex, and piriform cortex of the mouse. *J. Neurosci.* 32, 8173–8185. doi: 10.1523/JNEUROSCI.0928-12.2012

Cserr, H. F. (1971). Physiology of the choroid plexus. *Physiol. Rev.* 51, 273–311. doi: 10.1152/physrev.1971.51.2.273

Daneman, R., and Prat, A. (2015). The blood-brain barrier. *Cold Spring Harbor Perspect. Biol.* 7, a020412. doi: 10.1101/cshperspect.a020412

Dávalos, D.,Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., et al. (2005). ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat. Neurosci.* 6, 752–758. doi: 10.1038/nn1472

Dawson, M. R., Polito, A., Levine, J. M., and Reynolds, R. (2003). NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. *Mol. Cell. Neurosci.* 24, 476–488. doi: 10.1016/S1044-7431(03)00210-0

Del Rio, P. (1932). "Microglia," in *Cytology and Cellular Pathology of the Nervous System*, eds W. Penfield (New York, NY: Hoeber), 481–584.

Demirjian, C., Grossman, R., Meyer, R., and Katzman, R. (1976). The catecholamine pontine cellular group locus coeruleus A4, subcoeruleus in the primate. *Cebus Spella Brain Res.* 115, 395–411. doi: 10.1016/0006-8993(76)90357-7

Dimou, L., and Gallo, V. (2015). NG2-glia and their functions in the central nervous system. *Glia*. 63, 1429–1451. doi: 10.1002/glia.22859

Edwards, M. A., Yamamoto, M., and Caviness, V. S. (1990). Organization of radial glia and related cells in the developing murine CNS. An analysis based upon a new monoclonal antibody marker. *Neuroscience.* 36, 121–144. doi: 10.1016/0306-4522(90)90356-9

Farmer, W. T., and Murai, K. (2017). Resolving astrocyte heterogeneity in the CNS. *Front. Cell Neurosci.* 11, 300. doi: 10.3389/fncel.2017.00300

Fogwe, D. T., and Mesfin, F. B. (2018). "Neuroanatomy, anterior inferior cerebellar arteries," in *StatPearls* (Treasure Island, FL: StatPearls Publishing).

García-Marín, V., García-López, P., and Freire, M. (2007). Cajal's contributions to glia research. *Trends Neurosci.* 30, 479–487. doi: 10.1016/j.tins.2007.06.008

Gómez-González, G. B., and Martínez-Torres, A. (2021). Inter-fastigial projections along the roof of the fourth ventricle. *Brain Struct. Funct.* 226, 901–917. doi: 10.1007/s00429-021-02217-8

Gómez-Nicola, D., and Perry, V. H. (2015). Microglial dynamics and role in the healthy and diseased brain: a paradigm of functional plasticity. *Neurosci. Rev. J. Bring. Neurobiol. Neurol. Psychiatry* 21, 169–184. doi: 10.1177/1073858414530512

González-González, M. A., Gómez-González, G. B., Becerra-González, M., and Martínez-Torres, A. (2017). Identification of novel cellular clusters define a specialized area in the cerebellar periventricular zone. *Sci. Rep.* 7, 40768. doi: 10.1038/srep40768

Grzanna, R., and Mottiver, M. E. (1980). The locus coeruleus in the rat: an immunohistochemical delineation. *Neurosci.* 5, l—40. doi: 10.1016/0306-4522(80)90068-8

Hanisch, U. K., and Kettenmann, H. (2007). Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat. Neurosci.* 11, 1387–1394. doi: 10.1038/nn1997

Hoffmann, C. J., Harms, U., Rex, A., Szulzewsky, F., Wolf, S. A., Grittner, U., et al. (2015). Vascular signal transducer and activator of transcription-3 promotes angiogenesis and neuroplasticity long-term after stroke. *Circulation* 131, 1772–1782. doi: 10.1161/CIRCULATIONAHA.114.013003

Holash, J. A., Sugamori, K., and Stewart, P. A. (1990). The difference in vascular volume between cerebrum and cerebellum is in the pia mater. *J. Cereb. Blood Flow Metab.* 10, 432–434. doi: 10.1038/jcbfm.1990.75

Huang, W., Bai, X., Stopper, L., Catalin, B., Cartarozzi, L. P., Scheller, A., et al. (2018). During development NG2 glial cells of the spinal cord are restricted to the oligodendrocyte lineage, but generate astrocytes upon acute injury. *Neuroscience* 385, 154–165. doi: 10.1016/j.neuroscience.2018.06.015

Hughes, A. L., Pakhomova, A., and Brown, P. D. (2010). Regulatory volume increase in epithelial cells isolated from the mouse fourth ventricle choroid plexus involves Na(+)-H(+) exchange but not Na(+)-K(+)-2Cl(-) cotransport. *Brain Res.* 1323, 1–10. doi: 10.1016/j.brainres.2009.12.094

Ide, C., Kitada, M., Chakrabortty, S., Taketomi, M., Matsumoto, N., Kikukawa, S., et al. (2001). Grafting of choroid plexus ependymal cells promotes the growth of regenerating axons in the dorsal funiculus of rat spinal cord: a preliminary report. *Exp. Neurol.* 167, 242–251. doi: 10.1006/exnr.2000.7566

Itokazu, Y., Kitada, M., Dezawa, M., Mizoguchi, A., Matsumoto, N., Shimizu, A., et al. (2006). Choroid plexus ependymal cells host neural progenitor cells in the rat. *Glia*. 53, 32–42. doi: 10.1002/glia.20255

Javed, K., Reddy, V., and Lui, F. (2022). "Neuroanatomy, choroid plexus," in *StatPearls* (Treasure Island, FL: StatPearls Publishing). Available online at: https://www.ncbi.nlm.nih.gov/books/NBK538156/ (accessed May 23, 2022).

Kanekiyo, K., Nakano, N., Noda, T., Yamada, Y., Suzuki, Y., Ohta, M., et al. (2016). Transplantation of choroid plexus epithelial cells into contusion-injured spinal cord of rats.Restor Neurol *Neurosci.* 34, 347–366. doi: 10.3233/RNN-150546

Keep, R. F., and Jones, H. C. (1990). A morphometric study on the development of the lateral ventricle choroid plexus, choroid plexus capillaries and ventricular ependyma in the rat. *Brain Res. Dev. Brain Res.* 56, 47–53. doi: 10.1016/0165-3806(90)90163-S

Keep, R. F., Jones, H. C., and Cawkwell, R. D. (1986). A morphometric analysis of the development of the fourth ventricle choroid plexus in the rat. *Brain Res.* 92, 77–85. doi: 10.1016/0165-3806(86)90234-8

Kettenmann, H., and Ransom, B. R. (2005). *Neuroglia. 2nd Edn*. New York, NY: Oxford.

Kibble, J.D., Trezise, A.E., Brown, P.D. (1996). Properties of the cAMP-activated C1- current in choroid plexus epithelial cells isolated from the rat. *J. Physiol.* 496, 69–80. doi: 10.1113/jphysiol.1996.sp021666

Kitada, M., Chakrabortty, S., Matsumoto, N., Taketomi, M., and Ide, C. (2001). Differentiation of choroid plexus ependymal cells into astrocytes after grafting into the pre-lesioned spinal cord in mice. *Glia.* 36, 364–374. doi: 10.1002/glia.1123

Kiyoshi, C. M., Du, Y., Zhong, S., Wang, W., Taylor, A. T., Xiong, B., et al. (2018). Syncytial isopotentiality: a system-wide electrical feature of astrocytic networks in the brain. *Glia*. 66, 2756–2769. doi: 10.1002/glia.23525

Krizhanovsky, V., and Ben-Arie, N. (2006). A novel role for the choroid plexus in BMP-mediated inhibition of differentiation of cerebellar neural progenitors. *Mech. Dev.* 123, 67–75. doi: 10.1016/j.mod.2005.09.005

Kulik, T., Kusano, Y., Aronhime, S., Sandler, A. L., and Winn, H. R. (2008). Regulation of cerebral vasculature in normal and ischemic brain. *Neuropharmacology* 55, 281–288. doi: 10.1016/j.neuropharm.2008.04.017

Labrada-Moncada, F. E., Martínez-Torres, A., and Reyes-Haro, D. (2020). GABAA receptors are selectively expressed in NG2 glia of the cerebellar white matter. *Neuroscience* 433, 132–143. doi: 10.1016/j.neuroscience.2020.03.003

Landis, D. M., and Reese, T. S. (1982). Regional organization of astrocytic membranes in cerebellar cortex. *Neuroscience* 7, 937–950. doi: 10.1016/0306-4522(82)90053-7

Lawson, L. J., Perry, V. H., Dri, P., and Gordon, S. (1990). Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* 39, 151–170. doi: 10.1016/0306-4522(90)90229-W

Leonhardt, H., Krisch, B., and Erhardt, H. (1987). "Organization of the neuroglia in the midsagittal plane of the central nervous system: a speculative report," in *Functional Morphology of Neuroendocrine Systems*, eds B. Scharrer, H. W. Korf, H. G. Hartwig (Berlin; Heidelberg: Springer).

Levine, J. M., and Card, J. P. (1987). Light and electron microscopic localization of a cell surface antigen (NG2) in the rat cerebellum:

association with smooth protoplasmic astrocytes. *J. Neurosci.* 7, 2711–2720. doi: 10.1523/JNEUROSCI.07-09-02711.1987

Levine, S., and Saltzman, A. (2003). Choroidal bodies: a new structure in the fourth ventricular choroid plexus of the rat and mouse. *Brain Res.* 981, 210–212. doi: 10.1016/S0006-8993(03)02891-9

Li, S., Zhang, Y., Shao, G., Yang, M., Niu, J., Lv, G., et al. (2013). Hypoxic preconditioning stimulates angiogenesis in ischemic penumbra after acute cerebral infarction. *Neural. Regen. Res.* 8, 2895–2903. doi: 10.3969/j.issn.1673-5374.2013.31.002

Lin, S. C., Huck, J. H., Roberts, J. D., Macklin, W. B., Somogyi, P., and Bergles, D. E. (2005). Climbing fiber innervation of NG2-expressing glia in the mammalian cerebellum. *Neuron* 5, 773–785. doi: 10.1016/j.neuron.2005.04.025

Lippman, J., Lordkipanidze, T., Buell, M. E., Yoon, S. O., and Dunaevsky, A. (2008). Morphogenesis and regulation of Bergmann glial processes during Purkinje cell dendritic spine ensheathment and synaptogenesis. *Glía* 56, 1463–1477. doi: 10.1002/glia.20712

Lippman, J., Lordkipanidze, T., Cobb, N., and Dunaevsky, A. (2010). Bergmann glial ensheathment of dendritic spines regulates synapse number without affecting spine motility. *Neuron Glia Biol.* 6, 193–200. doi: 10.1017/S1740925X10000165

Lomoio, S., Necchi, D., and Scherini, E. (2012). Uneven distribution of NG2 cells in the rat cerebellar vermis and changes in aging. *Eur. J. Histochem.* 56, 27. doi: 10.4081/ejh.2012.e27

Louvi, A., Alexandre, P., Métin, C., Wurst, W., and Wassef, M. (2003). The isthmic neuroepithelium is essential for cerebellar midline fusion. *Development* 130, 5319–5330. doi: 10.1242/dev.00736

Lu, Q., and Kim, J. Y. (2021). Mammalian circadian networks mediated by the suprachiasmatic nucleus. *FEBS J*. doi: 10.1111/febs.16233. [Epub ahead of print].

Lun, M. P., Johnson, M. B., Broadbelt, K. G., Watanabe, M., Kang, Y. J., Chau, K. F., et al. (2015). Spatially heterogeneous choroid plexus transcriptomes encode positional identity and contribute to regional CSF production. *J. Neurosci.* 35, 4903–4916. doi: 10.1523/JNEUROSCI.3081-14.2015

Marcet, B., Chevalier, B., Luxardi, G., Coraux, C., Zaragosi, L. E., Cibois, M., et al. (2011). Control of vertebrate multiciliogenesis by miR-449 through direct repression of the Delta/Notch pathway. *Nat. Cell Biol.* 13, 693–699. doi: 10.1038/ncb2241

Mathis, L., and Nicolas, J. F. (2003). Progressive restriction of cell fates in relation to neuroepithelial cell mingling in the mouse cerebellum. *Dev. Biol.* 258, 20–31. doi: 10.1016/S0012-1606(03)00098-8

Matsumoto, N., Taguchi, A., Kitayama, H., Watanabe, Y., Ohta, M., Yoshihara, T., et al. (2010). Transplantation of cultured choroid plexus epithelial cells *via* cerebrospinal fluid shows prominent neuroprotective effects against acute ischemic brain injury in the rat. *Neurosci. Lett.* 469, 283–288. doi: 10.1016/j.neulet.2009.09.060

McConnell, P., Berry, M., Rees, E. L., and Sievers, J. (1984). The injury response of nerve fibers in the anterior medullary velum of the adult rat. *Brain Res.* 323, 257–268. doi: 10.1016/0006-8993(84)90296-8

Miale, I. L., and Sidman, R. L. (1961). An autoradiographic analysis of histogénesis in the mouse cerebellum. *Exp. Neurol.* 4, 277–296. doi: 10.1016/0014-4886(61)90055-3

Mirzadeh, Z., Kusne, Y., Duran-Moreno, M., Cabrales, E., Gil-Perotin, S., Ortiz, C., et al. (2017). Bi- and uniciliated ependymal cells define continuous floor-plate-derived tanycytic territories. *Nat. Commun.* 8, 13759. doi: 10.1038/ncomms13759

Mittelbronn, M., Dietz, K., Schluesener, H. J., and Meyermann, R. (2001). Local distribution of microglia in the normal adult human central nervous system differs by up to one order of magnitude. *Acta Neuropathol.* 3, 249–255. doi: 10.1007/s004010000284

Myung, J., Schmal, S., Hong, S., Tsukizawa, Y., Rose, P., Zhang, Y., et al. (2018). The choroid plexus is an important circadian clock component. *Nat. Commun.* 9, 1062. doi: 10.1038/s41467-018-03507-2

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 308, 1314–1318. doi: 10.1126/science.1110647

Nishiyama, A., Serwanski, D. R., and Pfeiffer, F. (2021). Many roles for oligodendrocyte precursor cells in physiology and pathology. *Neuropathology* 41, 161–173. doi: 10.1111/neup.12732

Nishiyama, A., Suzuki, R., and Zhu, X. (2014). NG2 cells (polydendrocytes) in brain physiology and repair. *Front. Neurosci.* 8, 133. doi: 10.3389/fnins.2014.00133

Nishiyama, A., Watanabe, M., Yang, Z., and Bu, J. (2002). Identity, distribution and development of polydendrocytes: NG2-expressing glial cells. *J. Neurocytol.* 31, 437–455. doi: 10.1023/A:1025783412651

Nisimaru, N., and Kawaguchi, Y. (1984). Excitatory effects on renal sympathetic nerve activity induced by stimulation at two distinctive sites in the fastigial nucleus of rabbits. *Brain Res.* 304, 372–376. doi: 10.1016/0006-8993(84)90342-1

Nolte, C., Matyash, M., Pivneva, T., Schipke, C. G., Ohlemeyer, C., Hanisch, U. K., et al. (2001). GFAP promoter-controlled EGFP-expressing transgenic mice: a tool to visualize astrocytes and astrogliosis in living brain tissue. *Glia*. 33, 72–86. doi: 10.1002/1098-1136(20010101)33:1<72::aid-glia1007>3.0.co;2-a

Oda, Y., and Nakanishi, I. (1987). Ultrastructure of the caudal portion of the fourth ventricular roof in the mouse. *J. Comp. Neurol.* 256, 299–307. doi: 10.1002/cne.902560209

Palay, S., and Chan-Palay, V. (1974). *Cerebellar Cortex*. Berlin; Heidelberg; New York, NY: Springer-Verlag, 75–77.

Pascual, O., Achour,. S., Rostaing, P., Triller, A., and Bessis, A. (2012). Microglia activation triggers astrocyte-mediated modula- tion of excitatory neurotransmission. *Proc. Natl. Acad. Sci. U. S. A.* 4, E197–E205. doi: 10.1073/pnas.1111098109

Pétriz, A., Reyes-Haro, D., González-González, M. A., Miledi, R., and Martínez-Torres, A. (2014). GABAρ subunits confer a bicuculline-insensitive component to GFAP^{+} cells of cerebellum. *Proc. Natl. Acad. Sci.* 111, 17522–17527. doi: 10.1073/pnas.1419632111

Prinz, M., and Mildner, A. (2011). Microglia in the CNS: immigrants from another world. *Glia* 59, 177–187. doi: 10.1002/glia.21104

Prinz, M., and Priller, J. (2014). Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. *Nat. Rev. Neurosci.* 15, 300–312. doi: 10.1038/nrn3722

Prinz, M., and Priller, J. (2017). The role of peripheral immune cells in the CNS in steady state and disease. *Nat. Neurosci.* 2, 136–144. doi: 10.1038/nn.4475

Quintela, T., Albuquerque, T., Lundkvist, G., Belin, A. C., Talhada, D., Gonçalves, I., et al. (2018). The choroid plexus harbors a circadian oscillator modulated by estrogens. *Chronobiol. Int.* 35, 270–279. doi: 10.1080/07420528.2017.1400978

Quintela, T., Furtado, A., Duarte, A. C., Gonçalves, I., Myung, J., and Santos, C. R. A. (2021). The role of circadian rhythm in choroid plexus functions. *Prog. Neurobiol.* 205, 102129. doi: 10.1016/j.pneurobio.2021.102129

Quintela, T., Gonçalves, I., Carreto, L. C., Santos, M. A., Marcelino, H., Patriarca, F. M., et al. (2013). Analysis of the effects of sex hormone background on the rat choroid plexus transcriptome by cDNA. *Microarrays.* 8, e60199. doi: 10.1371/journal.pone.0060199

Rakic, P. (1972). Mode of cell migration to the superficial layers of fetal monkey neocortex. *J. Comp. Neurol.* 145, 61–84. doi: 10.1002/cne.901450105

Rakic, P. (2003). Elusive radial glial cells: historical and evolutionary perspective. *Glia* 43, 19–32. doi: 10.1002/glia.10244

Ramóny Cajal, S. (1916). El proceder del oro-sublimado para la coloración de la neuroglia. *Trab. Lab. Invest. Biol. Univ. Madrid* 14, 155–162.

Redshaw, N., Wheeler, G., Hajihosseini, M. K., and Dalmay, T. (2009). microRNA-449 is a putative regulator of choroid plexus development and function. *Brain Res.* 23, 20–26. doi: 10.1016/j.brainres.2008.11.020

Reyes-Haro, D., González-González, M. A., Pétriz, A., Rosas-Arellano, A., Kettenmann, H., Miledi, R., et al. (2013). γ-Aminobutyric acid-ρ expression in ependymal glial cells of the mouse cerebellum. *J. Neurosci. Res.* 91, 527–534. doi: 10.1002/jnr.23183

Rivers, L. E., Young, K. M., Rizzi, M., Jamen, F., Psachoulia, K., Wade, A., et al. (2008). PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. *Nat. Neurosci.* 11, 1392–1401. doi: 10.1038/nn.2220

Robinson, F. R., Straube, A., and Fuchs, A. F. (1993). Role of the caudal fastigial nucleus in saccade generation II. Effects of muscimol inactivation. *J. Neurophysiol.* 70, 1741–1758. doi: 10.1152/jn.1993.70.5.1741

Rodríguez-Arzate, C. A., Martínez-Mendoza, M. L., Rocha-Mendoza, I., Luna-Palacios, Y., Licea-Rodríguez, J., and Martínez-Torres, A. (2021). Morphological and calcium signaling alterations of neuroglial cells in cerebellar cortical dysplasia induced by carmustine. *Cells* 10, 1581. doi: 10.3390/cells10071581

Saab, A. S., Neumeyer, A., Jahn, H. M., Cupido, A., Simek, A. A., Boele, H. J., et al. (2012). Bergmann glial AMPA receptors are required for fine motor coordination. *Science* 337, 749–753. doi: 10.1126/science.1221140

Sánchez-González, R., Salvador, N., and López-Mascaraque, L. (2020). Unraveling the adult cell progeny of early postnatal progenitor cells. *Sci. Rep.* 10, 19058. doi: 10.1038/s41598-020-75973-y

Sellers, D. L., Maris, D. O., and Horner, P. J. (2009). Postinjury niches induce temporal shifts in progenitor fates to direct lesion repair after spinal cord injury. *J. Neurosci.* 29, 6722–6733. doi: 10.1523/JNEUROSCI.4538-08.2009

Sherafat, A., Pfeiffer, F., Reiss, A. M., Wood, W. M., and Nishiyama, A. (2021). Microglial neuropilin-1 promotes oligodendrocyte expansion during development and remyelination by trans-activating platelet-derived growth factor receptor. *Nat. Commun.* 12, 2265. doi: 10.1038/s41467-021-22532-2

Sliney, I., Berry, M., Sievers, J., and Mangold, U. (1981). CNS axons in the anterior medullary velum of the rat. *J. Anat.* 133, 656.

Spassky, N., Merkle, F. T., Flames, N., Tramontin, A. D., García-Verdugo, J. M., and Alvarez-Buylla, A. (2005). Adult ependymal cells are postmitotic and are derived from radial glial cells during embryogenesis. *J. Neurosci.* 25, 10–18. doi: 10.1523/JNEUROSCI.1108-04.2005

Speake, T., and Brown, P. D. (2004). Ion channels in epithelial cells of the choroid plexus isolated from the lateral ventricle of rat brain. *Brain Res.* 1005, 60–66. doi: 10.1016/j.brainres.2004.01.034

Stowell, D., Wong, E. L., Batchelor, H., Mendes, M., Lamantia, C., Whitelaw, B., et al. (2017). Cerebellar microglia are dynamically unique and survey Purkinje Neurons *In Vivo*. *Dev. Neurobiol.* 78, 627–644. doi: 10.1002/dneu.22572

Takahashi, M., Sugiuchi, Y., and Shinoda, Y. (2014). Convergent synaptic inputs from the caudal fastigial nucleus and the superior colliculus onto pontine and pontomedullary reticulospinal neurons. *J. Neurophysiol.* 111, 849–867. doi: 10.1152/jn.00634.2013

Tata, M., Ruhrberg, C., and Fantin, A. (2015). Vascularisation of the central nervous system. *Mech. Dev.* 138, 26–36. doi: 10.1016/j.mod.2015.07.001

Tay, T. L., Mai, D., Dautzenberg, J., Fernández-Klett, F., Lin, G., Sagar, D. M., et al. (2017). A new fate mapping system reveals context-dependent random or clonal expansion of microglia. *Nat. Neurosci.* 20, 793–803. doi: 10.1038/nn.4547

Telano, L. N., and Baker, S. (2021). "Physiology, cerebral spinal fluid," in *StatPearls* (Treasure Island, FL: StatPearls Publishing). Available online at: https://www.ncbi.nlm.nih.gov/books/NBK519007/ (accessed May 23, 2022).

Trotter, J., Karram, K., and Nishiyama, A. (2010). NG2 cells: properties, progeny and origin. *Brain Res. Rev.* 63, 72–82. doi: 10.1016/j.brainresrev.2009.12.006

Uusisaari, M., Obata, K., and Knöpfel, T. (2007). Morphological and electrophysiological properties of GABAergic and non-GABAergic cells in the deep cerebellar nuclei. *J. Neurophysiol.* 97, 901–911. doi: 10.1152/jn.00974.2006

Verkhratsky, A., and Butt, A. (2007). *Glial Neurobiology: A Textbook*. England: Wiley, 21–25.

Vígh, B., Manzano e Silva, M. J., Frank, C. L., Vincze, C., Czirok, S. J., Szabó, A., et al. (2004). The system of cerebrospinal fluid-contacting neurons. Its supposed role in the nonsynaptic signal transmission of the brain. *Histol. Histopathol.* 19, 607–628. doi: 10.14670/HH-19.607

Vigh-Teichmann, I., and Vigh, B. (1983). The system of cerebrospinal fluid-contacting neurons. *Arch. Histol. Jpn.* 46, 427–468. doi: 10.1679/aohc.46.427

Voogd, J. (1967). comparative aspects of the structure and fibre connexions of the mammalian cerebellum. *Prog. Brain Res.* 25, 94–134. doi: 10.1016/S0079-6123(08)60963-2

Voogd, J., and Glickstein, M. (1998). The anatomy of the cerebellum. *Trends Neurosci.* 21, 370–375. doi: 10.1016/S0166-2236(98)01318-6

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., and Nabekura, J. (2009). Resting microglia directly monitor the functional state of synapses *in vivo* and determine the fate of ischemic terminals. *J. Neurosci.* 13, 3974–3980. doi: 10.1523/JNEUROSCI.4363-08.2009

Walberg, F., Pompeiano, O., Brodal, A., and Jansen, J. (1962). The fastigio-vestibular projection in the cat. An experimental study with silver impregnation methods. *J. Comp. Neurol.* 8, 49–76. doi: 10.1002/cne.901180105

Wang, G., Zhang, J., Hu, X., Zhang, L., Mao, L., Jiang, X., et al. (2013). Microglia/macrophage polarization dynamics in white matter after traumatic brain injury. *J. Cereb. Blood Flow Metab.* 33, 1864–1874. doi: 10.1038/jcbfm.2013.146

Watanabe, M., Toyama, Y., and Nishiyama, A. (2002). Differentiation of proliferated NG2-positive glial progenitor cells in a remyelinating lesion. *J. Neurosci. Res.* 69, 826–836. doi: 10.1002/jnr.10338

Welle, A., Kasakow, C. V., Jungmann, A. M., Gobbo, D., Stopper, L., Nordström, K., et al. (2021). Epigenetic control of region-specific transcriptional programs in mouse cerebellar and cortical astrocytes. *Glia.* 9, 2160–2177. doi: 10.1002/glia.24016

Yamada, K., Fukaya, M., Shibata, T., Kurihara, H., Tanaka, K., Inocue, Y., et al. (2000). Dynamic transformation of Bergmann glial fibers proceeds in correlation with dendritic outgrowth and synapse formation of cerebellar Purkinje cells. *J. Comp. Neurol.* 418, 106–120. doi: 10.1002/(SICI)1096-9861(20000228)418:1<106::AID-CNE8>3.0.CO;2-N

Yamaguchi, T., Hamada, T., Matsuzaki, T., and Iijima, N. (2020). Characterization of the circadian oscillator in the choroid plexus of rats. *Biochem. Biophys. Res. Commun.* 524, 497–501. doi: 10.1016/j.bbrc.2020.01.125

Yamamoto, M., Kim, M., Imai, H., Itakura, Y., and Ohtsuki, G. (2019). Microglia-triggered plasticity of intrinsic excitability modulates psychomotor behaviors in acute cerebellar inflammation. *Cell Rep.* 28, 2923–2938.e8. doi: 10.1016/j.celrep.2019.07.078

Zawadzka, M., Rivers, L. E., Fancy, S. P., Zhao, C., Tripathi, R., Jamen, F., et al. (2010). CNS-resident glial progenitor/stem cells produce Schwann cells as well as oligodendrocytes during repair of CNS demyelination. *Cell Stem Cell* 6, 578–590. doi: 10.1016/j.stem.2010.04.002

Zeisel, A., Hochgerner, H., Lönnerberg, P., Johnsson, A., Memic, F., van der Zwan, J., et al. (2018). Molecular architecture of the mouse nervous system. *Cell* 174, 999–1014.e22. doi: 10.1016/j.cell.2018.06.021

Zhan, Y., Paolicelli, R. C., Sforazzini, F., Weinhard, L., Bolasco, G., Pagani, F., et al. (2014). Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. *Nat. Neurosci.* 17, 400–406. doi: 10.1038/nn.3641

Zhang, X., Liu, Y., Hong, X., Li, X., Meshul, C. K., Moore, C., et al. (2021). NG2 glia-derived GABA release tunes inhibitory synapses and contributes to stress-induced anxiety. *Nat. Commun.* 12, 5740. doi: 10.1038/s41467-021-25956-y

Zhu, X., Hill, R. A., and Nishiyama, A. (2008). NG2 cells generate oligodendrocytes and gray matter astrocytes in the spinal cord. *Neuron Glia Biol.* 4, 19–26. doi: 10.1017/S1740925X09000015

frontiers | Frontiers in Cellular Neuroscience

TYPE Mini Review
PUBLISHED 08 August 2022
DOI 10.3389/fncel.2022.983577

OPEN ACCESS

EDITED BY
Gertrudis Perea,
Cajal Institute (CSIC), Spain

REVIEWED BY
Artur Palasz,
Medical University of Silesia, Poland

*CORRESPONDENCE
Daniel Reyes-Haro
dharo@unam.mx

SPECIALTY SECTION
This article was submitted to Non-Neuronal Cells, a section of the journal Frontiers in Cellular Neuroscience

RECEIVED 01 July 2022
ACCEPTED 18 July 2022
PUBLISHED 08 August 2022

CITATION
Reyes-Haro D (2022) Glial cells in anorexia.
Front. Cell. Neurosci. 16:983577.
doi: 10.3389/fncel.2022.983577

Glial cells in anorexia

Daniel Reyes-Haro*

Instituto de Neurobiología, Universidad Nacional Autónoma de México - Campus Juriquilla, Querétaro, Mexico

Anorexia is a loss of appetite or an inability to eat and is often associated with eating disorders. However, animal anorexia is physiologically regulated as a part of the life cycle; for instance, during hibernation, migration or incubation. Anorexia nervosa (AN), on the other hand, is a common eating disorder among adolescent females that experience an intense fear of gaining weight due to body image distortion that results in voluntary avoidance of food intake and, thus, severe weight loss. It has been shown that the neurobiology of feeding extends beyond the hypothalamus. The prefrontal cortex (PFC) is involved in food choice and body image perception, both relevant in AN. However, little is known about the neurobiology of AN, and the lack of effective treatments justifies the use of animal models. Glial cells, the dominant population of nerve cells in the central nervous system, are key in maintaining brain homeostasis. Accordingly, recent studies suggest that glial function may be compromised by anorexia. In this review, we summarize recent findings about anorexia and glial cells.

KEYWORDS

astrocytes, microglia, eating disorders, neuroinflammation, cytokines, glutamate transporters, glutamine, prefrontal cortex

Introduction

Feeding is a basic need that helps maintain energy balance and body weight. The hypothalamus, a brain region that integrates somatosensory signals and adaptive responses (neuroendocrine, autonomic and behavioral), is involved in the homeostatic regulation of feeding. The cerebral cortex is another brain region involved in feeding. Particularly, the prefrontal cortex (PFC) integrates cognitive information about food perception (odor, flavor and texture) and updates associative learning to promote (hunger) or inhibit (satiety) food-seeking behavior. The medial prefrontal cortex (mPFC) and orbitofrontal cortex (OFC) are subregions of the PFC linked with feeding, motivation for eating, foraging (Petrovich et al., 2007; Gaykema et al., 2014; Jennings et al., 2019) and anorexia (Reyes-Ortega et al., 2020). Eating disorders include anorexia nervosa (AN), a serious mental and physical illness that involves a self-distorted body image, damaging relationships with food and an obsessive desire to lose weight by refusing to eat. Adaptive anorexia, also known as animal anorexia, is part of the life cycle of vertebrates. Some examples include hibernation (bears), migration (whales) or incubation (penguins) (Mrosovsky and Sherry, 1980). Another type of adaptive anorexia

is dehydration-induced anorexia (DIA), where reduced food intake occurs to ameliorate hyperosmolaemia. DIA reproduces weight loss despite food availability, and it is often used as a murine model to explore the neurobiology of anorexia. Studies using this model reported a pro-inflammatory environment associated with glial cells, which correlated with disrupted glutamate-glutamine homeostasis and neurodegeneration in the PFC of young female rats (Reyes-Ortega et al., 2022).

Anorexia

Appetite is regulated by neural ensembles involved in homeostatic and hedonic aspects of feeding. The neurobiology of appetite is clinically relevant for eating disorders like AN. However, the term anorexia describes any loss of appetite with a concomitant reduction in food intake that occurs in the presence of readily accessible food sources. Adaptive anorexia occurs regularly at specific stages of the life cycle of animals, whereas pathological anorexia comprises AN and cachexia. Thus, neural ensembles operating in adaptive anorexia or AN may converge in a common set of synaptic circuits controlling feeding behavior.

Adaptive anorexia

Animals can adapt to predictable periods when food access is limited in nature and food intake is reduced, which often results in weight loss. However, adaptive anorexia also occurs when animals engage in other important activities that compete with feeding, even when plentiful food is available. Indeed, feeding can be disrupted by hibernation, incubation, molting, migration and defense of the harem or territory (**Figure 1A**). Thus, adaptive anorexia is also known as animal anorexia and it occurs regularly, at specific stages of the life cycle (Mrosovsky and Sherry, 1980). Adaptive anorexia is also evident in physiological challenges, such as cellular dehydration by water deprivation or drinking hypertonic solution (Watts, 1999).

Anorexia nervosa

AN is a psychiatric disorder in which eating is anxiogenic and food restriction alleviates the fear of gaining weight. The onset of this mental illness occurs during adolescence and is prevalent among females (90–95%). AN is accompanied by severe weight loss, a distorted self-image and amenorrhea (**Figure 1A**). Moreover, it has the highest suicide rate of any psychiatric disorder, and full recovery is observed in only 21% of patients (Accurso et al., 2020).

Murine models of anorexia

Dehydration-induced anorexia model

Anorexia that accompanies the drinking of hypertonic saline is a critical adaptive behavioral mechanism that ameliorates hyperosmolaemia by reducing food intake (Watts and Boyle, 2010). Thus, DIA is a murine model that limits fluid loss, reproduces weight loss and reduces food intake despite its availability (**Figure 1B**). This model is different from other models of anorexia that impose food restriction. The effects of fasting over dehydration are distinguished with a pair-fed group under forced food restriction (FFR). The hypothalamic–pituitary–thyroid (HPT) axis adjusts to the negative energy balance induced by fasting, and the hypothalamic paraventricular nucleus promotes this adaptation by reducing the mRNA expression of thyrotropin-releasing hormone. Local T3, orexigenic and anorexigenic peptides regulate the expression of this hormone. The survival rate of the individual enhances as the serum content of thyrotropin and thyroid hormones decreases. This adaptation is observed in the FFR group, but HPT axis function fails to decrease and thyrotropin levels remain high in the DIA group of rats (Jaimes-Hoy et al., 2008; García-Luna et al., 2010, 2017). On the other hand, decreased leptin, estradiol, insulin, thyroid hormones and POMC expression, together with increased Neuropeptide Y and corticosterone serum levels, are common metabolic changes observed in FFR and DIA experimental groups (Jaimes-Hoy et al., 2008; García-Luna et al., 2010, 2017). A main feature of AN is voluntary avoidance of food, which is mimicked by DIA. Another important feature of the DIA model is that feeding and weight gain are rapidly promoted by restoring water availability (Watts, 1999; de Gortari et al., 2009; Reyes-Ortega et al., 2022). Thus, the simplicity of the DIA model allows researchers to study the cellular reorganization of ensembles in specific brain regions involved in eating disorders.

Activity-based anorexia model

Activity-based anorexia (ABA) is a murine model that results in weight loss. This model combines food restriction to a few hours a day (1–3 h) with free access to a running wheel, resulting in a compulsive behavior that may lead to death. Food-anticipatory activity is a typical pattern observed prior feeding. The ABA model mimics some features of AN such as weight loss promoted by hyperactivity, amenorrhea, hypoleptinemia and alterations in the hypothalamus—hypophysis—adrenal axis (reviewed by Scharner and Stengel, 2021; Frintrop et al., 2022). ABA is also known to affect hormone levels; for example, cortisol, vasopressin and ghrelin concentrations are augmented while oxytocin and leptin are reduced. Insulin sensitivity is elevated because of hypoglycemia and hypoinsulinemia, and

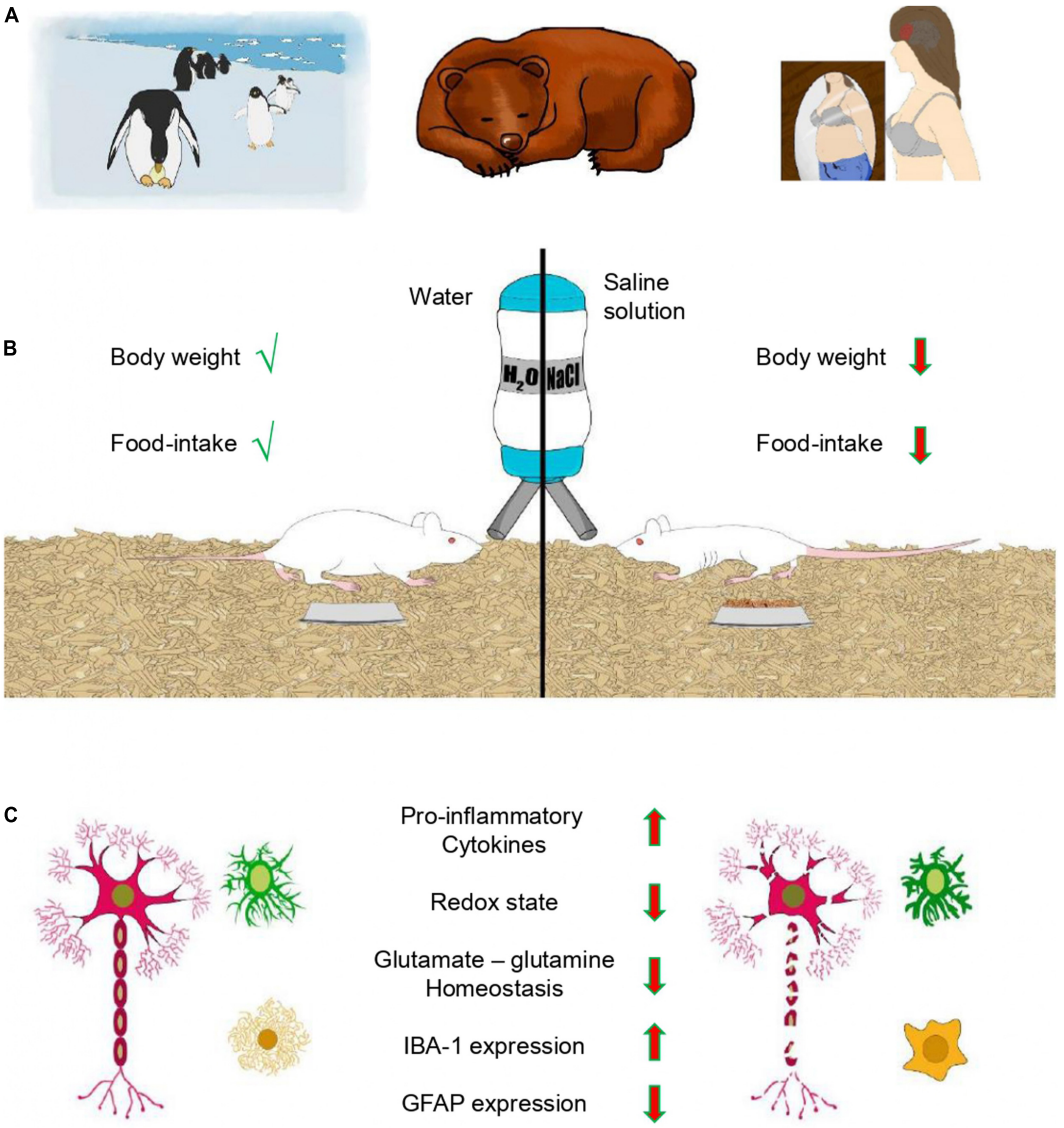


FIGURE 1
Anorexia and glial cells. **(A)** Examples of different types of anorexia. Male penguins during incubation and bears in hibernation experience adaptive anorexia (animal anorexia), which is physiologically regulated and part of the life cycle. In contrast, anorexia nervosa (AN) is pathological. **(B)** Dehydration-induced anorexia (DIA) is another example of adaptive anorexia, where animals reduce food intake in response to dehydration resulting in loss of body weight. **(C)** DIA and glial cells. Feeding provides nutrients and glial cells maintain the homeostasis of the brain. DIA induces a pro-inflammatory phenotype of glial cells with a de-ramified morphology, resulting in augmented expression of pro-inflammatory cytokines (TNFα, IL-1β, and IL-6) and the microglial marker IBA-1. These changes correlate with a decreased redox state, disrupted glutamate-glutamine homeostasis and diminished expression of the astroglial marker GFAP in the prefrontal cortex of young female rats.

the reproductive system is disturbed by hormonal changes that disrupt the estrous cycle.

Limitations of activity-based anorexia and dehydration-induced anorexia

The onset of AN is typically linked to psychosocial factors observed during adolescence, and relapses are common. In contrast, murine models of anorexia fail to reproduce these features, and caution must be taken when translating the results to human patients. However, ABA does reproduce hyperactivity commonly observed in AN, but it does not represent all cases and is not a diagnostic criterion. Physical activity is not prominent in DIA, but food restriction is not imposed, and this model mimics the voluntary avoidance of food intake observed in AN. Overall, both models share a negative energy balance as the main trigger of brain alterations. ABA and DIA may be

complementary murine models that could help to understand the neurobiology of AN.

Glial cells and feeding

Agouti-related peptide (AgRP) neurons of the arcuate nucleus (ARC) in the ventral floor of the mediobasal hypothalamus (MBH) are part of the circuit that drives feeding. Astrocytes modulate their activity through purinergic gliotransmission. The chemogenetic manipulation of astrocytes through hM3Dq DREADD activation showed both reduced (Yang et al., 2015) and augmented food intake (Chen et al., 2015) when the whole MBH or only the ARC was targeted, suggesting that modulation of AgRP neuron excitability by astrocytes is region-specific (Varela et al., 2021). Interestingly, Yang et al. (2015) observed anorexigenic effects after AgRP neuron inhibition through adenosine A1 receptors that were activated by adenosine derived from astrocytic release of ATP.

On the other hand, activation of hypothalamic microglia through Toll-like receptors (TLRs) elicited anorexia. These receptors participate in the innate immune system that recognizes pathogens and inflammatory signals. Interestingly, intracerebroventricular injection of the TLR2 ligand Pam3CSK4 resulted in microglia activation in the ARC. Microglial-TLR2 signaling diminished inhibitory GABAergic inputs and augmented excitatory glutamatergic inputs onto POMC neurons, resulting in increased POMC excitability (Jin et al., 2016).

Glial cells and leptin signaling

Leptin regulates appetite and energy balance through signaling in the hypothalamus. The lack of this anorexic adipokine or the corresponding receptors results in obesity. Neurons and glial cells of the hypothalamus are known to express leptin receptors (LepRs).

Transcytosis of adipokine leptin into the brain is mediated by tanycytes through activation of LepRs that trigger calcium waves and target protein phosphorylation of the LepR-EGFR complex. In addition, EGFR-dependent activation of ERK is required for leptin transcytosis. This mechanism was confirmed after selective deletion of LepR in tanycytes because leptin entry to the brain was blocked, and increased food intake and lipogenesis were observed, as well as glucose intolerance promoted by attenuated insulin secretion from pancreatic β-cells and an imbalance of the sympathetic nervous tone. These results suggest that leptin signaling in the CNS depends on tanycyte-mediated transcytosis (Duquenne et al., 2021). LepRs expressed by dendrites in the median eminence and oligodendrocyte precursor cells (OPCs) form a metabolic ensemble that maintains leptin and weight balance. Indeed, genetic and pharmacological ablation of OPCs abolish the anorexigenic action of leptin and induce obesity in mice (Djogo et al., 2016). Dendrites at the median eminence belong to neurons of the ARC and melanocortin neurons in this hypothalamic nucleus have astrocytic coverage that regulates neuronal excitability and feeding behavior. Thus, selective ablation of LepRs in astrocytes reduced hypothalamic astrogenesis and diminished synaptic coverage due to the retraction of astrocytic processes, resulting in diet-induced obesity (Kim et al., 2014; Rottkamp et al., 2015; Wang et al., 2015). Lack of LepRs in microglia impairs phagocytosis in the paraventricular nucleus, which contributes to the decrease of POMC neurons, diminishes α-MSH projection from the ARC to the paraventricular nucleus, and increases food intake and body weight gain in mice (Gao et al., 2017). Overall, leptin signaling in glial cells is necessary for proper energy balance.

Prefrontal cortex and anorexia

PFC activation is diminished when eating responses are inhibited in women, suggesting that this brain region may directly contribute to severe, out-of-control, maladaptive eating behaviors (Berner et al., 2022). Different fMRI studies have reported that PFC is involved in food reward and AN induces structural alterations, such as volume reduction of the mPFC (Uher et al., 2004; Mühlau et al., 2007; Brooks et al., 2011) and OFC (Lavagnino et al., 2018). The representation of taste and health attributes of food was explored in fMRI studies in the human OFC of healthy or anorexic women. The information of healthiness and tastiness was decodable from activity patterns in the OFC in both healthy and anorexic women. The representation of healthy food was stronger in anorexic women, suggesting a maladaptive over-consideration of food healthiness during deliberation about what to eat (Xue et al., 2022). The OFC is a major reward-processing hub that contains neural ensembles that respond differently to feeding or social stimuli. The activity of OFC neural ensembles, after coupling genetically encoded activity imaging with optogenetics, was monitored and manipulated at the single-cell level in real time during rewarding experiences. The results showed neural ensembles that selectively responded to either caloric rewards or social stimuli and found that optogenetic stimulation of feeding-responsive neurons was causally linked to increased feeding behavior. In contrast, optogenetic stimulation of social-responsive neurons inhibited feeding. Thus, neural ensembles within the OFC can engage to bidirectionally control feeding behavior by social stimuli (Jennings et al., 2019). On the other hand, the mPFC is known to control reward and extinction associated memories. Chemogenetic inactivation of food-reward ensembles decreased food seeking, while inactivation of the extinction ensembles promoted food-seeking behavior (Warren et al., 2016). The involvement of mPFC in hunger-

and thirst-related behaviors with hydrated food and water was examined by electrophysiological recording of extracellular activity in mice. Neuronal ensembles of the mPFC distinguished hydrated food and water with similar effectiveness in hunger and thirst, and responses were strongest for the need-appropriate outcome. Thus, the mPFC was required for evaluating action-outcome relationships to inform decision-making when need states where uncertain, under variable hunger and thirst states (Eiselt et al., 2021). Considering this information, one wonders whether cellular organization of the PFC is affected by anorexia.

Anorexia and glial cells of the prefrontal cortex

AN is linked to abnormalities of the neuroendocrine and immune systems. Leptin is a modulator of the immune response *via* microglia-induced inflammation through increased expression of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) (Lafrance et al., 2010). The mRNA expression of TNF-α and IL-6 was increased in the whole blood of eleven female patients with AN (Kahl et al., 2004). Furthermore, these cytokines (TNF-α, IL-1β and IL-6) are known to inhibit feeding (Kapás and Krueger, 1992; Fantino and Wieteska, 1993; Plata-Salamán et al., 1996). Augmented expression of them correlated with increased hippocampal-microglial density in the DIA model (Ragu-Varman et al., 2019), suggesting that cognitive brain areas involved in feeding may experience neuroinflammation associated with glial cells. This option was tested in the PFC with the DIA model, and western blot studies showed an increased expression of the microglial marker IBA-1 and the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 (**Figure 1C**). A similar result with the FFR group indicates that increased expression of IBA-1 and pro-inflammatory cytokines is due to starvation and not dehydration (Reyes-Ortega et al., 2020). This pro-inflammatory environment resulted in a selective increase of microglial density, promoted a de-ramified morphology and augmented the de-ramified/ramified ratio in the mPFC and OFC, but not in the secondary motor cortex (M2) (Reyes-Ortega et al., 2020). Double immunofluorescence experiments with Fluoro-Jade C and the neuronal marker NeuN showed selective neurodegeneration in the mPFC (−21%) and OFC (−30%), but not in M2 (−1%). In contrast, neuronal damage was practically absent in the mPFC, OFC and M2 of the control group (**Figure 1C**). Thus, DIA induces a pro-inflammatory environment that involves microglia and possibly also astrocytes. Indeed, astrocytes react to starvation with a pro-inflammatory response that correlates with the expression of pro-inflammatory genes (Kogel et al., 2021). Furthermore, DIA reduced the expression of glial fibrillary acidic protein (GFAP, a classical astrocytic marker) and the number of GFAP + cells in the corpus callosum and hippocampus (Reyes-Haro et al., 2015, 2016). Similar results were reported in the corpus callosum and cortex with the ABA model (Frintrop et al., 2018, 2019), suggesting that astrocyte density in the PFC may also be reduced by DIA. Astrocytes are part of the tripartite synapse and regulate synaptic transmission by removing neurotransmitters from the synaptic cleft and releasing gliotransmitters that modulate neuronal activity (Araque et al., 1999). Neurons receive metabolic support from astrocytes (Rouach et al., 2008), and the glutamate-glutamine cycle between them is a major metabolic pathway that requires ∼80% of the brain's total glucose consumption (Magistretti and Pellerin, 1999; Shen et al., 1999). Glutamate, the main excitatory neurotransmitter in the brain is removed from the synaptic cleft by high-affinity transporters expressed mainly in astrocytes. This mechanism ends synaptic transmission and prevents excitotoxicity (Reyes-Haro and Salceda-Sacanelles, 2000; Danbolt, 2001). Astrocytic glutamate transporters are sodium dependent, and their function is coupled to augmented metabolism (Magistretti and Pellerin, 1999). Thus, glutamate is removed from the synaptic cleft by astrocytes and rapidly amidated to glutamine by ATP-dependent glutamine synthetase. Glutamine is released through system N amino acid transport and incorporated by neurons through system A sodium-coupled amino acid transport, and converted to glutamate by mitochondrial glutaminase (Bröer and Brookes, 2001). In addition, glutamate can be oxidized for energy *via* the tricarboxylic acid (TCA) cycle and pyruvate recycling pathway (McKenna, 2013). Anorexia depletes energy reservoirs, and glutamate-glutamine catabolism may help to contend with the metabolic requirements of the brain. In support of this hypothesis, DIA reduced the redox state, as well as the endogenous levels of glutamate and glutamine in the PFC. Furthermore, DIA also diminished the expression of GFAP, glutamate transporters (GLT-1 and GLAST) and glutamine synthetase; all of them are astrocytic markers associated with glutamate-glutamine homeostasis (Reyes-Ortega et al., 2022) (**Figure 1C**). Accordingly, astrocytic density was selectively reduced and a de-ramified morphology was promoted by DIA in the mPFC and OFC but not in M2 (Reyes-Ortega et al., 2022) (**Figure 1C**). The model of DIA, a type of adaptive anorexia, shows that glial cells are central to understanding the cellular mechanisms linked to starvation in cognitive brain regions associated with feeding, such as the OFC and mPFC.

Discussion

The PFC belongs to the mesocorticolimbic reward circuit and OFC participates in the decision to eat (Gutierréz et al., 2010), while the mPFC regulates food intake (Land et al., 2014; Prado et al., 2016). Accordingly, the DIA model induces neurodegeneration associated with pro-inflammatory microglia in the mPFC and OFC (Reyes-Ortega et al., 2020). This in agreement with *postmortem* studies in girls diagnosed with

anorexia showing signs of degeneration in cortical neurons (Neumärker et al., 1997). The pro-inflammatory environment induced by DIA correlated with reduced astrocyte density (Reyes-Ortega et al., 2020, 2022). Similarly, cortical astrocyte density was diminished by ABA (Frintrop et al., 2018; Hurley et al., 2022), suggesting that astrocyte loss may be relevant for the neurobiology of anorexia. In support of this hypothesis, recent studies showed that DIA promoted de-ramification of astrocytes and reduced the expression of glutamate transporters (GLAST and GLT-1). Moreover, suppression of hyperactivity correlated with augmented expression of GLT-1, minimizing the severity of ABA (Bilash et al., 2021). Thus, reverting glial cell-mediated neuroinflammation and boosting the expression of glutamate transporters may ameliorate DIA/ABA vulnerability. Overall, we propose that glial cells should be considered as a potential therapeutic target for AN, based on the experimental evidence provided by murine models of anorexia.

Author contributions

DR-H wrote the manuscript, designed the content of the manuscript, and approved the submitted version.

Funding

This work was supported by the Consejo Nacional de Ciencia y Tecnología (CONACYT-319209) to DR-H, through the program Ciencia de Frontera Paradigmas y Controversias de la Ciencia; Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica—Universidad Nacional Autónoma de México (PAPIIT-UNAM) to DR-H (IN209121).

Acknowledgments

The author is grateful to Eduardo Demetrio Marcial Montalvo (EDMM) for illustrating the figure included in this publication. EDMM received a fellowship from Consejo Nacional de Ciencia y Tecnología (CONACYT) through grant program Ciencia de Frontera-Paradigmas y Controversias de la Ciencia (319209) to DR-H. The author acknowledges Jessica Norris for proofreading the English version of the manuscript. The author is grateful to R. Arellano, E. Garay, and A. Cisneros-Mejorado for academic and technical support.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Accurso, E. C., Sim, L., Muhlheim, L., and Levow, J. (2020). Parents know best: Caregiver perspectives on eating disorder recovery. *Eat. Disord.* 8, 1252–1260. doi: 10.1002/eat.23200

Araque, A., Parpura, V., Sanzgiri, R. P., and Haydon, P. G. (1999). Tripartite synapses: Glia, the unacknowledged partner. *Trends Neurosci.* 22, 208–215. doi: 10.1016/s0166-2236(98)01349-6

Berner, L., Winter, S., Ayaz, H., Shewokis, P., Izzetoglu, M., Marsh, R., et al. (2022). Altered prefrontal activation during the inhibition of eating responses in women with bulimia nervosa. *Psychol. Med.*[Epub ahead of print]. doi: 10.1017/S0033291722000198

Bilash, O. M., Actor-Engel, H. S., Sherpa, A. D., Chen, Y. W., and Aoki, C. (2021). Suppression of food restriction-evoked hyperactivity in activity-based anorexia animal model through glutamate transporters GLT-1 at excitatory synapses in the hippocampus. *Synapse* 75:e22197. doi: 10.1002/syn.22197

Bröer, S., and Brookes, N. (2001). Transfer of glutamine between astrocytes and neurons. *J. Neurochem.* 77, 705–719. doi: 10.1046/j.1471

Brooks, S. J., Barker, G. J., O'Daly, O. G., Brammer, M., Williams, S. C. R., Benedict, C., et al. (2011). Restraint of appetite and reduced regional brain volumes in anorexia nervosa: A voxel-based morphometric study. *BMC Psychiatry* 11:179. doi: 10.1186/1471

Chen, Y., Lin, Y. C., Kuo, T. W., and Knight, Z. A. (2015). Sensory detection of food rapidly modulates arcuate feeding circuits. *Cell* 160, 829–841. doi: 10.1016/j.cell.2015.01.033

Danbolt, N. C. (2001). Glutamate uptake. *Prog. Neurobiol.* 65, 1–105. doi: 10.1016/s0301-0082(00)00067-8

de Gortari, P., Mancera, K., Cote-Vélez, A., Amaya, M. I., Martínez, M. A., Jaimes-Hoy, L., et al. (2009). Involvement of CRH-R2 receptor in eating behavior and in the response of the HPT axis in rats subjected to dehydration-induced anorexia. *Psychoneuroendocrinology* 34, 259–272. doi: 10.1016/j.psyneuen.2008.09.010

Djogo, T., Robins, S. C., Schneider, S., Kryzskaya, D., Liu, X., Mingay, A., et al. (2016). Adult NG2-glia are required for median eminence-mediated leptin sensing and body weight control. *Cell Metab.* 23, 797–810. doi: 10.1016/j.cmet.2016.04.013

Duquenne, M., Folgueira, C., Bourouh, C., Millet, M., Silva, A., Clasadonte, J., et al. (2021). Leptin brain entry via a tanycytic LepR:EGFR shuttle controls lipid metabolism and pancreas function. *Nat. Metab.* 3, 1071–1090. doi: 10.1038/s42255-021-00432-5

Eiselt, A. K., Chen, S., Chen, J., Arnold, J., Pachitariu, M., and Sternson, S. M. (2021). Hunger or thirst state uncertainty is resolved by outcome evaluation in medial prefrontal cortex to guide decision-making. *Nat. Neurosci.* 24, 907–912. doi: 10.1038/s41593-021-00850-4

Fantino, M., and Wieteska, L. (1993). Evidence for a direct central anorectic effect of tumor-necrosis-factor-alpha in the rat. *Physiol. Behav.* 53, 477–483. doi: 10.1016/0031-9384(93)90141-2

Frintrop, L., Liesbrock, J., Paulukat, L., Johann, S., Kas, M. J., Tolba, R., et al. (2018). Reduced astrocyte density underlying brain volume reduction in activity-based anorexia rats. *World J. Biol. Psychiatry* 19, 225–235. doi: 10.1080/15622975.2016.1273552

Frintrop, L., Trinh, S., Liesbrock, J., Leunissen, C., Kempermann, J., Etdöger, S., et al. (2019). The reduction of astrocytes and brain volume loss in anorexia nervosa-the impact of starvation and refeeding in a rodent model. *Transl. Psychiatry* 9:159. doi: 10.1038/s41398-019

Frintrop, L., Trinh, S., Seitz, J., and Kipp, M. (2022). The role of glial cells in regulating feeding behavior: Potential relevance to anorexia nervosa. *J. Clin. Med.* 11:186. doi: 10.3390/jcm11010186

Gao, Y., Layritz, C., Legutko, B., Eichmann, T. O., Laperrousaz, E., Moulle, V. S., et al. (2017). Disruption of Lipid Uptake in Astroglia Exacerbates Diet-Induced Obesity. *Diabetes* 66, 2555–2563. doi: 10.2337/db16-1278

García-Luna, C., Amaya, M. I., Alvarez-Salas, E., and de Gortari, P. (2010). Prepro-orexin and feeding-related peptide receptor expression in dehydration-induced anorexia. *Regul. Pept.* 159, 54–60. doi: 10.1016/j.regpep.2009.09.011

Garcí,a-Luna, C., Soberanes-Chávez, P., and de Gortari, P. (2017). Impaired hypothalamic cocaine- and amphetamine-regulated transcript expression in lateral hypothalamic area and paraventricular nuclei of dehydration-induced anorexic rats. *J. Neuroendocrinol* 29, doi: 10.1111/jne.12541

Gaykema, R. P. A., Nguyen, X.-M. T., Boehret, J. M., Lambeth, P. S., Joy-Gaba, J., Warthen, D. M., et al. (2014). Characterization of excitatory and inhibitory neuron activation in the mouse medial prefrontal cortex following palatable food ingestion and food driven exploratory behavior. *Front. Neuroanat.* 8:60. doi: 10.3389/fnana.2014.00060

Gutierréz, R., Simon, S. A., and Nicolelis, M. A. L. (2010). Licking-induced synchrony in the taste-reward circuit improves cue discrimination during learning. *J. Neurosci.* 30, 287–303. doi: 10.1523/JNEUROSCI.0855-09.2010

Hurley, M. M., Collica, S. C., Aston, S. A., Wiles, L. J., Weiner, R. C., Biswas, A., et al. (2022). Adolescent female rats prone to the activity based anorexia (ABA) paradigm have altered hedonic responses and cortical astrocyte density compared to resistant animals. *Appetite* 168:105666. doi: 10.1016/j.appet.2021.105666

Jaimes-Hoy, L., Joseph-Bravo, P., and de Gortari, P. (2008). Differential response of TRHergic neurons of the hypothalamic paraventricular nucleus (PVN) in female animals submitted to food-restriction or dehydration-induced anorexia and cold exposure. *Horm. Behav.* 53, 366–377. doi: 10.1016/j.yhbeh.2007.11.003

Jennings, J. H., Kim, C. K., Marshel, J. H., Raffiee, M., Ye, L., Quirin, S., et al. (2019). Interacting neural ensembles in orbitofrontal cortex for social and feeding behaviour. *Nature* 565, 645–649. doi: 10.1038/s41586-018-0866-8

Jin, S., Kim, J. G., Park, J. W., Koch, M., Horvath, T. L., and Lee, B. J. (2016). Hypothalamic TLR2 triggers sickness behavior via a microglia-neuronal axis. *Sci. Rep.* 6, 1–13. doi: 10.1038/srep29424

Kahl, K. G., Kruse, N., Rieckmann, P., and Schmidt, M. H. (2004). Cytokine mRNA expression patterns in the disease course of female adolescents with anorexia nervosa. *Psychoneuroendocrinology* 29, 13–20. doi: 10.1016/S0306-4530(02)00131-2

Kapás, L., and Krueger, J. M. (1992). Tumor necrosis factor-beta induces sleep, fever, and anorexia. *Am. J. Physiol.* 263:R703–R707. doi: 10.1152/ajpregu.1992.263.3.R703

Kim, J. G., Suyama, S., Koch, M., Jin, S., Argente-Arizon, P., Argente, J., et al. (2014). Leptin signaling in astrocytes regulates hypothalamic neuronal circuits and feeding. *Nat. Neurosci.* 17, 908–910. doi: 10.1038/nn.3725

Kogel, V., Trinh, S., Gasterich, N., Beyer, C., and Seitz, J. (2021). Long-term glucose starvation induces inflammatory responses and phenotype switch in primary cortical rat astrocytes. *J. Mol. Neurosci.* 71, 2368–2382. doi: 10.1007/s12031-0210-180-02

Lafrance, V., Inoue, W., Kan, B., and Luheshi, G. N. (2010). Leptin modulates cell morphology and cytokine release in microglia. *Brain Behav. Immun.* 24, 358–365. doi: 10.1016/j.bbi.2009.11.003

Land, B. B., Narayanan, N. S., Liu, R.-J., Gianessi, C. A., Brayton, C. E., Grimaldi, D. M., et al. (2014). Medial prefrontal D1 dopamine neurons control food intake. *Nat. Neurosci.* 17, 248–253. doi: 10.1038/nn.3625

Lavagnino, L., Mwangi, B., Cao, B., Shott, M. E., Soares, J. C., and Frank, G. K. W. (2018). Cortical thickness patterns as state biomarker of anorexia nervosa. *Int. J. Eat. Disord.* 51, 241–249. doi: 10.1002/eat.22828

Magistretti, P. J., and Pellerin, L. (1999). Astrocytes couple synaptic activity to glucose utilization in the brain. *News Physiol. Sci.* 14, 177–182. doi: 10.1152/physiologyonline.1999.14.5.177

McKenna, M. C. (2013). Glutamate pays its own way in astrocytes. *Front. Endocrinol.* 4:191. doi: 10.3389/fendo.2013.00191

Mrosovsky, N., and Sherry, D. F. (1980). Animal Anorexias. *Science* 207, 837–842. doi: 10.1126/science.6928327

Mühlau, M., Gaser, C., Ilg, R., Conrad, B., Leibl, C., Cebulla, M. H., et al. (2007). Gray matter decrease of the anterior cingulate cortex in anorexia nervosa. *Am. J. Psychiatry* 164, 1850–1857. doi: 10.1176/appi.ajp.2007.06111861

Neumärker, K. J., Dudeck, U., Meyer, U., Neumärker, U., Schulz, E., and Schönheit, B. (1997). Anorexia nervosa and sudden death in childhood: Clinical data and results obtained from quantitative neurohistological investigations of cortical neurons. *Eur. Arch. Psychiatry Clin. Neurosci.* 247, 16–22. doi: 10.1007/BF02916248

Petrovich, G. D., Ross, C. A., Holland, P. C., and Gallagher, M. (2007). Medial prefrontal cortex is necessary for an appetitive contextual conditioned stimulus to promote eating in sated rats. *J. Neurosci.* 27, 6436–6441. doi: 10.1523/JNEUROSCI.5001-06.2007

Plata-Salamán, C. R., Sonti, G., Borkoski, J. P., Wilson, C. D., and French-Mullen, J. M. H. (1996). Anorexia induced by chronic central administration of cytokines at estimated pathophysiological concentrations. *Physiol. Behav.* 60, 867–875. doi: 10.1016/0031-9384(96)00148-5

Prado, L., Luis-Islas, J., Sandoval, O. I., Puron, L., Gil, M. M., Luna, A., et al. (2016). Activation of Glutamatergic Fibers in the Anterior NAc Shell Modulates Reward Activity in the aNAcSh, the Lateral Hypothalamus, and Medial Prefrontal Cortex and Transiently Stops Feeding. *J. Neurosci.* 36, 12511–12529. doi: 10.1523/JNEUROSCI.1605-16.2016

Ragu-Varman, D., Macedo-Mendoza, M., Labrada-Moncada, F. E., Reyes-Ortega, P., Morales, T., Martínez-Torres, A., et al. (2019). Anorexia increases microglial density and cytokine expression in the hippocampus of young female rats. *Behav. Brain Res.* 363, 118–125. doi: 10.1016/j.bbr.2019.01.042

Reyes-Haro, D., Labrada-Moncada, F. E., Miledi, R., and Martínez-Torres, A. (2015). Dehydration-induced anorexia reduces astrocyte density in the rat Corpus Callosum. *Neural Plast* 2015:474917. doi: 10.1155/2015/474917

Reyes-Haro, D., Labrada-Moncada, F. E., Varman, D. R., Krüger, J., Morales, T., Miledi, R., et al. (2016). Anorexia reduces GFAP+ cell density in the rat hippocampus. *Neural Plast* 2016:2426413. doi: 10.1155/ 2016/2426413

Reyes-Haro, D., and Salceda-Sacanelles, R. (2000). The high affinity glutamate transporters in the nervous system: Structure, function and physiological relevance. *Arch. Neurocienc.* 5, 96–102.

Reyes-Ortega, P., Ragu Varman, D., Rodríguez, V. M., and Reyes-Haro, D. (2020). Anorexia induces a microglial associated pro-inflammatory environment and correlates with neurodegeneration in the prefrontal cortex of young female rats. *Behav. Brain Res.* 392:112606. doi: 10.1016/j.bbr.2020.112606

Reyes-Ortega, P., Soria-Ortiz, M. B., Rodríguez, V. M., Vázquez-Martínez, E. O., Díaz-Muñoz, M., and Reyes-Haro, D. (2022). Anorexia disrupts glutamate-glutamine homeostasis associated with astroglia in the prefrontal cortex of young female rats. *Behav. Brain Res.* 420:113715. doi: 10.1016/j.bbr.2021.113715

Rottkamp, D. M., Rudenko, I. A., Maier, M. T., Roshanbin, S., Yulyaningsih, E., Perez, L., et al. (2015). Leptin potentiates astrogenesis in the developing hypothalamus. *Mol. Metab.* 4, 881–889. doi: 10.1016/j.molmet.2015.08.005

Rouach, N., Koulakoff, A., Abudara, V., Willecke, K., and Giaume, C. (2008). Astroglia metabolic networks sustain hippocampal synaptic transmission. *Science* 322, 1551–1555. doi: 10.1126/science.1164022

Scharner, S., and Stengel, A. (2021). Animal models for Anorexia Nervosa – A systematic review. *Front. Hum. Neurosci.* 14:596381. doi: 10.3389/fnhum.2020.596381

Shen, J., Petersen, K. F., Behar, K. L., Brown, P., Nixon, T. W., Mason, G. F., et al. (1999). Determination of the rate of the glutamate/glutamine cycle in the human brain by in vivo 13C NMR. *Proc. Natl. Acad. Sci. U.S.A.* 96, 8235–8240. doi: 10.1073/pnas.96.14.8235

Uher, R., Murphy, T., Brammer, M. J., Dalgleish, T., Phillips, M. L., Ng, V. W., et al. (2004). Medial prefrontal cortex activity associated with symptom provocation in eating disorders. *Am. J. Psychiatry* 161, 1238–1246. doi: 10.1176/appi.ajp.161.7.1238

Varela, L., Stutz, B., Song, J. E., Kim, J. G., Liu, Z. W., Gao, X. B., et al. (2021). Hunger-promoting AgRP neurons trigger an astrocyte-mediated feed-forward autoactivation loop in mice. *J. Clin. Invest.* 131:e144239. doi: 10.1172/JCI144239

Wang, Y., Hsuchou, H., He, Y., Kastin, A. J., and Pan, W. (2015). Role of Astrocytes in Leptin Signaling. *J. Mol. Neurosci.* 56, 829–839. doi: 10.1007/s12031-015-0518-5

Warren, B. L., Mendoza, M. P., Cruz, F. C., Leao, R. M., Caprioli, D., Rubio, F. J., et al. (2016). Distinct Fos-expressing neuronal ensembles in the ventromedial prefrontal cortex mediated food reward and extinction memories. *J. Neurosci.* 36, 6691–6703. doi: 10.1523/JNEUROSCI.0140-16.2016

Watts, A. G. (1998). Dehydration-associated anorexia: Development and rapid reversal. *Physiol. Behav.* 65, 871–878. doi: 10.1016/S0031-9384(98)00244-3

Watts, A. G., and Boyle, C. N. (2010). The functional architecture of dehydration-anorexia. *Physiol. Behav.* 100, 472–477.

Xue, A. M., Foerde, K., Walsh, B. T., Steinglass, J. E., Shohamy, D., and Bakkour, A. (2022). Neural representations of food-related attributes in the human orbitofrontal cortex during choice deliberation in anorexia nervosa. *J. Neurosci.* 42, 109–120. doi: 10.1523/JNEUROSCI.0958-21.2021

Yang, L., Qi, Y., and Yang, Y. (2015). Astrocytes control food intake by inhibiting AGRP neuron activity via adenosine A1 receptors. *Cell Rep.* 11, 798–807. doi: 10.1016/j.celrep.2015.04.002

frontiers | Frontiers in Cellular Neuroscience

TYPE Mini Review
PUBLISHED 25 August 2022
DOI 10.3389/fncel.2022.967496

OPEN ACCESS

EDITED BY
Adrian Rodriguez-Contreras,
Northwestern University, United States

REVIEWED BY
Maria Grazia Mola,
University of Bari Aldo Moro, Italy

*CORRESPONDENCE
Lenin David Ochoa-de la Paz
lochoa@unam.mx

SPECIALTY SECTION
This article was submitted to Non-Neuronal Cells, a section of the journal Frontiers in Cellular Neuroscience

RECEIVED 13 June 2022
ACCEPTED 03 August 2022
PUBLISHED 25 August 2022

CITATION
Ochoa-de la Paz LD and Gulias-Cañizo R (2022) Glia as a key factor in cell volume regulation processes of the central nervous system.
Front. Cell. Neurosci. 16:967496.
doi: 10.3389/fncel.2022.967496

Glia as a key factor in cell volume regulation processes of the central nervous system

Lenin David Ochoa-de la Paz[1,2]* and Rosario Gulias-Cañizo[3]

[1]Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico, [2]Asociación para Evitar la Ceguera en México (APEC), Unidad de Investigación APEC-UNAM, Mexico, [3]Centro de Investigación en Ciencias de la Salud, Universidad Anáhuac México, Mexico

Brain edema is a pathological condition with potentially fatal consequences, related to cerebral injuries such as ischemia, chronic renal failure, uremia, and diabetes, among others. Under these pathological states, the cell volume control processes are fully compromised, because brain cells are unable to regulate the movement of water, mainly regulated by osmotic gradients. The processes involved in cell volume regulation are homeostatic mechanisms that depend on the mobilization of osmolytes (ions, organic molecules, and polyols) in the necessary direction to counteract changes in osmolyte concentration in response to water movement. The expression and coordinated function of proteins related to the cell volume regulation process, such as water channels, ion channels, and other cotransport systems in the glial cells, and considering the glial cell proportion compared to neuronal cells, leads to consider the astroglial network the main regulatory unit for water homeostasis in the central nervous system (CNS). In the last decade, several studies highlighted the pivotal role of glia in the cell volume regulation process and water homeostasis in the brain, including the retina; any malfunction of this astroglial network generates a lack of the ability to regulate the osmotic changes and water movements and consequently exacerbates the pathological condition.

KEYWORDS

brain edema, glia, cell volume regulation, astrocytes, Müller cells

The cell volume and the regulation processes

Most cells behave like perfect osmometers when the osmotic change begins, since several mechanisms that allow the regulation of cell volume is immediately activated. Extracellular osmolarity alterations induce anisosmotic changes, as in chronic renal failure, while isosmotic changes are due to an alteration in the intracellular content of solutes, such as the mobilization of ionic gradients on both sides of the membrane, under physiological (synaptic transmission) or pathophysiological (ischemic) conditions (McManus et al., 1995).

Under physiological conditions, the cell maintains its volume because of the orchestrated operation of two membrane transport systems: (1) the Na^+-K^+ ATPase, which maintains intracellular ion concentrations by expelling Na^+ and

exchanging it for K^+, thus compensating for the osmotic gradient generated by the concentration of impermeable molecules; and (2) the selective permeability of the cell membrane to K^+, which results in an output current of this cation through ion channels coupled to Cl^- a flow which functions as an accompanying ion to counteract the asymmetric equilibrium of impermeable organic ions (Armstrong, 2003; Lang, 2007).

Mobilization of ions (K^+, Na^+, and Cl^-) and organic osmolytes (amino acids, polyalcohols) regulates cell volume. Cells respond to cell volume changes through two mechanisms: Regulatory Volume Decrease (RVD) refers to the mobilization of osmolytes towards the extracellular space in response to a volume increase, and Regulatory Volume Increase (RVI) which means an intracellular accumulation of osmolytes in response to a decrease in cell volume. Anisosmotic conditions activate both mechanisms; however, the activation of each one depends on the nature of the osmotic change (**Figure 1**).

In both cases, the process of regulating cell volume is divided into: (1) detection of volume changes through a volume sensor; (2) generation of signaling cascades in response to the activation of the volume sensor; and (3) activation and/or regulation of pathways responsible for the mobilization of osmolytes to compensate osmotic changes (Hoffmann and Pedersen, 2006).

During RVD, initially, there is an activation of transport systems responsible for mobilizing K^+ and Cl^-, amino acids, polyalcohols, and methylamines. The transport systems that participate in the mobilization of K^+ are mainly ion channels that differ in type depending on the cell type (Calloe et al., 2007; Lotshaw, 2007). Different pharmacological and biophysical data indicate a common pathway for the mobilization of Cl^- and organic osmolytes (amino acids) through volume-regulated anion channels (VRAC). However, there is evidence that suggests that the routes of mobilization of Cl^- and organic osmolytes differ depending on the nature of the osmolyte (Franco, 2003). In the case of RVI, volume compensation is mainly due to Na^+ mobilization through specific transport systems such as the Na^+/H^+ exchanger, the $Na^+/K^+/Cl^-$ co-transporter, ion channels, and the amino acid transporters dependent on Na^+ concentration (Franchi-Gazzola et al., 2006; Pedersen et al., 2006; Lang, 2007).

Cell volume and brain edema

Water transport is an essential function associated with different cellular processes in the central nervous system (CNS). At a cellular level, water transport is associated with cell volume regulation and, therefore, with control of extracellular space dimensions. Considering the physical imposition that involves the skull on the brain, the processes associated with RVD require complete control for the proper functioning of the CNS. The movement of water through the membranes of neural and glial cells affects, mainly, the intra- and extra-cellular concentration of ions, which impacts synaptic physiology (Kimelberg, 2004).

In physiopathological conditions, the movement of water in the CNS is vital given the mortality and morbidity caused by cerebral edema. Cerebral edema is classified as vasogenic edema, where the permeability of the blood-brain barrier is compromised, generating an accumulation of extracellular water; and cytotoxic or cellular edema, due to a constant entry of water into the intracellular space (Unterberg et al., 2004; **Figure 2**). A large amount of experimental evidence indicates that this phenomenon represents the final point of different neurological factors where there are structural, functional, cellular, and molecular changes in the blood-brain barrier, changes in microcirculation, and alterations in the mechanisms of cell volume regulation (Vajda et al., 2002; Manley et al., 2004).

In the brain, as in other tissues, water is mobilized through plasma membranes by aquaporins, co-transport systems coupled to the mobilization of ions and organic molecules (e.g., amino acids), and a simple diffusion mechanism that has an intrinsic very slow diffusion speed. The contribution of these mechanisms depends on factors such as density, expression level, and the capacity of water flow through aquaporins and co-transporters. However, to understand the complexity of water transport in the brain, it is necessary to consider that water movement relates directly or indirectly to neuronal activity, which generates temporal changes in the extracellular space.

Glial cells and cell volume homeostasis

In the CNS, glial cells are classified into astrocytes, oligodendrocytes, microglia, and ependymal cells. Müller cells (MC) are part of the glial component in the retina along with astrocytes and microglia. Even though all cells are perfect osmometers, glial cells and particularly astrocytes and MC play a pivotal role in the mobilization of osmolytes and water in the direction required to counteract the osmotic change. Astrocytes and MC are strategically located between the vasculature and the synaptic structure, sharing functional and neurochemical properties with neuronal cells and regulating the homeostasis of extracellular fluids through several membrane proteins responsible for the active and passive transport of ions, organic osmolytes, and osmotically obligated water (Bormann and Kettenmann, 1988; O'Neill, 1999; Simard and Nedergaard, 2004; Pasantes-Morales and Vázquez-Juárez, 2012; Reed and Blazer-Yost, 2022). In this work, we are considering the systems responsible for cell volume control points in astrocytes and MC as key players in water homeostasis in the brain and retina (Nicchia et al., 2004; Simard and Nedergaard, 2004; Bringmann et al., 2005; Kuhrt et al., 2008).

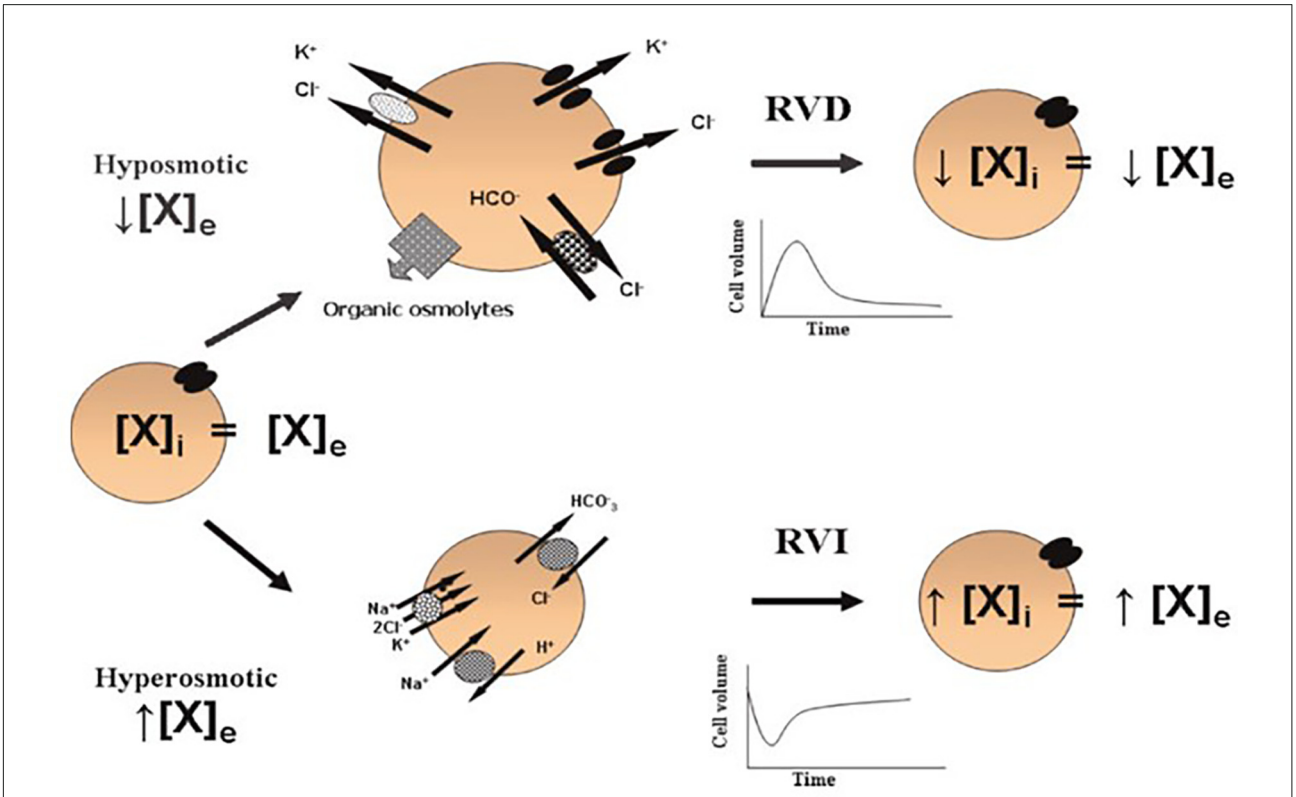


FIGURE 1
Cell volume regulation. In isosmotic conditions, the extracellular $(X)_e$ and intracellular $(X)_i$ concentrations of osmolytes are in equilibrium. Acute cell volume regulation is accomplished by two mechanisms: (1) regulatory volume decrease (RVD) mediated by the net loss of intracellular osmolytes in response to cell swelling induced by a hypoosmotic environment [a reduced $(X)_e$ compared to the $(X)_i$]; and (2) regulatory volume increase (RVI) mediated by the net accumulation of active solutes in response to cell shrinkage induced by a hyperosmotic environment [an increased $(X)_e$ compared to the $(X)_i$]. These phenomena allow the cells to partially recover their original dimensions and in this way, prevent the deleterious effects of changes in cell volume (taken from Franco, 2003).

Cell volume regulation, ion channels, and aquaporins

K^+ channels

Astrocytes form a syncytium for rapid redistribution of extracellular K^+ concentration in areas of high synaptic activity, through the high permeability they have to this cation (Sontheimer, 1992; Larsen et al., 2014). Kir 4.1 is a weak rectifying channel with the highest conductance in astrocytes, and allows bidirectional movement of K^+ depending on the transmembrane gradient of this cation; this enables and allows astroglial K^+ reuptake from the extracellular space, after high neuronal activity or ion accumulation by the Na^+-K^+ ATPase pump (Butt and Kalsi, 2006; Larsen et al., 2014). Kir 4.1 expression has been observed in the astrocytic projection that is in contact with synaptic areas, blood vessels, and the pia mater (Higashi et al., 2001). These features make these channels the main pathway to mobilize K^+ in response to a cell volume change under pathological and physiological conditions. Kir 4.1 heterodimerizes with Kir 5.1 mouse neocortex glial cells, whereas in the hippocampus Kir 4.1 is found as a homomer (Hibino et al., 2004). The relevance of these types of interactions is not yet clear. In the stratum radiatum, the presence of two-pore K^+ channels, TREK and TWIK subtypes were determined. These channels activate in a wide range of membrane potentials, which could contribute to the high conductance that astrocytes have towards K^+, increasing their capacity to mobilize this cation (Zhou et al., 2009).

A major function of MC in the retina is to regulate its ionic and osmotic balance. As with astrocytes, MC buffering of K^+ concentration occurs predominantly through Kir channels. Kir 4.1 expression has been demonstrated in the perivascular projections of MC (Kofuji et al., 2002), and Kir 2.1 has been observed "accompanying" Kir 4.1; however, the functional significance of Kir 2.1 is still unknown, even though its location would suggest participation in the control of extracellular K^+ concentration in the end-feet of MC (Kofuji et al., 2002). Skatchkov et al. (2006) determined that two-pore K^+ channels (TASK-1 and TASK-2) participate in the cell volume

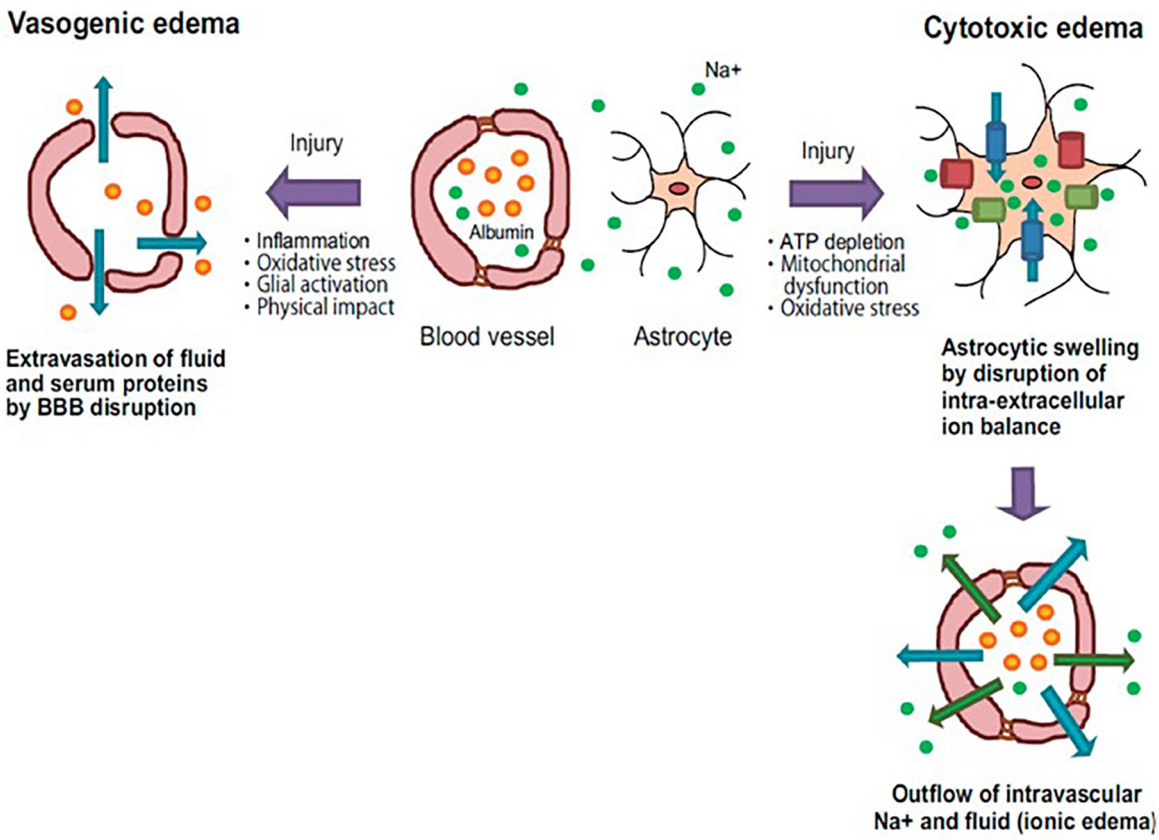


FIGURE 2
Pathology of vasogenic and cytotoxic edema. Vasogenic edema: after brain injury, endothelial tight junctions are disrupted by inflammatory reactions and oxidative stress. These events cause fluid and albumin extravasation, leading to the extracellular accumulation of fluid into the cerebral parenchyma. Cytotoxic edema: brain insults induce intracellular ATP depletion, resulting in mitochondrial dysfunction and oxidative stress. These events cause a disturbance of intra-extracellular ion balance. As a result, excessive inflows of extracellular fluid and Na^+ into cells are induced, leading to cell swelling. Blue arrows: water flow; Green arrows: Na^+ flow; Orange spheres: albumin; Green spheres: Na^+; Blue columns: water channel; Green columns: ion transporter; Red columns: ion channel (taken and modified from Michinaga and Koyama, 2015).

regulation of MC buffering extracellular K^+, and maintaining the membrane potential when Kir channels are blocked or downregulated under ischemic conditions or retinal detachment (Bringmann et al., 2006; Wurm et al., 2006).

Cl^- channels

Cl^- is the most abundant anion in animal cells and different transport systems use it as an accompanying ion to neutralize cation movements. Crépel et al. (1998) characterized an outwardly rectifying anion current in astrocytes, activated by hyposmolarity and regulated by tyrosine kinases. These Cl^- currents were identified in different cell types, including astrocytes, called "ICl, swell" (Nilius et al., 1994; Jentsch et al., 2002). This osmosensitive anion current is induced by VRAC, involved in the astrocyte's RVD processes (Parkerson and Sontheimer, 2004). VRAC contributes to glutamate release from astrocytes during the spreading depression event in the cortical zone of the brain (Basarsky et al., 1999) or ischemia, exacerbating the damage by excitotoxicity (Abdullaev et al., 2006). Two independent groups determined the molecular identity of VRACs. These channels are formed by subunits of the LRRC8A protein (Leucine-Rich Repeats Containing 8a Subunit A), which are part of the LRRC8E family related to pannexins (Qiu et al., 2014; Voss et al., 2014). Reports indicate a passive flow of organic osmolytes (taurine, aspartate, glutamate, glutamine, and polyols) also generated through the hypoosmotic activation of VRACs-containing LRRC8A subunits (Strange et al., 1993; Parkerson and Sontheimer, 2003; Murphy et al., 2017; Formaggio et al., 2019).

In MC, the activation of Cl^- currents by γ-aminobutyric acid (GABA) have similar characteristics to those observed in neurons (Bormann and Kettenmann, 1988). Other Cl^- currents have been associated with voltage changes, such as the ClC-2 channel, activated by hyperpolarization

(Thiemann et al., 1992). There is no protein characterization of VRAC channels in MC; however, Netti et al. (2018) showed that VRAC mediated the Ca^{2+}-independent release of taurine and glutamate during the RVD processes. MIO-M1, the human MC line, showed that Cl^- currents modulate the membrane potential in the RVD response, accompanied by a passive flux of K^+, indicating that the RVD response in MC depends on the magnitude of hyperosmolarity (Fernández et al., 2013).

TRPV4 channels

Transient Receptor Potential Vanilloid type 4 (TRPV4), is a nonselective cation channel activated by heat, mechanical stimuli, and changes in cell volume; it plays an important role in physiological processes and is upregulated in a variety of pathological conditions (Vennekens et al., 2008; Kumar et al., 2018). TRPV4 is expressed in adult cortical and hippocampal astrocytes (Benfenati et al., 2007a), and despite the extensive study of TRPV4 channels and their participation in astrocytic cell volume regulation, their contribution to this event remains controversial in some cases. In an ischemic animal model with $trpv4^{-/-}$ mice, TRPV4 channels are involved in astrocytic volume regulation only *in vitro* experiments, not *in situ* (Pivonkova et al., 2018). TRPV4 channels have a protective role during ischemia-induced edema if Ca^{2+} influx was blocked with a TRPV4 antagonist; however, the RVD processes are not affected (Butenko et al., 2012; Pivonkova et al., 2018). The role of TRPV4 in the RVD could be related to the differentiation of astrocytes and the expression level of other proteins such as aquaporin 4 (AQP4; Mola et al., 2021).

TRPV4 channels associated with K^+ channels regulate the resting membrane potential and the voltage changes occurring during the RVD in MC (Netti et al., 2017). This TRPV4 regulation of voltage membrane and RVD depends on the threshold activated by Ca^{2+} and phospholipase A_2 activity (Toft-Bertelsen et al., 2019). Jo et al. (2015) proposed that TRPV4 is an influx pathway for Ca^{2+} to modulate the RVD, swelling, and AQP4 expression in MC. This observation confirms an interaction between the TRPV4 channel and AQP4 in MC and astrocytes associated with persistent swelling of the CNS and retina (Thrane et al., 2011; Jo et al., 2015).

Aquaporins

Jung et al. (1994) were the first to show the presence of water channels in the CNS. None of the other members of the aquaporin family showed such a broad expression pattern in the CNS as type 4. Six isoforms of AQP4 have been characterized, all related to cell volume regulation (Moe et al., 2008). This water channel is located in the ependymal layer of the ventricular system and astrocytes (Rash et al., 1998). In the glial projections of astrocytes, AQP4 associated with α-syntrophin allows the bidirectional mobilization of water between plasma and the CNS (Amiry-Moghaddam et al., 2004). The presence of AQP4 in glial endings that are found in the abluminal membrane of the cerebral capillaries (Nielsen et al., 1997) suggests that water transport between the systemic circulation and the CNS is modulated by the membrane permeability of the astrocytic cells (Amiry-Moghaddam et al., 2003). AQP4 participates in the astrocyte swelling causally related to neural activity, promoting the re-distribution of extracellular K^+ and osmotically obligated water, between the plasma and the cerebrospinal fluid during periods of high neural activity (MacVicar et al., 2002; Kitaura et al., 2009). Since water flow through AQP4 is bidirectional and only occurs in response to osmotic gradients, the perivascular presence of this aquaporin could have adverse effects on pathophysiological conditions that involve water accumulation in the CNS (Amiry-Moghaddam et al., 2004). For example, AQP4-knockout mice exhibit reduced post-ischemic cerebral edema and a decrease in a glial cell volume increase in response to anisosmotic conditions (Manley et al., 2000). Astrocytes from AQP4-knockout mice and astrocytes with low expression levels of TRPV4 channels lack the Ca^{2+}-dependent RVD processes, suggesting that AQP4 and TRPV4 interaction are essential to initiate the RVD in astrocytes (Benfenati et al., 2011). Downregulating AQP4 by miR-29b overexpression ameliorates damage after stroke in humans and mice with cerebral ischemia, an event associated with astrocytic swelling (Wang et al., 2015). Conversely, AQP4 deletion exacerbates water accumulation and induces higher intracranial pressures in AQP4-knockout mice (Papadopoulos et al., 2004).

In mammalian retinas, AQP4 is expressed on the MC projections facing capillaries, near the vitreoretinal border region, and on high synaptic activity areas in the retina (plexiform layers), regulating water flow and ion homeostasis from the inner retina into the vitreous body and retinal capillaries (Nagelhus et al., 1998; Kofuji and Newman, 2004). This localization of AQP4 in MC is consistent with the presence of dystrophin and a-syntrophin known to be involved in AQP4 polarization compared to cortical astrocytes (Enger et al., 2012). AQP4-knockout mice showed blood-barrier impairment in the blood vessels in contact with MC projections, but not in the areas with a stronger gliotic response and where blood vessels are covered by astrocytic projections (Nicchia et al., 2016). In MC endfeet, AQP4 colocalizes with the TRPV4 channel; AQP4 and TRPV4 interact synergically in cell volume regulation, and both regulate each other's expression (Jo et al., 2015). Hyperglycemic conditions induce an increase in AQP4 expression in MC, and AQP4 knockdown in diabetic animals exacerbates diabetic retinopathy (Cui et al., 2012; Qin et al., 2012; Picconi et al., 2019).

Aquaporins and ion channel interactions

Considering astrocytic cells as multifunctional regulatory "units" of neuronal activity, it is not surprising to consider that both ion and water channels have a polarized and co-localized distribution to form macromolecular complexes capable of regulating specific cellular processes (Nagelhus et al., 1999; Amiry-Moghaddam et al., 2003). As mentioned before, AQP4 and TRPV4 channels regulate cell volume in astrocytes and MC; however, there are other channels besides TRPV4 that interact with AQP4. Since AQP4 is predominantly expressed in astrocytes and MC, it has been proposed as an important element associated with Kir 4.1 to facilitate the movement of water through the membrane during the process of compensation of extracellular K^+ concentration through Kir 4.1 (Nagelhus et al., 2004). Experimental work reinforces the idea of molecular interaction between AQP4 and Kir 2.1 through the dystrophin complex. AQP4 is a water channel associated with dystrophin through α-syntrophin (Amiry-Moghaddam and Ottersen, 2003); Kir 4.1 shows co-localization with α-syntrophin in astrocytes and MC (Guadagno and Moukhles, 2004; Noël et al., 2005; Connors and Kofuji, 2006). The interaction of α-syntrophin is crucial for the formation of the dystrophin-Kir 4.1 complexes in the CNS and retina (Connors et al., 2004; Connors and Kofuji, 2006). Mice with α-syntrophin deletion show delocalization of AQP4, associated with an imbalance in the regulation of the extracellular K^+ concentration in the CNS under hypothermia (Amiry-Moghaddam et al., 2003). It is proposed that AQP4 functions as a "transducer" at the membrane level for the detection of cell volume and during the osmotic response; however, this role is not clear yet.

Despite the physiological importance of VRAC channels and AQPs in the physiology and physiopathology of glial cells, there is very little information about the functional interaction between these two channels. Benfenati et al. (2007b) used RNAi against AQP4 in a primary culture of cortical astrocytes type 1 and observed a considerable decrease in the conductance generated by Cl^- through VRAC, without affecting voltage-activated K^+ currents.

The process of cell volume regulation is a fundamental homeostatic mechanism for cellular physiology and consequently for the organism. In the case of the CNS, this phenomenon is essential due to the physical delimitation imposed by the skull; therefore, any alteration of this mechanism leads to brain damage and, in extreme cases, coma and death. This is why the study and understanding of the mechanisms involved in the regulation of cell volume in the brain are vital. In other tissues such as the retina, which is part of the CNS and lacks a physical delimitation, the regulation of water homeostasis is necessary for the correct function of the visual system. At a cellular level, astrocytes and MC, besides actively participating in synaptic physiology and metabolism, are key factors in the maintenance of water homeostasis in the brain and retina. The expression of different proteins involved in ion (Kir 4.1, 2.1, TRPV4), organic osmolytes (VRAC), and water mobilization (AQP4) in their membranes, provides an effective ability to maintain a stable cell volume under physiological and pathological conditions. On the other hand, the characterization of proteins and the understanding of the processes involved in osmotic control (sensor, signaling, and effector), will allow the development of therapies aimed at controlling or avoiding the cerebral and retinal edema that occurs in different disorders.

Author contributions

LO-P: conceived the manuscript, literature review, resources, and draft of the manuscript. RG-C: performed writing, literature review, and editing. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by grants PAPIIT-UNAM IN221820 and Presupuesto Interno Facultad de Medicina, UNAM.

Acknowledgments

We wold like to thank the Departamento de Investigación, Asociación para Evitar la Ceguera en México IAP Hospital "Dr. Luis Sánchez Bulnes" for all the support provided in the writing of this article.

Conflict of interest

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

Publisher's note

References

Abdullaev, I. F., Rudkouskaya, A., Schools, G. P., Kimelberg, H. K., and Mongin, A. A. (2006). Pharmacological comparison of swelling-activated excitatory amino acid release and Cl^- currents in cultured rat astrocytes. *J. Physiol.* 572, 677–689. doi: 10.1113/jphysiol.2005.103820

Amiry-Moghaddam, M., Frydenlund, D. S., and Ottersen, O. P. (2004). Anchoring of aquaporin-4 in brain: molecular mechanisms and implications for the physiology and pathophysiology of water transport. *Neuroscience* 129, 999–1010. doi: 10.1016/j.neuroscience.2004.08.049

Amiry-Moghaddam, M., Otsuka, T., Hurn, P. D., Traystman, R. J., Haug, F. M., Froehner, S. C., et al. (2003). An α-syntrophin-dependent pool of AQP4 in astroglial end-feet confers bidirectional water flow between blood and brain. *Proc. Natl. Acad. Sci. U S A* 100, 2106–2111. doi: 10.1073/pnas.0437946100

Amiry-Moghaddam, M., and Ottersen, O. P. (2003). The molecular basis of water transport in the brain. *Nat. Rev. Neurosci.* 4, 991–1001. doi: 10.1038/nrn1252

Armstrong, C. M. (2003). The Na/K pump, Cl ion and osmotic stabilization of cells. *Proc. Natl. Acad. Sci. U S A* 100, 6257–6262. doi: 10.1073/pnas.0931278100

Basarsky, T. A., Feighan, D., and MacVicar, B. A. (1999). Glutamate release through volume-activated channels during spreading depression. *J. Neurosci.* 19, 6439–6445. doi: 10.1523/JNEUROSCI.19-15-06439.1999

Benfenati, V., Amiry-Moghaddam, M., Caprini, M., Mylonakou, M. N., Rapisarda, C., Ottersen, O. P., et al. (2007a). Expression and functional characterization of transient receptor potential vanilloid-related channel 4 (TRPV4) in rat cortical astrocytes. *Neuroscience* 148, 876–892. doi: 10.1016/j.neuroscience.2007.06.039

Benfenati, V., Nicchia, G. P., Svelto, M., Rapisarda, C., Frigeri, A., and Ferroni, S. (2007b). Functional down-regulation of volume-regulated anion channels in AQP4 knockdown cultured rat cortical astrocytes. *J. Neurochem.* 100, 87–104. doi: 10.1111/j.1471-4159.2006.04164.x

Benfenati, V., Caprini, M., Dovizio, M., Mylonakou, M. N., Ferroni, S., Ottersen, O. P., et al. (2011). An aquaporin-4/transient receptor potential vanilloid 4 (AQP4/TRPV4) complex is essential for cell-volume control in astrocytes. *Proc. Natl. Acad. Sci. U S A* 108, 2563–2568. doi: 10.1073/pnas.1012867108

Bormann, J., and Kettenmann, H. (1988). Patch-clamp study of gamma-aminobutyric acid receptor Cl- channels in cultured astrocytes. *Proc. Natl. Acad. Sci. U S A* 85, 9336–9340. doi: 10.1073/pnas.85.23.9336

Bringmann, A., Pannicke, T., Grosche, J., Francke, M., Wiedemann, P., Skatchkov, S. N., et al. (2006). Müller cells in the healthy and diseased retina. *Prog. Retin. Eye Res.* 25, 397–424. doi: 10.1016/j.preteyeres.2006.05.003

Bringmann, A., Uckermann, O., Pannicke, T., Iandiev, I., Reichenbach, A., and Wiedemann, P. (2005). Neuronal versus glial cell swelling in the ischaemic retina. *Acta Ophthalmol. Scand.* 83, 528–538. doi: 10.1111/j.1600-0420.2005.00565.x

Butenko, O., Dzamba, D., Benesova, J., Honsa, P., Benfenati, V., Rusnakova, V., et al. (2012). The increased activity of TRPV4 channel in the astrocytes of the adult rat hippocampus after cerebral hypoxia/ischemia. *PLoS One* 7:e39959. doi: 10.1371/journal.pone.0039959

Butt, A. M., and Kalsi, A. (2006). Inwardly rectifying potassium channels (Kir) in central nervous system glia: a special role for Kir4.1 in glial functions. *J. Cell Mol. Med.* 10, 33–44. doi: 10.1111/j.1582-4934.2006.tb00289.x

Calloe, K., Nielsen, M. S., Grunnet, M., Schmitt, N., and Jorgensen, N. K. (2007). KCNQ channels are involved in the regulatory volume decrease response in primary neonatal rat cardiomyocytes. *Biochim. Biophys. Acta* 1773, 764–773. doi: 10.1016/j.bbamcr.2007.02.008

Connors, N. C., Adams, M. E., Froehner, S. C., and Kofuji, P. (2004). The potassium channel Kir4.1 associates with the dystrophin-glycoprotein complex via α-syntrophin in glia. *J. Biol. Chem.* 279, 28387–28392. doi: 10.1074/jbc.M402604200

Connors, N. C., and Kofuji, P. (2006). Potassium channel Kir4.1 macromolecular complex in retinal glial cells. *Glia* 53, 124–131. doi: 10.1002/glia.20271

Crépel, V., Panenka, W., Kelly, M. E., and MacVicar, B. A. (1998). Mitogen-activated protein and tyrosine kinases in the activation of astrocyte volume-activated chloride current. *J. Neurosci.* 18, 1196–1206. doi: 10.1523/jneurosci.18-04-01196.1998

Cui, B., Sun, J. H., Xiang, F. F., Liu, L., and Li, W. J. (2012). Aquaporin 4 knockdown exacerbates streptozotocin-induced diabetic retinopathy through aggravating inflammatory response. *Exp. Eye Res.* 98, 37–43. doi: 10.1016/j.exer.2012.02.013

Enger, R., Gundersen, G. A., Haj-Yasein, N. N., Eilert-Olsen, M., Thoren, A. E., Vindedal, G. F., et al. (2012). Molecular scaffolds underpinning macroglial polarization: an analysis of retinal Müller cells and brain astrocytes in mouse. *Glia* 60, 2018–2026. doi: 10.1002/glia.22416

Fernández, J. M., Di Giusto, G., Kalstein, M., Melamud, L., Rivarola, V., Ford, P., et al. (2013). Cell volume regulation in cultured human retinal Müller cells is associated with changes in transmembrane potential. *PLoS One* 8:e57268. doi: 10.1371/journal.pone.0057268

Formaggio, F., Saracino, E., Mola, M. G., Rao, S. B., Amiry-Moghaddam, M., Muccini, M., et al. (2019). LRRC8A is essential for swelling-activated chloride current and for regulatory volume decrease in astrocytes. *FASEB J.* 33, 101–113. doi: 10.1096/fj.201701397RR

Franchi-Gazzola, R., Dall'Asta, V., Sala, R., Visigalli, R., Bevilacqua, E., Gaccioli, F., et al. (2006). The role of the neutral amino acid transporter SNAT2 in cell volume regulation. *Acta Physiol. (Oxf).* 187, 273–283. doi: 10.1111/j.1748-1716.2006.01552.x

Franco, R. (2003). Osmosensitive taurine release: does taurine share the same efflux pathway with chloride and other amino acid osmolytes? *Adv. Exp. Med. Biol.* 526, 189–196. doi: 10.1007/978-1-4615-0077-3_24

Guadagno, E., and Moukhles, H. (2004). Laminin-induced aggregation of the inwardly rectifying potassium channel, Kir4.1 and the water-permeable channel, AQP4, via a dystroglycan-containing complex in astrocytes. *Glia* 47, 138–149. doi: 10.1002/glia.20039

Hibino, H., Fujita, A., Iwai, K., Yamada, M., and Kurachi, Y. (2004). Differential assembly of inwardly rectifying K^+ channel subunits, Kir4.1 and Kir5.1, in brain astrocytes. *J. Biol. Chem.* 279, 44065–44073. doi: 10.1074/jbc.M405985200

Higashi, K., Fujita, A., Inanobe, A., Tanemoto, M., Doi, K., Kubo, T., et al. (2001). An inwardly rectifying K^+ channel, Kir4.1, expressed in astrocytes surrounds synapses and blood vessels in brain. *Am. J. Physiol. Cell Physiol.* 281, C922–C931. doi: 10.1152/ajpcell.2001.281.3.C922

Hoffmann, E. K., and Pedersen, S. F. (2006). Sensors and signal transduction pathways in vertebrate cell volume regulation. *Contrib. Nephrol.* 152, 54–104. doi: 10.1159/000096318

Jentsch, T. J., Stein, V., Weinreich, F., and Zdebik, A. A. (2002). Molecular structure and physiological function of chloride channels. *Physiol. Rev.* 82, 503–568. doi: 10.1152/physrev.00029.2001

Jo, A. O., Ryskamp, D. A., Phuong, T. T., Verkman, A. S., Yarishkin, O., MacAulay, N., et al. (2015). TRPV4 and AQP4 channels synergistically regulate cell volume and calcium homeostasis in retinal Müller glia. *J. Neurosci.* 35, 13525–13537. doi: 10.1523/JNEUROSCI.1987-15.2015

Jung, J. S., Bhat, R. V., Preston, G. M., Guggino, W. B., Baraban, J. M., and Agre, P. (1994). Molecular characterization of an aquaporin cDNA from brain: candidate osmoreceptor and regulator of water balance. *Proc. Natl. Acad. Sci. U S A* 91, 13052–13056. doi: 10.1073/pnas.91.26.13052

Kimelberg, H. K. (2004). Water homeostasis in the brain: basic concepts. *Neuroscience* 129, 851–860. doi: 10.1016/j.neuroscience.2004.07.033

Kitaura, H., Tsujita, M., Huber, V. J., Kakita, A., Shibuki, K., Sakimura, K., et al. (2009). Activity-dependent glial swelling is impaired in aquaporin-4 knockout mice. *Neurosci. Res.* 64, 208–212. doi: 10.1016/j.neures.2009.03.002

Kofuji, P., Biedermann, B., Siddharthan, V., Raap, M., Iandiev, I., Milenkovic, I., et al. (2002). Kir potassium channel subunit expression in retinal glial cells: implications for spatial potassium buffering. *Glia* 39, 292–303. doi: 10.1002/glia.10112

Kofuji, P., and Newman, E. A. (2004). Potassium buffering in the central nervous system. *Neuroscience* 129, 1045–1056. doi: 10.1016/j.neuroscience.2004.06.008

Kuhrt, H., Wurm, A., Karl, A., Iandiev, I., Wiedemann, P., Reichenbach, A., et al. (2008). Müller cell gliosis in retinal organ culture mimics gliotic alterations after ischemia *in vivo*. *Int. J. Dev. Neurosci.* 26, 745–751. doi: 10.1016/j.ijdevneu.2008.07.003

Kumar, H., Lee, S. H., Kim, K. T., Zeng, X., and Han, I. (2018). TRPV4: a sensor for homeostasis and pathological events in the CNS. *Mol. Neurobiol.* 55, 8695–8708. doi: 10.1007/s12035-018-0998-8

Lang, F. (2007). Mechanisms and significance of cell volume regulation. *J. Am. Coll. Nutr.* 26, 613S–623S. doi: 10.1080/07315724.2007.10719667

Larsen, B. R., Assentoft, M., Cotrina, M. L., Hua, S. Z., Nedergaard, M., Kaila, K., et al. (2014). Contributions of the Na^+/K^+-ATPase, NKCC1 and Kir4.1 to hippocampal K^+; clearance and volume responses. *Glia* 62, 608–622. doi: 10.1002/glia.22629

Lotshaw, D. P. (2007). Biophysical, pharmacological and functional characteristics of cloned and native mammalian two-pore domain K^+ channels. *Cell Biochem. Biophys.* 47, 209–256. doi: 10.1007/s12013-007-0007-8

MacVicar, B. A., Feighan, D., Brown, A., and Ransom, B. (2002). Intrinsic optical signals in the rat optic nerve: role for K^+ uptake via NKCC1 and swelling of astrocytes. *Glia* 37, 114–123. doi: 10.1002/glia.10023

Manley, G. T., Binder, D. K., Papadopoulos, M. C., and Verkman, A. S. (2004). New insights into water transport and edema in the central nervous system from phenotype analysis of aquaporin-4 null mice. *Neuroscience* 129, 983–991. doi: 10.1016/j.neuroscience.2004.06.088

Manley, G. T., Fujimura, M., Ma, T., Noshita, N., Filiz, F., Bollen, A. W., et al. (2000). Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. *Nat. Med.* 6, 159–163. doi: 10.1038/72256

McManus, M. L., Churchwell, K. B., and Strange, K. (1995). Regulation of cell volume in health and disease. *N Engl. J. Med.* 333, 1260–1266. doi: 10.1056/NEJM199511093331906

Michinaga, S., and Koyama, Y. (2015). Pathogenesis of brain edema and investigation into anti-edema drugs. *Int. J. Mol. Sci.* 16, 9949–9975. doi: 10.3390/ijms16059949

Moe, S. E., Sorbo, J. G., Sogaard, R., Zeuthen, T., Petter Ottersen, O., and Holen, T. (2008). New isoforms of rat Aquaporin-4. *Genomics* 91, 367–377. doi: 10.1016/j.ygeno.2007.12.003

Mola, M. G., Saracino, E., Formaggio, F., Amerotti, A. G., Barile, B., Posati, T., et al. (2021). Cell volume regulation mechanisms in differentiated astrocytes. *Cell. Physiol. Biochem.* 55, 196–212. doi: 10.33594/000000469

Murphy, T. R., Binder, D. K., and Fiacco, T. A. (2017). Turning down the volume: astrocyte volume change in the generation and termination of epileptic seizures. *Neurobiol. Dis.* 104, 24–32. doi: 10.1016/j.nbd.2017.04.016

Nagelhus, E. A., Horio, Y., Inanobe, A., Fujita, A., Haug, F. M., Nielsen, S., et al. (1999). Immunogold evidence suggests that coupling of K^+ siphoning and water transport in rat retinal Müller cells is mediated by a coenrichment of Kir4.1 and AQP4 in specific membrane domains. *Glia* 26, 47–54. doi: 10.1002/(SICI)1098-1136(199903)26:13c47::AID-GLIA53e3.0.CO;2-5

Nagelhus, E. A., Mathiisen, T. M., and Ottersen, O. P. (2004). Aquaporin-4 in the central nervous system: cellular and subcellular distribution and coexpression with KIR4.1. *Neuroscience* 129, 905–913. doi: 10.1016/j.neuroscience.2004.08.053

Nagelhus, E. A., Veruki, M. L., Torp, R., Haug, F. M., Laake, J. H., Nielsen, S., et al. (1998). Aquaporin-4 water channel protein in the rat retina and optic nerve: polarized expression in Müller cells and fibrous astrocytes. *J. Neurosci.* 18, 2506–2519. doi: 10.1523/JNEUROSCI.18-07-02506.1998

Netti, V., Fernández, J., Kalstein, M., Pizzoni, A., Di Giusto, G., Rivarola, V., et al. (2017). TRPV4 contributes to resting membrane potential in retinal Müller cells: implications in cell volume regulation. *J. Cell. Biochem.* 118, 2302–2313. doi: 10.1002/jcb.25884

Netti, V., Pizzoni, A., Pérez-Domínguez, M., Ford, P., Pasantes-Morales, H., Ramos-Mandujano, G., et al. (2018). Release of taurine and glutamate contributes to cell volume regulation in human retinal Müller cells: differences in modulation by calcium. *J. Neurophysiol.* 120, 973–984. doi: 10.1152/jn.00725.2017

Nicchia, G. P., Nico, B., Camassa, L. M., Mola, M. G., Loh, N., Dermietzel, R., et al. (2004). The role of aquaporin-4 in the blood-brain barrier development and integrity: studies in animal and cell culture models. *Neuroscience* 129, 935–945. doi: 10.1016/j.neuroscience.2004.07.055

Nicchia, G. P., Pisani, F., Simone, L., Cibelli, A., Mola, M. G., Dal Monte, M., et al. (2016). Glio-vascular modifications caused by Aquaporin-4 deletion in the mouse retina. *Exp. Eye Res.* 146, 259–268. doi: 10.1016/j.exer.2016.03.019

Nielsen, S., Nagelhus, E. A., Amiry-Moghaddam, M., Bourque, C., Agre, P., and Ottersen, O. P. (1997). Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J. Neurosci.* 17, 171–180. doi: 10.1523/JNEUROSCI.17-01-00171.1997

Nilius, B., Sehrer, J., and Droogmans, G. (1994). Permeation properties and modulation of volume-activated Cl^--currents in human endothelial cells. *Br. J. Pharmacol.* 112, 1049–1056. doi: 10.1111/j.1476-5381.1994.tb13189.x

Noël, G., Belda, M., Guadagno, E., Micoud, J., Klöcker, N., and Moukhles, H. (2005). Dystroglycan and Kir4.1 coclustering in retinal Müller glia is regulated by laminin-1 and requires the PDZ-ligand domain of Kir4.1. *J. Neurochem.* 94, 691–702. doi: 10.1111/j.1471-4159.2005.03191.x

O'Neill, W. C. (1999). Physiological significance of volume-regulatory transporters. *Am. J. Physiol.* 276, C995–C1011. doi: 10.1152/ajpcell.1999.276.5.C995

Papadopoulos, M. C., Manley, G. T., Krishna, S., and Verkman, A. S. (2004). Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. *FASEB J.* 18, 1291–1293. doi: 10.1096/fj.04-1723fje

Parkerson, K. A., and Sontheimer, H. (2003). Contribution of chloride channels to volume regulation of cortical astrocytes. *Am. J. Physiol. Cell Physiol.* 284, C1460–C1467. doi: 10.1152/ajpcell.00603.2002

Parkerson, K. A., and Sontheimer, H. (2004). Biophysical and pharmacological characterization of hypotonically activated chloride currents in cortical astrocytes. *Glia* 46, 419–436. doi: 10.1002/glia.10361

Pasantes-Morales, H., and Vázquez-Juárez, E. (2012). Transporters and channels in cytotoxic astrocyte swelling. *Neurochem. Res.* 37, 2379–2387. doi: 10.1007/s11064-012-0777-2

Pedersen, S. F., O'Donnell, M. E., Anderson, S. E., and Cala, P. M. (2006). Physiology and pathophysiology of Na^+/H^+ exchange and Na^+-K^+-$2Cl^-$ cotransport in the heart, brain and blood. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 291, R1–R25. doi: 10.1152/ajpregu.00782.2005

Picconi, F., Parravano, M., Sciarretta, F., Fulci, C., Nali, M., Frontoni, S., et al. (2019). Activation of retinal Müller cells in response to glucose variability. *Endocrine* 65, 542–549. doi: 10.1007/s12020-019-02017-5

Pivonkova, H., Hermanova, Z., Kirdajova, D., Awadova, T., Malinsky, J., Valihrach, L., et al. (2018). The contribution of TRPV4 channels to astrocyte volume regulation and brain edema formation. *Neuroscience* 394, 127–143. doi: 10.1016/j.neuroscience.2018.10.028

Qin, Y., Ren, H., Hoffman, M. R., Fan, J., Zhang, M., and Xu, G. (2012). Aquaporin changes during diabetic retinopathy in rats are accelerated by systemic hypertension and are linked to the renin-angiotensin system. *Invest. Ophthalmol. Vis. Sci.* 53, 3047–3053. doi: 10.1167/iovs.11-9154

Qiu, Z., Dubin, A. E., Mathur, J., Tu, B., Reddy, K., Miraglia, L. J., et al. (2014). SWELL1, a plasma membrane protein, is an essential component of volume-regulated anion channel. *Cell* 2, 447–458. doi: 10.1016/j.cell.2014.03.024

Rash, J. E., Yasumura, T., Hudson, C. S., Agre, P., and Nielsen, S. (1998). Direct immunogold labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in rat brain and spinal cord. *Proc. Natl. Acad. Sci. U S A* 95, 11981–11986. doi: 10.1073/pnas.95.20.11981

Reed, M. M., and Blazer-Yost, B. (2022). Channels and transporters in astrocyte volume regulation in health and disease. *Cell. Physiol. Biochem.* 56, 12–30. doi: 10.33594/000000495

Simard, M., and Nedergaard, M. (2004). The neurobiology of glia in the context of water and ion homeostasis. *Neuroscience* 129, 877–896. doi: 10.1016/j.neuroscience.2004.09.053

Skatchkov, S. N., Eaton, M. J., Shuba, Y. M., Kucheryavykh, Y. V., Derst, C., Veh, R. W., et al. (2006). Tandem-pore domain potassium channels are functionally expressed in retinal (Müller) glial cells. *Glia* 53, 266–276. doi: 10.1002/glia.20280

Sontheimer, H. (1992). Astrocytes, as well as neurons, express a diversity of ion channels. *Can. J. Physiol. Pharmacol.* 70, S223–S238. doi: 10.1139/y92-266

Strange, K., Morrison, R., Shrode, L., and Putman, R. (1993). Mechanism and regulation of swelling-activated inositol efflux in brain glia cells. *Am. J. Physiol.* 265, C244–C256. doi: 10.1152/ajpcell.1993.265.1.C244

Thiemann, A., Gründer, S., Pusch, M., and Jentsch, T. J. (1992). A chloride channel widely expressed in epithelial and non-epithelial cells. *Nature* 356, 57–60. doi: 10.1038/356057a0

Thrane, A. S., Rappold, P. M., Fujita, T., Torres, A., Bekar, L. K., Takano, T., et al. (2011). Critical role of aquaporin-4 (AQP4) in astrocytic Ca^{2+} signaling events elicited by cerebral edema. *Proc. Natl. Acad. Sci. U S A* 108, 846–851. doi: 10.1073/pnas.1015217108

Toft-Bertelsen, T. L., Yarishkin, O., Redmon, S., Phuong, T., Križaj, D., and MacAulay, N. (2019). Volume sensing in the transient receptor potential vanilloid 4 ion channel is cell type-specific and mediated by an N-terminal volume-sensing domain. *J. Biol. Chem.* 294, 18421–18434. doi: 10.1074/jbc.RA119.011187

Unterberg, A. W., Stover, J., Kress, B., and Kiening, K. L. (2004). Edema and brain trauma. *Neuroscience* 129, 1021–1029. doi: 10.1016/j.neuroscience.2004.06.046

Vajda, Z., Pedersen, M., Füchtbauer, E. M., Wertz, K., Stødkilde-Jørgensen, H., Sulyok, E., et al. (2002). Delayed onset of brain edema and mislocalization of aquaporin-4 in dystrophin-null transgenic mice. *Proc. Natl. Acad. Sci. U S A* 99, 13131–13136. doi: 10.1073/pnas.192457099

Vennekens, R., Owsianik, G., and Nilius, B. (2008). Vanilloid transient receptor potential cation channels: an overview. *Curr. Pharm. Des.* 14, 18–31. doi: 10.2174/138161208783330763

Voss, F. K., Ullrich, F., Münch, J., Lazarow, K., Lutter, D., Mah, N., et al. (2014). Identification of LRRC8 heteromers as an essential component of the volume-regulated anion channel VRAC. *Science* 344, 634–638. doi: 10.1126/science.1252826

Wang, Y., Huang, J., Ma, Y., Tang, G., Liu, Y., Chen, X., et al. (2015). MicroRNA-29b is a therapeutic target in cerebral ischemia associated with aquaporin 4. *J. Cereb. Blood Flow Metab.* 35, 1977–1984. doi: 10.1038/jcbfm.2015.156

Wurm, A., Pannicke, T., Iandiev, I., Bühner, E., Pietsch, U. C., Reichenbach, A., et al. (2006). Changes in membrane conductance play a pathogenic role in osmotic glial cell swelling in detached retinas. *Am. J. Pathol.* 169, 1990–1998. doi: 10.2353/ajpath.2006.060628

Zhou, M., Xu, G., Xie, M., Zhang, X., Schools, G. P., Ma, L., et al. (2009). TWIK-1 and TREK-1 are potassium channels contributing significantly to astrocyte passive conductance in rat hippocampal slices. *J. Neurosci.* 29, 8551–8564. doi: 10.1523/JNEUROSCI.5784-08.2009

frontiers | Frontiers in Cellular Neuroscience

TYPE Perspective
PUBLISHED 07 September 2022
DOI 10.3389/fncel.2022.972827

OPEN ACCESS

EDITED BY
Alejandro López-Juárez,
The University of Texas Rio Grande Valley, United States

REVIEWED BY
Francisco Mercado,
National Institute of Psychiatry Ramon de la Fuente Muñiz (INPRFM), Mexico

*CORRESPONDENCE
Mónica López-Hidalgo
lopezhidalgo@unam.mx

SPECIALTY SECTION
This article was submitted to Non-Neuronal Cells, a section of the journal Frontiers in Cellular Neuroscience

RECEIVED 19 June 2022
ACCEPTED 15 August 2022
PUBLISHED 07 September 2022

CITATION
Higinio-Rodríguez F, Rivera-Villaseñor A, Calero-Vargas I and López-Hidalgo M (2022) From nociception to pain perception, possible implications of astrocytes. *Front. Cell. Neurosci.* 16:972827. doi: 10.3389/fncel.2022.972827

From nociception to pain perception, possible implications of astrocytes

Frida Higinio-Rodríguez[1,2], Angélica Rivera-Villaseñor[1,2], Isnarhazni Calero-Vargas[1,2] and Mónica López-Hidalgo[1]*

[1]Escuela Nacional de Estudios Superiores, Universidad Nacional Autónoma de México, Querétaro, Mexico, [2]Instituto de Neurobiología, Universidad Nacional Autónoma de México, Querétaro, Mexico

Astrocytes are determinants for the functioning of the CNS. They respond to neuronal activity with calcium increases and can in turn modulate synaptic transmission, brain plasticity as well as cognitive processes. Astrocytes display sensory-evoked calcium responses in different brain structures related to the discriminative system of most sensory modalities. In particular, noxious stimulation evoked calcium responses in astrocytes in the spinal cord, the hippocampus, and the somatosensory cortex. However, it is not clear if astrocytes are involved in pain. Pain is a private, personal, and complex experience that warns us about potential tissue damage. It is a perception that is not linearly associated with the amount of tissue damage or nociception; instead, it is constructed with sensory, cognitive, and affective components and depends on our previous experiences. However, it is not fully understood how pain is created from nociception. In this perspective article, we provide an overview of the mechanisms and neuronal networks that underlie the perception of pain. Then we proposed that coherent activity of astrocytes in the spinal cord and pain-related brain areas could be important in binding sensory, affective, and cognitive information on a slower time scale.

KEYWORDS

astrocytes, sensory, nociception, perception, pain, calcium activity

Introduction

From nociception to pain

According to the International Association for the Study of Pain (IASP), pain is defined as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage (Raja et al., 2020). It is a complex experience defined by biological, psychological, and social factors with sensorial, emotional, and cognitive components. It is important to make clear that pain and nociception are not the same phenomena; nociception is related to the activation, transfer, and processing of noxious stimuli within the CNS (Loeser and Treede, 2008). Instead, pain is an individual experience that, in

physiological conditions, warns us about potential or real tissue damage. Although pain can be induced by different modalities, here we will focus on nociception and pain evoked by the somatosensory system.

When you harm yourself, the noxious stimulus is detected in the periphery by receptors called nociceptors. This information arrives at the dorsal horn of the spinal cord (Dubin and Patapoutian, 2010) and is transmitted to the thalamus directly through the spinothalamic tract or indirectly through the spinoreticular and spinomesencephalic tracts (Giesler et al., 1981; Willis and Westlund, 1997). From the canonical view, thalamic neurons from the ventral posterolateral (VPL) and ventral posteromedial nucleus (VPM) relay nociceptive information from the body and the head, respectively, to the primary somatosensory cortex (SI) to process sensory components such as quality, intensity, and location of nociceptive stimuli (Backonja, 1996; Monconduit et al., 2006; Kim et al., 2019). From there, the information spread to hierarchically higher cortical regions to transform nociception into pain perception.

However, growing evidence shows that nociceptive information is processed in parallel in multiple brain areas (**Figure 1A**) and that is necessary to bind sensory, affective, and cognitive components to generate the experience of pain (Coghill, 2020). The thalamus, more than a sensory relay, is a key brain structure interplaying cortical and subcortical brain structures to integrate the components of the pain experience. The thalamic nuclei that directly received nociceptive information from the periphery and relay it to the primary sensory cortex are classified as first-order (VPL and VPM); high-order thalamic nucleus (i.e., posterior nucleus) receive information from the deep cortical layer from S1 and send it back to S1, secondary somatosensory cortex (S2), and primary motor cortex to modulate the motor response to pain (Liao and Yen, 2008; Zhang and Bruno, 2019). The thalamus is also connected with the amygdala, the anterior cingulate, and the insular cortex, brain structures involved in the affective component of pain; it also projects to the association cortices such as the prefrontal and the parietal cortices related to the cognitive component of pain (Petrovic et al., 2000; Yen and Lu, 2013). This is relevant because although several brain structures are active during nociception, the activity of these structures by themselves cannot explain the pain experience, instead, the perception of pain requires the coordinated activity from many of these brain regions, and the thalamus is well located to accomplish this function.

The parabrachial nucleus (PBN) receives direct information from nociceptive neurons located in the superficial lamina of the dorsal horn of the spinal cord through the spinoparabrachial tract. It processes visceral pain due to noxious thermal stimuli and inflammatory process (Willis, 1985; Bester et al., 1997) and conveys several types of information such as a taste, pain, and aspects of autonomic control like respiration, blood pressure, fluid balance, and thermoregulation (Chiang et al., 2020). It has reciprocal connections with the central nucleus of the amygdala, the nucleus of the bed of the stria terminalis, and hypothalamic nuclei. The PBN have an important role in the autonomic, motivational and affective responses to pain (Schaible and Grubb, 1993; Millan, 1999) regulating the emotional and autonomic aspects of pain experience (Bourgeais et al., 2001).

In S1, the neuronal activity is somatotopically organized (Mancini et al., 2012) providing information about the location of the noxious stimulation (Jin et al., 2018). Its neuronal activity correlates with the properties of the stimulus including the intensity (Kenshalo et al., 2000; Bufalari et al., 2007; Iwamoto et al., 2021). These neurons are highly modulated by prior experiences or cognitive factors such as attention. For example, when a subject is diverted from a painful stimulus, the nociceptive-evoked neuronal responses are reduced (Backonja, 1996; Bushnell et al., 1999). On the other side, neurons from S2 respond bilaterally to the noxious stimuli (Davis et al., 1998) coding its intensity with increases in neuronal activity. Although this region is also involved in nociceptive processing (Davis et al., 1998; Coghill et al., 1999), the activation of S2 is better associated with the scores of a sensory discriminative component induced by different pain modalities (Maihöfner et al., 2006).

The emotional component of pain is related to the unpleasantness of the nociceptive stimuli. This is by definition, an essential component for the categorization of an experience as painful. It can be associated with the stimulus intensity (Wilcox et al., 2015), but also be independent of it and related to individual, contextual, or cultural aspects. Some of the brain areas involved in this pain-related affective system include the anterior cingulate cortex (Tölle et al., 1999), the amygdala (Straube et al., 2011), the insula (Kim et al., 2017), and the hippocampus (Angenstein et al., 2013).

The basolateral amygdala receives sensory information from the thalamus and cortical areas (LeDoux et al., 1990; Shi and Davis, 1999) whereas the central amygdala receives nociceptive information directly from the spinal cord (Cliffer et al., 1991; Burstein and Potrebic, 1993) and parabrachial area (Bernard and Besson, 1990; Gauriau and Bernard, 2002). The anterior cingulate cortex is connected to the thalamus, prefrontal cortex (orbitofrontal and the medial portion), and amygdala. They mediate fight behaviors in response to noxious stimuli (Gao et al., 2004). The cingulate and the prefrontal cortex are also active when the subject perceives pain from others and has been linked to prosocial behavior and empathy (Kim et al., 2021).

The hippocampus receives nociceptive inputs from the periphery through the spinothalamic and parabrachial pathways and directly from the spinal cord through the septo-hippocampal (Mokhtari et al., 2019). It works together with the anterior cingulate, the insula, the amygdala, the nucleus accumbens, and the prefrontal cortex (Thompson and Neugebauer, 2019) to combine emotional and cognitive

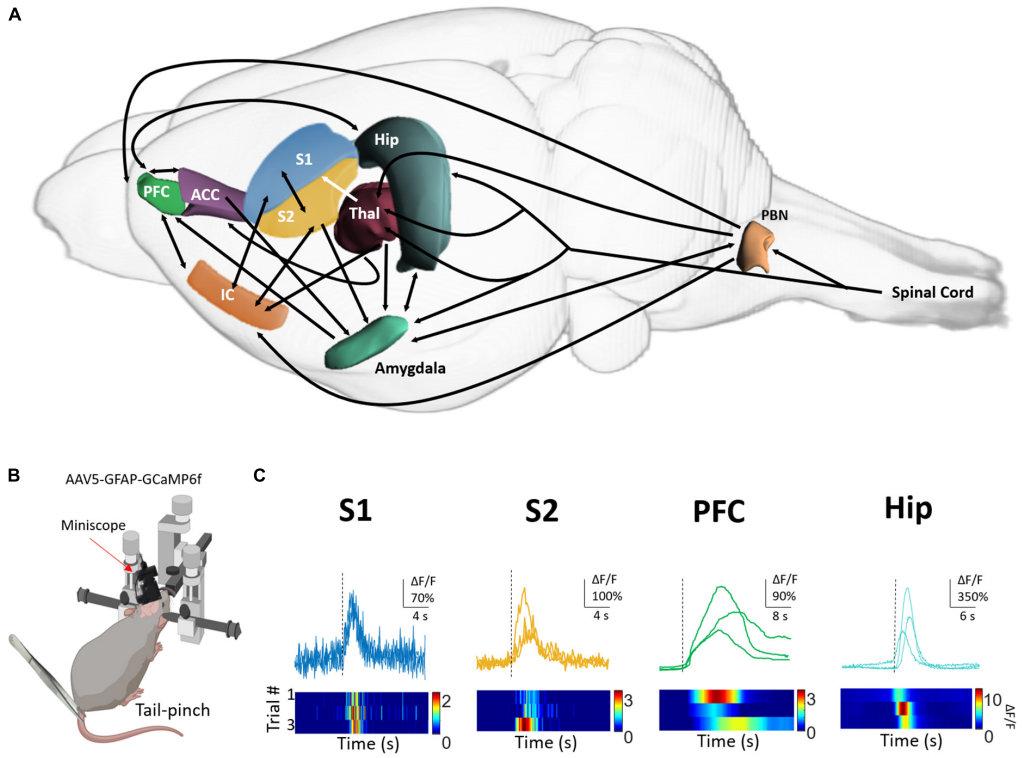


FIGURE 1
Astrocytes respond to tail-pinched stimulation with calcium increases in pain-related brain areas. **(A)** Diagram showing regions (in color) and connections (arrows) of the nervous system that process nociceptive stimuli and are involved in pain perception. PFC, prefrontal cortex; ACC, anterior cingulate area; Hip, Hippocampus; S2, secondary somatosensory cortex; S1, primary somatosensory cortex; IC, insular cortex; Thal, thalamus. **(B)** Tail-pinch was applied with forceps to lightly anesthetized mice (0.5% isoflurane) expressing GCaMP6f in astrocytes located in S1, S2, PFC, and Hip. Representative traces of astrocyte calcium dynamics were monitored with one-photon Miniscope. Tail-pinch stimulation is indicated with a dotted black line **(C)**. The color maps show the calcium responses before, during, and after tail pinch stimulation. Basal fluorescence was considered as the calcium activity observed before the sensory stimulation.

aspects of pain perception. For example, remembering a painful moment can induce pain by itself (Babel, 2017), the novelty of a painful stimulus is determinant for the percept of pain (a process known as a "pain alarm"). The importance of the activity of this pain matrix is also relevant during the expectancy-based modulation of pain in placebo and hypnosis-induced analgesia (Bushnell et al., 2013; Geuter et al., 2013; Keller et al., 2018), as well as during "pain catastrophizing," where the subject tends to magnify a possible threat of painful stimulus, with constant ruminant thoughts associated with pain anxiety and helpless (Quartana et al., 2009).

Individual pain experiences depend on the activation of regions and networks that are spatially distributed through the brain that engage during nociception (Kim and Davis, 2020) commonly referred to as "pain matrix." The integration of pain components depends on the synchronization of neuronal activity in parallel but also complex temporal patterns of brain activity to allow communication in a long-range scale (Hauck et al., 2008; Barardi et al., 2014; Peng and Tang, 2016). Binding oscillatory activity, in particular gamma oscillations, in the pain matrix has been proposed as the mechanism to integrate these components to generate a complete perceptual representation of pain (Sedley and Cunningham, 2013; Ghiani et al., 2021). In particular, nociceptive stimulation can induce by itself, coherent gamma activity (Tan et al., 2019) in sensory (Gross et al., 2007; Kim et al., 2015) and affective-cognitive pathways (Hauck et al., 2017; Liberati et al., 2018; Xiao et al., 2019), that have a direct relation with pain ratings in humans (Ploner et al., 2017; May et al., 2019). Although the neural mechanism related to the generation of gamma oscillations is still under investigation, it has been proposed that the activity of fast-spiking inhibitory interneurons, and neuromodulator systems are essential for gamma rhythm (Kim and Davis, 2020).

Astrocytes respond to nociceptive stimulation

Astrocytes are electrically non-excitable cells that exhibit changes in cytosolic calcium concentration as a form of excitability in response to neuronal activity (Parpura and Verkhratsky, 2012; Zorec et al., 2012;

Bazargani and Attwell, 2016). Calcium events are observed throughout the astrocyte due to the activation of ionotropic calcium-permeable receptors or metabotropic receptors linked to phospholipase C/inositol trisphosphate receptors (IP_3R) (Foskett et al., 2007; Shigetomi et al., 2012; Okubo, 2020; Sherwood et al., 2021). Astrocyte-calcium events are diverse in terms of the spatial extension, they can be localized to some subcellular domains (López-Hidalgo et al., 2017), extended to the entire astrocyte or throughout gap junctions forming an astrocyte network (Hirase et al., 2004; Hoogland et al., 2009).

Astrocytes respond to different sensory modalities in cortical and subcortical regions involved in sensory processing (**Figures 1B,C**) such as the spinal cord, olfactory bulb, visual, auditory, and somatosensory cortex (Petzold et al., 2008; Schummers et al., 2008; Ghosh et al., 2013; Otsu et al., 2015; Sekiguchi et al., 2016; Lopez-Hidalgo et al., 2019; Lines et al., 2020). In these structures, evoked-calcium responses encode the intrinsic properties of the stimulus such as duration, intensity, location, and modality and share the topographical organization of neuronal maps (Ghosh et al., 2013; López-Hidalgo and Schummers, 2014).

In the somatosensory system, astrocytes respond to tactile and noxious stimuli in regions related to nociception and the perception of pain. In a seminal work by Sekiguchi et al. (2016), sensory-evoked calcium activity of dorsal horn astrocytes was not correlated with the duration and intensity of the mechanical stimulus whereas the underlying neuronal activity was positively correlated suggesting that astrocyte activity is not secondary to the activation of neurons of the pain matrix. Astrocytes respond to low and medium pressure-amplitude applied to the tail of freely moving mice, with increases in the frequency of astrocyte calcium events, however, this activity was mostly restricted within the astrocytes. On the other side, high mechanical pressure induced large-scale synchronized calcium activity in astrocytes but not in neurons. Although it is not clear if non-nociceptive or nociceptive fibers are activated during low/medium or high pressures, respectively, this suggests that somatosensory-evoked calcium responses on astrocytes depend on the sensory modality more than the stimulus intensity (Sekiguchi et al., 2016).

In the somatosensory cortex, astrocyte calcium responses encode different parameters of the somatosensory stimuli (Wang et al., 2006; Winship et al., 2007; Stobart et al., 2018; Lines et al., 2020). The stimulation of the peripheral receptive field of S1 (Winship et al., 2007; Stobart et al., 2018) and barrel cortex induced evoked calcium responses in astrocytes that depend on the intensity and the frequency of the stimulus (Wang et al., 2006; Thrane et al., 2012; Stobart et al., 2018). However, as occurs in the spinal cord, low-intensity electrical stimulation (0.4–0.6 mA) induces spatially restricted calcium responses within the astrocytes that do not extend in the field of view (Ghosh et al., 2013; Zhang et al., 2016). However, high-intensity electrical stimulation (1–3 mA) evoked large-scale calcium responses in astrocytes (Gu et al., 2018; Lines et al., 2020) which reinforces the idea that nociceptive information recruited astrocytes networks.

Noxious stimulation (footshock) can induce reliable calcium activity in astrocytes in other brain areas related to pain perception such as the hippocampus (Zhang et al., 2021). Moreover, auditory cortical astrocytes are more responsive to footshock (67%) in comparison to the number of astrocytes activated by the natural sensory stimuli, sound (8%). Here, astrocytes respond with a coordinated large-scale activity that is partially mediated by gap junctions and depends on the nicotinic acetylcholine receptors (Zhang et al., 2021). This supports the relevant role of neuromodulatory systems in mediating nociceptive-evoked responses in astrocytes throughout the nervous system. Furthermore, this evidence highlights the importance of nociception in shaping the activity of brain cortical circuits and hence the behavior. In this case, Zhang et al. (2021) showed that footshock-evoked calcium activity in auditory cortical astrocytes is induced in fear memory and its extinction goes in parallel with the extinction of the behavior.

Data obtained in our laboratory from astrocytes expressing GCaMP6f in anesthetized mice (**Figure 1B**) extend the previous evidence regarding the nociceptive-evoked calcium responses (tail pinch) in pain-related areas. Here, we provide evidence that astrocytes located in S2 and prefrontal cortex also respond to nociceptive stimulation as occurs in astrocytes from S1 and hippocampus (**Figure 1C**). Although there are differences in the amplitude, the delay, and the duration of nociceptive-evoked calcium responses in astrocytes, one common characteristic among these regions is that nociceptive stimulation recruited a large portion of astrocytes (Gu et al., 2018; Lines et al., 2020). In this context, it is tempting to propose that nociceptive stimuli activate coherent activity of astrocytes located in pain-related brain areas acting as an "astrocyte pain matrix" that in conjunction with the neural activity would construct pain perception. Global widespread activation of astrocytes can be induced by the increase in glutamate and K^+ levels in the synaptic space due to synchronic neuronal activity (De Pittà et al., 2008). The activation of astrocytes can lead to gliotransmitter release acting as a paracrine signal to activate nearby astrocytes generating a "domino effect" to spread calcium signaling in large-scale proportions (Pereira and Furlan, 2009; Goldberg et al., 2010; Lallouette et al., 2019). Another possibility is that global astrocyte activity is not secondary to the activation of neurons, instead, nociceptive stimulation could open gap junctions located in the astrocytes to allow the activation of the syncytium. In fact, connexin 30, 43, and 32 are

highly expressed in pain-related brain areas such as the spinal cord, thalamus, S1, prefrontal cortex, and cingulate cortex (Hirase et al., 2004; Houades et al., 2008; Ernst et al., 2011; Zhang et al., 2013; Fujii et al., 2017). In any case, the activity of the coordinated astrocytes could in turn feeds back to the neuronal circuit (Pereira et al., 2013) increasing the synchronization (Makovkin et al., 2022) and hence contributing to the binding of the information and pain perception.

Perspective

Cutaneous tactile stimulation evoked sparser calcium activity in astrocytes (Stobart et al., 2018). This favors local neuron-astrocyte interaction facilitating the location of the stimulus in places with a topographic organization. Instead, nociception informs the nervous system of real or potential damage and produces pain to protect the harmed area. In this scenario, the cortical representation of the location of the stimulus extends to nearby areas developing a state of hyperalgesia around the damaged zone to ensure the protection of the area while the integrity of the tissue is restored. In this context, global astrocytes activity in the cortex could be involved in short and long-term plasticity to ensure this state of hyperalgesia.

Although nociceptive processing occurs unconsciously, emotional, socio-cultural, and cognitive factors (such as attention) are relevant in producing pain perception; therefore, a state of consciousness is necessary to construct the experience of pain. However, an important question in neuroscience that remains to be answered is how pain is created from nociception? How are the elements that compose the percept of pain binding to provide a painful experience? Here, we provide evidence about global and synchronous calcium activity in astrocytes evoked by nociceptive stimulation in areas related to the pain experience. It seems plausible that a coherent activity of astrocytes in the brain and the spinal cord could be important to bind sensory, affective, and cognitive information on a slower time scale forming an astrocyte pain matrix. According to this, Pereira and Furlan (2010) proposed that individual astrocytes could operate as a "local hub" integrating information within their local domain. However, the astrocyte pain matrix would communicate astrocytes within the pain network (Pereira and Furlan, 2010; Lallouette et al., 2014) acting as a "Master Hub" integrating information from several brain areas that could be involved in the process of perception as occurs with pain. Furthermore, because astrocytes can in turn increase the synchronization of neuronal networks (Makovkin et al., 2022), it could be involved in directly modulating gamma activity and hence in the binding process. Another possibility is that astrocytes respond directly to neuromodulators such as acetylcholine, norepinephrine, and dopamine (Jennings et al., 2017; Covelo and Araque, 2018) that are released by nociceptive stimulation (Wahis and Holt, 2021) resulting in changes in the excitability and synchrony of neural networks (Sardinha et al., 2017; Adamsky et al., 2018; Bellot-Saez et al., 2018) that are necessary for the perception of pain.

Brain oscillations in the frequencies of the gamma band have been linked to the perception of pain (Tan et al., 2019; Kim and Davis, 2020). These oscillations are present throughout the brain (Buzsáki and Draguhn, 2004) including regions such as the sensory, prefrontal, insula, and anterior cingulate cortex (Gross et al., 2007; Kim et al., 2015; Hauck et al., 2017; Liberati et al., 2018; Xiao et al., 2019). In 2020, Lines and colleagues showed that sensory stimulation in the paw induces an increase in gamma activity in the primary somatosensory cortex that correlates with the intensity of the stimulus (Lines et al., 2020). In parallel, sensory stimulation induces global calcium responses in cortical astrocytes with a delay in the order of seconds. Moreover, the manipulation of calcium activity in astrocytes was inversely correlated with gamma activity demonstrating that activation of astrocytes with DREADDs is sufficient to decrease gamma oscillations. On the other side, Lee et al. (2014) observed during a spatial memory task (Y maze), that calcium elevations in hippocampal astrocytes precede the onset of gamma activity in the hippocampus. This could imply a different role of astrocytes regulating gamma activity depending on the level of arousal (anesthetized vs. awake), brain structures (somatosensory cortex vs. hippocampus) as well as underlying neural activity (natural activity in behaving animal vs. sensory stimulation).

High-order thalamic nucleus plays an important role in making consciousness of an experience thanks to the connections with cortical and subcortical regions, where it acts as a sensory activity filter and synchronized neuronal activity (Ward, 2011). Although it is unknown if thalamic astrocytes respond to nociceptive stimulation with calcium activity and the extension of these responses, thalamic astrocytes regulate the sensory acuity of mice in a tactile-discriminatory task by releasing GABA (Kwak et al., 2020). In this scenario, it would be interesting to analyze if GABA release by astrocytes could set an inhibitory tone that regulates gamma activity as occurs with inhibitory interneurons and hence modulate gamma activity and its impact on perceptions.

There are still fundamental questions in neuroscience that are still poorly understood despite all the technical advances and progress in neurobiology such as the process of creating perception from sensations. Thus, is tempting to propose that astrocytes are well equipped, located, and connected to regulate, in parallel, multiple neuronal networks allowing individual pain experiences. However, further work integrating astrocytes in this field is required.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This animal study was reviewed and approved by Instituto de Neurobiología at Universidad Nacional Autónoma de México (No. 043).

Author contributions

All authors drafted, edited, and approved the final version of the manuscript.

Funding

This work was supported by grants from Consejo Nacional de Ciencia y Tecnología (CONACyT) Ciencia de Frontera 171874 (to ML-H), PAPIIT-DGAPA IA208120 (to ML-H), and PAPIIT-DGAPA IA208022 (to ML-H). AR-V and IC-V were doctoral students from the Programa de Doctorado en Ciencias Biomédicas, from Universidad Nacional Autónoma de México, and received CONACyT Fellowships 788790 and 786125. FH-R was master student from the Programa de Maestría en Ciencias (Neurobiología) from UNAM and received CONACyT Fellowship 778405.

Acknowledgments

We thank Dr. Rafael Olivares Moreno and Dr. Nuri Aranda for providing technical assistance.

Conflict of interest

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

Publisher's note

References

Adamsky, A., Kol, A., Kreisel, T., Doron, A., Ozeri-Engelhard, N., Melcer, T., et al. (2018). Astrocytic activation generates de novo neuronal potentiation and memory enhancement. *Cell* 174, 59–71.e14. doi: 10.1016/j.cell.2018.05.002

Angenstein, F., Krautwald, K., Wetzel, W., and Scheich, H. (2013). Perforant pathway stimulation as a conditioned stimulus for active avoidance learning triggers BOLD responses in various target regions of the hippocampus: A combined fMRI and electrophysiological study. *NeuroImage* 75, 213–227. doi: 10.1016/j.neuroimage.2013.03.007

Babel, P. (2017). The Influence of State and Trait Anxiety on the Memory of Pain. *Pain Med.* 18, 2340–2349. doi: 10.1093/pm/pnw354

Backonja, M. (1996). Primary somatosensory cortex and pain perception. *Pain Forum* 5, 174–180. doi: 10.1016/s1082-3174(96)80026-2

Barardi, A., Sancristóbal, B., and Garcia-Ojalvo, J. (2014). Phase-Coherence Transitions and Communication in the Gamma Range between Delay-Coupled Neuronal Populations. *PLoS Comput. Biol.* 10:e1003723. doi: 10.1371/journal.pcbi.1003723

Bazargani, N., and Attwell, D. (2016). Astrocyte calcium signaling: The third wave. *Nat. Neurosci.* 19, 182–189. doi: 10.1038/nn.4201

Bellot-Saez, A., Cohen, G., van Schaik, A., Ooi, L. W., Morley, J., and Buskila, Y. (2018). Astrocytic modulation of cortical oscillations. *Sci. Rep.* 8:11565. doi: 10.1038/s41598-018-30003-w

Bernard, J. F., and Besson, J. M. (1990). The spino(trigemino)pontoamygdaloid pathway: Electrophysiological evidence for an involvement in pain processes. *J. Neurophysiol.* 63, 473–490. doi: 10.1152/jn.1990.63.3.473

Bester, H., Matsumoto, N., Besson, J. M., and Bernard, J. F. (1997). Further evidence for the involvement of the spinoparabrachial pathway in nociceptive processes: A c-Fos study in the rat. *J. Comp. Neurol.* 383, 439–458.

Bourgeais, L., Monconduit, L., Villanueva, L., and Bernard, J. F. (2001). Parabrachial internal lateral neurons convey nociceptive messages from the deep laminas of the dorsal horn to the intralaminar thalamus. *J. Neurosci.* 21, 2159–2165. doi: 10.1523/JNEUROSCI.21-06-02159.2001

Bufalari, I., Aprile, T., Avenanti, A., Di Russo, F., and Aglioti, S. M. (2007). Empathy for pain and touch in the human somatosensory cortex. *Cereb. Cortex* 17, 2553–2561. doi: 10.1093/cercor/bhl161

Burstein, R., and Potrebic, S. (1993). Retrograde labeling of neurons in the spinal cord that project directly to the amygdala or the orbital cortex in the rat. *J. Comp. Neurol.* 335, 469–485. doi: 10.1002/cne.903350402

Bushnell, M. C., Ceko, M., and Low, L. A. (2013). Cognitive and emotional control of pain and its disruption in chronic pain. *Nat. Rev. Neurosci.* 14, 502–511. doi: 10.1038/nrn3516

Bushnell, M. C., Duncan, G. H., Hofbauer, R. K., Ha, B., Chen, J. I., and Carrier, B. (1999). Pain perception: Is there a role for primary somatosensory cortex?. *Proc. Natl. Acad. Sci. U. S. A.* 96, 7705–7709. doi: 10.1073/pnas.96.14.7705

Buzsáki, G., and Draguhn, A. (2004). Neuronal oscillations in cortical networks. *Science* 304, 1926–1929. doi: 10.1126/science.1099745

Chiang, M. C., Nguyen, E. K., Canto-Bustos, M., Papale, A. E., Oswald, A. M., and Ross, S. E. (2020). Divergent Neural Pathways Emanating from the Lateral Parabrachial Nucleus Mediate Distinct Components of the Pain Response. *Neuron* 106, 927–939.e5. doi: 10.1016/j.neuron.2020.03.014

Cliffer, K. D., Burstein, R., and Giesler, G. J. (1991). Distributions of spinothalamic, spinohypothalamic, and spinotelencephalic fibers revealed by

anterograde transport of PHA-L in rats. *J. Neurosci.* 11, 852–868. doi: 10.1523/JNEUROSCI.11-03-00852.1991

Coghill, R. C. (2020). The Distributed Nociceptive System: A Framework for Understanding Pain. *Trends Neurosci.* 43, 780–794. doi: 10.1016/j.tins.2020.07.004

Coghill, R. C., Sang, C. N., Maisog, J. M., and Iadarola, M. J. (1999). Pain Intensity Processing Within the Human Brain: A Bilateral, Distributed Mechanism. *J. Neurophysiol.* 82, 1934–1943. doi: 10.1152/jn.1999.82.4.1934

Covelo, A., and Araque, A. (2018). Neuronal activity determines distinct gliotransmitter release from a single astrocyte. *Elife* 7:e32237. doi: 10.7554/eLife.32237

Davis, K. D., Kwan, C. L., Crawley, A. P., and Mikulis, D. J. (1998). Functional MRI Study of Thalamic and Cortical Activations Evoked by Cutaneous Heat, Cold, and Tactile Stimuli. *J. Neurophysiol.* 80, 1533–1546. doi: 10.1152/jn.1998.80.3.1533

De Pittà, M., Volman, V., Levine, H., Pioggia, G., De Rossi, D., and Ben-Jacob, E. (2008). Coexistence of amplitude and frequency modulations in intracellular calcium dynamics. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* 77:030903. doi: 10.1103/PhysRevE.77.030903

Dubin, A. E., and Patapoutian, A. (2010). Nociceptors: The sensors of the pain pathway. *J. Clin. Investig.* 120, 3760–3772. doi: 10.1172/JCI42843

Ernst, C., Nagy, C., Kim, S., Yang, J. P., Deng, X., Hellstrom, I. C., et al. (2011). Dysfunction of astrocyte connexins 30 and 43 in dorsal lateral prefrontal cortex of suicide completers. *Biol. Psychiatry* 70, 312–319. doi: 10.1016/j.biopsych.2011.03.038

Foskett, J. K., White, C., Cheung, K. H., and Mak, D. O. (2007). Inositol trisphosphate receptor Ca2+ release channels. *Physiol. Rev.* 87, 593–658. doi: 10.1152/physrev.00035.2006

Fujii, Y., Maekawa, S., and Morita, M. (2017). Astrocyte calcium waves propagate proximally by gap junction and distally by extracellular diffusion of ATP released from volume-regulated anion channels. *Sci. Rep.* 7:13115. doi: 10.1038/s41598-017-13243-0

Gao, Y. J., Ren, W. H., Zhang, Y. Q., and Zhao, Z. Q. (2004). The anterior cingulate cortex and amygdala contribute to pain- and fear-conditioned place avoidance in rats. *Pain* 110, 343–353. doi: 10.1016/j.pain.2004.04.030

Gauriau, C., and Bernard, J. F. (2002). Pain pathways and parabrachial circuits in the rat. *Exp. Physiol.* 87, 251–258. doi: 10.1113/eph8702357

Geuter, S., Eippert, F., Hindi Attar, C., and Büchel, C. (2013). Cortical and subcortical responses to high and low effective placebo treatments. *NeuroImage* 67, 227–236. doi: 10.1016/j.neuroimage.2012.11.029

Ghiani, A., Maniglia, M., Battaglini, L., Melcher, D., and Ronconi, L. (2021). Binding Mechanisms in Visual Perception and Their Link With Neural Oscillations: A Review of Evidence From tACS. *Front. Psychol.* 12:643677. doi: 10.3389/fpsyg.2021.643677

Ghosh, A., Wyss, M. T., and Weber, B. (2013). Somatotopic astrocytic activity in the somatosensory cortex. *Glia* 61, 601–610. doi: 10.1002/glia.22458

Giesler, G. J. Jr., Yezierski, R. P., Gerhart, K. D., and Willis, W. D. (1981). Spinothalamic tract neurons that project to medial and/or lateral thalamic nuclei: Evidence for a physiologically novel population of spinal cord neurons. *J. Neurophysiol.* 46, 1285–1308. doi: 10.1152/jn.1981.46.6.1285

Goldberg, M., De Pittà, M., Volman, V., Berry, H., and Ben-Jacob, E. (2010). Nonlinear gap junctions enable long-distance propagation of pulsating calcium waves in astrocyte networks. *PLoS Comput. Biol.* 6:e1000909. doi: 10.1371/journal.pcbi.1000909

Gross, J., Schnitzler, A., Timmermann, L., and Ploner, M. (2007). Gamma oscillations in human primary somatosensory cortex reflect pain perception. *PLoS Biol.* 5:e133. doi: 10.1371/journal.pbio.0050133

Gu, X., Chen, W., Volkow, N. D., Koretsky, A. P., Du, C., and Pan, Y. (2018). Synchronized Astrocytic Ca2+ Responses in Neurovascular Coupling during Somatosensory Stimulation and for the Resting State. *Cell Rep.* 23, 3878–3890. doi: 10.1016/j.celrep.2018.05.091

Hauck, M., Lorenz, J., and Engel, A. K. (2008). Role of synchronized oscillatory brain activity for human pain perception. *Rev. Neurosci.* 19, 441–450. doi: 10.1515/revneuro.2008.19.6.441

Hauck, M., Schröder, S., Meyer-Hamme, G., Lorenz, J., Friedrichs, S., Nolte, G., et al. (2017). Acupuncture analgesia involves modulation of pain-induced gamma oscillations and cortical network connectivity. *Sci. Rep.* 7:16307. doi: 10.1038/s41598-017-13633-4

Hirase, H., Qian, L., Barthó, P., and Buzsáki, G. (2004). Calcium Dynamics of Cortical Astrocytic Networks In Vivo. *PLoS Biol.* 2:e96. doi: 10.1371/journal.pbio.0020096

Hoogland, T. M., Kuhn, B., Göbel, W., Huang, W., Nakai, J., Helmchen, F., et al. (2009). Radially expanding transglial calcium waves in the intact cerebellum. *Proc. Natl. Acad. Sci. U. S. A.* 106, 3496–3501. doi: 10.1073/pnas.0809269106

Houades, V., Koulakoff, A., Ezan, P., Seif, I., and Giaume, C. (2008). Gap junction-mediated astrocytic networks in the mouse barrel cortex. *J. Neurosci.* 28, 5207–5217. doi: 10.1523/JNEUROSCI.5100-07.2008

Iwamoto, S., Tamura, M., Sasaki, A., and Nawano, M. (2021). Dynamics of neuronal oscillations underlying nociceptive response in the mouse primary somatosensory cortex. *Sci. Rep.* 11:1667. doi: 10.1038/s41598-021-81067-0

Jennings, A., Tyurikova, O., Bard, L., Zheng, K., Semyanov, A., Henneberger, C., et al. (2017). Dopamine elevates and lowers astroglial Ca^{2+} through distinct pathways depending on local synaptic circuitry. *Glia* 65, 447–459. doi: 10.1002/glia.23103

Jin, Q. Q., Wu, G. Q., Peng, W. W., Xia, X. L., Hu, L., and Iannetti, G. D. (2018). Somatotopic Representation of Second Pain in the Primary Somatosensory Cortex of Humans and Rodents. *J. Neurosci.* 38, 5538–5550. doi: 10.1523/JNEUROSCI.3654-17.2018

Keller, A., Akintola, T., and Colloca, L. (2018). Placebo Analgesia in Rodents: Current and Future Research. *Int. Rev. Neurobiol.* 138, 1–15. doi: 10.1016/bs.irn.2018.02.001

Kenshalo, D. R., Iwata, K., Sholas, M., and Thomas, D. A. (2000). Response properties and organization of nociceptive neurons in area 1 of monkey primary somatosensory cortex. *J. Neurophysiol.* 84, 719–729. doi: 10.1152/jn.2000.84.2.719

Kim, J. A., and Davis, K. D. (2020). Neural Oscillations: Understanding a Neural Code of Pain. *Neuroscientist* 27, 544–570. doi: 10.1177/1073858420958629

Kim, J. H., Chien, J. H., Liu, C. C., and Lenz, F. A. (2015). Painful cutaneous laser stimuli induce event-related gamma-band activity in the lateral thalamus of humans. *J. Neurophysiol.* 113, 1564–1573. doi: 10.1152/jn.00778.2014

Kim, J. H., Choi, S. H., Jang, J. H., Lee, D. H., Lee, K. J., Lee, W. J., et al. (2017). Impaired insula functional connectivity associated with persistent pain perception in patients with complex regional pain syndrome. *PLoS One* 12:e0180479. doi: 10.1371/journal.pone.0180479

Kim, S. W., Kim, M., and Shin, H. S. (2021). Affective empathy and prosocial behavior in rodents. *Curr. Opin. Neurobiol.* 68, 181–189. doi: 10.1016/j.conb.2021.05.002

Kim, Y. R., Kim, C. E., Yoon, H., Kim, S. K., and Kim, S. J. (2019). S1 Employs Feature-Dependent Differential Selectivity of Single Cells and Distributed Patterns of Populations to Encode Mechanosensations. *Front. Cell. Neurosci.* 13:132. doi: 10.3389/fncel.2019.00132

Kwak, H., Koh, W., Kim, S., Song, K., Shin, J. I., Lee, J. M., et al. (2020). Astrocytes Control Sensory Acuity via Tonic Inhibition in the Thalamus. *Neuron* 108, 691–706. doi: 10.1016/j.neuron.2020.08.013

Lallouette, J., De Pittà, M., Ben-Jacob, E., and Berry, H. (2014). Sparse short-distance connections enhance calcium wave propagation in a 3D model of astrocyte networks. *Front. Comput. Neurosci.* 8:45. doi: 10.3389/fncom.2014.00045

Lallouette, J., De Pittà, M., and Berry, H. (2019). "Astrocyte networks and intercellular calcium propagation," in *Computational glioscience*, eds M. De Pittà and H. Berry (Cham: Springer), 177–210.

LeDoux, J. E., Cicchetti, P., Xagoraris, A., and Romanski, L. M. (1990). The lateral amygdaloid nucleus: Sensory interface of the amygdala in fear conditioning. *J. Neurosci.* 10, 1062–1069. doi: 10.1523/JNEUROSCI.10-04-01062.1990

Lee, H. S., Ghetti, A., Pinto-Duarte, A., Wang, X., Dziewczapolski, G., Galimi, F., et al. (2014). Astrocytes contribute to gamma oscillations and recognition memory. *Proc. Natl. Acad. Sci. U. S. A.* 111, E3343–E3352. doi: 10.1073/pnas.1410893111

Liao, C. C., and Yen, C. T. (2008). Functional connectivity of the secondary somatosensory cortex of the rat. *Anat. Rec.* 291, 960–973. doi: 10.1002/ar.20696

Liberati, G., Klöcker, A., Algoet, M., Mulders, D., Maia Safronova, M., Ferrao Santos, S., et al. (2018). Gamma-Band Oscillations Preferential for Nociception can be Recorded in the Human Insula. *Cereb. Cortex* 28, 3650–3664. doi: 10.1093/cercor/bhx237

Lines, J., Martin, E. D., Kofuji, P., Aguilar, J., and Araque, A. (2020). Astrocytes modulate sensory-evoked neuronal network activity. *Nat. Commun.* 11:3689. doi: 10.1038/s41467-020-17536-3

Loeser, J. D., and Treede, R. D. (2008). The Kyoto protocol of IASP Basic Pain Terminology. *Pain* 137, 473–477. doi: 10.1016/j.pain.2008.04.025

López-Hidalgo, M., Kellner, V., and Schummers, J. (2017). Astrocyte Calcium Responses to Sensory Input: Influence of Circuit Organization and Experimental Factors. *Front. Neural Circuits* 11:16. doi: 10.3389/fncir.2017.00016

Lopez-Hidalgo, M., Kellner, V., and Schummers, J. (2019). Astrocyte subdomains respond independently to neural activity in vivo. *bioRxiv* [Preprint]. doi: 10.1101/675769

López-Hidalgo, M., and Schummers, J. (2014). Cortical maps: A role for astrocytes? *Curr. Opin. Neurobiol.* 24, 176–189. doi: 10.1016/j.conb.2013.11.001

Maihöfner, C., Herzner, B., and Otto Handwerker, H. (2006). Secondary somatosensory cortex is important for the sensory-discriminative dimension of pain: A functional MRI study. *Eur. J. Neurosci.* 23, 1377–1383. doi: 10.1111/j.1460-9568.2006.04632.x

Makovkin, S., Kozinov, E., Ivanchenko, M., and Gordleeva, S. (2022). Controlling synchronization of gamma oscillations by astrocytic modulation in a model hippocampal neural network. *Sci. Rep.* 12:6970. doi: 10.1038/s41598-022-10649-3

Mancini, F., Haggard, P., Iannetti, G. D., Longo, M. R., and Sereno, M. I. (2012). Fine-grained nociceptive maps in primary somatosensory cortex. *J. Neurosci.* 32, 17155–17162. doi: 10.1523/JNEUROSCI.3059-12.2012

May, E. S., Nickel, M. M., Ta Dinh, S., Tiemann, L., Heitmann, H., Voth, I., et al. (2019). Prefrontal gamma oscillations reflect ongoing pain intensity in chronic back pain patients. *Hum. Brain Mapp.* 40, 293–305. doi: 10.1002/hbm.24373

Millan, M. J. (1999). The induction of pain: An integrative review. *Prog. Neurobiol.* 57, 1–164.

Mokhtari, T., Tu, Y., and Hu, L. (2019). Involvement of the hippocampus in chronic pain and depression. *Sci. Adv.* 5, 288–298. doi: 10.26599/bsa.2019.9050025

Monconduit, L., Lopez-Avila, A., Molat, J., Chalus, M., and Villanueva, L. (2006). Corticofugal Output from the Primary Somatosensory Cortex Selectively Modulates Innocuous and Noxious Inputs in the Rat Spinothalamic System. *J. Neurosci.* 26, 8441–8450. doi: 10.1523/jneurosci.1293-06.2006

Okubo, Y. (2020). Astrocytic Ca2+ signaling mediated by the endoplasmic reticulum in health and disease. *J. Pharmacol. Sci.* 144, 83–88. doi: 10.1016/j.jphs.2020.07.006

Otsu, Y., Couchman, K., Lyons, D. G., Collot, M., Agarwal, A., Mallet, J. M., et al. (2015). Calcium dynamics in astrocyte processes during neurovascular coupling. *Nat. Neurosci.* 18, 210–218. doi: 10.1038/nn.3906

Parpura, V., and Verkhratsky, A. (2012). The astrocyte excitability brief: From receptors to gliotransmission. *Neurochem. Int.* 61, 610–621. doi: 10.1016/j.neuint.2011.12.001

Peng, W., and Tang, D. (2016). Pain Related Cortical Oscillations: Methodological Advances and Potential Applications. *Front. Comput. Neurosci.* 10:9. doi: 10.3389/fncom.2016.00009

Pereira, A. Jr., Dos Santos, R. P., and Barros, R. F. (2013). The calcium wave model of the perception-action cycle: Evidence from semantic relevance in memory experiments. *Front. Psychol.* 4:252. doi: 10.3389/fpsyg.2013.00252

Pereira, A. Jr., and Furlan, F. A. (2009). On the role of synchrony for neuron-astrocyte interactions and perceptual conscious processing. *J. Biol. Phys.* 35, 465–480. doi: 10.1007/s10867-009-9147-y

Pereira, A. Jr., and Furlan, F. A. (2010). Astrocytes and human cognition: Modeling information integration and modulation of neuronal activity. *Prog. Neurobiol.* 92, 405–420. doi: 10.1016/j.pneurobio.2010.07.001

Petrovic, P., Petersson, K. M., Ghatan, P. H., Stone-Elander, S., and Ingvar, M. (2000). Pain-related cerebral activation is altered by a distracting cognitive task. *Pain* 85, 19–30. doi: 10.1016/s0304-3959(99)00232-8

Petzold, G. C., Albeanu, D. F., Sato, T. F., and Murthy, V. N. (2008). Coupling of neural activity to blood flow in olfactory glomeruli is mediated by astrocytic pathways. *Neuron* 58, 897–910. doi: 10.1016/j.neuron.2008.04.029

Ploner, M., Sorg, C., and Gross, J. (2017). Brain Rhythms of Pain. *Trends Cogn. Sci.* 21, 100–110. doi: 10.1016/j.tics.2016.12.001

Quartana, P. J., Campbell, C. M., and Edwards, R. R. (2009). Pain catastrophizing: A critical review. *Expert Rev. Neurother.* 9, 745–758. doi: 10.1586/ern.09.34

Raja, S. N., Carr, D. B., Cohen, M., Finnerup, N. B., Flor, H., Gibson, S., et al. (2020). The revised International Association for the Study of Pain definition of pain: Concepts, challenges, and compromises. *Pain* 161, 1976–1982. doi: 10.1097/j.pain.0000000000001939

Sardinha, V. M., Guerra-Gomes, S., Caetano, I., Tavares, G., Martins, M., Reis, J. S., et al. (2017). Astrocytic signaling supports hippocampal-prefrontal theta synchronization and cognitive function. *Glia* 65, 1944–1960. doi: 10.1002/glia.23205

Schaible, H. G., and Grubb, B. D. (1993). Afferent and spinal mechanisms of joint pain. *Pain* 55, 5–54.

Schummers, J., Yu, H., and Sur, M. (2008). Tuned responses of astrocytes and their influence on hemodynamic signals in the visual cortex. *Science* 320, 1638–1643. doi: 10.1126/science.1156120

Sedley, W., and Cunningham, M. (2013). Do cortical gamma oscillations promote or suppress perception? An under-asked question with an over-assumed answer. *Front. Hum. Neurosci.* 7:595. doi: 10.3389/fnhum.2013.00595

Sekiguchi, K. J., Shekhtmeyster, P., Merten, K., Arena, A., Cook, D., Hoffman, E., et al. (2016). Imaging large-scale cellular activity in spinal cord of freely behaving mice. *Nat. Commun.* 7:11450. doi: 10.1038/ncomms11450

Sherwood, M. W., Arizono, M., Panatier, A., Mikoshiba, K., and Oliet, S. (2021). Astrocytic IP3Rs: Beyond IP3R2. *Front. Cell. Neurosci.* 15:695817. doi: 10.3389/fncel.2021.695817

Shi, C., and Davis, M. (1999). Pain pathways involved in fear conditioning measured with fear-potentiated startle: Lesion studies. *J. Neurosci.* 19, 420–430. doi: 10.1523/JNEUROSCI.19-01-00420.1999

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

Stobart, J. L., Ferrari, K. D., Barrett, M., Stobart, M. J., Looser, Z. J., Saab, A. S., et al. (2018). Long-term In Vivo Calcium Imaging of Astrocytes Reveals Distinct Cellular Compartment Responses to Sensory Stimulation. *Cereb. Cortex* 28, 184–198. doi: 10.1093/cercor/bhw366

Straube, T., Lipka, J., Sauer, A., Mothes-Lasch, M., and Miltner, W. H. (2011). Amygdala activation to threat under attentional load in individuals with anxiety disorder. *Biol. Mood Anxiety Disord.* 1:12. doi: 10.1186/2045-5380-1-12

Tan, L., Oswald, M., Heinl, C., Retana Romero, O., Kaushalya, S., Monyer, H., et al. (2019). Gamma oscillations in somatosensory cortex recruit prefrontal and descending serotonergic pathways in aversion and nociception. *Nat. Commun.* 10:983. doi: 10.1038/s41467-019-08873-z

Thompson, J. M., and Neugebauer, V. (2019). Cortico-limbic pain mechanisms. *Neurosci. Lett.* 702, 15–23. doi: 10.1016/j.neulet.2018.11.037

Thrane, A. S., Rangroo Thrane, V., Zeppenfeld, D., Lou, N., Xu, Q., Nagelhus, E. A., et al. (2012). General anesthesia selectively disrupts astrocyte calcium signaling in the awake mouse cortex. *Proc. Natl. Acad. Sci. U. S. A.* 109, 18974–18979. doi: 10.1073/pnas.1209448109

Tölle, T. R., Kaufmann, T., Siessmeier, T., Lautenbacher, S., Berthele, A., Munz, F., et al. (1999). Region-Specific Encoding of Sensory and Affective Components of Pain in the Human Brain: A Positron Emission Tomography Correlation Analysis. *Ann. Neurol.* 45, 40–47. doi: 10.1002/1531-8249(199901)45:1<40::aid-art8<3.0.co;2-l

Wahis, J., and Holt, M. G. (2021). Astrocytes, Noradrenaline, α1-Adrenoreceptors, and Neuromodulation: Evidence and Unanswered Questions. *Front. Cell. Neurosci.* 15:645691. doi: 10.3389/fncel.2021.645691

Wang, X., Lou, N., Xu, Q., Tian, G. F., Peng, W. G., Han, X., et al. (2006). Astrocytic Ca2+ signaling evoked by sensory stimulation in vivo. *Nat. Neurosci.* 9, 816–823. doi: 10.1038/nn1703

Ward, L. M. (2011). The thalamic dynamic core theory of conscious experience. *Conscious. Cogn.* 20, 464–486. doi: 10.1016/j.concog.2011.01.007

Wilcox, C. E., Mayer, A. R., Teshiba, T. M., Ling, J., Smith, B. W., Wilcox, G. L., et al. (2015). The Subjective Experience of Pain: An FMRI Study of Percept-Related Models and Functional Connectivity. *Pain Med.* 16, 2121–2133. doi: 10.1111/pme.12785

Willis, W. D. Jr. (1985). Pain pathways in the primate. *Prog. Clin. Biol. Res.* 176, 117–133.

Willis, W. D., and Westlund, K. N. (1997). Neuroanatomy of the pain system and of the pathways that modulate pain. *J. Clin. Neurophysiol.* 14, 2–31. doi: 10.1097/00004691-199701000-00002

Winship, I. R., Plaa, N., and Murphy, T. H. (2007). Rapid astrocyte calcium signals correlate with neuronal activity and onset of the hemodynamic response in vivo. *J. Neurosci.* 27, 6268–6272. doi: 10.1523/JNEUROSCI.4801-06.2007

Xiao, Z., Martinez, E., Kulkarni, P. M., Zhang, Q., Hou, Q., Rosenberg, D., et al. (2019). Cortical Pain Processing in the Rat Anterior Cingulate Cortex and

Primary Somatosensory Cortex. *Front. Cell. Neurosci.* 13:165. doi: 10.3389/fncel.2019.00165

Yen, C. T., and Lu, P. L. (2013). Thalamus and pain. *Acta Anaesthesiol. Taiwan*. 51, 73–80. doi: 10.1016/j.aat.2013.06.011

Zhang, K., Chen, C., Yang, Z., He, W., Liao, X., Ma, Q., et al. (2016). Sensory Response of Transplanted Astrocytes in Adult Mammalian Cortex In Vivo. *Cereb. cortex* 26, 3690–3704. doi: 10.1093/cercor/bhw213

Zhang, K., Förster, R., He, W., Liao, X., Li, J., Yang, C., et al. (2021). Fear learning induces α7-nicotinic acetylcholine receptor-mediated astrocytic responsiveness that is required for memory persistence. *Nat. Neurosci*. 24, 1686–1698. doi: 10.1038/s41593-021-00949-8

Zhang, W., and Bruno, R. M. (2019). High-order thalamic inputs to primary somatosensory cortex are stronger and longer lasting than cortical inputs. *eLife* 8:e44158. doi: 10.7554/eLife.44158

Zhang, X., Cao, B., Wang, J., Liu, J., Tung, V. O. V., Lam, P. K. S., et al. (2013). Neurotoxicity and Reactive Astrogliosis in the Anterior Cingulate Cortex in Acute Ciguatera Poisoning. *Neuromolecular Med*. 15, 310–323. doi: 10.1007/s12017-013-8220-7

Zorec, R., Araque, A., Carmignoto, G., Haydon, P. G., Verkhratsky, A., and Parpura, V. (2012). Astroglial excitability and gliotransmission: An appraisal of Ca2+ as a signalling route. *ASN Neuro* 4:e00080. doi: 10.1042/AN20110061

frontiers | Frontiers in Cellular Neuroscience

TYPE Mini Review
PUBLISHED 20 September 2022
DOI 10.3389/fncel.2022.987212

OPEN ACCESS

EDITED BY
Adrian Rodriguez-Contreras,
Northwestern University, United States

REVIEWED BY
Barbara Monti,
University of Bologna, Italy

*CORRESPONDENCE
Octavio García
ogarciag@unam.mx

SPECIALTY SECTION
This article was submitted to Non-Neuronal Cells, a section of the journal Frontiers in Cellular Neuroscience

RECEIVED 05 July 2022
ACCEPTED 31 August 2022
PUBLISHED 20 September 2022

CITATION
García O and Flores-Aguilar L (2022) Astroglial and microglial pathology in Down syndrome: Focus on Alzheimer's disease.
Front. Cell. Neurosci. 16:987212.
doi: 10.3389/fncel.2022.987212

Astroglial and microglial pathology in Down syndrome: Focus on Alzheimer's disease

Octavio García[1]* and Lisi Flores-Aguilar[2]

[1]Facultad de Psicología, Unidad de Investigación en Psicobiología y Neurociencias, Universidad Nacional Autónoma de México, Ciudad de México, Mexico, [2]Department of Pathology and Laboratory Medicine, University of California, Irvine, Irvine, CA, United States

Down syndrome (DS) arises from the triplication of human chromosome 21 and is considered the most common genetic cause of intellectual disability. Glial cells, specifically astroglia and microglia, display pathological alterations that might contribute to DS neuropathological alterations. Further, in middle adulthood, people with DS develop clinical symptoms associated with premature aging and Alzheimer's disease (AD). Overexpression of the amyloid precursor protein (*APP*) gene, encoded on chromosome 21, leads to increased amyloid-β (Aβ) levels and subsequent formation of Aβ plaques in the brains of individuals with DS. Amyloid-β deposition might contribute to astroglial and microglial reactivity, leading to neurotoxic effects and elevated secretion of inflammatory mediators. This review discusses evidence of astroglial and microglial alterations that might be associated with the AD continuum in DS.

KEYWORDS

Down syndrome, Alzheimer's disease, astrocytes, microglia, β-amyloid, neuroinflammation, aging, cytokines

Introduction

Down syndrome (DS) or trisomy 21 is the most common autosomal aneuploidy in human births, and it is the single most common genetic cause of intellectual disability (Antonarakis, 2017). Individuals with DS display different phenotypic characteristics, including craniofacial alterations, skeletal anomalies, hypotonia, congenital heart disease, gastrointestinal anomalies, seizures, sleep apnea, deficits in immune response, and leukemia (Antonarakis et al., 2020). Although these characteristics can lead to premature death, in recent years, people with DS have increased life expectancy, gradually approaching that of the general population (Glasson et al., 2016; Carr and Collins, 2018). However, upon reaching middle adulthood, people with DS commonly experience clinical symptoms associated with older age (Gensous et al., 2020), such as premature menopause, skin wrinkles, presbycusis, alopecia, premature graying of the hair, congestive heart failure, hypogonadism, hypothyroidism, osteoporosis atherosclerosis, diabetes, hypercholesterolemia, hypertension, and visual and auditory decline. In addition to the physiological manifestations of premature aging, people with DS present early cognitive impairment characterized by a decrease in memory skills (Godfrey and Lee, 2018), language skills (Vicari et al., 1994), social communication, motor skills, personal life, and community life skills (Hawkins et al., 2003). By the age

of ∼50 years, individuals with full triplication of chromosome 21 develop Alzheimer's disease (AD) dementia (Holland et al., 1998; Margallo-Lana et al., 2007; Zigman, 2013; Covelli et al., 2016; Fortea et al., 2021; Iulita et al., 2022) with an incidence of 88–100% in those older than 65 years (McCarron et al., 2017). The development of AD in DS could be associated with an overexpression of several genes located on chromosome 21, including the amyloid precursor protein (*APP)* gene, which encodes the amyloid-beta precursor protein (AβPP) (Wiseman et al., 2015), leading to an increase in Aβ production. Adults with DS over age 40 display overt AD neuropathology characterized by the deposition of extracellular amyloid plaques and the formation of neurofibrillary tangles (Wisniewski et al., 1985; Lemere et al., 1996; Mori et al., 2002; Davidson et al., 2018; Lott and Head, 2019).

Postmortem and *in vivo* studies in DS brains show pathological changes associated with AD, including brain atrophy (Mann and Esiri, 1989; Sadowski et al., 1999; Annus et al., 2016; Head et al., 2016), endosomal/lysosomal abnormalities (Cataldo et al., 2000), degeneration of cholinergic basal forebrain neurons, neurotrophin deregulation (Iulita et al., 2014), oxidative stress (Busciglio and Yanker, 1995), cerebral amyloid angiopathy (Carmona-Iragui et al., 2017; Head et al., 2017), white matter lesions (Lao et al., 2020), and neuroinflammation (Wilcock et al., 2015; Flores-Aguilar et al., 2020). Several studies have demonstrated the importance of inflammatory processes in AD pathogenesis (Liu et al., 2022), suggesting that Aβ could induce glial cell dysfunction (Uddin and Lim, 2022) and subsequent neurodegeneration. This review describes the pathological changes in astrocytes and microglia of individuals with DS and we discuss the implications of glial dysfunction in the AD continuum in DS.

Astrocytes

Astrocytes constitute the largest glial cell population in the central nervous system (CNS). Astrocytes can be as heterogeneous as neurons at the genetic, physiological, and functional levels (Torres-Ceja and Olsen, 2022). Astrocytes play an essential role in the development and maintenance of the CNS. They provide structural support and participate in neuronal pathfinding and metabolism, ionic homeostasis, neurotransmitter release, and regulation of the blood-brain barrier (Allen, 2014). Astrocytes release trophic molecules, including thrombospondins (TSPs), cholesterol, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophins 3 and 4 (NT3, NT4), and tumor necrosis factor-α (TNF-α) (Clarke and Barres, 2013) involved in the modulation of synaptic transmission and synaptogenesis.

Astrocytes in Down syndrome

Several studies have described neuropathological changes in the DS brain (Dierssen, 2012). In contrast, studies focusing on glial cell changes have received little attention. The number of glial cells, especially astrocytes, is altered during brain development in DS (Ponroy Bally and Murai, 2021). An increase in astrocytes and radial glial cells has been described in the frontal lobe of fetuses with DS at 18–20 weeks of gestation (Zdaniuk et al., 2011). Another study reported a lower expression of the glial fibrillary acidic protein (GFAP) in the hippocampus and temporal lobe of fetuses with DS only during the middle pregnancy period (Kanaumi et al., 2013). On the other hand, the fusiform gyrus and inferior temporal gyrus of fetuses with DS at 17–21 gestational weeks showed a higher percentage of GFAP-positive astrocytes (Guidi et al., 2018). Disruption of the interlaminar astroglial processes has been reported across the lifespan of individuals with DS (Colombo et al., 2005). A decrease in the number of astroglial interlaminar processes occurs within the first year of age, accompanied by an increase in immature astroglial cells in different regions of the DS brain (Colombo et al., 2005). Disruptions of the cortical astroglial architecture are more pronounced during adulthood. The absence of astroglial palisade has been reported in the dorsolateral region and the striate cortex of a 23-year-old individual with DS (Colombo et al., 2005). Degeneration of the interlaminar glial palisade progresses until adulthood, and instead, an increase in astrogliosis has been observed and associated with AD pathology in DS brains (Colombo et al., 2005).

In middle-aged and adults with DS (15–45 years old), there is a decrease in GFAP gene expression in the superior prefrontal cortex (Goodison et al., 1993). In contrast, GFAP protein expression increases after 30 years of age in the molecular layer of the hippocampus (Mito and Becker, 1993). In adults between the ages of 61 and 80, a partial reduction in the number of astrocytes and oligodendrocytes in the mediodorsal thalamic nucleus has been reported (Karlsen et al., 2014).

Such differential GFAP expression may be region specific and may change according to the progression of AD neuropathology.

In Ts1Cje mice, a mouse model of DS, the cerebellum displays a continuous increase of GFAP from youth to old age (Creaú et al., 2016), suggesting defective astrocyte production during aging. Some evidence indicates that GFAP expression is associated with increased micro-RNA-125b (miRNA-125b) levels in the temporal cortex of adults with DS between 63 and 73 years of age (Pogue et al., 2010). Micro RNAs (miRNAs) are post-transcriptional modulators of gene expression that regulate the stability and translation of their target messenger RNAs,

suggesting that miRNA-125b contributes to astrogliosis during aging in DS.

On the other hand, several studies support the importance of the surrounding environment in DS neurological alterations. Nelson et al. (1997), used the DS (Ts16) mouse model to co-culture neurons and astrocytes, and demonstrated that wild-type neurons co-cultured with Ts16 astrocytes showed a decrease in choline acetyltransferase activity and a decrease in cholinergic neuron number, while Ts16 neurons co-cultured with wild-type astrocytes showed regular cholinergic activity (Nelson et al., 1997). In agreement with these results, studies have demonstrated that deficits of thrombospondin-1 (TSP-1, an extracellular matrix component involved in cell–cell and cell–matrix communication) in DS astrocytes can cause the alterations of dendritic spines and synapses similar to those reported in DS neurons (Garcia et al., 2010; Torres et al., 2018). In addition, astrocyte-conditioned cell media from DS astroglia causes toxicity to neurons and fails to promote neuronal ion channel maturation and synaptic formation (Chen et al., 2014). On the other hand, induced pluripotent stem cells (iPSCs) derived from individuals with DS show an increase in the number of astrocytes (Briggs et al., 2013). Down syndrome astrocytes differentiated from iPSCs exhibit high levels of GFAP, S-100β, and reactive oxygen species (Chen et al., 2014). Induced pluripotent stem cells-derived astrocytes negatively regulate secreted factors that can promote the formation of synapses, including TSP-1 and TSP-2, and alter the mTOR pathway in neurons (Araujo et al., 2017), suggesting a role of astrocytes in DS neuropathology.

Ca^{2+} signaling in DS astrocytes

Hippocampal and cortical astrocytes obtained from Ts16 and Ts65Dn DS mouse models show impaired Ca^{2+} signaling. In the resting state, DS astrocytes show a higher concentration of cytoplasmic Ca^{2+} compared to euploid astrocytes (Bambrick et al., 1997; Muller et al., 1997). On the other hand, the inhibition of Ca^{2+} reservoirs in the endoplasmic reticulum induced a transient increase in cytoplasmic Ca^{2+} of 1,200 nM in Ts16 astrocytes compared to only 500 nM in euploid astrocytes (Bambrick et al., 1997). However, a stimulus-induced by serotonin or glutamate evoked transient Ca^{2+} increases from 400 to 600 nM in euploid astrocytes and from 20 to 150 nM in trisomic astrocytes, suggesting alterations in intracellular Ca^{2+} homeostasis in DS (Muller et al., 1997). Defects in Ca^{2+} homeostasis in astrocytes could affect cell proliferation and neuronal maturation (Bambrick et al., 2003). Finally, an increase in reactive astrogliosis has been described in basal ganglia calcification in DS (Takashima and Becker, 1985). Basal ganglia calcification may indicate premature aging (Mann, 1988).

Oxidative stress in DS astrocytes

Altered mitochondrial activity and oxidative stress have long been associated with DS (Coskun and Busciglio, 2012). Several genes involved in the cell redox state are triplicated in DS, the most prominent being Cu^{2+}/Zn^{2+} superoxide dismutase 1 (SOD1) (Pagano and Castello, 2012). Superoxide dismutase 1 scavenges reactive free radicals by catalyzing the dismutation of oxide anion into molecular oxygen and hydrogen peroxide. A high expression of SOD1 has been observed in the brains of people with AD between 59 and 97 years of age and in the temporal lobe of individuals with DS between 59 and 70 (Furuta et al., 1995). Mainly, SOD1 has been closely related to GFAP-positive cell processes (Furuta et al., 1995). Down syndrome brain tissue is susceptible to oxidative injury, mostly because increased SOD1 activity is not followed by an adaptive rise in hydrogen peroxide metabolism (Pagano and Castello, 2012). In neurons and astrocytes from human fetal DS cerebral cortical tissue, exposure to hydrogen peroxide shows that astrocytes are more resistant to oxidative damage (Sebastia et al., 2004). Down syndrome astrocytes show a lower basal content of superoxide ions and a higher clearance of hydrogen peroxide from the culture medium (Sebastia et al., 2004). In the presence of hydrogen peroxide, DS astrocytes maintain their concentration of intracellular superoxide and hydroperoxides at a lower level than normal astrocytes (Sebastia et al., 2004). Neurons co-cultured with DS astrocytes have greater protection against hydrogen peroxide injury than neurons co-cultured with normal astrocytes (Sebastia et al., 2004). Down syndrome astrocytes show a greater antioxidant capacity against hydrogen peroxide than normal astrocytes, and they partially counteract the oxidative vulnerability of trisomic neurons in cultures (Sebastia et al., 2004). In addition, microarray analyses showed that genes overexpressed in DS astrocytes are involved in energy metabolism and oxidative stress (Helguera et al., 2013). Interestingly, in this study, the authors show that when mitochondrial function is restored, there is increased production of reactive oxygen species and cellular damage, suggesting that reduced DS mitochondrial activity is an adaptive response to avoid injury and preserve essential cellular functions.

DS astrocytes and S100β

S-100β is a protein-enriched in astrocytes implicated in neuronal growth and differentiation, intracellular calcium signaling transduction, and proliferation and morphogenesis of astrocytes (Michetti et al., 2018). Like the SOD gene, the S100β gene is encoded on chromosome 21 (Allore et al., 1988), and overexpression of S100β during development may be associated with neurological abnormalities described in DS (Marks and Allore, 1990). Indeed, the overexpression

of S100β has been found in the temporal lobe of fetuses with DS at 18–19 gestational weeks, neonates and infants (Griffin et al., 1989), in the frontal and temporal lobes of adults with DS (Jorgensen et al., 1990; Mito and Becker, 1993; Royston et al., 1999), and the cerebellum of middle-aged and old Ts1Cje mice, a DS model (Creaú et al., 2016). Moreover, an increase in S100β has been observed in AD brains (Jorgensen et al., 1990). In addition, some studies show that overexpression of astrocytic S100β is positively associated with the number of APP overexpressing neurons and in neurites with abnormal growth (Royston et al., 1999), suggesting that overexpression of S100β could be associated with amyloid plaque formation and AD progression in DS (Griffin et al., 1998). In addition, S100β and APP increase astrogliogenesis and reduce neurogenesis (Guidi et al., 2008; Lu et al., 2011; Coronel et al., 2019), contributing to astrocyte dysfunction in DS.

Astrocytes and AD pathology in DS

The development of AD-type neuropathology in DS individuals has been observed from 12 years old (Wisniewski et al., 1985). However, the contribution of astrocytes in this process is not completely known. Abundant GFAP-positive astrocytes associated with Aβ42 plaques were reported in the frontal cortex of 15 and 29-years-old individuals with DS (Stoltzner et al., 2000). Another study has reported that astrocytes from the hippocampus and cerebral cortex displayed Aβ1–28 and Aβ40 in their cell bodies and in processes that extended to the vicinity of blood vessels (Gyure et al., 2001). The detection of Aβ in astrocytes may reflect either increased synthesis of Aβ by these cells or enhanced clearance of the peptide by astrocytes (Gyure et al., 2001). In addition, an increase in astrocytic apolipoprotein E expression has been reported in the gray matter of the DS frontal cortex from fetal stages to 24-years-old (Arai et al., 1995). In contrast, a decrease of astrocytic apolipoprotein E was reported in the white matter of the same individuals (Arai et al., 1995), suggesting that apolipoprotein E might be produced in astrocytes at the early phase of the pathological process lead to AD in DS people.

Down syndrome astrocytes also show alterations in mitochondrial transmembrane potential, mitochondrial redox activity, mitochondrial morphology, and ATP levels (Busciglio et al., 2002; Coskun and Busciglio, 2012; Helguera et al., 2013). The metabolic alterations in DS astrocytes could directly influence the processing of APP, resulting in increased levels of AβPP and reduced levels of secreted AβPP (AβPPs). This pattern of AβPP processing can be recapitulated in control astrocytes by inhibiting the mitochondrial metabolism, suggesting that DS astrocytes have altered mitochondrial function caused by AβPP overexpression (Busciglio et al., 2002). Interestingly, it has been shown that the survival of DS neurons increases by astrocyte-produced AβPPs, suggesting that AβPPs may be a neuronal survival factor (Busciglio et al., 2002). The mitochondrial dysfunction in DS may lead to increase intracellular expression of Aβ42 and reduced levels of AβPPs, leading to increased neuronal vulnerability (Busciglio et al., 2002). Altered mitochondrial membrane potential, low levels of ATP, and increased reactive oxygen species have also been found in cortical astrocytes obtained from TsCje mice (Shukkur et al., 2006). Furthermore, mitochondrial dysfunction might be associated with an increase in Tau hyperphosphorylation without NFTs formation through an increase in GSK3β (glycogen synthase kinase 3β) and JNK/SAPK (Jun amino-terminal kinase/stress-activated protein kinase) activities (Shukkur et al., 2006).

It has been established that neuroinflammation and glial activation play a significant role in the development and progression of AD (Wilcock, 2012). Astrocytes from the frontal cortex of fetuses, neonates, children, and adults with DS display upregulation of the inflammatory cytokines IL-1 and S100β (Griffin et al., 1989). Further studies have shown that IL-1 regulates the synthesis of AβPP (Batarseh et al., 2016), suggesting that inflammatory processes participate in the pathogenesis of AD in DS (Martini et al., 2022).

Microglial cells

Microglial cells, the resident macrophages of the CNS, account for 5–12% of the total number of cells in the human brain (Lawson et al., 1990). Microglial cells are derived from yolk sac primitive myeloid progenitors that migrate to the CNS during embryogenesis (Ginhoux et al., 2010). Their brain distribution is heterogeneous, and high microglial cell numbers are observed in specific anatomic regions (Lawson et al., 1990). During brain development, microglial cells are more proliferative than in adult stages and typically display an amoeboid morphology (Perez-Pouchoulen et al., 2015). Such morphology has been associated with its early role in shaping neuronal circuits, tissue remodeling, and cell phagocytosis (Prinz et al., 2019).

In the adult brain and under physiological conditions, microglial cells display a resting or surveying state (Nimmerjahn et al., 2005). Adult microglia are characterized by a small soma size and elongated processes that allow them to constantly probe their surrounding environment and interact with neighboring cells (Nimmerjahn et al., 2005). In response to a harmful stimulus, microglial cells undergo different intermediate stages displaying changes in their morphology, motility, phagocytic status, and gene and protein expression (Prinz et al., 2019). Microglial cells become reactive upon sensing acute injury, retracting their processes, and adopting an amoeboid morphology (Stence et al., 2001).

This fully activated state has been associated with microglial phagocytic functions.

Microglia in DS

Microglial chronic activation is a common feature of neurodegenerative disorders. Such chronic activation interferes with microglial homeostatic functions and contributes to the exacerbated neuroinflammatory profile observed in neurological conditions. In the brains of fetuses with DS (17–22 gestational weeks), microglial cells, including amoeboid and ramified microglia, emerge at the same timepoint as in control brains (Wierzba-Bobrowicz et al., 1999). However, an increase in ramified microglial cells has been reported in the frontal lobe, mesencephalon, and cerebellum from fetuses with DS (Wierzba-Bobrowicz et al., 1999). Further studies have shown that CD68 and HLA-DR positive cells are detectable in the germinal layers from control and DS fetal brains at 14 gestational weeks (Kanaumi et al., 2013). However, an increase in these cells was also observed in DS fetal brains. Furthermore, the increase in CD68+ microglial cells in the germinal matrix is higher in DS than in control brains, suggesting that microglial phagocytic activity might be associated with defects in neurogenesis and apoptosis (Kanaumi et al., 2013). Another study found that microglial cells from fetuses, neonates, children, and adults with DS were immunoreactive to IL-1β, a pro-inflammatory cytokine, suggesting increased neuroinflammation at the fetal stages (Griffin et al., 1989). Similarly, increased production of IL-1β and superoxide anion was reported in microglial cells from a DS mouse model at the fetal stage (embryonic day 15) (Colton et al., 1990, 1991). Interestingly, analysis of cell-free mRNA in the amniotic fluid suggests an increase in oxidative stress signaling pathways in DS fetuses (Slonim et al., 2009). Such an increase may interfere with glial cell modulatory processes and negatively impact brain development in DS. Further research is needed to fully understand the role of microglial and astroglial cells during brain development in DS.

During infancy, morphological analyses have revealed that microglial cells from children with DS displayed an increased soma size and soma size-to-process-length ratio than age-matched controls, suggesting that microglial cells might be reactive (Flores-Aguilar et al., 2020).

Microglia and Alzheimer's disease in Down syndrome

Microglial activation is also present in adolescents and young adults with DS and becomes exacerbated in adults with overt AD pathology (Stoltzner et al., 2000; Head et al., 2001; Flores-Aguilar et al., 2020; Martini et al., 2020; Palmer et al., 2021). Notably, a transcriptomic signature related to AD and aging has been reported in DS microglial cells before the development of full-blown AD pathology (Palmer et al., 2021). This microglial signature is characterized by an increased expression of C1q-complement-related genes (Palmer et al., 2021). In line with this, an increase in cytokine gene and protein expression has been reported in fetuses, children, and young adults with DS, well-before developing a full-blown AD pathology (Wilcock et al., 2015; Flores-Aguilar et al., 2020). Moreover, the overexpression of IFN-related genes encoded in chromosome 21 might impact microglial reactivity and neuroinflammation in DS (Wilcock and Griffin, 2013). In addition, individuals with DS display a biphasic expression of the triggering receptor expressed on myeloid cells 2 (TREM2) (Raha-Chowdhury et al., 2018; Weber et al., 2020). Triggering receptor expressed on myeloid cells 2 is involved in microglial phagocytosis (Krasemann et al., 2017; Mazaheri et al., 2017), and its serum and plasma levels are increased in young adults with DS (Raha-Chowdhury et al., 2018; Weber et al., 2020). In contrast, downregulation of TREM2 expression has been reported in serum and the frontal cortex of adults with DS (Raha-Chowdhury et al., 2018). Such biphasic expression, also reported in AD (Kleinberger et al., 2014; Suarez-Calvet et al., 2016), might influence microglial activity at different stages of AD in DS, as exemplified in an AD mouse model (Jay et al., 2017). Moreover, older adults with DS display increased CD64 and CD86 (Wilcock et al., 2015). The expression of these markers has been associated with the formation of immune complexes in the brain and subsequent microglial activation (Edwards et al., 2006; Sudduth et al., 2013). Therefore, microglial activation might also be associated with cerebrovascular dysfunction and the extravasation of serum proteins into the brain (Wilcock et al., 2015).

Microglial dystrophy has been reported in older adults with DS (Xue and Streit, 2011; Flores-Aguilar et al., 2020; Martini et al., 2020). Dystrophic microglia in DS brains are characterized by process swelling, bead formation, and cell rupture. Such dystrophic phenotype might be associated with advanced stages of AD neuropathology commonly present in the brains of older adults with DS.

Interestingly, the brains of individuals with DS also display an increase in rod-like microglial cells (Flores-Aguilar et al., 2020; Martini et al., 2020). Higher rod-like microglia counts have been reported in the frontal cortex of young adults and adults with DS and in the posterior cingulate cortex of adults with DS and AD (Flores-Aguilar et al., 2020; Martini et al., 2020). Rod-like microglial cells have also been observed in the CA1 region of the hippocampus in DS brains (Head et al., 2001). Moreover, rod-like microglial cells might align to p-tau positive neurons in the DS frontal cortex (Flores-Aguilar et al., 2020). Rod-like microglial cells have been observed in

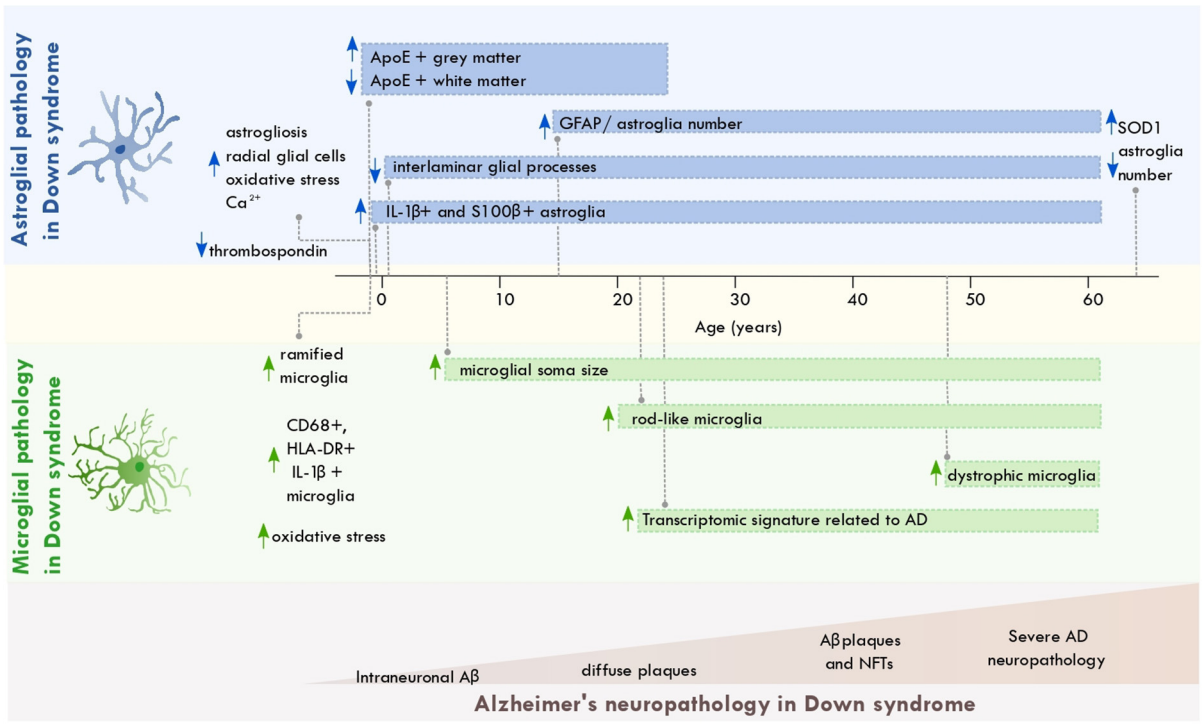


FIGURE 1
Astroglial and microglial pathology across the lifespan of individuals with Down syndrome. Scheme depicting the most significant changes in astrocytes and microglia from individuals with Down syndrome across aging and along with the progression of AD neuropathology. The brain regions where these disruptions occur are described in the main text. Aβ, amyloid beta; NFT, neurofibrillary tangles; ApoE, apolipoprotein E; AD, Alzheimer's disease; SOD, superoxide dismutase 1; GFAP, glial fibrillary acidic protein.

other neurodegenerative disorders such as AD (Bachstetter et al., 2015) and might be linked to the appearance of tau pathology (Adaikkan et al., 2019; Malcolm et al., 2019). However, their exact function is not well-understood (Giordano et al., 2021).

Studies in DS animal models highlight the critical role that microglial cells exert in DS. Microglial activation has been observed in the Ts65Dn and Dp(16) DS mouse models (Hunter et al., 2004; Lockrow et al., 2011; Rueda et al., 2018; Illouz et al., 2019; Hamlett et al., 2020; Pinto et al., 2020). Microglial depletion in the Dp(16) DS mouse model improves cognition in juvenile mice and neuronal spine and activity (Pinto et al., 2020). Further, anti-inflammatory treatments in DS mouse models promote microglial homeostasis and rescue cognitive deficits (Hamlett et al., 2020; Pinto et al., 2020). Such observations support that microglial chronic activation and neuroinflammation might have a detrimental role in DS. As mentioned above, AD neuropathology might also be involved in the life-long microglial activation observed in DS brains (Flores-Aguilar et al., 2020). DNA vaccination against the $A\beta_{1-11}$ fragment in the Ts65Dn DS mouse model restored microglial homeostasis, rescued cognitive deficits, and reduced neurodegeneration and Aβ levels (Illouz et al., 2019).

Conclusion

Astroglial and microglial cells display pathological alterations across the lifespan of individuals with DS. Down syndrome astrocytes show metabolic alterations involving pathways associated with oxidative stress, calcium regulation, and Aβ production. These alterations might result from the trisomic condition that occurs in these cells (Figure 1). Astrocytic dysfunction might interfere with neuronal excitability and the balance of excitatory and inhibitory transmission (Cresto et al., 2019). Such dysfunction could contribute to learning and memory impairments observed in DS (Fernández-Blanco and Dierssen, 2020).

On the other hand, microglial cells display several reactive states across the lifespan of individuals with DS. Such activation states might be associated with AD neuropathology, increased CNS and peripheral inflammation, and the triplication of immune-related genes encoded in chromosome 21 (Wilcock and Griffin, 2013). Given the involvement of microglial cells in the development of AD pathology, chronic microglial activation in DS might feed the progression of AD in this population.

Further studies are needed to fully understand the pathological alterations of glial cells in DS and their contribution to AD pathology. This could lead to identifying potential therapeutics targeting dysfunctional astroglial and microglial cells in DS.

Author contributions

Both authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

This work was supported by PAPIIT IN-304817 and PAPIME-PE302320 (OG).

Conflict of interest

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

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Adaikkan, C., Middleton, S. J., Marco, A., Pao, P. C., Mathys, H., Kim, D. N., et al. (2019). Gamma entrainment binds higher-order brain regions and offers neuroprotection. *Neuron* 102, 929.e8–943.e8. doi: 10.1016/j.neuron.2019.04.011

Allen, N. J. (2014). Astrocyte regulation of synaptic behavior. *Annu. Rev. Cell Dev. Biol.* 30, 439–463. doi: 10.1146/annurev-cellbio-100913-013053

Allore, R., O'Hanlon, D., Price, R., Neilson, K., Willard, H. F., Cox, D. R., et al. (1988). Gene encoding the beta subunit of S100 protein is on chromosome 21: implications for Down syndrome. *Science* 239, 1311–1313. doi: 10.1126/science.2964086

Annus, T., Wilson, L. R., Hong, Y. T., Acosta-Cabronero, J., Fryer, T. D., Cardenas-Blanco, A., et al. (2016). The pattern of amyloid accumulation in the brains of adults with Down syndrome. *Alzheimers Dement.* 12, 538–545. doi: 10.1016/j.jalz.2015.07.490

Antonarakis, S. E. (2017). Down syndrome and the complexity of genome dosage imbalance. *Nat. Rev. Genet.* 18, 147–163. doi: 10.1038/nrg.2016.154

Antonarakis, S. E., Skotko, B. G., Rafii, M. S., Strydom, A., Pape, S. E., Bianchi, D. W., et al. (2020). Down syndrome. *Nat. Rev. Dis. Primers* 6:9. doi: 10.1038/s41572-019-0143-7

Arai, Y., Mizuguchi, M., Ikeda, K., and Takashima, S. (1995). Developmental changes of apolipoprotein E immunoreactivity in Down syndrome brains. *Dev. Brain Res.* 87, 228–233. doi: 10.1016/0165-3806(95)00066-M

Araujo, B. H. S., Kaid, C., De Souza, J. S., Gomes da Silva, S., Goulart, E., Caires, L. C. J., et al. (2017). Down syndrome iPSC-Derived astrocytes impair neuronal synaptogenesis and the mTOR pathway *in vitro*. *Mol. Neurobiol.* 55, 5962–5975. doi: 10.1007/s12035-017-0818-6

Bachstetter, A. D., Van Eldik, L. J., Schmitt, F. A., Neltner, J. H., Ighodaro, E. T., Webster, S. J., et al. (2015). Disease-related microglia heterogeneity in the hippocampus of Alzheimer's disease, dementia with Lewy bodies, and hippocampal sclerosis of aging. *Acta Neuropathol. Commun.* 3, 32. doi: 10.1186/s40478-015-0209-z

Bambrick, L. L., Golovina, V. A., Blaustein, M. P., Yarowsky, P. J., and Krueger, B. K. (1997). Abnormal calcium homeostasis in astrocytes from the trisomy 16 mouse. *Glia* 19, 352–358. doi: 10.1002/(SICI)1098-1136(199704)19:4<352::AID-GLIA8>3.0.CO;2-z

Bambrick, L. L., Yarowsky, P. J., and Krueger, B. K. (2003). Altered astrocyte calcium homeostasis and proliferation in the Ts65Dn mouse, a model of Down syndrome. *J. Neurosci. Res.* 73, 89–94. doi: 10.1002/jnr.10630

Batarseh, Y. S., Duong, Q. V., Mousa, Y. M., Al Rihani, S. B., Elfakhri, K., and Kaddoumi, A. (2016). Amyloid-β and astrocytes interplay in amyloid-β related disorders. *Int. J. Mol. Sci.* 17:338. doi: 10.3390/ijms17030338

Briggs, J. A., Sun, J., Shepherd, J., Ovchinnikov, D. A., Chung, T. L., Nayler, S. P., et al. (2013). Integration-free induced pluripotent stem cells model genetic and neuronal developmental features of Down syndrome etiology. *Stem Cells* 31, 647–478. doi: 10.1002/stem.1297

Busciglio, J., Pelsman, A., Wong, C., Pigino, G., Yuan, M., Mori, H., et al. (2002). Altered metabolism of the amyloid-beta precursor protein is associated with mitochondrial dysfunction in Down's syndrome. *Neuron* 33, 677–688. doi: 10.1016/S0896-6273(02)00604-9

Busciglio, J., and Yanker, B. A. (1995). Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons *in vitro*. *Nature* 378, 776–779. doi: 10.1038/378776a0

Carmona-Iragui, M., Balasa, M., Benejam, B., Alcolea, D., Fernandez, S., Videla, L., et al. (2017). Cerebral amyloid angiopathy in Down syndrome and sporadic and autosomal-dominant Alzheimer's disease. *Alzheimers Dement.* 13, 1251–1260. doi: 10.1016/j.jalz.2017.03.007

Carr, J., and Collins, S. (2018). 50 years with Down syndrome: a longitudinal study. *J. Appl. Res. Intellect. Disabil.* 31, 743–750. doi: 10.1111/jar.12438

Cataldo, A. M., Peterhoff, C. M., Troncoso, J. C., Gomez-Isla, T., Hyman, B. T., and Nixon, R. A. (2000). Endocytic pathway abnormalities precede amyloid beta deposition in sporadic Alzheimer's disease and Down syndrome: differential effects of APOE genotype and presenilin mutations. *Am. J. Pathol.* 157, 277–286. doi: 10.1016/S0002-9440(10)64538-5

Chen, C., Jiang, P., Xue, H., Peterson, S. E., Tran, H. T., McCann, A. E., et al. (2014). Role of astroglia in Down's syndrome revealed by patient-derived human-induced pluripotent stem cells. *Nat. Commun.* 5:4430. doi: 10.1038/ncomms5430

Clarke, L. E., and Barres, B. A. (2013). Emerging roles of astrocytes in neural circuit development. *Nat. Rev. Neurosci.* 14, 311–321. doi: 10.1038/nrn3484

Colombo, J. A., Reisin, H. D., Jones, M., and Bentham, C. (2005). Development of interlaminar astroglial processes in the cerebral cortex of control and Down's syndrome human cases. *Exp. Neurol.* 193, 207–217. doi: 10.1016/j.expneurol.2004.11.024

Colton, C. A., Yao, J., Taffs, R. E., Keri, J. E., and Oster-Granite, M. L. (1991). Abnormal production of interleukin-1 by microglia from trisomy 16 mice. *Neurosci. Lett.* 132, 270–274. doi: 10.1016/0304-3940(91)90318-N

Colton, C. A., Yao, J. B., Gilbert, D., and Oster-Granite, M. L. (1990). Enhanced production of superoxide anion by microglia from trisomy 16 mice. *Brain Res.* 519, 236–242. doi: 10.1016/0006-8993(90)90083-N

Coronel, R., Lachgar, M., Bernabeu-Zornoza, A., Palmer, C., Domínguez-Alvaro, M., Revilla, A., et al. (2019). Neuronal and glial differentiation of human neural stem cells is regulated by Amyloid Precursor Protein (APP) levels. *Mol. Neurobiol.* 56, 1248–1261. doi: 10.1007/s12035-018-1167-9

Coskun, P. E., and Busciglio, J. (2012). Oxidative stress and mitochondrial dysfunction in Down's syndrome: relevance to aging and dementia. *Curr. Gerontol. Geriatr. Res*. 2012:383170. doi: 10.1155/2012/383170

Covelli, V., Raggi, A., Meucci, P., Paganelli, C., and Leonardi, M. (2016). Ageing of people with Down's syndrome: a systematic literature review from 2000 to 2014. *Int. J. Rehabil. Res*. 39, 20–28. doi: 10.1097/MRR.0000000000000147

Creaú, N., Cabet, E., Daubigney, F., Souchet, B., Bennai, S., and Delabar, J. (2016). Specific age-related molecular alterations in the cerebellum of Down syndrome mouse models. *Brain Res*. 1646, 342–353. doi: 10.1016/j.brainres.2016.06.003

Cresto, N., Pillet, L. E., Billuart, P., and Rouach, N. (2019). Do astrocytes play a role in intellectual disabilities? *Trends Neurosci*. 42, 518–527. doi: 10.1016/j.tins.2019.05.011

Davidson, Y. S., Robinson, A., Prasher, V. P., and Mann, D. M. A. (2018). The age of onset and evolution of Braak tangle stage and Thal amyloid pathology of Alzheimer's disease in individuals with Down syndrome. *Acta Neuropathol. Commun*. 6:56. doi: 10.1186/s40478-018-0559-4

Dierssen, M. (2012). Down syndrome: the brain in trisomic mode. *Nat. Rev. Neurosci*. 13, 844–858. doi: 10.1038/nrn3314

Edwards, J. P., Zhang, X., Frauwirth, K. A., and Mosser, D. M. (2006). Biochemical and functional characterization of three activated macrophage populations. *J. Leukoc. Biol*. 80, 1298–1307. doi: 10.1189/jlb.0406249

Fernández-Blanco, Á., and Dierssen, M. (2020). Rethinking intellectual disability from neuro- to astro-pathology. *Int. J. Mol. Sci*. 21:9039. doi: 10.3390/ijms21239039

Flores-Aguilar, L., Iulita, M. F., Kovecses, O., Torres, M. D., Levi, S. M., Zhang, Y., et al. (2020). Evolution of neuroinflammation across the lifespan of individuals with Down syndrome. *Brain* 143, 3653–3671. doi: 10.1093/brain/awaa326

Fortea, J., Zaman, S. H., Hartley, S., Rafii, M. S., Head, E., and Carmona-Iragui, M. (2021). Alzheimer's disease associated with Down syndrome: a genetic form of dementia. *Lancet Neurol*. 20, 930–942. doi: 10.1016/S1474-4422(21)00245-3

Furuta, A., Price, D. L., Pardo, C. A., Troncoso, J. C., Xu, Z.-S., Taniguchi, N., et al. (1995). Localization of superoxide dismutase in Alzheimer disease and Down's syndrome neocortex and hippocampus. *Am. J. Pathol*. 146, 357–366.

Garcia, O., Torres, M., Helguera, P., Coskun, P., and Busciglio, J. (2010). A role for thrombospondin-1 deficits in astrocyte-mediated spine and synaptic pathology in Down's syndrome. *PLoS ONE* 5:e14200. doi: 10.1371/journal.pone.0014200

Gensous, N., Bacalini, M. G., Franceschi, C., and Garagnani, P. (2020). Down syndrome, accelerated aging and immunosenescence. *Semin. Immunopathol*. 42, 635–645. doi: 10.1007/s00281-020-00804-1

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., et al. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330, 841–845. doi: 10.1126/science.1194637

Giordano, K. R., Denman, C. R., Dubisch, P. S., Akhter, M., and Lifshitz, J. (2021). An update on the rod microglia variant in experimental and clinical brain injury and disease. *Brain Commun*. 3:fcaa227. doi: 10.1093/braincomms/fcaa227

Glasson, E. J., Jacques, A., Wong, K., Bourke, J., and Leonard, H. (2016). Improved survival in Down syndrome over the last 60 years and the impact of perinatal factors in recent decades. *J. Pediatr*. 169, 214.e1–220.e1. doi: 10.1016/j.jpeds.2015.10.083

Godfrey, M., and Lee, N. R. (2018). Memory profiles in Down syndrome across development: a review of memory abilities through the lifespan. *J. Neurodev. Disord*. 10:5. doi: 10.1186/s11689-017-9220-y

Goodison, K. L., Parhad, I. M., Whit, C. L. III, Sima, A. A., and Clark, A. W. (1993). Neuronal and glial gene expression in neocortex of Down's syndrome and Alzheimer's disease. *J. Neuropathol. Exp. Neurol*. 52, 192–198. doi: 10.1097/00005072-199305000-00002

Griffin, W. S. T., Sheng, J. G., Mckenzie, J. E., Royston, M. C., Gentleman, S. M., Brumback, R. A., et al. (1998). Life-long overexpression of S100B in Down syndrome: implications for Alzheimer pathogenesis. *Neurobiol. Aging* 19, 401–405. doi: 10.1016/S0197-4580(98)00074-8

Griffin, W. S. T., Stanley, L. C., Ling, C., White, L., MacLeod, V., Perrot, L. P., et al. (1989). Brain interleukin 1 and S100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A*. 86, 7611–7615. doi: 10.1073/pnas.86.19.7611

Guidi, S., Bonasoni, P., Ceccarelli, C., Santini, D., Gualtieri, F., Ciani, E., et al. (2008). Neurogenesis impairment and increased cell death reduce total neuron number in the hippocampal region of fetuses with Down syndrome. *Brain Pathol*. 18, 180–197. doi: 10.1111/j.1750-3639.2007.00113.x

Guidi, S., Giacomini, A., Stagni, F., Emili, M., Uguagliati, B., Bonasoni, M. P., et al. (2018). Abnormal development of the inferior temporal region in fetuses with Down syndrome. *Brain Pathol*. 28, 986–998. doi: 10.1111/bpa.12605

Gyure, K. A., Durham, R., Stewart, W. F., Smialek, J. E., and Troncoso, J. C. (2001). Intraneuronal AβB-amyloid precedes development of amyloid plaques in Down syndrome. *Arch. Pathol. Lab. Med*. 125, 489–492. doi: 10.5858/2001-125-0489-IAAPDO

Hamlett, E. D., Hjorth, E., Ledreux, A., Gilmore, A., Schultzberg, M., and Granholm, A. C. (2020). RvE1 treatment prevents memory loss and neuroinflammation in the Ts65Dn mouse model of Down syndrome. *Glia* 68, 1347–1360. doi: 10.1002/glia.23779

Hawkins, B. A., Eklund, S. J., James, D. R., and Foose, A. K. (2003). Adaptive behavior and cognitive function of adults with Down syndrome: modeling change with age. *Ment. Retard*. 41, 7–28. doi: 10.1352/0047-6765(2003)041<0007:ABACFO>2.0.CO;2

Head, E., Azizeh, B. Y., Lott, I. T., Tenner, A. J., Cotman, C. W., and Cribbs, D. H. (2001). Complement association with neurons and beta-amyloid deposition in the brains of aged individuals with down syndrome. *Neurobiol. Dis*. 8, 252–265. doi: 10.1006/nbdi.2000.0380

Head, E., Lott, I. T., Wilcock, D. M., and Lemere, C. A. (2016). Aging in Down syndrome and the development of Alzheimer's disease neuropathology. *Curr. Alzheimer Res*. 13, 18–29. doi: 10.2174/1567205012666151020114607

Head, E., Phelan, M. J., Doran, E., Kim, R. C., Poon, W. W., Schmitt, F. A., et al. (2017). Cerebrovascular pathology in Down syndrome and Alzheimer disease. *Acta Neuropathol. Commun*. 5:93. doi: 10.1186/s40478-017-0499-4

Helguera, P., Seiglie, J., Rodríguez, J., Hanna, M., Helguera, G., and Busciglio, J. (2013). Adaptative Downregulation of mitochondrial function in Down syndrome. *Cell Metab*. 17, 132–140. doi: 10.1016/j.cmet.2012.12.005

Holland, A. J., Hon, J., Huppert, F. A., Stevens, F., and Watson, P. (1998). Population-based study of the prevalence and presentation of dementia in adults with Down's syndrome. *Br. J. Psychiatry* 172, 493–498. doi: 10.1192/bjp.172.6.493

Hunter, C. L., Bachman, D., and Granholm, A. C. (2004). Minocycline prevents cholinergic loss in a mouse model of Down's syndrome. *Ann. Neurol*. 56, 675–688. doi: 10.1002/ana.20250

Illouz, T., Madar, R., Biragyn, A., and Okun, E. (2019). Restoring microglial and astroglial homeostasis using DNA immunization in a Down Syndrome mouse model. *Brain Behav. Immun*. 75, 163–180. doi: 10.1016/j.bbi.2018.10.004

Iulita, M. F., Do Carmo, S., Ower, A. K., Fortress, A. M., Aguilar, L. F., Hanna, M., et al. (2014). Nerve growth factor metabolic dysfunction in Down's syndrome brains. *Brain*. 137, 860–872. doi: 10.1093/brain/awt372

Iulita, M. F., Garzón Chavez, D., Klitgaard Christensen, M., Valle Tamayo, N., Plana-Ripoll, O., Rasmussen, S. A., et al. (2022). Association of Alzheimer disease with life expectancy in people with Down syndrome. *JAMA Netw. Open*. 5:e2212910. doi: 10.1001/jamanetworkopen.2022.12910

Jay, T. R., Hirsch, A. M., Broihier, M. L., Miller, C. M., Neilson, L. E., Ransohoff, R. M., et al. (2017). Disease progression-dependent effects of TREM2 deficiency in a mouse model of Alzheimer's disease. *J. Neurosci*. 37, 637–647. doi: 10.1523/JNEUROSCI.2110-16.2016

Jorgensen, O. S., Brooksbank, B. W., and Balazs, R. (1990). Neuronal plasticity and astrocytic reaction in Down syndrome and Alzheimer disease. *J. Neurol. Sci*. 98, 63–79. doi: 10.1016/0022-510X(90)90182-M

Kanaumi, T., Milenkovic, I., Adle-Biassette, H., Aronica, E., and Kovacs, G. C. (2013). Non-neuronal cell responses differ between normal and Down syndrome developing brains. *Int. J. Dev. Neurosci*. 31, 796–803. doi: 10.1016/j.ijdevneu.2013.09.011

Karlsen, A. S., Korbo, S., Uylings, H. B. M., and Pakkenberg, B. (2014). A stereological study of the mediodorsal thalamic nucleus in Down syndrome. *Neuroscience* 279, 253–259. doi: 10.1016/j.neuroscience.2014.08.046

Kleinberger, G., Yamanishi, Y., Suarez-Calvet, M., Czirr, E., Lohmann, E., Cuyvers, E., et al. (2014). TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. *Sci. Transl. Med*. 6:243ra286. doi: 10.1126/scitranslmed.3009093

Krasemann, S., Madore, C., Cialic, R., Baufeld, C., Calcagno, N., El Fatimy, R., et al. (2017). The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. *Immunity* 47, 566.e569–581.e569. doi: 10.1016/j.immuni.2017.08.008

Lao, P. J., Gutierrez, J., Keator, D., Rizvi, B., Banerjee, A., Igwe, K. C., et al. (2020). Alzheimer-related cerebrovascular disease in Down syndrome. *Ann. Neurol*. 88, 1165–1177. doi: 10.1002/ana.25905

Lawson, L. J., Perry, V. H., Dri, P., and Gordon, S. (1990). Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* 39, 151–170. doi: 10.1016/0306-4522(90)90229-W

Lemere, C. A., Blusztajn, J. K., Yamaguchi, H., Wisniewski, T., Saido, T. C., and Selkoe, D. J. (1996). Sequence of deposition of heterogeneous amyloid beta-peptides and APO E in Down syndrome: implications for initial events in amyloid plaque formation. *Neurobiol. Dis.* 3, 16–32. doi: 10.1006/nbdi.1996.0003

Liu, P., Wang, Y., Sun, Y., and Peng, G. (2022). Neuroinflammation as a potential therapeutic target in Alzheimer's disease. *Clin. Interv. Aging*. 17, 665–674. doi: 10.2147/CIA.S357558

Lockrow, J., Boger, H., Gerhardt, G., Aston-Jones, G., Bachman, D., and Granholm, A. C. (2011). A noradrenergic lesion exacerbates neurodegeneration in a Down syndrome mouse model. *J. Alzheimers Dis.* 23, 471–489. doi: 10.3233/JAD-2010-101218

Lott, I. T., and Head, E. (2019). Dementia in Down syndrome: unique insights for Alzheimer disease research. *Nat. Rev. Neurol.* 15, 135–147. doi: 10.1038/s41582-018-0132-6

Lu, J., Esposito, G., Scuderi, C., Steardo, L., Delli-Bovi, L. C., Hecht, J. L., et al. (2011). S100B and APP promote a gliocentric shift and impaired neurogenesis in Down syndrome neuronal progenitors. *PLoS ONE* 6:e22126. doi: 10.1371/journal.pone.0022126

Malcolm, J. C., Breuillaud, L., Do Carmo, S., Hall, H., Welikovitch, L. A., Macdonald, J. A., et al. (2019). Neuropathological changes and cognitive deficits in rats transgenic for human mutant tau recapitulate human tauopathy. *Neurobiol. Dis*. 127, 323–338. doi: 10.1016/j.nbd.2019.03.018

Mann, D. M. (1988). Calcification of the basal ganglia in Down's syndrome and Alzheimer's disease. *Acta Neuropathol*. 76, 595–598. doi: 10.1007/BF00689598

Mann, D. M., and Esiri, M. M. (1989). The pattern of acquisition of plaques and tangles in the brains of patients under 50 years of age with Down's syndrome. *J. Neurol. Sci*. 89, 169–179. doi: 10.1016/0022-510X(89)90019-1

Margallo-Lana, M. L., Moore, P. B., Kay, D. W., Perry, R. H., Reid, B. E., Berney, T. P., et al. (2007). Fifteen-year follow-up of 92 hospitalized adults with Down's syndrome: incidence of cognitive decline, its relationship to age and neuropathology. *J. Intellect. Disabil. Res.* 51(Pt. 6), 463–477. doi: 10.1111/j.1365-2788.2006.00902.x

Marks, A., and Allore, R. (1990). S100 protein and Down syndrome. *Bioessays* 12, 381–383. doi: 10.1002/bies.950120806

Martini, A. C., Gross, T. J., Head, E., and Mapstone, M. (2022). Beyond amyloid: Immune, cerebrovascular, and metabolic contributions to Alzheimer disease in people with Down syndrome. *Neuron* 110, 2063–2079. doi: 10.1016/j.neuron.2022.04.001

Martini, A. C., Helman, A. M., McCarty, K. L., Lott, I. T., Doran, E., Schmitt, F. A., et al. (2020). Distribution of microglial phenotypes as a function of age and Alzheimer's disease neuropathology in the brains of people with Down syndrome. *Alzheimers Dement*. 12:e12113. doi: 10.1002/dad2.12113

Mazaheri, F., Snaidero, N., Kleinberger, G., Madore, C., Daria, A., Werner, G., et al. (2017). TREM2 deficiency impairs chemotaxis and microglial responses to neuronal injury. *EMBO Rep*. 18, 1186–1198. doi: 10.15252/embr.201743922

McCarron, M., McCallion, P., Reilly, E., Dunne, P., Carroll, R., and Mulryan, N. (2017). A prospective 20-year longitudinal follow-up of dementia in persons with Down syndrome. *J. Intellect. Disabil. Res*. 61, 843–852. doi: 10.1111/jir.12390

Michetti, F., D'Ambrosio, N., Toesca, A., Puglisi, M. A., Serrano, A., Marchese, E., et al. (2018). The S100β story: from biomarker to active factor in neuronal injury. *J. Neurochem*. 148, 168–187. doi: 10.1111/jnc.14574

Mito, T., and Becker, L. E. (1993). Developmental changes of S-100 protein and glial fibrillary acid protein in the brain in Down syndrome. *Exp. Neurol*. 120, 170–176. doi: 10.1006/exnr.1993.1052

Mori, C., Spooner, E. T., Wisniewsk, K. E., Wisniewski, T. M., Yamaguch, H., Saido, T. C., et al. (2002). Intraneuronal Abeta42 accumulation in Down syndrome brain. *Amyloid* 9, 88–102. doi: 10.3109/13506120208995241

Muller, W., Heinemann, U., and Schuchmenn, S. (1997). Impaired Ca-signaling in astrocytes from the Ts16 mouse model of Down syndrome. *Neurosci. Lett*. 223, 81–84. doi: 10.1016/S0304-3940(97)13406-1

Nelson, P. G., Fitzgerald, S., Rapoport, S. L., Neale, E. A., Galdzicki, Z., Dulap, V., et al. (1997). Cerebral cortical astroglia from the trisomy 16 mouse, a model for Down syndrome produce neuronal cholinergic deficits in cell culture. *Proc. Nat. Acad. Sci. U.S.A*. 94, 12644–12648. doi: 10.1073/pnas.94.23.12644

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 308, 1314–1318. doi: 10.1126/science.1110647

Pagano, G., and Castello, G. (2012). Oxidative stress and mitochondrial dysfunction in Down syndrome. *Adv. Exp. Med. Biol*. 724, 291–299. doi: 10.1007/978-1-4614-0653-2_22

Palmer, C. R., Liu, C. S., Romanow, W. J., Lee, M. H., and Chun, J. (2021). Altered cell and RNA isoform diversity in aging Down syndrome brains. *Proc. Natl. Acad. Sci. U.S.A*. 118:e2114326118. doi: 10.1073/pnas.2114326118

Perez-Pouchoulen, M., VanRyzin, J. W., and McCarthy, M. M. (2015). Morphological and phagocytic profile of microglia in the developing rat cerebellum. *eNeuro* 2:ENEURO.0036-15.2015. doi: 10.1523/ENEURO.0036-15.2015

Pinto, B., Morelli, G., Rastogi, M., Savardi, A., Fumagalli, A., Petretto, A., et al. (2020). Rescuing over-activated microglia restores cognitive performance in juvenile animals of the Dp(16) mouse model of Down syndrome. *Neuron* 108, 887.e12–904.e12. doi: 10.1016/j.neuron.2020.09.010

Pogue, A. I., Cui, J. G., Li, Y. Y., Zhao, Y., Culicchia, F., and Lukiw, W. J. (2010). Micro RNA-125b (miRNA-125b) function in astrogliosis and glial cell proliferation. *Neurosci. Lett*. 476, 18–22. doi: 10.1016/j.neulet.2010.03.054

Ponroy Bally, B., and Murai, K. K. (2021). Astrocytes in Down syndrome across the lifespan. *Front. Cell. Neurosci*. 15:702685. doi: 10.3389/fncel.2021.702685

Prinz, M., Jung, S., and Priller, J. (2019). Microglia biology: one century of evolving concepts. *Cell*. 179, 292–311. doi: 10.1016/j.cell.2019.08.053

Raha-Chowdhury, R., Henderson, J. W., Raha, A. A., Stott, S. R. W., Vuono, R., Foscarin, S., et al. (2018). Erythromyeloid-derived TREM2: a major determinant of Alzheimer's disease pathology in Down syndrome. *J. Alzheimers Dis*. 61, 1143–1162. doi: 10.3233/JAD-170814

Royston, M. C., Mckenzie, J. E., Gentleman, S. M., Sheng, J. G., Mann, D. M. A., Griffin, W. S. T., et al. (1999). Overexpression of S100β in Down's syndrome: correlation with patient age and with B-amyloid deposition. *Neuropathol. Appl. Neurobiol*. 25, 387–393. doi: 10.1046/j.1365-2990.1999.00196.x

Rueda, N., Vidal, V., Garcia-Cerro, S., Narcis, J. O., Llorens-Martin, M., Corrales, A., et al. (2018). Anti-IL17 treatment ameliorates Down syndrome phenotypes in mice. *Brain Behav. Immun*. 73, 235–251. doi: 10.1016/j.bbi.2018.05.008

Sadowski, M., Wisniewski, H. M., Tarnawski, M., Kozlowski, P. B., Lach, B., and Wegiel, J. (1999). Entorhinal cortex of aged subjects with Down's syndrome shows severe neuronal loss caused by neurofibrillary pathology. *Acta Neuropathol*. 97, 156–164. doi: 10.1007/s004010050968

Sebastia, J., Cristofol, R., Pertusa, M., Vilchez, D., Torán, N., Barambio, S., et al. (2004). Down's syndrome astrocytes have greater antioxidant capacity that euploid astrocytes. *Eur. J. Neurosci*. 20, 2355–2366. doi: 10.1111/j.1460-9568.2004.03686.x

Shukkur, E. A., Shimohata, A., Akagi, T., Yu, W., Yamaguchi, M., Murayama, M., et al. (2006). Mitochondrial dysfunction and Tau hyperphosphorilation in Ts1Cje, a mouse model for Down syndrome. *Hum. Mol. Genet*. 15, 2752–2762. doi: 10.1093/hmg/ddl211

Slonim, D. K., Koide, K., Johnson, K. L., Tantravahi, U., Cowan, J. M., Jarrah, Z., et al. (2009). Functional genomic analysis of amniotic fluid cell-free mRNA suggests that oxidative stress is significant in Down syndrome fetuses. *Proc. Natl. Acad. Sci. U.S.A*. 106, 9425–9429. doi: 10.1073/pnas.0903909106

Stence, N., Waite, M., and Dailey, M. E. (2001). Dynamics of microglial activation: a confocal time-lapse analysis in hippocampal slices. *Glia* 33, 256–266. doi: 10.1002/1098-1136(200103)33:3<256::AID-GLIA1024>3.0.CO;2-J

Stoltzner, S. E., Grenfell, T. J., Mori, C., Wisniewski, K. E., Wisniewski, T. M., Selkoe, D. J., et al. (2000). Temporal accrual of complement proteins in amyloid plaques in Down's syndrome with Alzheimer's disease. *Am. J. Pathol*. 156, 489–499. doi: 10.1016/S0002-9440(10)64753-0

Suarez-Calvet, M., Kleinberger, G., Araque Caballero, M. A., Brendel, M., Rominger, A., Alcolea, D., et al. (2016). sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. *EMBO Mol. Med*. 8, 466–476. doi: 10.15252/emmm.201506123

Sudduth, T. L., Greenstein, A., and Wilcock, D. M. (2013). Intracranial injection of Gammagard, a human IVIg, modulates the inflammatory response of the brain and lowers Aβ in APP/PS1 mice along a different time course than anti-Aβ antibodies. *J. Neurosci*. 33, 9684–9692. doi: 10.1523/JNEUROSCI.1220-13.2013

Takashima, S., and Becker, L. E. (1985). Basal ganglia calcification in Down's syndrome. *J. Neurol. Neurosurg. Psychol*. 48, 61–64. doi: 10.1136/jnnp.48.1.61

Torres, M. D., Garcia, O., Tang, C., and Busciglio, J. (2018). Dendritic spine pathology and thrombospondin-1 deficits in Down syndrome. *Free Radic. Biol. Med*. 114, 10–14. doi: 10.1016/j.freeradbiomed.2017.09.025

Torres-Ceja, B., and Olsen, M. L. (2022). A closer look at astrocyte morphology: development, heterogeneity, and plasticity at astrocyte leaflets. *Curr. Opin. Neurobiol*. 74:102550. doi: 10.1016/j.conb.2022.102550

Uddin, M. S., and Lim, L. W. (2022). Glial cells in Alzheimer's disease: from neuropathological changes to therapeutic implications. *Ageing Res. Rev*. 78:101622. doi: 10.1016/j.arr.2022.101622

Vicari, S., Nocentini, U., and Caltagirone, C. (1994). Neuropsychological diagnosis of aging in adults with Down syndrome. *Dev. Brain Dysfun*. 7, 340–348.

Weber, G. E., Koenig, K. A., Khrestian, M., Shao, Y., Tuason, E. D., Gramm, M., et al. (2020). An altered relationship between soluble TREM2 and inflammatory markers in young adults with down syndrome: a preliminary report. *J. Immunol.* 204, 1111–1118. doi: 10.4049/jimmunol.1901166

Wierzba-Bobrowicz, T., Lewandowska, E., Schmidt-Sidor, B., and Gwiazda, E. (1999). The comparison of microglia maturation in CNS of normal human fetuses and fetuses with Down's syndrome. *Folia Neuropathol.* 37, 227–234.

Wilcock, D. M. (2012). Neuroinflammation in the aging Down syndrome brain: lessons from Alzheimer disease. *Curr. Gerontol. Geriatr. Res.* 2012:170276. doi: 10.1155/2012/170276

Wilcock, D. M., and Griffin, W. S. (2013). Down's syndrome, neuroinflammation, and Alzheimer neuropathogenesis. *J. Neuroinflamm.* 10:84. doi: 10.1186/1742-2094-10-84

Wilcock, D. M., Hurban, J., Helman, A. M., Sudduth, T. L., McCarty, K. L., Beckett, T. L., et al. (2015). Down syndrome individuals with Alzheimer's disease have a distinct neuroinflammatory phenotype compared to sporadic Alzheimer's disease. *Neurobiol. Aging* 36, 2468–2474. doi: 10.1016/j.neurobiolaging.2015.05.016

Wiseman, F. K., Al-Jabani, T., Hardy, J., Kamiloff-Smith, A., Nizetic, D., Tybulewicz, V. L., et al. (2015). A genetic cause of Alzheimer disease: mechanistic insights from Down syndrome. *Nat. Rev. Neurosci.* 16, 564–574. doi: 10.1038/nrn3983

Wisniewski, K. E., Wisniewski, H. M., and Wen, G. Y. (1985). Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. *Ann. Neurol.* 17, 278–282. doi: 10.1002/ana.410170310

Xue, Q. S., and Streit, W. J. (2011). Microglial pathology in Down syndrome. *Acta Neuropathol.* 122, 455–466. doi: 10.1007/s00401-011-0864-5

Zdaniuk, G., Wierzba-Bobrowicz, T., Szpak, G. M., and Stepien, T. (2011). Astroglia disturbances during development of the central nervous system in fetuses with Down's syndrome. *Folia Neuropathol.* 49, 109–114.

Zigman, W. B. (2013). Atypical aging in Down syndrome. *Dev. Disabil. Res. Rev.* 18, 51–67. doi: 10.1002/ddrr.1128

frontiers | Frontiers in Cellular Neuroscience

TYPE Original Research
PUBLISHED 25 October 2022
DOI 10.3389/fncel.2022.989945

OPEN ACCESS

EDITED BY
Daniel Reyes-Haro,
Universidad Nacional Autónoma de México, Mexico

REVIEWED BY
Francisco G. Vázquez-Cuevas,
Universidad Nacional Autónoma de México, Mexico
Gerardo Bernabé Ramirez-Rodriguez,
National Institute of Psychiatry Ramon de la Fuente Muñiz (INPRFM), Mexico

*CORRESPONDENCE
Hui Liu
huiliu@cqmu.edu.cn

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Non-Neuronal Cells, a section of the journal Frontiers in Cellular Neuroscience

RECEIVED 09 July 2022
ACCEPTED 21 September 2022
PUBLISHED 25 October 2022

CITATION
Zheng Y, Yang M, Chen X, Zhang G, Wan S, Zhang B, Huo J and Liu H (2022) Decreased tubulin-binding cofactor B was involved in the formation disorder of nascent astrocyte processes by regulating microtubule plus-end growth through binding with end-binding proteins 1 and 3 after chronic alcohol exposure. *Front. Cell. Neurosci.* 16:989945. doi: 10.3389/fncel.2022.989945

Decreased tubulin-binding cofactor B was involved in the formation disorder of nascent astrocyte processes by regulating microtubule plus-end growth through binding with end-binding proteins 1 and 3 after chronic alcohol exposure

Yin Zheng[1,2†], Mei Yang[1†], Xiaoqiao Chen[1†], Gaoli Zhang[3], Shanshan Wan[4], Bingqiu Zhang[1], Jiechao Huo[5] and Hui Liu[1*]

[1]Institute of Neuroscience, Chongqing Medical University, Chongqing, China, [2]Department of Basic Medicine, Chongqing College of Traditional Chinese Medicine, Chongqing, China, [3]Institute for Viral Hepatitis, Second Affiliated Hospital of Chongqing Medical University, Chongqing, China, [4]Department of Blood Transfusion, Sichuan Cancer Hospital and Institute, Chengdu, China, [5]Fujian Province University Engineering Research Center of Mindong She Medicine, Medical College, Ningde Normal University, Ningde, China

Fetal alcohol syndrome (FAS) is a neurological disease caused by excessive drinking during pregnancy and characterized by congenital abnormalities in the structure and function of the fetal brain. This study was proposed to provide new insights into the pathogenesis of FAS by revealing the possible mechanisms of alcohol-induced astrocyte injury. First, a chronic alcohol exposure model of astrocytes was established, and the formation disorder was found in astrocyte processes where tubulin-binding cofactor B (TBCB) was decreased or lost, accompanied by disorganized microtubules (MT). Second, to understand the relationship between TBCB reduction and the formation disorder of astrocyte processes, TBCB was silenced or overexpressed. It caused astrocyte processes to retract or lose after silencing, while the processes increased with expending basal part and obtuse tips after overexpressing. It confirmed that TBCB was one of the critical factors for the formation of astrocyte processes through regulating MT plus-end and provided a new view on the pathogenesis of FAS. Third, to explore the mechanism of TBCB regulating MT plus-ends, we first proved end-binding proteins 1 and 3 (EB1/3) were bound at MT plus-ends in astrocytes. Then, through interference experiments, we found that both EB1 and EB3, which formed in heterodimers, were necessary to mediate TBCB binding to MT plus-ends and thus regulated the formation of astrocyte processes. Finally, the regulatory mechanism was studied and the ERK1/2 signaling pathway was found as one of the main pathways regulating the expression of TBCB in astrocytes after alcohol injury.

KEYWORDS

end binding proteins, ERK1/2 signaling pathway, fetal alcohol syndrome, microtubules, tubulin-binding cofactor B

Introduction

Fetal alcohol syndrome (FAS), caused by alcohol exposure during pregnancy, is one of the most common causes of nonhereditary lifelong disability worldwide, characterized by widespread impairments in fetal brain structure and function (Carter et al., 2008; Patten et al., 2014). Symptom severity of FAS varies greatly with timing, duration, and dose of alcohol exposure and genetic and metabolic factors (Burd et al., 2003). *In vitro* and *in vivo* models have successfully summarized multiple aspects of the disorder, including morphological and behavioral defects—still, the molecular and genetic mechanisms underlying FAS are poorly understood (Fischer et al., 2021). Astrocytes are the most abundant glial cells in the brain (Han et al., 2020), whose main functions include providing nutrients to the neuron, maintaining the extracellular ion balance, releasing transmitters, regulating synaptic plasticity, maintaining synaptic connection, and so on (Acosta et al., 2017; Palmer and Ousman, 2018; Munger et al., 2019). Astrocytes could not grow normally when cultured in a medium containing alcohol (Renau-Piqueras et al., 1989), and even moderate alcohol could delay their growth and maturation (Renau-Piqueras et al., 1989; Davies and Cox, 1991). They were more sensitive to alcohol than neurons (Lokhorst and Druse, 1993) and were found as one of the main targets of alcoholism during the central nervous system development (Guerri and Renau-Piqueras, 1997). Thus, the morphological and functional changes in astrocytes caused by alcohol toxicity should certainly be related to the pathogenesis of FAS. However, there are few reports about it, and it is worth studying.

Tubulin folding cofactor B (TBCB) participates in the conformational folding of α-tubulin to promote formations of α/β-tubulin heterodimers (Lopez-Fanarraga et al., 2001; Baffet et al., 2012), thus regulating the assembly, growth, and dynamic stability of microtubules (MTs) (Tian et al., 1997; Feierbach et al., 1999; Kortazar et al., 2007; Fleming et al., 2013), which is essential for cell viability. In addition, TBCB has been implicated in neurodegenerative processes (Wang et al., 2005), neurodevelopmental malformations (Tian et al., 2010), human cancer (Vadlamudi et al., 2005), and schizophrenia (Martins-de-Souza et al., 2009). Feltes et al. (2014) also found that the expression of RNA of TBCB was upregulated in adult FAS mice, suggesting that there might be some links between TBCB and the pathogenesis of FAS.

MTs are intrinsically polar filaments with two structurally and functionally distinct ends, the minus- and the plus-end (Desai and Mitchison, 1997; Howard and Hyman, 2003). MT minus-ends connected with the microtubule-organizing center grow slowly, while the plus-ends grow fast and are known as dynamic instability in the dynamic change of polymerization and depolymerization (Mitchison and Kirschner, 1984; Desai and Mitchison, 1997; Nogales and Wang, 2006). TBCB was shown to play a role at MT plus-ends (Lopez-Fanarraga et al., 2007). It was found to co-express with the most abundant MT at the early oocyte edge and (Baffet et al., 2012) to increase neuronal growth cones, which were characterized by active growth of MT plus-end (Lopez-Fanarraga et al., 2007). These data suggested the critical role of TBCB in MT plus-ends. However, TBCB could not directly bind to MT. How does it affect the MT plus-ends?

End-binding proteins (EBS) were key members of MT plus-end tracking proteins (+TIPS) that could directly bind to MT plus-ends and could connect with other proteins, which affected MT plus-ends (Akhmanova and Steinmetz, 2010; Galjart, 2010; Kumar and Wittmann, 2012), including EB1, EB2, and EB3. Among them, EB1 and EB3, but not EB2, promoted continuous microtubule growth (Komarova et al., 2009). TBCB mainly contains three domains, a ubiquitin-like (UBL) domain, a coiled-coil, and a Cytoskeleton-Associated Protein Glycine-rich (CAP-Gly) domain in the C-terminus (Lytle et al., 2004), which can interact with the EEY/F amino acid sequence at the C-terminus of EBs (Carranza et al., 2013). EB1 was preferentially bound to and produced comet-like streaks on the growing MT plus-ends (Sandblad et al., 2006; Dixit et al., 2009) and was found to combine with the mutation of TBCB (TBCBD3) (Carranza et al., 2013). through the EEY/F structure of EB1 and the CAP-Gly domains of TBCB (Honnappa et al., 2006; Steinmetz and Akhmanova, 2008). Similar to EB1, EB3 also has the C-terminal EEY structure (Nehlig et al., 2017) and can theoretically bind with TBCB. Our previous co-immunoprecipitation experiments on Cos7 cells had proved that TBCB could combine with EB3 (data not shown). Therefore, we reasonably speculated that TBCB may affect MT plus-ends through binding to the EB1/EB3 and finally affect the morphology and function of astrocytes. Indeed, it is necessary to conduct a systematic and in-depth study on whether TBCB regulates MT plus-ends through binding with EB1/EB3.

Mitogen-activated protein kinases (MAPK) include extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 protein kinase (Zhu et al., 2013). Previous studies showed that ethanol can impact the expression of some proteins in neurons (Zamora-Martinez and Edwards, 2014; Qiao et al., 2021), microglia (Gofman et al., 2016), and other cells (Aroor and Shukla, 2004). Alcohol, as an exogenous stimulant, was associated with the MAPK pathway (Zamora-Martinez and Edwards, 2014; Gofman et al., 2016; Qiao et al., 2021), and the ERK1/2 signaling pathway was found sensitive to alcohol (Peng et al., 2009). In addition, long-term alcohol consumption could inhibit the phosphorylation of ERK1/2 but not JNK or p38 in the mesocorticolimbic system (Zhu et al., 2013). In summary, the specific role of the MAPK signaling pathway in TBCB regulation after alcohol exposure needs to be further studied.

To sum up, we speculated that some of the neurological symptoms of FAS should be related to the morphological and

functional change of astrocytes caused by TBCB changes, which can regulate MT plus-ends through binding with EB1/EB3 and can be regulated by the MAPK signaling pathway after alcoholic injury. To confirm this, we carried out the following four parts of experiments: first, to study how the astrocytes and TBCB changed and the relationship between their changes after chronic alcohol exposure; second, to confirm whether the astrocytes change is caused by the change in TBCB through silencing or overexpressing TBCB; third, to confirm whether TBCB regulated the MT plus-ends through binding with EB1/EB3 by interrupting their expression and finally led to the morphologic change of astrocytes; and finally, to find and confirm the regulatory pathway of TBCB in astrocytes after chronic alcoholic injury.

Materials and methods

Ethics statement

All animals were obtained from the Animal Center of Chongqing Medical University and were approved by the Ethics Committee of Animal Care of Chongqing Medical University. All animal experiments in this study conformed to the standards of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). The license number for using laboratory animals is SCXK(Chongqing)2018-0003. The experimental animals were killed immediately after purchase, and the primary astrocytes were extracted; all the cells were randomized and blinded. Experimenter blinding was sufficient to control for selection bias. The sample size was evaluated using Power Analysis and Sample Size 2022 (PASS 2022, USA). Furthermore, sample sizes for experiments were sufficient for normality, variance homogeneity, and statistical analyses.

Primary astrocyte cultures

Mice primary astrocytes were cultured from the cerebral cortices of C57BL/6 mice at postnatal day 0 (Mccarthy and De Vellis, 1980). Briefly, newborn mice were sterilized alive by immersion in 75% ethanol alive, and then the brain tissue was removed and the cerebral membranes were carefully removed. The cerebral cortex was cut into pieces and digested with 0.25% trypsin at 37°C for 10 min, then stopped digestion by a mixture of Dulbecco's modified Eagle's medium with Ham's F-12 medium (LD1223, DMEM/F12, Hyclone) supplemented with 10% fetal bovine serum (900108, FBS, Gemini). After centrifugation, discarded supernate and resuspended the pellet in a mixture of DMEM/F12 supplemented with 10% FBS. Then, the cells' suspension was filtered with a 200-mesh filter (Saimike) and were planted in flasks and incubated at 37°C with 5% CO_2 and 95% air. The culture medium was replaced every 2 days. After 7 days, the cells were transferred to 6-well plates for western blotting (WB) or Reverse transcription real-time PCR (RT-PCR), and on 12-well plates covered with glass slides for immunofluorescence staining (IF). All experiments were performed on secondary cultures after being grown for 1 day. Cultures of at least 95% astrocytes were used in the following study, as confirmed by immunofluorescent staining (IF) for glial fibrillary acidic protein (GFAP, Supplementary Figure 1A).

Establishment of chronic alcohol exposure model

Mice secondary-generation astrocytes were randomly divided into a blank control group (Con) and chronic alcohol exposure groups (30, 100 mM). In the chronic alcohol exposure group, secondary astrocytes were cultured in an alcohol-containing medium for 7 days. At 7 days, cells in each group were collected and used for IF ($n = 6$), WB ($n = 6$), and RT-PCR ($n = 6$). The medium with the same alcohol concentration was changed regularly everyday to maintain the alcohol concentration (Ethanol evaporation after 24 h was 10–20%) (Fischer et al., 2021). To analyze the concentration-dependent effect of alcohol, cells were exposed to varying concentrations of alcohol (0, 30,100 mM) (Tomas et al., 2005). The final 30-mM alcohol concentration is similar to the blood alcohol level reported by chronic pregnant drinkers or women weighing about 60 kg when drinking 3–5 glasses of wine within 1 h (Eckardt et al., 1998). Some cells were exposed to 100 mM ethanol for 7 days to analyze the efficacy of high ethanol concentrations (Guasch et al., 2003; Tomas et al., 2003). The alcohol concentrations used are in the range of the blood alcohol levels (BAL) found among alcoholics (Jones and Sternebring, 1992).

Small-interfering RNA (siRNA) transfection

All small-interfering RNAs (siRNAs) scrambled sequences were synthesized by Chongqing Maobai Technology Co. (Chongqing, China). There was no significant change between the negative siRNA control group and the blank group, so only the negative siRNA control group was displayed as the control group. Astrocytes were transfected with TBCB siRNA or negative oligonucleotides in 6-well or 24-well plates for 6 h using the Lipofectamine™ 3000 transfection kit (L3000015, Invitrogen, USA). Each well of a 6-well plate contained 0.8 × 10^6 cells, 5 μl siRNA, 3.75 μl Lipofectamine 3000, and 250 μl Opti-MEM (31985062, Gibco, USA). Each well of 24-well plates contained 0.6 × 105 cells per well, 1.25 μl

siRNA, 0.75 μl Lipofectamine 3000, and 50 μl Opti-MEM. They were replaced with fresh and DMEM/F12 supplemented with 10% FBS after 6 h. After 48 h following transfection, cells were collected and analyzed by RT-PCR ($n = 6$). After 72 h following transfection, cells were collected and analyzed by WB ($n = 6$) and IF ($n = 6$). All operations were carried out in strict accordance with the commodity instructions. The target siRNA sequences (5 → 3′) used in this study were as follows: TBCB: forward GCAUGGAACUGGAGCUGUATT, reverse UGGUCAAUGACAUGG AUGCTT; EB1: forward GCAGAGGAUUGUAGAUAUUTT, reverse AAUAUCUACAAUCCUCUGCTT; EB3: forward GACGCAAACUAUGAUGGAATT, reverse UUCCAUCAUAGUUUGCGUCTT; and Negative control siRNA: forward UUCUCCGAACGUGUCACGUTT, reverse ACGUGACACGUUCGGAGAATT.

Lentiviral infection

Mice secondary-generation astrocytes were seeded in 6-well plates with 0.6×10^6 cells. There was no significant change between the no-load lentivirus control group and the blank group, so only the lentivirus no-load control group was displayed as the control group. Astrocytes were randomly assigned to two groups: The control group, cultured in a medium containing 8-μl no-load lentivirus (L2019-358SH GenePharma, Shanghai); and the virus groups cultured in a medium containing 8-μl TBCB overexpress virus (L2019-358SH, EF-1aF/ GFP & Puro, GenePharma, Shanghai) or EB3 overexpress virus (L2019-358SH, EF-1aF/m-Cherry& Puro; GenePharma, Shanghai). After 24 h, a 2-ml DMEM/F12 supplemented with 10% FBS was replaced. After 8 days of infection, cells were collected and analyzed by WB ($n = 6$) and IF ($n = 6$). All operations were carried out in strict accordance with the commodity instructions.

ERK1/2 signaling pathway interference assay

To confirm whether the ERK1/2 signaling pathway regulated the expression of TBCB after alcohol exposure, the ERK1/2 interference experiment was carried out. Astrocytes were randomly divided into three groups: the solvent control group (Con), cultured in a medium containing 2 μl dissolved in dimethyl sulfoxide (DMSO, Saimike; Zhang et al., 2019); ERK1/2 agonist group (TPA), cultured in a medium containing 200 μM TAP (ERK1/2 agonist, CST; Zhang et al., 2019) dissolved in 2 μl DMSO; ERK1/2 inhibitor group (U0126), cultured in medium containing 10 mM U0126 (MEK1/2 inhibitor, Selleck; Zhang et al., 2019) dissolved in 2 μl DMSO. After 1 h, the drugged medium was removed, and DMEM/F12 supplemented with 10% FBS was added. After 12 h, the three groups of cells were collected separately and analyzed by WB ($n = 6$).

Cell counting kit-8 multiplication experiment

Cell viability was evaluated using the Cell Counting Kit-8 (CCK-8) assay (AR1199, Boster, China). Mice secondary-generation astrocytes were inoculated on 96-well plates at a concentration of 5×10^3 cells/well ($n = 6$) in 100 μl DMEM-F12 medium containing 10% FBS and cultured at 37°C with 5% CO2 and 95% air. After chronic alcohol exposure, discard the culture medium. A complete medium of 100 μl containing 10 volumes of CCK8 reagent was added to each well and then incubated in the cell incubator for 1 h at 37°C in the dark. The absorbance at 450 nm was measured by the enzyme labeling instrument. Cell viability was calculated using the following equation: cell viability (%) = average OD in the study group/average OD in the control group × 100%.

Western blotting

The harvested astrocytes were lysed on ice in RIPA cleavage buffer containing 1% PMSF (ST506, Beyotime, Guangzhou, China), and total protein concentration was measured with a BCA protein assay kit (P0012S, Beyotime, China). After dilution in the sample loading buffer, 20 μg of protein was added to each lane. According to the molecular weight of the protein, suitable electrophoresis and wet transfer conditions were selected. The proteins were then separated on a 10% SDS-PAGE gel and transferred to a 0.2-μm polyvinylidene difluoride (PVDF) membrane (IPVH00010, Millipore, USA). The membranes were blocked with a blocking buffer (P0220, Beyotime, China) at room temperature for 30 min. Then, they were probed with the primary antibodies properly diluted overnight at 4°C and then with the HRP-labeled antimouse or antirabbit IgG secondary antibody (ZB-2305 or ZB2301, ZSGBBIO, China) for 1.5 h at room temperature. Then, they were visualized by Western Bright ECL (K-12045-D20, Advansta, USA) and imaged using a Western blotting detection system (Bio-Rad, USA) or X-ray film. There are six repeats, which are three biological repeats multiplied by two technical repeats. Then, the densities of the bands in each image were quantified three times by Quantity-One software. The value of the target protein was normalized to the value of housekeeper protein from the same sample within the same blotting. Then, all the corresponding values from different groups were performed statistically analyzed by GraphPad Prism 6.0 software (GraphPad Software, USA).

The locations of all the proteins detected by the first antibody used in WB were shown in the full-length blotting (Supplementary Figures 1B–N). The location of

the first antibody was consistent with that provided by the manufacturer, and the specificity of the first antibody was verified by subsequent silencing or overexpression experiments. The primary antibodies used in WB were listed as follows: Anti-TBCB (1:500, A13248, ABclonal, China), Anti-MAPRE1 (1:1,000, NHA2107, Novogene, China), Anti-MAPRE3 (1:1,000, #AP52003-100, Abcepta, China), Anti-p38 (1:1,000, #8690, Cell Signaling Technology, USA), Anti-pp38 (1:800, #4511, Cell Signaling Technology, USA), Anti-JNK (1:1,000, #9252, Cell Signaling Technology, USA), Anti-p-JNK (1:1,000, #4668, Cell Signaling Technology, USA), Anti-PAK1 (1:1,000, #2602, Cell Signaling Technology, USA), Anti-pPAK1 (1:1,000, #2606, Thermo Fisher Scientific, USA), Anti-β-actin (1:5,000,20536-1-AP, Proteintech, China), Anti-α-T (1: 5,000, GTX628802, GeneTex, USA), Anti-β-T (1:5,000, TA503129, OriGene, USA), Anti-ERK1/2 (1:1,000, #4695, Cell Signaling Technology, USA), Anti-pERK1/2 (1:1,000, #4370, Cell Signaling Technology, USA), and Anti-GAPDH (1:5,000, 60004-1-lg, Proteintech, China).

Reverse transcription real-time PCR

Total RNA was extracted from harvested astrocytes ($n = 6$) by using RANiso plus (#9108, TaKaRa, China), and the concentration of RNA was measured by spectrophotometer. A total of 1 μg RNA was reverse transcribed to generate cDNA using the PrimeScript™ II 1st Strand cDNA Synthesis Kit (6210A, TaKaRa, China). The expression of messenger of TBCB as a housekeeping gene was assessed by real-time PCR. PCR amplification was performed using a T100 thermal cycler (BIO-RAD) and Premix Taq™ (RR901Q, TaKaRa, China). The PCR reaction mixture consisted of 1 μl of each primer, 25 μl of Premix Taq, and 1 μl of cDNA in a final volume of 50 μl. The PCR conditions were as follows: denaturation at 94°C for 3 min, followed by 34 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. All qPCRs were run on a CFX96 real-time system (Bio-Rad). The $2^{-\Delta\Delta Ct}$ method was used to calculate the fold change of RNA or miRNA level compared to control samples. The primers sequences ($5' \rightarrow 3'$) were listed as follows: TBCB, forward ATGGAGCAGACGACAAGTTCT, reverse CCGTCATCCACAGGATAGGAG, product size (77 bp); EB1, forward AAGCTAGAACACGAGTACATCCA, reverse AGTTTCTTGACCTTGTCTGGC, product size (210 bp); EB3, forward ATGGCCGTCAATGTGTACTCC, reverse GTTTGGCCTGGAACTTCACTT, product size (199 bp); and β-actin (control), forward CAGCCTTCCTTCTTGGGTA, reverse TTTACGGATGTCAACGTCACAC, product size (87 bp). All operations were carried out in strict accordance with the commodity instructions.

Immunofluorescent staining

The astrocytes were cultured on glass coverslips in a 24-well plate and fixed in −20° precooled acetone and methanol (1:1) for 5 min and then were blocked with 5% bovine serum albumin (BSA) at room temperature for 30 min. The cells were probed with the primary antibodies (anti-TBCB, 1:50, A13248, ABclonal, China; anti-TBCB, 1:250, sc-377139, Santa Cruz, USA; Anti-MAPRE1, 1:1,000, NHA2107, Novogene, China; Anti-MAPRE1, ab53358, Abcam, UK; Anti-MAPRE3, 1:1,000, #AP52003-100, Abcepta, China; anti-α-T, 1: 5,000, GTX628802, GeneTex, USA; GFAP, 1:1,000, P107217, KleanAB, China) properly diluted 4° overnight. Then, the cells were incubated with secondary antibodies (FITC goat antirabbit IgG, 1:200, E031220-01, EARTH, China; Cy3 goat antimouse IgG, 1:200, Abbkine, China) and stained with DAPI (C1005, Beyotime, China). Subsequently, the cells were mounted in Fluorescence Mounting Medium (ab104135, Abcam, UK) and sealed with nail polish. Images were obtained by Inverted fluorescence microscope (Leica DMI8, Germany), and the intensity of fluorescence was analyzed by ImageJ (1.53c) software. The astrocyte processes in high magnification images were also counted by ImageJ (1.53c) software (according to the results, about 50 cells were counted: six 200-fold visual fields were randomly selected in each group, and the cells were counted from the center of the visual field to the periphery). It was mainly used to observe the changes in cell morphology and protein distribution in the study.

Statistical analysis

Statistical analyses were performed and the corresponding graphs were drawn using GraphPad Prism 6.0 software (GraphPad Software, USA). Data were expressed as means ± standard deviation (SD). The related changes in alcohol intervention groups and control groups were evaluated using a one-way ANOVA. The related changes between other treatment groups and control groups were evaluated using a two-tailed unpaired t-test. The Shapiro–Wilk normality test was performed to analyze the data distribution for each group. Homogeneity of variance was evaluated using Brown–Forsythe and F test for ANOVA and unpaired t-test analyses, respectively. All the data presented in the manuscript passed both tests and were analyzed as normally distributed with equal variances (Supplementary Table 1). Investigators who performed the experiments and the statistical calculations were blinded to the experimental protocol. Values were considered statistically significant when $p < 0.05$ ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$).

Results

Alcohol inhibited the formation of astrocyte processes and TBCB expression in nascent processes

To reveal the changes and the relationship between astrocytes and TBCB after chronic alcohol exposure, low (30 mM) and high (100 mM) ethanol concentrations ethanol were used for establishing chronic alcohol exposure models. Astrocytes in the control group grew well and had good viability (Figure 1D), with flat and plump cell bodies and plenty of processes (Figure 1A). TBCB was mainly not only diffusely distributed in the cytoplasm but also could be arranged in filaments along with MT. It was especially abundant in nascent processes (Figure 1A, arrows), suggesting TBCB played an essential role in the formation of astrocyte processes. Most of the MTs, labeled by α-tubulin, radiated from the organizing center around the nucleus to the edge of the cell cortex and were arranged in dense and regular lines (Figure 1A, arrows). They were especially enhanced in the nascent processes, colocalized with TBCB, hinting at the close relationship between MT and TBCB in this study.

The cell injury was similar in the two alcohol groups, and cell viability decreased (Figure 1D), but the damage degree was concentration-dependent (Figures 1B–D). The astrocytic body collapsed, and the cortical volume and nascent process numbers decreased significantly (Figures 1B,C,E), indicating that alcohol could inhibit astrocyte process formation. The intracellular MT was significantly decreased (Figures 1E,F) with the irregular arrangement, lower density, and more intertwined bundles (Figures 1B,C), especially with the curly plus-ends in the processes (Figures 1B,C, arrowheads), suggesting the irregular growth in MT plus-ends in this study. The expression of TBCB was decreased significantly (Figures 1F,G), particularly in nascent astrocyte processes (Figures 1B,C, arrows, Figure 1E), which were originally in the expression of high-density (Figure 1A, arrows), indicating that alcohol could cause the decrease in the expression of TBCB, especially in astrocytes nascent processes. Moreover, it suggests that the change in the expression of TBCB in this study was closely related to the formation and the growth of nascent astrocyte processes.

TBCB was involved in the formation of astrocyte processes

To confirm the relationship between the decreased expression of TBCB after alcohol exposure and the inhibition of process formation on astrocytes, we used siRNA to silence TBCB. Positive cells transfected by siRNA showed green fluorescence, and the transfection efficiency was more than 90% (Supplementary Figure 2D). WB (Figure 2F) and PCR (Figure 2G) showed that TBCB protein and mRNA content were significantly decreased in the silent group, indicating that the silencing effect was excellent.

After TBCB silence, TBCB still could be observed along with MT (Figure 2B); however, the diffuse TBCB decreased significantly (Figures 2B,E), especially in the nascent astrocyte processes where the original highly expressed TBCB almost disappeared (Figure 2B, arrows, Figure 2E). As well, the intracellular MT content decreased (Figures 2E,F), with the sparse and irregular arrangement, intertwined bundles, and especially the curly plus-ends in nascent processes (Figure 2B, arrows), indicating the irregular growth in MT plus-ends and that the decrease or disappearance of TBCB in nascent processes had severe impacts on the growth of MT plus-ends. With the disappearance of TBCB from nascent processes of astrocytes, most already formed processes were also retracted or disappeared (Figure 2E; Supplementary Figure 2F, arrows). These results showed that TBCB decrease at astrocyte nascent processes did lead to the disorder of astrocyte process formation by regulating the plus-ends of MT.

To understand whether increasing the expression of TBCB had the opposite effect, we also overexpressed TBCB. Lentivirus was used as the vector, and the astrocyte infection rate reached more than 95% (Supplementary Figures 2G,H). WB (Figure 2I) and PCR (Figure 2J) showed that the TBCB protein and mRNA levels were significantly increased in the overexpression group of TBCB, indicating a successful infection. After TBCB was overexpressed, the expression of TBCB increased (Figures 2D,H; Supplementary Figure 2L, arrows), especially in nascent astrocyte processes (Figure 2D, arrows, Figure 2E), accompanied by increased content and dense arrangement of MT in the same place (Figures 2H,I,D, arrows). Meanwhile, astrocyte processes also increased significantly in numbers (Figures 2D,H; Supplementary Figure 2L); however, they lost the original sharp-angle-shape (Figure 2C, arrows), instead with the enlarged basal parts and obtuse tips with the longer extension (Figure 2D; Supplementary Figure 2L, arrows), which was similar to the huge axon-end in Giant Axonal Neuropathy (Wang et al., 2005; Ganay et al., 2011). It was suggested that the accumulation of TBCB could cause the huge processes of astrocytes. All the results of this part indicated that the concentration of TBCB within a certain range was critical for the normal morphology and function of astrocytes. In other words, TBCB was one of the essential factors affecting the formation and growth of astrocyte processes by regulating the growth of MT plus-ends. Combined with the first part experiment, it was proved that the decreased expression of TBCB in astrocyte processes caused by alcohol was one of the main factors leading to the decrease in astrocyte processes by regulating the growth of MT plus-ends. Although how TBCB banded to MT plus-ends and regulated them was still unclear, EB1 and EB3, as the

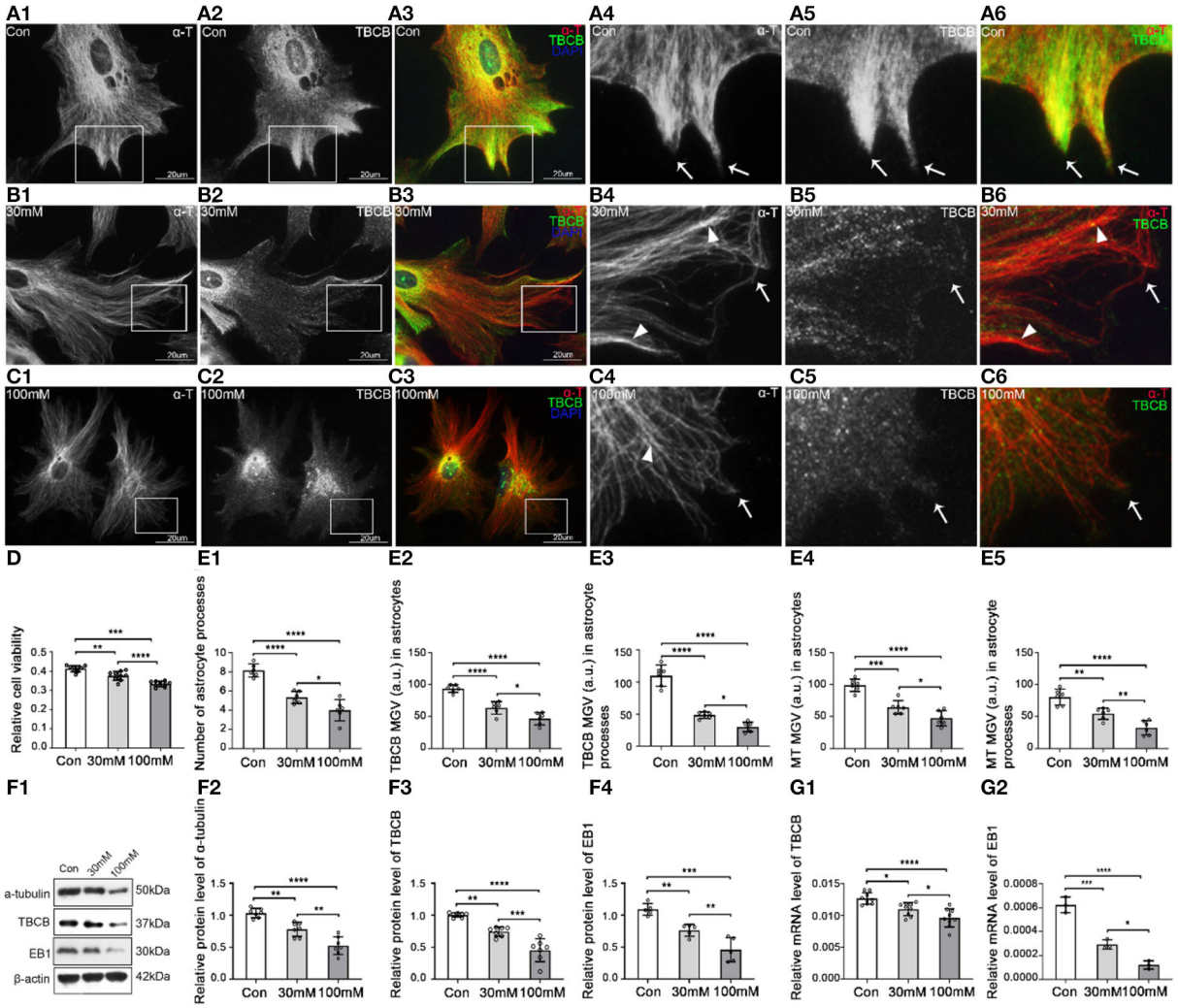


FIGURE 1
Morphological changes in astrocytes and expression levels of α-tubulin and TBCB after chronic alcohol exposure. **(A–C)** Immunofluorescence showed that α-tubulin (red signal) was co-expressed with TBCB (green signal) in astrocytes both in the control group [Con **(A)**] and the chronic alcohol exposure group [30 mM **(B)**; 100 mM **(C)**]. **(D)** Relative cell viability was detected by Cell Counting Kit-8 after chronic alcohol exposure. **(E)** The number of astrocyte processes and mean gray value (MGV) of TBCB and MT in astrocytes and its processes (MGV = Integrated Density/Area) after chronic alcohol exposure group. **(F)** Western blot and **(G)** RT-PCR analysis of the levels of the α-tubulin protein and TBCB/EB1 protein and its mRNA after chronic alcohol exposure compared with the control group ($n = 6$, $p < 0.05$). **(A–G)** Number of panels. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

necessary binding proteins, were the potential candidates based on the previous research.

EB1 and EB3 were bound to MT plus-ends in astrocytes

EB1 and EB3 were reported to bind with MT plus-ends in many kinds of cells (Jaworski et al., 2008), but there were still unclear in astrocytes. To confirm it and pave the way for further experiments, interference experiments were carried out on EB1 and EB3 mediated by siRNA transduction or lentivirus infection. Because that EB1 has been found to bind and regulate MT plus-ends in astrocytes (Bu and Su, 2001; Chiu et al., 2015) and to bind with TBCB directly (Carranza et al., 2013), and also based on the inhibitory results of TBCB and EB1 (Figures 1F,G) on MT plus-ends growth after alcohol injury, we only carried out the silence experiments on EB1 in the following studies. However, little was known about EB3 in the relationship between TBCB and MT plus-ends, so except for silenced experiments, the overexpression experiments of EB3 were also carried out in subsequent studies.

In all the transfection (silence), infection (overexpression), and control groups in our experiments,

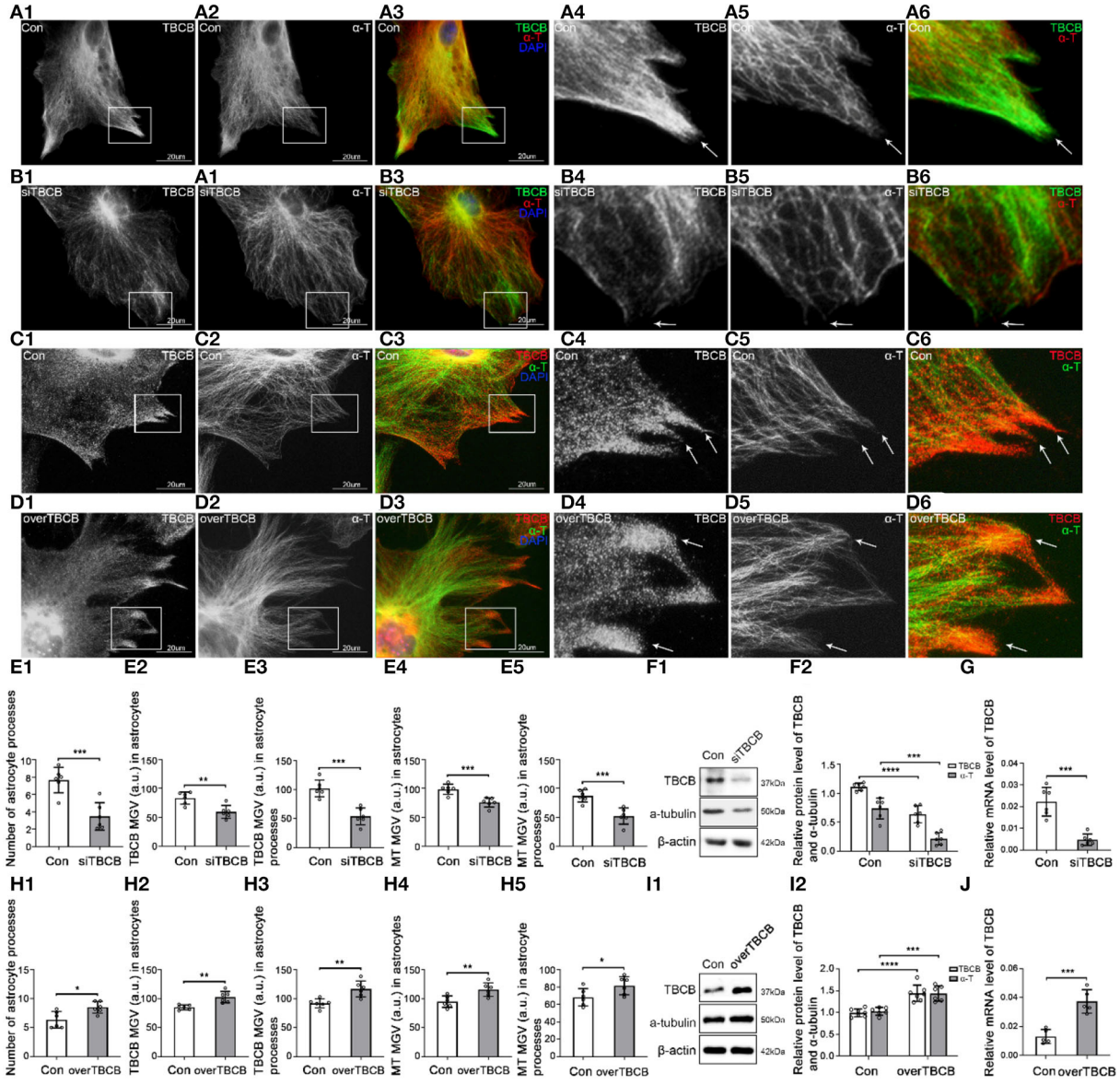


FIGURE 2
Morphological changes in astrocytes and expression levels of TBCB and α-tubulin after TBCB was silenced or overexpressed. **(A–D)** Immunofluorescence showed that TBCB [**(A,B)** green signal; **(C,D)** red signal] was co-expressed with α-tubulin [**(A,B)** red signal; **(C,D)** green signal] in astrocytes both in the control group [Con **(A)**] and in TBCB silence group **(B)** or TBCB overexpression group **(D)**. **(E,H)** The number of astrocyte processes and mean gray value (MGV) of TBCB and MT in astrocytes and its processes (MGV = Integrated Density/Area) after TBCB was silenced or overexpressed. **(F,I)** Western blot and **(G,J)** RT-PCR analysis of the levels of the α-tubulin protein and TBCB protein and its mRNA after TBCB was silenced or overexpressed compared with the control group ($n = 6$, $p < 0.05$). **(A–J)** Number of panels. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

the transfection and infection efficiencies were more than 90% (Supplementary Figures 2B,C) and 95% (Supplementary Figures 2I,J), respectively. The proteins and mRNA levels of EB1 and EB3 were significantly decreased after gene silencing (Figures 3H,I,K,L) and increased after gene overexpressing (Figures 3N,O), indicating that transfection and infection were successful.

In the control group, IF showed that most MT labeled by α-tubulin originated from perinuclear MT organizing centers and were arranged in a straight, radial, dense, and regular manner (Figures 3A,C,E, arrows), and a few were arranged in a circle along with the cell cortex edge (Figures 3A,C,E). Although EB1 and EB3 could be weakly distributed along with MT, they mainly concentrated on MT plus-ends as typical bright

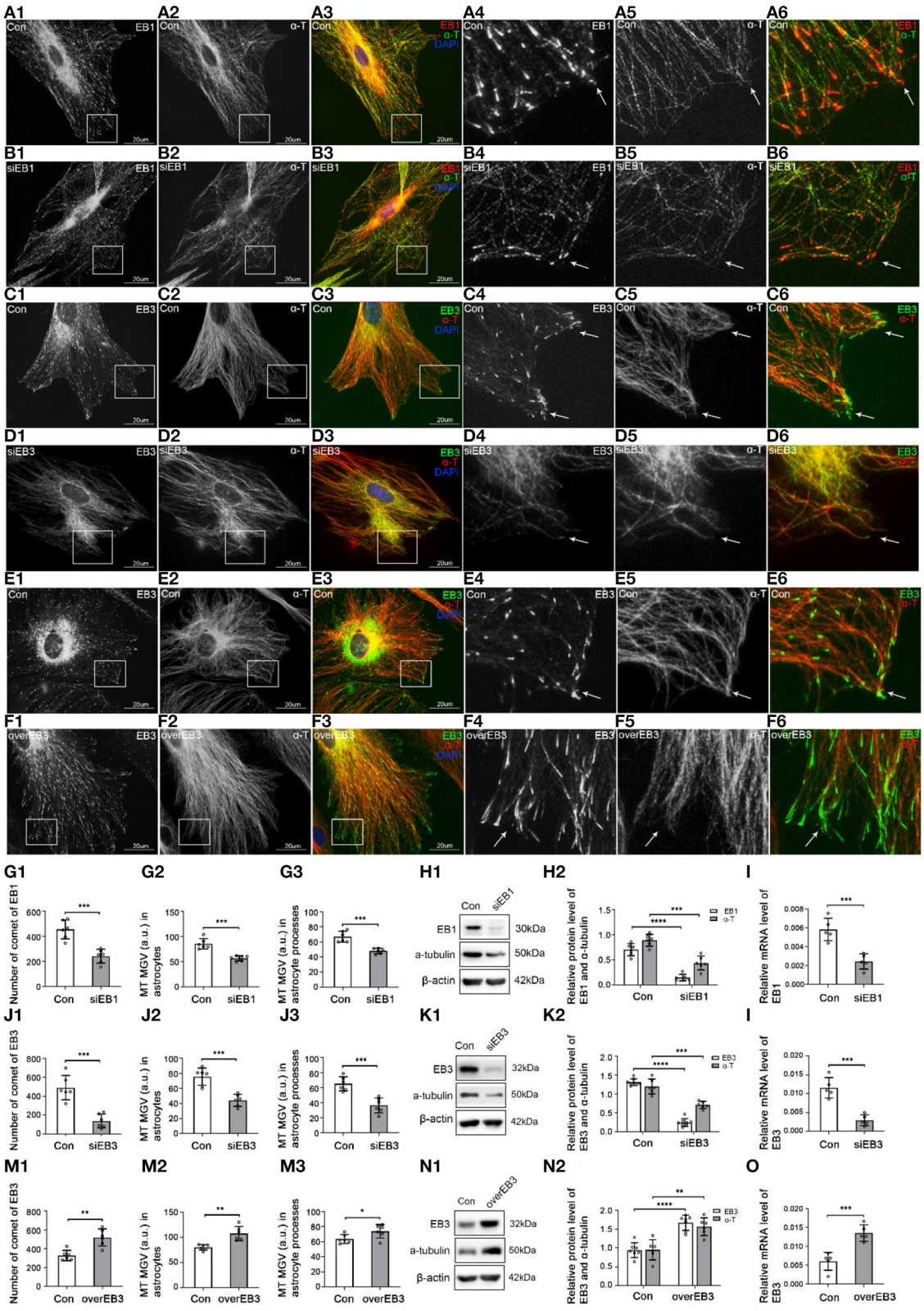


FIGURE 3
The expression of EB1/EB3 and α-tubulin after EB1/EB3 was silenced or EB3 was overexpressed in astrocytes. **(A–F)** Immunofluorescence showed that α-tubulin [**(A,B)** green signal; **(C–F)** red signal] was co-expressed with EB1 [**(A,B)** red signal] or EB3 [**(C–F)** green signal] in astrocytes both in the control group [Con **(A,C,E)**] and EB1/EB3 silence group **(B,D)** or EB3 overexpression group **(F)**. **(G,J,M)** The number of EB1/EB3

(Continued)

FIGURE 3 (Continued)
comets and mean gray value (MGV) of MT in astrocytes and its processes (MGV = Integrated Density/Area) after EB1/EB3 was silenced or EB3 was overexpressed. **(H,K,N)** Western blot and **(I,L,O)** RT-PCR analysis of the levels of the α-tubulin protein and EB1/EB3 protein and its mRNA after EB1/EB3 was silenced or EB3 was overexpressed compared with the control group ($n = 6$, $p < 0.05$). **(A–O)** Number of panels. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

comet-like streaks (Figures 3A,C,E, arrows). Most comets were arranged radially, with the comet-heads located at MT plus-ends and the comet-tails toward MT organizing centers, and a few were arranged on the circle MT plus-ends in the cell cortex. It indicated that EB1 and EB3 were localized at the plus-ends of MT in astrocytes.

After EB1 or EB3 silence, the comets were still located at MT plus-ends, but the numbers decreased significantly (EB1) or even lost (EB3), and changed into thinner, shorter, and smaller comets (Figures 3B,D, arrows, Figures 3G,J). In addition, the content (Figures 3G,I) and numbers (Figures 3B,D,G,J) of MT were decreased, with a sparse, irregular arrangement and curly plus-ends (Figures 3B,D, arrows). The astrocyte cortex was hollowed out and collapsed (Figures 3B,D). These results proved again EB1 localized at MT plus-ends and involved in the regulation of the astrocyte.

After the overexpression of EB3, the comet was still localized at MT plus-ends but significantly denser, longer, and stronger (Figures 3F,M); meanwhile, MT grew exuberantly, mainly in regularly radial straight lines, with obviously increased density and length (Figures 3F,M). The astrocytes were extended and grew dramatically compared with the control group (Figure 3F). Combined with the silence experiments data, EB3 was confirmed as a microtubule plus-end tracking protein in astrocytes and involved in regulating the growth of MT plus-ends.

As we all know, EB1 and EB3 were binding proteins and could not directly act on or regulate MT plus-ends. The regulation function of MT plus-ends they showed was attributed to the proteins they were linked. The above experiments had shown the ability of TBCB to regulate the MT plus-ends. Therefore, TBCB binding to MT plus-ends through EB1/EB3 affects astrocyte processes was our aim for the subsequent study.

EB1 was necessary to mediate TBCB binding to MT plus-ends

First, to determine whether EB1 was involved in TBCB binding with MT plus-ends in astrocytes, EB1 was silenced with siRNA. EB1 content was decreased (Figures 4C–E), and EB1 comets at MT plus-ends dramatically decreased and shortened into small dots (Figure 4B, arrows, Figure 4C). Furthermore, TBCB content decreased significantly (Figures 4C–E), especially in astrocyte processes, but did not disappear, accompanied by the number decrease in astrocyte processes (Figure 4B, arrows, Figure 4C), in which most of them lost their sharp shape (Figure 4B, arrows). These results indicated that EB1 was necessary to mediate TBCB binding to MT plus-ends, and through this binding, TBCB could regulate the growth of MT plus-ends, thus affecting the formation of the astrocyte process.

EB3 was necessary to mediate TBCB binding to MT plus-ends

Second, to determine whether EB3 mediated TBCB binding to MT plus-ends, EB3 silence and overexpression were carried out. After EB3 silence, similar to EB1, the expression of EB3 content was significantly decreased (Figures 5E–G), and the EB3 comets at MT plus-ends were reduced and shortened into small dots (Figure 5B, arrows). In addition, TBCB content decreased significantly, especially in astrocyte processes (Figures 5E,F), and the original high expression of diffused TBCB decreased significantly (Figure 5B, arrows), accompanied by the decrease in astrocyte processes (Figures 5B,E). It proved EB3 was needed for TBCB to perform its normal function on MT plus-ends.

Furthermore, after the overexpression of EB3, EB3 content (Figures 5H–J), and the length, size, and numbers of EB3 comets increased significantly (Figure 5D, arrows, Figure 5H). The expression of TBCB was also increased (Figures 5H–J), and the shape and processes of astrocytes showed no obvious change (Figure 5D). These results further prove that EB3 was necessary for TBCB binding to MT plus-ends and contributing to astrocyte processes.

EB1 and EB3 changed synchronously

Thirdly, to explore the possible relationship between the two EB proteins and understand the possible binding model between EB proteins and TBCB, we, respectively, silence and overexpress one EB protein and detect the changes of the other. In addition, EB3 comets co-labeled the tip of EB1 comets at MT plus-ends in the astrocyte control group (Figures 6A,D). After EB1 was silenced, the protein and gene levels of EB3 decreased synchronously with EB1 (Figures 6F–H), the EB3 comets shrank into small dots with the decreased number, and cells' bodies also shrank obviously (Figure 6B, arrows, Figure 6F). These data suggested that the distribution and role of EB3 in MT plus-ends were closely related to the expression of EB1. In addition, after EB3 silence, EB1 protein and gene levels also decreased (Figures 6J,K). Meanwhile, EB1 almost lost the comet

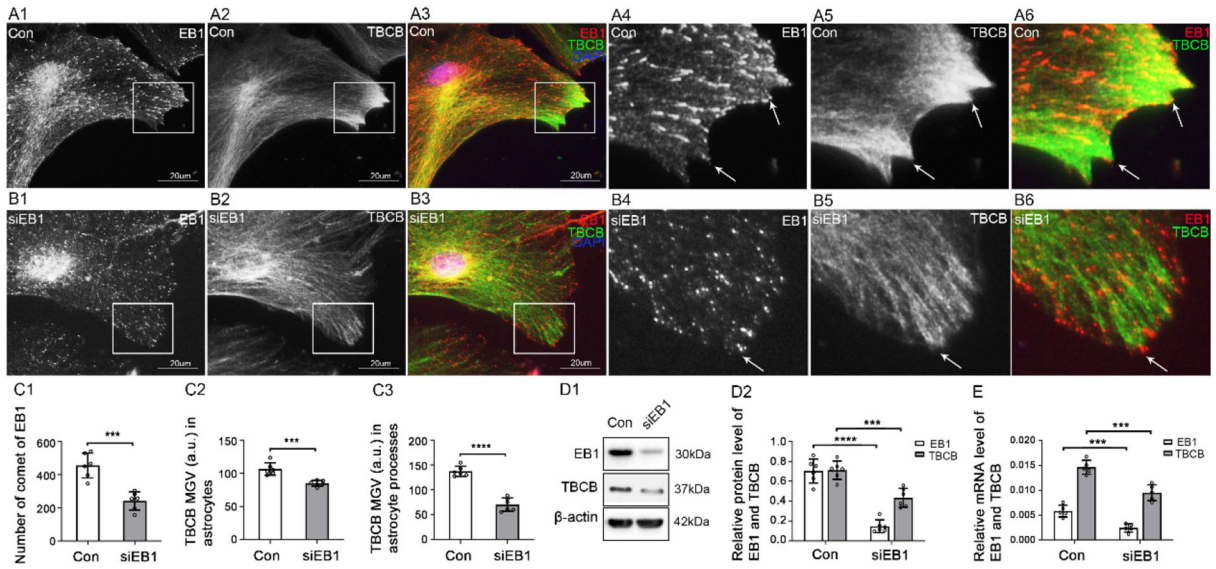


FIGURE 4
The expression of TBCB after EB1 was silenced in astrocytes. **(A,B)** Immunofluorescence showed that EB1 [**(A,B)** red signal] was co-expressed with TBCB [**(A,B)** green signal] in astrocytes in the negative control group [Con **(A)**] and EB1 silence group **(B)**. **(C)** The number of EB1 comets and mean gray value (MGV) of TBCB in astrocytes and its processes (MGV = Integrated Density/Area) after EB1 was silenced. **(D)** Western blot and **(E)** RT-PCR analysis of the protein and mRNA levels of EB1 and TBCB after EB1 was silenced compared with the control group ($n = 6$, $p < 0.05$). **(A–E)** Number of panels. ***$p < 0.001$, ****$p < 0.0001$.

shape that changed into small dots and decreased (Figure 6C, arrows, Figure 6I). Furthermore, after EB3 overexpress, the protein and gene levels of EB1 were increased (Figures 6M,N), and EB1 comets also increased significantly and changed longer and stronger (Figure 6E, arrows, Figure 6L). These data suggested that the distribution and role of EB1 in MT plus-ends were also closely related to the expression of EB3. They two changed synchronously and were interdependent, but not complementary, and necessary for each other.

The change in TBCB expression led to the synchronous change in EB1 and EB3

Fourthly, to further explore the relationship between TBCB and EB proteins, TBCB was silenced or overexpressed. After TBCB silence, the expression of TBCB decreased (Figures 7I–K) and almost disappeared in astrocyte processes (Figures 7B,F, arrows, Figure 7I). Conversely, it was abundant in processes after the overexpression of TBCB (Figures 7D,H, arrows, Figure 7L). The expression of EB1 and its mRNA both decreased in the TBCB silence group (Figures 7I–K) and increased in the overexpression group of TBCB (Figures 7L–N), and the comets decreased (Figures 7B,F,I), especially in the edge of astrocytes where the original processes were lost after silence (Figure 7B, arrowheads) or increased significantly in the nascent process after overexpression (Figure 7D, arrows, Figure 7L). It suggested that the change in EB1 was a passivity response to the disorder of the process formation due to the irregular growth of MT plus-ends caused by TBCB changes.

The expression of EB3 and its mRNA both decreased in the TBCB silence group (Figures 7I–K) and increased in the overexpression group of TBCB (Figures 7L–N). However, its comets were almost lost and changed into small dots after TBCB silencing (Figure 7F, arrows, Figure 7I) or increased obviously and changed bigger and longer after TBCB overexpressing (Figure 7H, arrows, Figure 7L). It suggested that EB3 was not only involved in binding with TBCB on MT plus-ends but also relied, at least partly, on TBCB to execute its normal function.

The above experiments confirmed that alcohol could cause TBCB decrease, impede the growth of MT plus-ends through binding with EB1 and EB3, and cause the deficient arrangement of astrocyte processes, finally leading to neurological symptoms. However, the signaling pathway that regulates the expression of TBCB after alcohol exposure is still unclear, and we did the following experiments to explore it.

ERK1/2 signaling pathway regulated the expression of TBCB

To determine whether the MAPK signaling pathway regulates the expression of TBCB, we detected MAPK phosphorylation levels after chronic alcohol exposure to

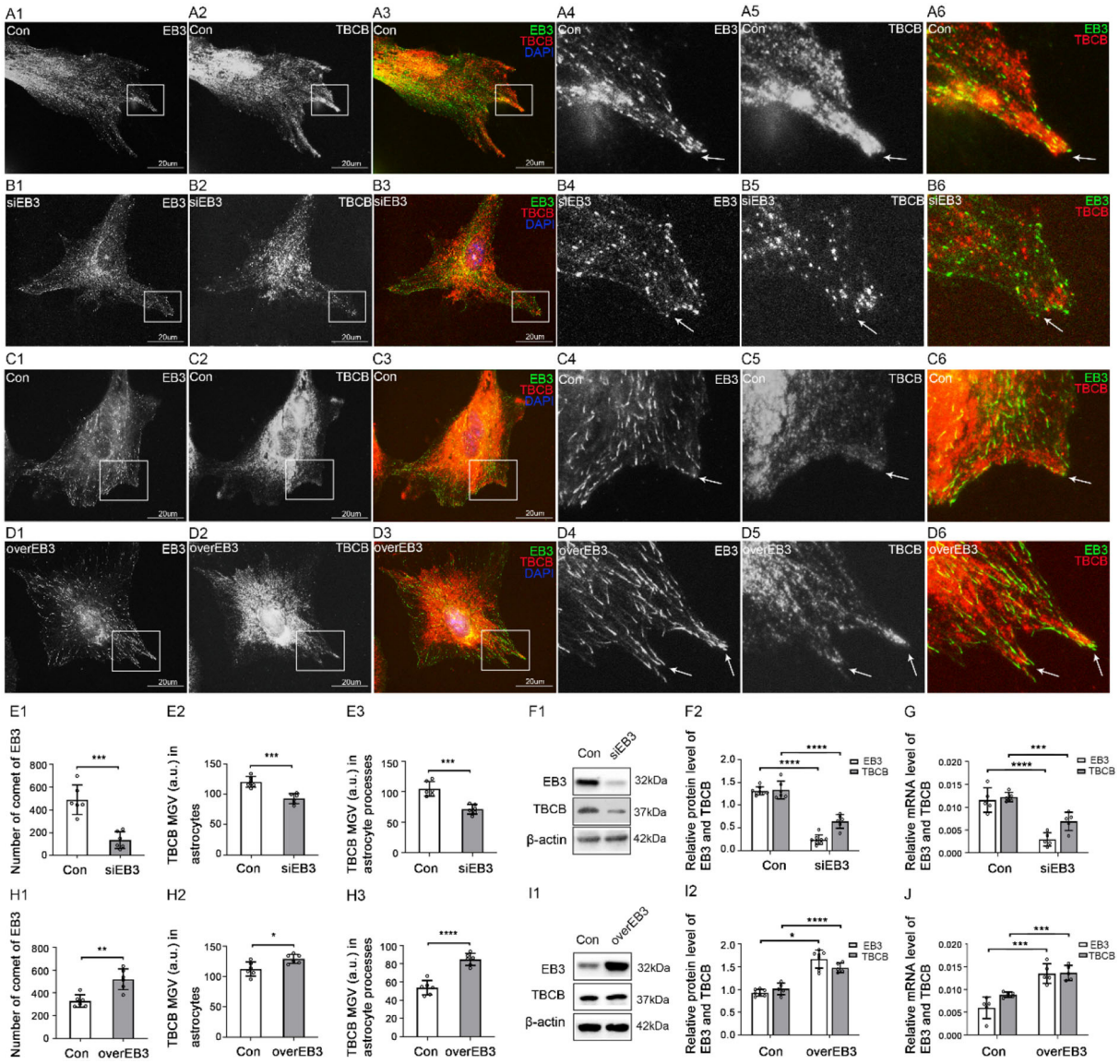


FIGURE 5
The expression of TBCB after EB3 was silenced or overexpressed in astrocytes. **(A–D)** Immunofluorescence showed that EB3 [**(A–D)** green signal] was co-expressed with TBCB [**(A–D)** red signal] in astrocytes both in the control group [Con **(A,C)**] and EB3 silence group **(B)** or EB3 overexpression group **(D)**. **(E,H)** The number of EB3 comets and mean gray value (MGV) of TBCB in astrocytes and its processes (MGV = Integrated Density/Area) after EB3 was silenced or overexpressed. **(F,I)** Western blot and **(G,J)** RT-PCR analysis of protein and mRNA levels of EB3 and TBCB after EB3 was silenced or overexpressed compared with the control group ($n = 6$, $p < 0.05$). **(A–J)** Number of panels. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

astrocytes (Figures 8E–G). Compared with the control group, the p-ERK1/2 protein level decreased after chronic alcohol exposure (Figure 8E, $p < 0.05$), which was consistent with the change of TBCB, while p-P38 and p-JNK showed no significant change (Figure 8E, $p > 0.05$). It suggested that the MAPK-ERK1/2 signaling pathway might interfere with the expression of TBCB after chronic alcohol exposure. Therefore, to further confirm the relationship between the ERK1/2 pathway and TBCB, the phosphorylation levels of MAPK were detected after silencing or overexpressing TBCB. Similar results were gathered: p-ERK1/2 was changed synchronously with that of the TBCB (Figures 8F,G, $p < 0.05$) but not of the p-P38 and p-JNK (Figures 8F,G, $p > 0.05$). It indicated that the ERK1/2 signaling pathway was involved in the regulation of the expression of TBCB after chronic alcohol exposure.

Moreover, to confirm that the ERK1/2 signaling pathway was one of the main signaling pathways regulating the expression of TBCB, astrocytes were treated, respectively, with ERK1/2 agonist

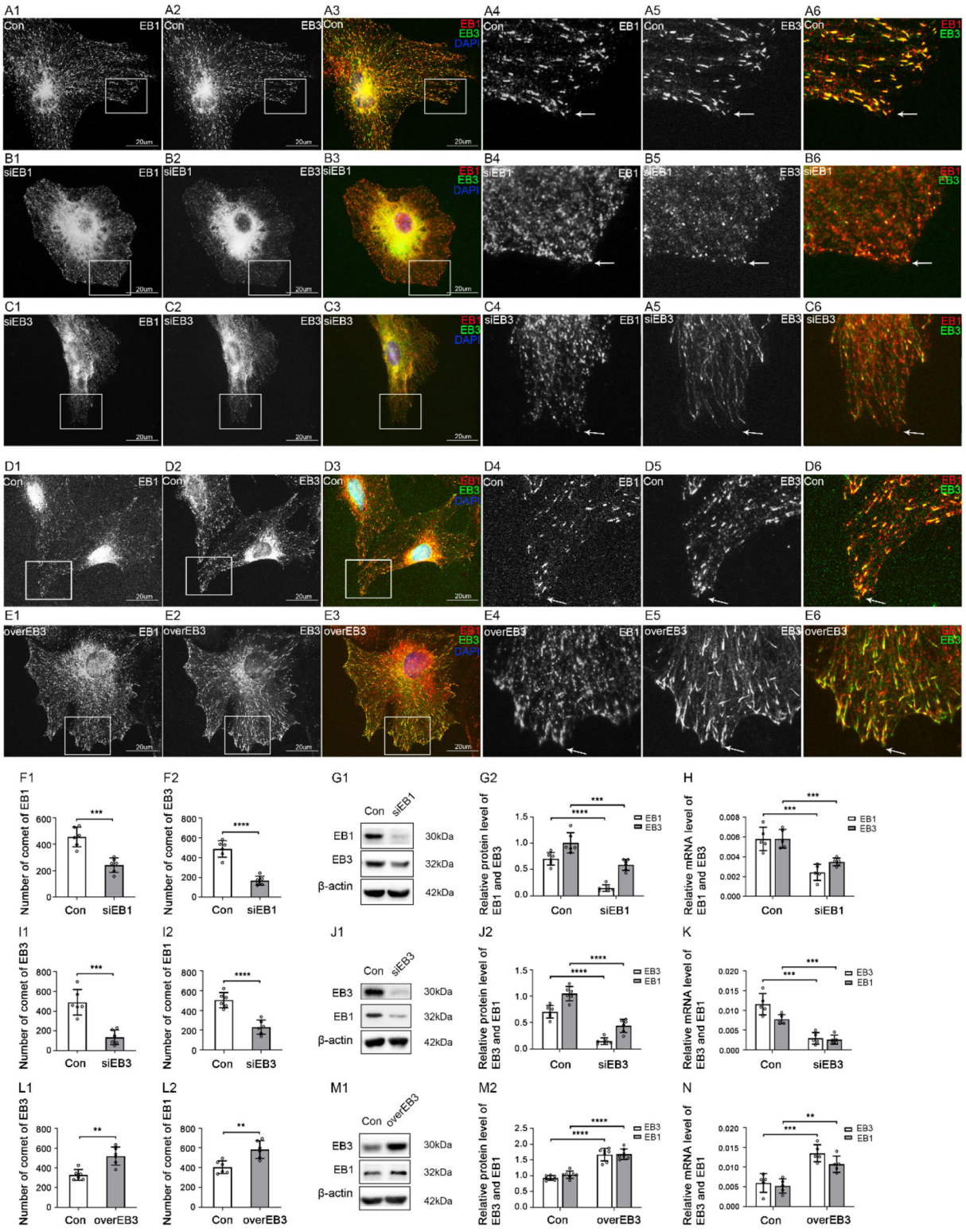


FIGURE 6
The expression of EB1 and EB3 after EB1/3 was silenced or EB3 was overexpressed in astrocytes. **(A–E)** Immunofluorescence showed that EB1 [**(A–E)** red signal] was co-expressed with EB3 [**(A–E)** green signal] in astrocytes in the control group [Con **(A,D)**], EB1 silence group **(B)**, and EB3 silence group **(C)** or overexpression group **(E)**. **(F,I,L)** The number of comets of EB1 and EB3 in astrocytes after EB1/EB3 was silenced or EB3 was overexpressed. **(G,J,M)** Western blot and **(H,K,N)** RT-PCR analysis of protein and mRNA levels of EB3 and EB1 after EB1/EB3 was silenced or EB3 was overexpressed compared with the control group ($n = 6$, $p < 0.05$). **(A–N)** Number of panels. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

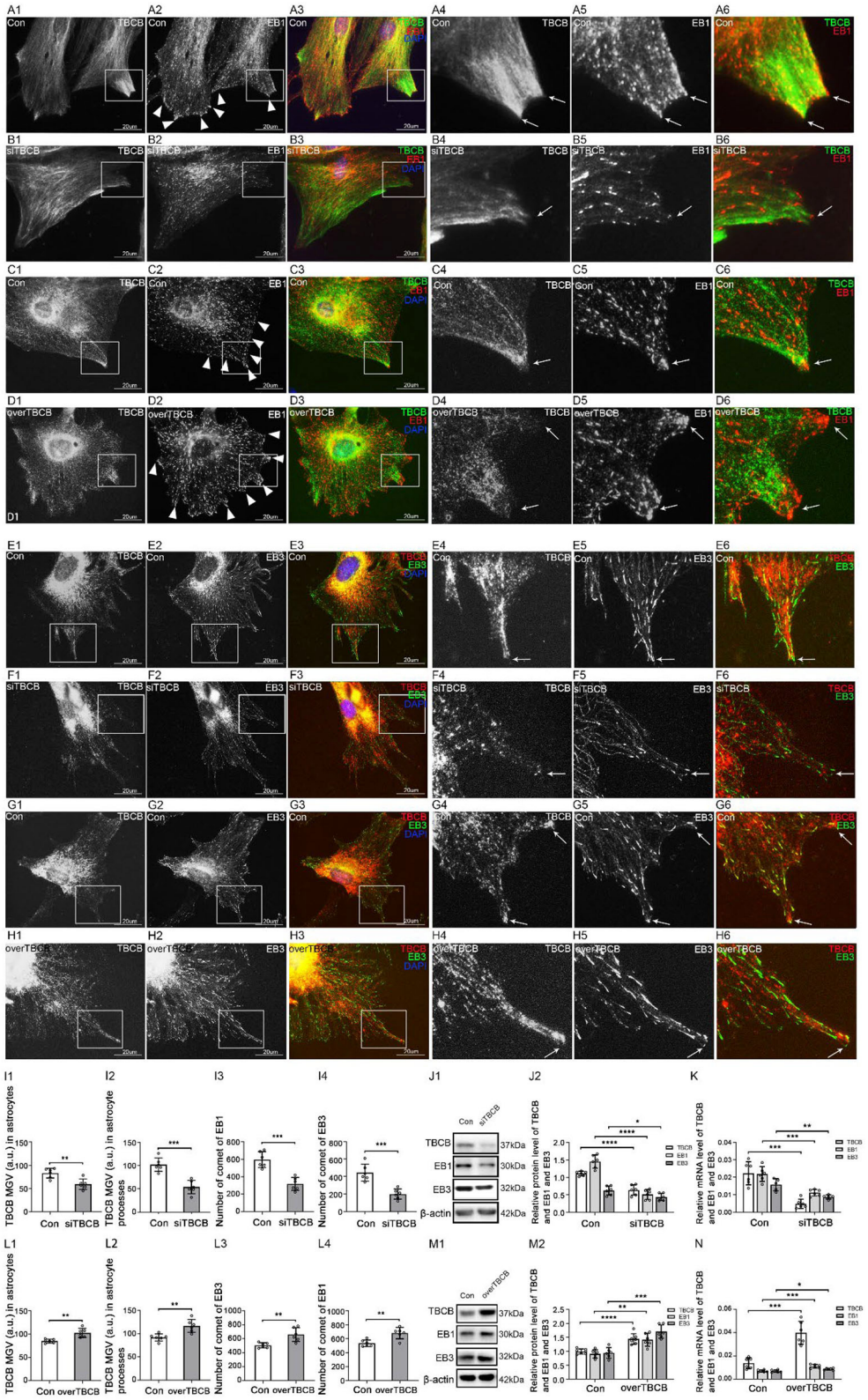


FIGURE 7
The expression of EB1/EB3 in astrocytes after TBCB was silenced or overexpressed. **(A–H)** Immunofluorescence showed that TBCB [**(A–D)** green signal; **(E–H)** red signal] was co-expressed with EB1 [**(A–D)** red signal] or EB3 [**(E–H)** red signal] in astrocytes both in the control group [Con **(A,C,E,G)**] and TBCB silence group **(B,F)** or TBCB overexpression group **(D,H)**. **(I,L)** The mean gray value (MGV) of TBCB in astrocytes and its processes (MGV = Integrated Density/Area) and the number of EB1/EB3 comets after EB1/EB3 was silenced or EB3 was overexpressed. **(J,M)** Western blot and **(K,N)** RT-PCR analysis of the levels of EB1/EB3 protein and TBCB protein and its mRNA after TBCB was silenced or overexpressed compared with the control group ($n = 6$, $p < 0.05$). **(A–N)** Number of panels. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

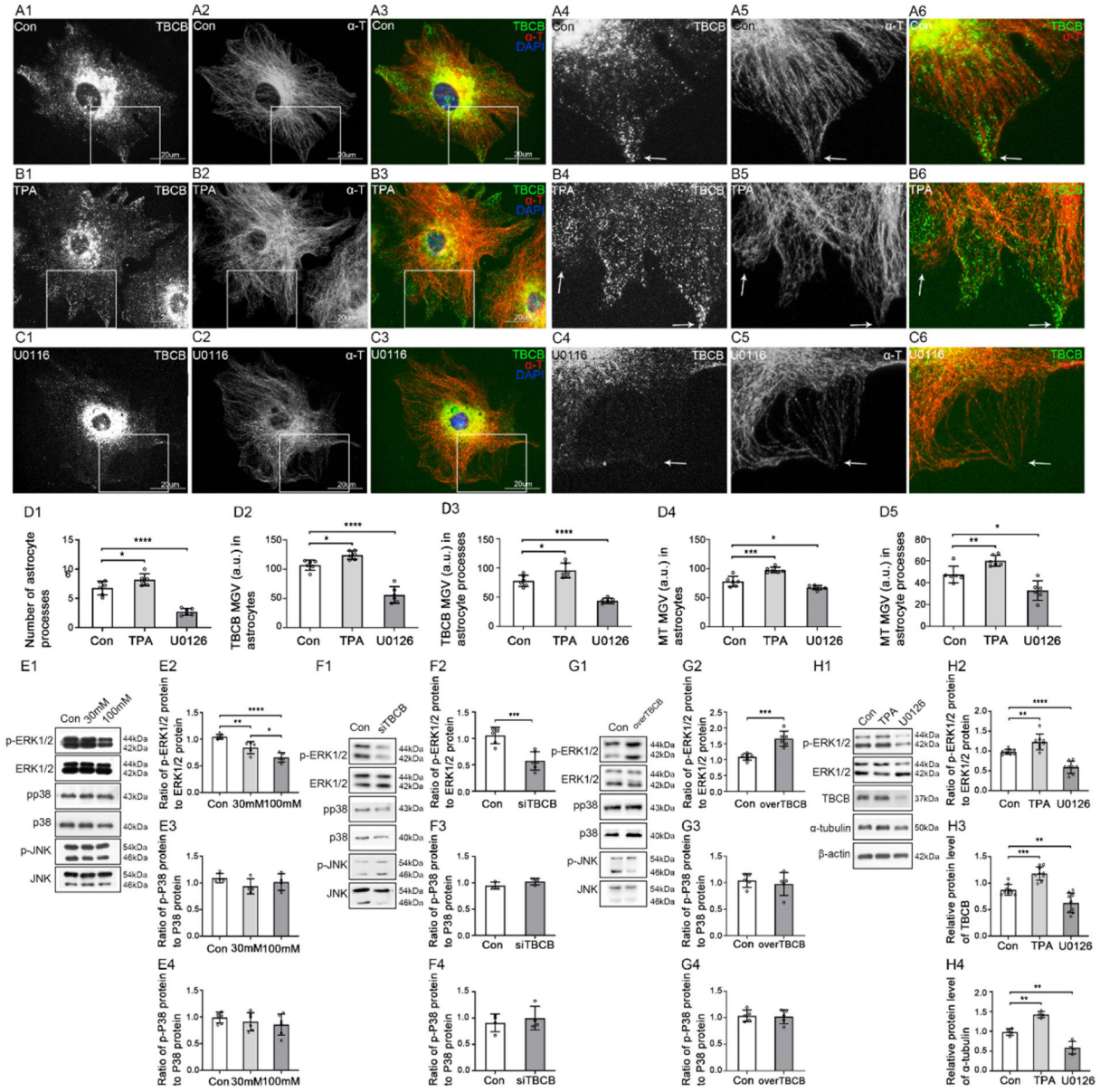


FIGURE 8
Changes in protein levels in MAPK signaling pathway after chronic alcohol exposure or TBCB interference, and distribution and protein levels of TBCB and α-tubulin in astrocytes after interfering with ERK1/2 signaling pathway. **(A–C)** Immunofluorescence showed that TBCB (green signal) was co-expressed with α-tubulin (red signal) in astrocytes both in the control group [Con **(A)**] and ERK1/2 agonist group [TPA **(B)**] or ERK1/2 inhibitor group [U0126, **(C)**]. **(D)** The number of astrocyte processes and mean gray value (MGV) of TBCB and MT in astrocytes and its processes (MGV = Integrated Density/Area) after interfering with the ERK1/2 signaling pathway. **(E–G)** Western blot analysis of phosphorylation levels and relative quantitative analysis of MAPK (ERK1/2, p38, JNK) signaling pathways in astrocytes under various interfering factors ($n = 6$, $p < 0.05$). **(H)** Western blot analysis of the ratio of p-ERK1/2 to ERK1/2, and protein levels of TBCB and α-tubulin in astrocytes after TPA pretreatment or after U0126 pretreatment ($n = 6$, $p < 0.05$). **(A–H)** Number of panels. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

(TPA) and MEK1/2 inhibitor (U0126) (Figures 8A–C,H). The expression of TBCB and α-tubulin upregulated (Figure 8H, $p < 0.05$) with special abundant TBCB and densely messy MT in newly formed processes (Figure 8B, arrows, Figure 8D) after activating the ERK1/2 pathway. In contrast, they were downregulated (Figure 8H, $p < 0.05$) with scarce TBCB and irregular MT in decreased astrocyte processes (Figure 8C, arrows, Figure 8D) after inhibiting the ERK1/2 pathway. These results proved that activation or inhibition of the ERK1/2 signaling pathway could change the expression of TBCB and

then affect the formation of astrocyte processes. To sum up, the MAPK-ERK1/2 signaling pathway was one of the main signaling pathways to regulate the expression of TBCB in astrocytes after alcohol exposure.

Discussion

In this study, we confirmed that the decreased TBCB was one of the critical factors for the formation and growth disorder of astrocyte processes after chronic alcohol exposure; TBCB, which could be regulated by ERR signaling pathway, regulated the growth of MT plus-ends through binding with EB1 and EB3, and thus regulated the formation and growth of astrocyte processes. Besides these, our study also showed the following interesting findings.

TBCB content in a certain range was critical for the normal morphology of astrocyte processes

Tubulin cofactor B was initially discovered as a protein required for proper tubulin folding and heterodimer formation (Lopez-Fanarraga et al., 2007; Baffet et al., 2012), transitory tubulin storage (Tian et al., 1997), tubulin degradation processes (Keller and Lauring, 2005; Kortazar et al., 2007), and the synthesis, growth, and metabolism of MT (Carranza et al., 2013; Tian and Cowan, 2013; Nithianantham et al., 2015; Al-Bassam, 2017). Previous experiments found two forms of the expression of TBCB: mainly distributed diffusely in the cytoplasm (Feierbach et al., 1999; Radcliffe et al., 1999; Kortazar et al., 2007; Lopez-Fanarraga et al., 2007) and partly overlaps with MT (Vadlamudi et al., 2005; Baffet et al., 2012), which were similar to our findings in astrocytes. TBCB was found localized on newly polymerized microtubules (Vadlamudi et al., 2005; Carranza et al., 2013) and might act on MT plus-ends in oocytes (Baffet et al., 2012), microglia (Fanarraga et al., 2009a,b), and neurons (Mitchison and Kirschner, 1984), thus affecting cell polarity, dynamic changes, and neurite growth. Our study also supplied similar data about astrocytes.

However, for the role of TBCB to MT plus-ends, maybe due to species or cells diversity and different degrees of TBCB interference or difference in technical approaches, there were inconsistent results: decreased or lost TBCB resulted in the decrease in α-tubulin (Radcliffe et al., 1999; Radcliffe and Toda, 2000; Baffet et al., 2012) vs. enhancing MT density (Lopez-Fanarraga et al., 2007; Fanarraga et al., 2009b); the overexpression of TBCB led to MT loss (Radcliffe et al., 1999; Wang et al., 2005; Kortazar et al., 2007; Fanarraga et al., 2009b; Ganay et al., 2011; Baffet et al., 2012) vs. obvious enhancement on the MT plus-ends growth in ours study; TBCB was not a destabilizing agent of MTs (Cleveland et al., 2009; Ganay et al., 2011). In this study, normally, TBCB was highly expressed in the new processes of astrocytes and co-expressed with MT plus-ends. In our study, silencing or overexpressing TBCB induced the synchronous change in MT plus-ends (Figure 3), which both led to the disorder of processes formation: the former impeded the formation or growth of astrocyte processes and the latter enhanced the formation or growth of astrocyte processes in the abnormal shapes with expanding basal parts and obtuse tips (Figure 2D; Supplementary Figure 2L, arrows), like the enlargement of the neuron axon in Giant Axonal Neuropathy (Ganay et al., 2011). Our data indicated that the balance of TBCB within a certain range was critical for the normal morphology and function of astrocytes; too low a concentration of TBCB was not conducive to the formation and growth of astrocyte processes, and too high a concentration would lead to expanding at the process base, which may be achieved by participating in the assembly and dissociation of MT plus-ends.

TBCB might affect the stability of MT minus-ends and MT walls

In most mammalian cells, microtubules grow from the microtubule-organizing center (MTOC) near the nucleus (usually the centrosome), where their minus-ends may be stably anchored (Schuyler and Pellman, 2001; Dammermann et al., 2003; Louie et al., 2004; Galjart, 2010). TBCB was found to localize at the centrosome of the base of the primary cilium (Bloodgood, 2009; Carranza et al., 2013) and the centrosome of Vero cells (Vadlamudi et al., 2005). When TBCB of astrocytes decreased due to alcohol interference and siRNA silencing, the perinuclear TBCB did not change significantly (Figures 1A–C, 2A–D), and there was still TBCB distributed along with the MT (Figures 1A–C, 2A–D), indicating that perinuclear TBCB and TBCB arranged along with MT might have an additional function. MT shaft was composed of GDP-tubulin and was intrinsically unstable (Akhmanova and Steinmetz, 2015). These results indicated that TBCB might be related to the stability of the MT wall and minus-ends, while the specific effect needs to be further discussed.

EB1 and EB3 might bind to MT plus-ends as a heterodimer in astrocytes

Our study showed that EB1 and EB3 were always changed synchronously in astrocytes. Silencing one of them, led the both to decrease with the typical comets losing or changing into small dots (Figures 6B,C, arrows); overexpressing EB3 led them both to upregulate with bigger, longer, and more comets (Figure 6E). Although EBs could compete with each other (Komarova et al., 2009) in astrocytes, there was undoubtedly not in this case.

In addition, although our results showed that they were both required for TBCB binding to MT plus-ends, they also did not show the character of compensating each other for binding with TBCB when one of them was decreased or lost. The findings that EB1 and EB3 could form heterodimers, which have a higher affinity for the growing of MT plus-ends than single monomers in cells (Komarova et al., 2009), could explain our results. Therefore, it can be concluded, based on our and the previous data, that EB1 and EB3 may bind to MT plus-ends as a heterodimer in astrocytes. This speculation also was confirmed by the results that silencing or overexpressing any one of these EBs always led to the synchronous change of TBCB (Figures 4, 5). It also hinted that TBCB connected with MT plus-ends might be through the heterodimer of EB1 and EB3. However, further experiments are still needed to verify it.

Accumulated TBCB, not EB3, was involved in the formation of the huge nascent processes in astrocyte

TBCB and EB3 were both increased obviously after the overexpression of TBCB or EB3. However, the shape of nascent astrocyte processes was different: maintaining the original shape after the overexpression of EB3 (Figures 5D, 6E) vs. changing into the expanded basal parts with the obtuse tips after the overexpression of TBCB (Figures 7D,H). After EB3 overexpressing, EB1 was increased obviously (Figure 6D) for forming the heterodimer with EB3, and all binding partners connected to MT plus-ends through EBs, including TBCB, were increased responsively and passively. These proteins increased proportionally, finally inducing more processes with the normal shape.

However, overexpressing TBCB led to the abnormal nascent process—"huge process," which has the expanded basal part with an obtuse tip (Figure 2D; Supplementary Figure 2L, arrows). Although EB1 and EB3 were increased responsively (Figure 7), other partners required to build the processes could not be regulated proportionally or could not compete with accumulated TBCB for the binding site, led to abnormal growth of MT plus-ends and finally caused the malformed nascent processes in astrocyte. These results also proved the special function of TBCB in regulating the formation and growth of nascent astrocyte processes.

TBCB was involved in the formation of EB3 comet

After TBCB silencing or overexpressing, the content and the comets number of EB1 and EB3 were changed synchronously (Figure 7), possibly because of the response to the change of TBCB. But interestingly, the shape of their comets was changed inconsistently. The EB1 comets still maintained their original shape (Figures 7B,D); however, most EB3 comets changed into smaller dots after TBCB silence (Figure 7F) or became more prominent and stronger after TBCB overexpressing (Figure 7H). These proved that TBCB might not involve in the comet structure formation of EB1 but might participate in EB3s. In other words, the formation of EB3 comets, a symbol of active function in normal (Mourino-Perez et al., 2013), might depend, at least partly, on the expression of TBCB. However, further and systemic research is still needed to reveal it.

Conclusion

Some of the neurological symptoms of FAS might be related to the formation disorder of astrocyte processes, and the altered organization of astrocytes reported in the FAS could be explained by the decrease in TBCB which could regulate the growth of MT plus-ends through binding with EB1/EB3 and by MAPK-ERK1/2 signaling pathway. These observations have relevance for understanding the mechanism underlying the astrocyte alterations that occurred in the pathogenesis of fetal alcohol syndrome.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by the Ethics Committee of Animal Care of the Chongqing Medical University.

Author contributions

HL and MY designed experiments. YZ and G-LZ performed experiments. YZ, S-AW, X-QC, B-QZ, and J-CH contributed to the data analysis. YZ wrote the main manuscript text. HL modified the manuscript. All authors reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Natural Science Foundation of China (81971230, 81500978, 81671312, and 81000566) and the Natural Science Foundation Project of Chong Qing (cstc2016jcyjA0229, cstc2017jcyjAX0414, cstc2015jcyja10018, and cstc2011jjA10093) and the Foundation of Chongqing Municipal Education Commission (KJ1600213).

Conflict of interest

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

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fncel.2022.989945/full#supplementary-material

References

Acosta, C., Anderson, H. D., and Anderson, C. M. (2017). Astrocyte dysfunction in Alzheimer disease. *J. Neurosci. Res.* 95, 2430–2447. doi: 10.1002/jnr.24075

Akhmanova, A., and Steinmetz, M. O. (2010). Microtubule +TIPs at a glance. *J. Cell Sci.* 123, 3415–3419. doi: 10.1242/jcs.062414

Akhmanova, A., and Steinmetz, M. O. (2015). Control of microtubule organization and dynamics: two ends in the limelight. *Nat. Rev. Mol. Cell Biol.* 16, 711–726. doi: 10.1038/nrm4084

Al-Bassam, J. (2017). Revisiting the tubulin cofactors and Arl2 in the regulation of soluble alphabeta-tubulin pools and their effect on microtubule dynamics. *Mol. Biol. Cell* 28, 359–363. doi: 10.1091/mbc.e15-10-0694

Aroor, A. R., and Shukla, S. D. (2004). MAP kinase signaling in diverse effects of ethanol. *Life Sci.* 74, 2339–2364. doi: 10.1016/j.lfs.2003.11.001

Baffet, A. D., Benoit, B., Januschke, J., Audo, J., Gourhand, V., Roth, S., et al. (2012). Drosophila tubulin-binding cofactor B is required for microtubule network formation and for cell polarity. *Mol. Biol. Cell* 23, 3591–3601. doi: 10.1091/mbc.e11-07-0633

Bloodgood, R. A. (2009). From central to rudimentary to primary: the history of an underappreciated organelle whose time has come. The primary cilium. *Methods Cell Biol.* 94, 2–52. doi: 10.1016/S0091-679X(08)94001-2

Bu, W., and Su, L. K. (2001). Regulation of microtubule assembly by human EB1 family proteins. *Oncogene* 20, 3185–3192. doi: 10.1038/sj.onc.1204429

Burd, L., Martsolf, J. T., Klug, M. G., and Kerbeshian, J. (2003). Diagnosis of FAS: a comparison of the fetal alcohol syndrome diagnostic checklist and the institute of medicine criteria for fetal alcohol syndrome. *Neurotoxicol. Teratol.* 25, 719–724. doi: 10.1016/j.ntt.2003.07.015

Carranza, G., Castano, R., Fanarraga, M. L., Villegas, J. C., Goncalves, J., Soares, H., et al. (2013). Autoinhibition of TBCB regulates EB1-mediated microtubule dynamics. *Cell. Mol. Life Sci.* 70, 357–371. doi: 10.1007/s00018-012-1114-2

Carter, J. J., Tong, M., Silbermann, E., Lahousse, S. A., Ding, F. F., Longato, L., et al. (2008). Ethanol impaired neuronal migration is associated with reduced aspartyl-asparaginyl-beta-hydroxylase expression. *Acta Neuropathol.* 116, 303–315. doi: 10.1007/s00401-008-0377-z

Chiu, C. T., Liao, C. K., Shen, C. C., Tang, T. K., Jow, G. M., Wang, H. S., et al. (2015). HYS-32-induced microtubule catastrophes in rat astrocytes involves the PI3K-GSK3beta signaling pathway. *PLoS ONE* 10, e0126217. doi: 10.1371/journal.pone.0126217

Cleveland, D. W., Yamanaka, K., and Bomont, P. (2009). Gigaxonin controls vimentin organization through a tubulin chaperone-independent pathway. *Hum. Mol. Genet.* 18, 1384–1394. doi: 10.1093/hmg/ddp044

Dammermann, A., Desai, A., and Oegema, K. (2003). The minus end in sight. *Curr. Biol.* 13, R614–624. doi: 10.1016/S0960-9822(03)00530-X

Davies, D. L., and Cox, W. E. (1991). Delayed growth and maturation of astrocytic cultures following exposure to ethanol: electron microscopic observations. *Brain Res.* 547, 62–68. doi: 10.1016/0006-8993(91)90573-E

Desai, A., and Mitchison, T. J. (1997). Microtubule polymerization dynamics. *Annu. Rev. Cell Dev. Biol.* 13, 83–117. doi: 10.1146/annurev.cellbio.13.1.83

Dixit, R., Barnett, B., Lazarus, J. E., Tokito, M., Goldman, Y. E., and Holzbaur, E. L. (2009). Microtubule plus-end tracking by CLIP-170 requires EB1. *Proc. Natl. Acad. Sci. USA.* 106, 492–497. doi: 10.1073/pnas.0807614106

Eckardt, M. J., File, S. E., Gessa, G. L., Grant, K. A., Guerri, C., Hoffman, P. L., et al. (1998). Effects of moderate alcohol consumption on the central nervous system. *Alcohol. Clin. Exp. Res.* 22, 998–1040. doi: 10.1111/j.1530-0277.1998.tb03695.x

Fanarraga, M., Villegas, J., Carranza, G., Castaño, R., and Zabala, J. (2009a). Tubulin cofactor B regulates microtubule densities during microglia transition to the reactive states. *Exp. Cell Res.* 315, 535–541. doi: 10.1016/j.yexcr.2008.10.045

Fanarraga, M. L., Villegas, J. C., Carranza, G., Castano, R., and Zabala, J. C. (2009b). Tubulin cofactor B regulates microtubule densities during microglia transition to the reactive states. *Exp. Cell Res.* 315, 535–541.

Feierbach, B., Nogales, E., Downing, K. H., and Stearns, T. (1999). Alf1p, a CLIP-170 domain-containing protein, is functionally and physically associated with α-tubulin. *J. Cell Biol.* 144, 113–124. doi: 10.1083/jcb.144.1.113

Feltes, B. C., De Faria Poloni, J., Nunes, I. J., and Bonatto, D. (2014). Fetal alcohol syndrome, chemo-biology and OMICS: ethanol effects on vitamin metabolism during neurodevelopment as measured by systems biology analysis. *OMICS* 18, 344–363. doi: 10.1089/omi.2013.0144

Fischer, M., Chander, P., Kang, H., Mellios, N., and Weick, J. P. (2021). Transcriptomic changes due to early, chronic intermittent alcohol exposure during forebrain development implicate WNT signaling, cell-type specification, and cortical regionalization as primary determinants of fetal alcohol syndrome. *Alcohol. Clin. Exp. Res.* 45, 979–995. doi: 10.1111/acer.14590

Fleming, J. R., Morgan, R. E., Fyfe, P. K., Kelly, S. M., and Hunter, W. N. (2013). The architecture of Trypanosoma brucei tubulin-binding cofactor B and implications for function. *FEBS J.* 280, 3270–3280. doi: 10.1111/febs.12308

Galjart, N. (2010). Plus-end-tracking proteins and their interactions at microtubule ends. *Curr. Biol.* 20, R528–537. doi: 10.1016/j.cub.2010.05.022

Ganay, T., Boizot, A., Burrer, R., Chauvin, J. P., and Bomont, P. (2011). Sensory-motor deficits and neurofilament disorganization in gigaxonin-null mice. *Mol. Neurodegener.* 6, 25. doi: 10.1186/1750-1326-6-25

Gofman, L., Fernandes, N. C., and Potula, R. (2016). Relative role of Akt, ERK and CREB in alcohol-induced microglia P2X4R receptor expression. *Alcohol Alcohol.* 51, 647–654. doi: 10.1093/alcalc/agw009

Guasch, R. M., Tomas, M., Minambres, R., Valles, S., Renau-Piqueras, J., and Guerri, C. (2003). RhoA and lysophosphatidic acid are involved in the actin cytoskeleton reorganization of astrocytes exposed to ethanol. *J. Neurosci. Res.* 72, 487–502. doi: 10.1002/jnr.10594

Guerri, C., and Renau-Piqueras, J. (1997). Alcohol, astroglia, and brain development. *Mol. Neurobiol.* 15, 65–81. doi: 10.1007/BF02740616

Han, X., Zhang, T., Liu, H., Mi, Y., and Gou, X. (2020). Astrocyte senescence and Alzheimer's disease: a review. *Front. Aging Neurosci.* 12, 148. doi: 10.3389/fnagi.2020.00148

Honnappa, S., Okhrimenko, O., Jaussi, R., Jawhari, H., Jelesarov, I., Winkler, F. K., et al. (2006). Key interaction modes of dynamic +TIP networks. *Mol. Cell* 23, 663–671. doi: 10.1016/j.molcel.2006.07.013

Howard, J., and Hyman, A. A. (2003). Dynamics and mechanics of the microtubule plus end. *Nature* 422, 753–758. doi: 10.1038/nature01600

Jaworski, J., Hoogenraad, C. C., and Akhmanova, A. (2008). Microtubule plus-end tracking proteins in differentiated mammalian cells. *Int. J. Biochem. Cell Biol.* 40, 619–637. doi: 10.1016/j.biocel.2007.10.015

Jones, A. W., and Sternebring, B. (1992). Kinetics of ethanol and methanol in alcoholics during detoxification. *Alcohol Alcohol.* 27, 641–647.

Keller, C. E., and Lauring, B. P. (2005). Possible regulation of microtubules through destabilization of tubulin. *Trends Cell Biol.* 15, 571–573. doi: 10.1016/j.tcb.2005.09.008

Komarova, Y., De Groot, C. O., Grigoriev, I., Gouveia, S. M., Munteanu, E. L., Schober, J. M., et al. (2009). Mammalian end binding proteins control persistent microtubule growth. *J. Cell Biol.* 184, 691–706. doi: 10.1083/jcb.200807179

Kortazar, D., Fanarraga, M. L., Carranza, G., Bellido, J., Villegas, J. C., Avila, J., et al. (2007). Role of cofactors B (TBCB) and E (TBCE) in tubulin heterodimer dissociation. *Exp. Cell Res.* 313, 425–436. doi: 10.1016/j.yexcr.2006.09.002

Kumar, P., and Wittmann, T. (2012). +TIPs: SxIPping along microtubule ends. *Trends Cell Biol.* 22, 418–428. doi: 10.1016/j.tcb.2012.05.005

Lokhorst, D. K., and Druse, M. J. (1993). Effects of ethanol on cultured fetal astroglia. *Alcohol. Clin. Exp. Res.* 17, 810–815. doi: 10.1111/j.1530-0277.1993.tb00846.x

Lopez-Fanarraga, M., Avila, J., Guasch, A., Coll, M., and Zabala, J. C. (2001). Review: postchaperonin tubulin folding cofactors and their role in microtubule dynamics. *J. Struct. Biol.* 135, 219–229. doi: 10.1006/jsbi.2001.4386

Lopez-Fanarraga, M., Carranza, G., Bellido, J., Kortazar, D., Villegas, J. C., and Zabala, J. C. (2007). Tubulin cofactor B plays a role in the neuronal growth cone. *J. Neurochem.* 100, 1680–1687. doi: 10.1111/j.1471-4159.2006.04328.x

Louie, R. K., Bahmanyar, S., Siemers, K. A., Votin, V., Chang, P., Stearns, T., et al. (2004). Adenomatous polyposis coli and EB1 localize in close proximity of the mother centriole and EB1 is a functional component of centrosomes. *J. Cell Sci.* 117, 1117–1128. doi: 10.1242/jcs.00939

Lytle, B. L., Peterson, F. C., Qiu, S. H., Luo, M., Zhao, Q., Markley, J. L., et al. (2004). Solution structure of a ubiquitin-like domain from tubulin-binding cofactor B. *J. Biol. Chem.* 279, 46787–46793. doi: 10.1074/jbc.M409422200

Martins-de-Souza, D., Gattaz, W. F., Schmitt, A., Rewerts, C., Maccarrone, G., Dias-Neto, E., et al. (2009). Prefrontal cortex shotgun proteome analysis reveals altered calcium homeostasis and immune system imbalance in schizophrenia. *Eur. Arch. Psychiatry Clin. Neurosci.* 259, 151–163. doi: 10.1007/s00406-008-0847-2

Mccarthy, K. D., and De Vellis, J. (1980). Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. *J. Cell Biol.* 85, 890–902. doi: 10.1083/jcb.85.3.890

Mitchison, T., and Kirschner, M. (1984). Dynamic instability of microtubule growth. *Nature* 312, 237–242. doi: 10.1038/312237a0

Mourino-Perez, R. R., Linacre-Rojas, L. P., Roman-Gavilanes, A. I., Lew, T. K., Callejas-Negrete, O. A., Roberson, R. W., et al. (2013). MTB-3, a microtubule plus-end tracking protein (+TIP) of *Neurospora crassa*. *PLoS ONE* 8, e70655. doi: 10.1371/journal.pone.0070655

Munger, E. L., Edler, M. K., Hopkins, W. D., Ely, J. J., Erwin, J. M., Perl, D. P., et al. (2019). Astrocytic changes with aging and Alzheimer's disease-type pathology in chimpanzees. *J. Comp. Neurol.* 527, 1179–1195. doi: 10.1002/cne.24610

Nehlig, A., Molina, A., Rodrigues-Ferreira, S., Honore, S., and Nahmias, C. (2017). Regulation of end-binding protein EB1 in the control of microtubule dynamics. *Cell. Mol. Life Sci.* 74, 2381–2393. doi: 10.1007/s00018-017-2476-2

Nithianantham, S., Le, S., Seto, E., Jia, W., Leary, J., Corbett, K. D., et al. (2015). Tubulin cofactors and Arl2 are cage-like chaperones that regulate the soluble alphabeta-tubulin pool for microtubule dynamics. *Elife* 4, e34. doi: 10.7554/eLife.08811.034

Nogales, E., and Wang, H. W. (2006). Structural mechanisms underlying nucleotide-dependent self-assembly of tubulin and its relatives. *Curr. Opin. Struct. Biol.* 16, 221–229. doi: 10.1016/j.sbi.2006.03.005

Palmer, A. L., and Ousman, S. S. (2018). Astrocytes and aging. *Front. Aging Neurosci.* 10, 337. doi: 10.3389/fnagi.2018.00337

Patten, A. R., Fontaine, C. J., and Christie, B. R. (2014). A comparison of the different animal models of fetal alcohol spectrum disorders and their use in studying complex behaviors. *Front. Pediatr.* 2, 93. doi: 10.3389/fped.2014.00093

Peng, J., Wagle, M., Mueller, T., Mathur, P., Lockwood, B. L., Bretaud, S., et al. (2009). Ethanol-modulated camouflage response screen in zebrafish uncovers a novel role for cAMP and extracellular signal-regulated kinase signaling in behavioral sensitivity to ethanol. *J. Neurosci.* 29, 8408–8418. doi: 10.1523/JNEUROSCI.0714-09.2009

Qiao, X., Sun, M., Chen, Y., Jin, W., Zhao, H., Zhang, W., et al. (2021). Ethanol-induced neuronal and cognitive/emotional impairments are accompanied by down-regulated NT3-TrkC-ERK in hippocampus. *Alcohol Alcohol.* 56, 220–229. doi: 10.1093/alcalc/agaa101

Radcliffe, P. A., Hirata, D., Vardy, L., and Toda, T. (1999). Functional dissection and hierarchy of tubulin-folding cofactor homologues in fission yeast. *Mol. Biol. Cell* 10, 2987–3001. doi: 10.1091/mbc.10.9.2987

Radcliffe, P. A., and Toda, T. (2000). Characterisation of fission yeast alp11 mutants defines three functional domains within tubulin-folding cofactor B. *Mol. Gen. Genet.* 263, 752–760. doi: 10.1007/s004380000252

Renau-Piqueras, J., Zaragoza, R., De Paz, P., Baguena-Cervellera, R., Megias, L., and Guerri, C. (1989). Effects of prolonged ethanol exposure on the glial fibrillary acidic protein-containing intermediate filaments of astrocytes in primary culture: a quantitative immunofluorescence and immunogold electron microscopic study. *J. Histochem. Cytochem.* 37, 229–240. doi: 10.1177/37.2.2642942

Sandblad, L., Busch, K. E., Tittmann, P., Gross, H., Brunner, D., and Hoenger, A. (2006). The Schizosaccharomyces pombe EB1 homolog Mal3p binds and stabilizes the microtubule lattice seam. *Cell* 127, 1415–1424. doi: 10.1016/j.cell.2006.11.025

Schuyler, S. C., and Pellman, D. (2001). Microtubule "plus-end-tracking proteins": the end is just the beginning. *Cell* 105, 421–424. doi: 10.1016/S0092-8674(01)00364-6

Steinmetz, M. O., and Akhmanova, A. (2008). Capturing protein tails by CAP-Gly domains. *Trends Biochem. Sci.* 33, 535–545. doi: 10.1016/j.tibs.2008.08.006

Tian, G., and Cowan, N. J. (2013). Tubulin-specific chaperones: components of a molecular machine that assembles the alpha/beta heterodimer. *Methods Cell Biol.* 115, 155–171. doi: 10.1016/B978-0-12-407757-7.00011-6

Tian, G., Jaglin, X. H., Keays, D. A., Francis, F., Chelly, J., and Cowan, N. J. (2010). Disease-associated mutations in TUBA1A result in a spectrum of defects in the tubulin folding and heterodimer assembly pathway. *Hum. Mol. Genet.* 19, 3599–3613. doi: 10.1093/hmg/ddq276

Tian, G., Lewis, S. A., Feierbach, B., Stearns, T., Rommelaere, H., Ampe, C., et al. (1997). Tubulin subunits exist in an activated conformational state generated and maintained by protein cofactors. *J. Cell Biol.* 138, 821–832. doi: 10.1083/jcb.138.4.821

Tomas, M., Lazaro-Dieguez, F., Duran, J. M., Marin, P., Renau-Piqueras, J., and Egea, G. (2003). Protective effects of lysophosphatidic acid (LPA) on chronic ethanol-induced injuries to the cytoskeleton and on glucose uptake in rat astrocytes. *J. Neurochem.* 87, 220–229. doi: 10.1046/j.1471-4159.2003.01993.x

Tomas, M., Marin, P., Megias, L., Egea, G., and Renau-Piqueras, J. (2005). Ethanol perturbs the secretory pathway in astrocytes. *Neurobiol. Dis.* 20, 773–784. doi: 10.1016/j.nbd.2005.05.012

Vadlamudi, R. K., Barnes, C. J., Rayala, S., Li, F., Balasenthil, S., Marcus, S., et al. (2005). p21-activated kinase 1 regulates microtubule dynamics by phosphorylating tubulin cofactor B. *Mol. Cell. Biol.* 25, 3726–3736. doi: 10.1128/MCB.25.9.3726-3736.2005

Wang, W., Ding, J., Allen, E., Zhu, P., Zhang, L., Vogel, H., et al. (2005). Gigaxonin interacts with tubulin folding cofactor B and controls its degradation through the ubiquitin-proteasome pathway. *Curr. Biol.* 15, 2050–2055. doi: 10.1016/j.cub.2005.10.052

Zamora-Martinez, E. R., and Edwards, S. (2014). Neuronal extracellular signal-regulated kinase (ERK) activity as marker and mediator of alcohol and opioid dependence. *Front. Integr. Neurosci.* 8, 24. doi: 10.3389/fnint.2014.00024

Zhang, G., Ma, P., Wan, S., Xu, J., Yang, M., Qiu, G., et al. (2019). Dystroglycan is involved in the activation of ERK pathway inducing the change of AQP4 expression in scratch-injured astrocytes. *Brain Res.* 1721, 146347. doi: 10.1016/j.brainres.2019.146347

Zhu, Y., Wang, Y., Zhao, B., Wei, S., Xu, M., Liu, E., et al. (2013). Differential phosphorylation of GluN1-MAPKs in rat brain reward circuits following long-term alcohol exposure. *PLoS ONE* 8, e54930. doi: 10.1371/journal.pone.0054930

frontiers | Frontiers in Cellular Neuroscience

TYPE Perspective
PUBLISHED 11 November 2022
DOI 10.3389/fncel.2022.1025429

OPEN ACCESS

EDITED BY
Dirk Feldmeyer,
Julich Research Center, Helmholtz Association of German Research Centres (HZ), Germany

REVIEWED BY
Patrick D. Parker,
Johns Hopkins University, United States

*CORRESPONDENCE
Adrián Rodríguez-Contreras
adrian.rodriguezcontreras@northwestern.edu

SPECIALTY SECTION
This article was submitted to Non-Neuronal Cells, a section of the journal Frontiers in Cellular Neuroscience

RECEIVED 22 August 2022
ACCEPTED 26 October 2022
PUBLISHED 11 November 2022

CITATION
Konecny L, Quadir R, Ninan A and Rodríguez-Contreras A (2022) Neurovascular responses to neuronal activity during sensory development.
Front. Cell. Neurosci. 16:1025429.
doi: 10.3389/fncel.2022.1025429

Neurovascular responses to neuronal activity during sensory development

Lukas Konecny[1], Rafid Quadir[1], Abel Ninan[1] and Adrián Rodríguez-Contreras[2]*

[1]Department of Biology, City College, The City University of New York, New York, NY, United States, [2]The Roxelyn and Richard Pepper Department of Communication Sciences and Disorders, School of Communication, Northwestern University, Evanston, IL, United States

Understanding the development of intercellular communication in sensory regions is relevant to elucidate mechanisms of physiological and pathological responses to oxygen shortage in the newborn brain. Decades of studies in laboratory rodents show that neuronal activity impacts sensory maturation during two periods of postnatal development distinguished by the maturation of accessory structures at the sensory periphery. During the first of these developmental periods, angiogenesis is modulated by neuronal activity, and physiological levels of neuronal activity cause local tissue hypoxic events. This correlation suggests that neuronal activity is upstream of the production of angiogenic factors, a process that is mediated by intermittent hypoxia caused by neuronal oxygen consumption. In this perspective article we address three theoretical implications based on this hypothesis: first, that spontaneous activity of sensory neurons has properties that favor the generation of intermittent tissue hypoxia in neonate rodents; second, that intermittent hypoxia promotes the expression of hypoxia inducible transcription factors (HIFs) in sensory neurons and astrocytes; and third, that activity-dependent production of angiogenic factors is involved in pathological oxygen contexts.

KEYWORDS

hypoxia, onset of hearing, neurovascular unit, astrocytes, hemodynamics

Introduction

Perinatal damage to the developing brain is a major cause of death and permanent neurodevelopmental disability in the world, with oxygen shortage being an important factor that affects preterm and term neonates (Watchel et al., 2019). For example, asphyxia and hypoxic-ischemic insult are associated with hearing loss and poor speech development in humans (Pham, 2017), and with auditory brainstem processing deficits in rodent models (Hall, 1964; Kaga et al., 1996; Rehn et al., 2002; Strata et al., 2005, 2010; Jiang et al., 2009). These observations indicate that part of the pathology derived from altered oxygen supply in the neonate brain involves sensory neurons, but its effect on other cell types remains relatively unexplored. Intriguingly, recent studies showed that local tissue hypoxic events are generated in response to physiological levels of neuronal activity in the somatosensory cortex of neonate rodents (Kozberg et al., 2016), and

that this occurs contemporary with a period in which angiogenesis is modulated by neuronal activity (Lacoste et al., 2014; Whiteus et al., 2014). This correlation suggests that neuronal activity is upstream of the production of angiogenic factors, a process that is mediated by intermittent hypoxia caused by neuronal oxygen consumption. In this perspective article we address three theoretical implications based on this hypothesis: first, that spontaneous activity of sensory neurons has properties that favor the generation of intermittent tissue hypoxia in neonate rodents; second, that intermittent hypoxia promotes the expression of hypoxia inducible transcription factors (HIFs) in sensory neurons and astrocytes; and third, that activity-dependent production of angiogenic factors is involved in pathological oxygen contexts (**Figure 1**).

Properties of spontaneous activity of sensory neurons that favor the generation of intermittent hypoxia in neonate rodents

In rodents, developmental changes in neuronal activity of sensory neurons are observed during two periods of postnatal development. In the first period, spontaneous bursts of action potentials that originate endogenously in sensory organs propagate to the brain through neuronal connections that are refined by activity-dependent and genetically encoded mechanisms. During the second period, development of accessory structures at the sensory periphery marks the onset of sensation and initiates critical periods where synaptic and intrinsic neuronal properties continue to recalibrate in response to activity changes driven by stimuli from the environment (Knudsen et al., 2000; Wang and Bergles, 2015; Seabrook et al., 2017; Cisneros-Franco et al., 2020; Rubio, 2020). For example, between birth (postnatal day 0, P0) and P12 cochlear inner hair cells in the auditory system fire spontaneous bursts of calcium action potentials that drive activity-dependent synaptic refinement and maturation of intrinsic properties in central auditory neurons (Tritsch et al., 2007, 2010; Kandler et al., 2009; Johnson et al., 2011, 2012; Clause et al., 2014; Sendin et al., 2014; Di Guilmi et al., 2019). In turn, around P10 in mice and P12 in rats, formation of the ear canal and clearance of mesenchyme from the middle ear cavity mark the beginning of experience-dependent plasticity in the auditory system (Sanes and Bao, 2009; de Villers-Sidani and Merzenich, 2011; Adise et al., 2014; Anthwal and Thompson, 2015).

Although different cellular mechanisms are involved in the generation of spontaneous neuronal activity in different sensory organs before P12, electrophysiology recordings showed that action potential bursts are the predominant firing pattern in individual visual and auditory neurons of neonate rodents (Mooney et al., 1996; Sonntag et al., 2009; Tritsch et al., 2010). It is important to note that during burst activity the firing rates of individual auditory neurons vary across three orders of magnitude. This means that although action potential bursts occur very infrequently (30–100 mHz), maximal firing-rates within bursts can reach between 10 Hz and up to 100 Hz for bursts that last from 2 to 12 s (Tritsch et al., 2010). In addition, multi-electrode recordings in the auditory brainstem of neonate rats showed evidence that the levels of ensemble neuronal activity increase from birth until hearing onset, reaching a maximum at P9 (Di Guilmi and Rodríguez-Contreras, 2021).

Classic studies in the mammalian visual system have shown that optical recordings of neuronal activity can provide detailed information about the spatiotemporal properties of spontaneous ensemble neuronal activity, complementing the information obtained with electrophysiological methods (Meister et al., 1991; Wong et al., 1993, 1995). In recent studies, transgenic mice that express genetically encoded calcium indicators in neurons were used to show that ensembles of sensory midbrain and cortical neurons exhibit highly synchronized activity with different modes of propagation. In the visual system, ensemble neuronal activity propagates through waves of irregular trajectories that resemble the waves of neuronal activity generated in the retina (Wong et al., 1995; Ackman et al., 2012). In the auditory system, ensemble neuronal activity is spatially restricted, and resembles the spatiotemporal activation of hair cells in the cochlea (Tritsch and Bergles, 2010; Babola et al., 2018). Additional calcium imaging studies in mouse auditory midbrain showed that spontaneous ensemble neuronal activity becomes more frequent and spatially refined between birth and P12 (Wang et al., 2021). Despite the different modes of propagation, spontaneous ensemble neuronal activity is stochastic, and highly variable in amplitude and duration, which implies participation of different numbers of cells during each activation event (Ackman et al., 2012; Babola et al., 2018). Altogether, these results raise new questions about the significance of the spatiotemporal features of spontaneous neuronal activity during postnatal development.

The significance of the spatiotemporal changes of neuronal activity before P12 has been addressed in the context of synaptic development, where neurons that fire together wire together (Butts et al., 2007). However, recent studies motivated to characterize the development of neurovascular responses in neonates have provided novel and intriguing results for alternative lines of investigation. Using genetic targeting of calcium indicators in neurons, Kozberg and colleagues showed that sensory stimulation reliably activates localized neuronal ensembles in the somatosensory cortex but fails to cause a significant increase in blood flow at ages P7–P8. In contrast, at ages P12–P15 and adulthood, sensory neuron activation leads to robust increases in blood flow due to neurovascular coupling (Kozberg et al., 2016). Furthermore, the authors obtained simultaneous optical measurements of

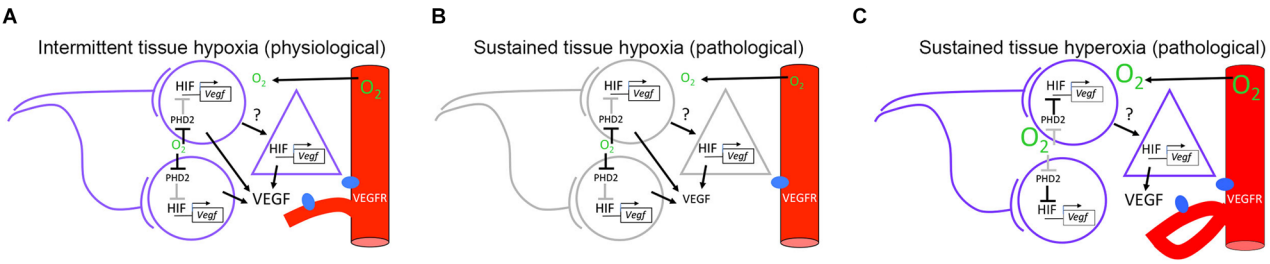


FIGURE 1
Hypothetical model for neuronal activity modulation of angiogenesis in neonate rodents. **(A)** Under environmental normoxic conditions, co-active neurons contribute to generate an intermittent hypoxic environment that favors proangiogenic signaling through the HIF/VEGF pathway. Neuron to astrocyte communication may contribute to VEGF production through unidentified signals. **(B)** Under environmental hypoxic conditions, a sustained hypoxic environment is imposed in the brain parenchyma. Sustained hypoxia inhibits electrical activity in neurons and potentially, signaling from neurons to astrocytes. Despite favorable conditions for HIF/VEGF signaling, VEGF levels are reduced resulting in a decrease in the angiogenic response. **(C)** Under environmental hyperoxic conditions, a sustained hyperoxic environment is imposed on the brain parenchyma. Sustained hyperoxia is favorable for neuronal activity but not for HIF/VEGF signaling in neurons. Altered VEGF release and aberrant angiogenic responses are hypothesized to originate from abnormal neuron to astrocyte signaling. Circles represent neurons. Triangles represent astrocytes. Red cylinders represent blood vessels expressing VEGF receptors (VEGFR), shown in blue. Active cells and pathways are shown in purple and black, respectively. Inactive cells and pathways are shown in gray. HIF/VEGF signaling pathway has been adapted from Leu et al. (2019). HIF, hypoxia inducible transcription factor; VEGF, vascular endothelial growth factor.

neuronal activity and local changes in oxygenated hemoglobin. This approach demonstrated the existence of local hypoxic events in P7–P8 mice, particularly during long periods of ensemble neuronal activity, whether they were initiated by sensory stimulation or occurred spontaneously (Kozberg et al., 2016; reviewed in Kozberg and Hillman, 2016).

Altogether, the predominance of burst firing in spontaneously active sensory neurons, the developmental increase in the number of co-active cells in ensembles, and the recent discovery that blood flow does not increase in response to neuronal activity in neonates before P12, imply that sensory neurons that fire together contribute to generate a tissue environment characterized by intermittent hypoxia, despite the fact that neonates breathe in a normoxic environment (**Figure 1A**).

Intermittent hypoxia favors the expression of hypoxia inducible factors (HIFs) in sensory neurons and astrocytes

Vascular development and homeostasis are partly regulated by vascular endothelial growth factor (VEGF), a secreted polypeptide that is produced by tissues in response to hypoxia. VEGF activates receptors on vascular endothelial cells that promote their survival, proliferation, and migration toward the VEGF source (Chung and Ferrara, 2011; Leu et al., 2019). Because transcription of the *Vegf* gene is controlled by HIFs, α–β heterodimeric transcription factors that are stabilized by tissue hypoxia (Semenza, 2014), different studies have used genetic manipulations of this signaling pathway in the retina and the brain of neonate mice to determine its effects on vascular development.

In the retina, the three vascular layers: the external, the deep and the intermediate plexuses, begin to form at birth, at P7 and at P11, respectively. Genetic activation or suppression of HIF/VEGF signaling in retinal horizontal or amacrine cells results in inversely modulated intermediate plexus vascularization (Usui et al., 2015). When genetic suppression of HIF/VEGF in neurons was compared to genetic suppression of HIF/VEGF in astrocytes, the results indicated that neurons and astrocytes are sources of VEGF that affect angiogenesis of the intermediate plexus. Interesting to us, broad suppression of VEGF also affected astrocyte migration, while suppression of VEGF only in astrocytes affected endothelial cell migration. This suggests that paracrine and autocrine VEGF have different effects in the proliferation and migration of retinal endothelial cells and the migration of astrocytes, respectively (Rattner et al., 2019).

Tissue hypoxia stabilizes the expression of HIFs by inhibiting the HIF suppressing action of prolyl-4-hydroxylase domain (PHD) enzymes (Rey and Semenza, 2010; Leu et al., 2019). PHD2 is the most abundant isoform in neurons of the mouse brain (Rabie et al., 2011; Segura et al., 2016), and has been identified to be the critical oxygen sensor setting the low steady-state levels of HIFs in normoxic conditions (Berra et al., 2003). Nasyrov and colleagues generated *Phd2*-deficient and *Hif1a/Hif2a*-deficient mice to demonstrate that perinatal activation or suppression of HIF signaling in excitatory forebrain neurons inversely modulates angiogenesis at age P7 but not at birth (Nasyrov et al., 2019). When *Phd2/Hif1a/Hif2a*-deficient animals were generated and analyzed, the authors found that in addition to HIF1a stabilization, VEGF mRNA levels were

increased not only in neurons but surprisingly also in astrocytes. Although the molecular mechanism was not identified, this result underscores the relevance of signaling between neurons and astrocytes (Nasyrov et al., 2019).

Altogether, the results of neuron and astrocyte targeted genetic manipulations in neonate mice show evidence that these cells express HIFs at the developmental period when spontaneous activity of sensory neurons is at its peak. Furthermore, these studies also show that specific types of neurons are a major source of VEGF for local angiogenesis, and that astrocytes can produce VEGF in response to neuronal signaling. Next, we address studies that demonstrate a relationship between neuronal activity and postnatal angiogenesis in physiological and pathological contexts.

Activity-dependent production of angiogenic factors is involved in pathological oxygen contexts

In the retina, the development of the external and deep vascular plexuses overlaps with a period of cholinergic neural activity driven by starburst amacrine cells (SAC), the only cholinergic neurons during the P0–P10 developmental stage (Seabrook et al., 2017). By using a combination of SAC ablation, pharmacological blockade of cholinergic activity, and chemogenetic inhibition of SAC activity from P3 to P9, Weiner and colleagues demonstrated that SAC activity is involved in vascularization of the deep retinal layer, but not the external layer by a decrease in VEGF (Weiner et al., 2019). Furthermore, in this study the authors showed that inhibiting cholinergic activity also reduced the vascular defects in a mouse model of oxygen-induced retinopathy (Weiner et al., 2019). The results of this study show that neuronal activity lies upstream of VEGF and highlight the important role of this signaling pathway in developmental and pathological angiogenesis (Kurihara et al., 2014; Weiner et al., 2019).

Discussion

In this perspective article, we hypothesized that neuronal activity is upstream of the production of angiogenic factors, a process that is mediated by intermittent hypoxia caused by neuronal oxygen consumption. Next, we discuss alternative ideas that support or argue against this hypothesis, and propose potential areas for future research.

Although we have argued that the spontaneous electrical activity of sensory neurons has properties that favor the generation of intermittent tissue hypoxia in postnatal mice, it is important to acknowledge that synaptic neurotransmission between neurons may play a substantive role in linking neuronal activity to responses from other cells of the neurovascular unit. This interpretation is supported by the studies of cholinergic SAC in the retina, which are cells that do not fire action potentials, and nevertheless play integral roles in synaptic communication with other SAC and with retinal ganglion cells (Seabrook et al., 2017; Weiner et al., 2019). Others have also proposed that the dependence of angiogenesis on neuronal activity may be restricted to specific circuits and CNS regions at different periods in development (Weiner et al., 2019).

We have also argued that neuronal activity promotes the expression of HIFs in sensory neurons and astrocytes by creating an environment of intermittent hypoxia. Others have proposed that developmental angiogenesis is dependent on additional energy consuming processes such as myelination (Yuen et al., 2014). Without a quantification of oxygen consumption by different cellular processes, it will be difficult to argue in favor of a general role of neuronal activity in different regions of the brain. It is clear however, that VEGF is a main factor regulating angiogenesis in the retina and the brain of neonates, and that VEGF is produced by specific types of neurons and by astrocytes (Usui et al., 2015; Nasyrov et al., 2019; Weiner et al., 2019). Additional signaling roles for oligodendrocytes, microglia, and mural cells cannot be excluded (Yuen et al., 2014; Biswas et al., 2020; Huang, 2020).

As participants of the tripartite synapse, astrocytes communicate with neurons and regulate neurotransmitter processes (Araque et al., 2014; Durque and Araque, 2019). Thus it is possible that synaptic communication could confer the hypoxic signal to neighboring cells through spillover of co-transmitters such as ATP, acetylcholine or to-be-identified transmitters released from neurons to astrocytes (Nasyrov et al., 2019; Weiner et al., 2019). Relevant examples of communication between sensory cells and glia exist in the developing auditory system. For example, glia-like supporting cells in the cochlea have been identified as key initiators of inner hair cell depolarization and calcium spiking *via* the extracellular release of potassium ions modulated by purinergic signaling (Tritsch et al., 2007; Babola et al., 2020). A recent calcium imaging study in the mouse auditory midbrain also showed that spontaneous bouts of ensemble neuronal activity triggered the co-activation of astrocytes by inducing calcium release from intracellular stores through the activation of metabotropic glutamate receptors, mGluR5, and mGluR3 (Kellner et al., 2021). The significance of these forms of co-activation between sensory cells and glial cells for HIF/VEGF signaling and postnatal angiogenesis remain to be established.

Lastly, we considered that activity-dependent production of angiogenic factors is altered in pathological oxygen contexts.

The severity of brain damage caused by altered oxygen levels may be a function of the developmental stage, the direction of oxygen change, and the spatiotemporal spread of the effect. We believe that distinction between intermittent physiological and sustained pathological oxygen changes is important. **Figures 1B,C** shows a hypothetical view of pathological contexts. First, because neurons have a high energy turnover and rely on aerobic metabolism, we can expect that an environmental decrease in oxygenation would lead to a sustained decrease in tissue oxygen levels, with subsequent inhibition of neuronal activity (**Figure 1B**, Lujan et al., 2021). If VEGF production was dependent on neuronal activity, this would lead to hypovascularization, although compensatory mechanisms could be observed. In contrast, neuronal activity may produce aberrant new vessels in the presence of increased oxygen levels (**Figure 1C**, Weiner et al., 2019). In addition, it is important to acknowledge that changes in oxygen levels may be accompanied by other factors such as aglycemia and inflammation during ischemic-hypoxic contexts, which could have different effects in gray matter compared to white matter regions (Tekkök et al., 2003).

Despite the above considerations, it remains to be determined how the effects of acute oxygen changes translate into long-term deficiencies in sensory processing, motor function, and cognition. Functional magnetic resonance imaging (fMRI) studies in the somatosensory system and the visual system of rats showed gradual increases in the magnitude of the blood oxygen level-dependent (BOLD) signal in response to sensory stimulation between P13 and adulthood (Colonnese et al., 2008; Chan et al., 2010), which were in agreement with earlier studies of sensory plasticity, where changes in regional manganese-enhanced MRI activity were detected in the auditory midbrain of mice reared in control and experimental sound conditions between P13 and P19 (Yu et al., 2007). Could alterations in activity-dependent angiogenesis during early postnatal development be related to long-term defects in neurovascular coupling? Although the mechanisms that trigger neurovascular coupling are under intense investigation (Kaplan et al., 2020), future work can focus in a detailed examination of the onset of neurovascular coupling and its relationship to recent angiogenic activity in relevant auditory and voice control regions of animal models, or in human subjects with non-invasive approaches.

Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Author contributions

AR-C conceived the idea and wrote the final version of the manuscript with input from all the authors. LK, RQ, AN, and AR-C discussed and drafted the manuscript. RQ and AR-C generated figures. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by startup funds from Northwestern University to AR-C.

Acknowledgments

We would like to thank Drs. Jason Tait Sanchez and Beverly A. Wright for their comments on a previous version of this manuscript.

Conflict of interest

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

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Ackman, J. B., Burbridge, T. J., and Crair, M. C. (2012). Retinal waves coordinate patterned activity throughout the developing visual system. *Nature* 490, 219–225. doi: 10.1038/nature11529

Adise, S., Saliu, A., Maldonado, N., Khatri, V., Cardoso, L., and Rodríguez-Contreras, A. (2014). Effect of maternal care on hearing onset induced by developmental changes in the auditory periphery. *J. Neurosci.* 34, 4528–4533. doi: 10.1523/JNEUROSCI.4188-13.2014

Anthwal, N., and Thompson, H. (2015). The development of the mammalian outer and middle ear. *J. Anat.* 228, 217–232. doi: 10.1111/joa.12344

Araque, A., Carmignoto, G., Haydon, P. G., Oliet, S. H., Robitaille, R., and Volterra, A. (2014). Gliotransmitters travel in time and space. *Neuron* 81, 728–739. doi: 10.1016/j.neuron.2014.02.007

Babola, T. A., Kersbergen, C. J., Wang, H. C., and Bergles, D. E. (2020). Purinergic signaling in cochlear supporting cells reduces hair cell excitability by increasing the extracellular space. *eLife* 9:e52160. doi: 10.7554/eLife.52160

Babola, T. A., Li, S., Gribizis, A., Lee, B. J., Issa, J. B., Wang, H. C., et al. (2018). Homeostatic control of spontaneous activity in the developing auditory system. *Neuron* 99, 511–524.e5. doi: 10.1016/j.neuron.2018.07.004

Berra, E., Benizri, E., Ginouvès, A., Volmat, V., Roux, D., and Pouysségur, J. (2003). HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1 alpha in normoxia. *EMBO J.* 22, 4082–4090. doi: 10.1093/emboj/cdg392

Biswas, S., Cottarelli, A., and Agalliu, D. (2020). Neuronal and glial regulation of CNS angiogenesis and barriergenesis. *Development* 147:dev182279. doi: 10.1242/dev.182279

Butts, D. A., Kanold, P. O., and Shatz, C. J. (2007). A burst-based "Hebbian" learning rule at retinogeniculate synapses links retinal waves to activity-dependent refinement. *PLoS Biol.* 5:e61. doi: 10.1371/journal.pbio.0050061

Chan, K. C., Xing, K. K., Cheung, M. M., Zhou, I. Y., and Wu, E. X. (2010). Functional MRI of postnatal visual development in normal and hypoxic-ischemic-injured superior colliculi. *Neuroimage* 49, 2013–2020. doi: 10.1016/j.neuroimage.2009.10.069

Chung, A. S., and Ferrara, N. (2011). Developmental and pathological angiogenesis. *Annu. Rev. Cell Dev. Biol.* 27, 563–584. doi: 10.1146/annurev-cellbio-092910-154002

Cisneros-Franco, J. M., Voss, P., Thomas, M. E., and de Villers-Sidani, E. (2020). Critical periods of brain development. *Handb. Clin. Neurol.* 173, 75–88. doi: 10.1016/B978-0-444-64150-2.00009-5

Clause, A., Kim, G., Sonntag, M., Weisz, C. J. C., Vetter, D. E., Rubsamen, R., et al. (2014). The precise temporal pattern of prehearing spontaneous activity is necessary for tonotopic map refinement. *Neuron* 82, 822–835. doi: 10.1016/j.neuron.2014.04.001

Colonnese, M. T., Phillips, M. A., Constantine-Paton, M., Kaila, K., and Jasanoff, A. (2008). Development of hemodynamic responses and functional connectivity in rat somatosensory cortex. *Nat. Neurosci.* 11, 72–79. doi: 10.1038/nn2017

de Villers-Sidani, E., and Merzenich, M. M. (2011). Lifelong plasticity in the rat auditory cortex: basic mechanisms and role of sensory experience. *Prog. Brain Res.* 191, 119–131. doi: 10.1016/B978-0-444-53752-2.00009-6

Di Guilmi, M. N., Boero, L. E., Castagna, V. C., Rodríguez-Contreras, A., Wedemeyer, C., Gómez-Casati, M. E., et al. (2019). Strengthening of the efferent olivocochlear system leads to synaptic dysfunction and tonotopy disruption of a central auditory nucleus. *J. Neurosci.* 39, 7037–7048. doi: 10.1523/JNEUROSCI.2536-18.2019

Di Guilmi, M. N., and Rodríguez-Contreras, A. (2021). Characterization of developmental changes in spontaneous electrical activity of medial superior olivary neurons before hearing onset with a combination of injectable and volatile anesthesia. *Front. Neurosci.* 15:654479. doi: 10.3389/fnins.2021.654479

Durque, C. A., and Araque, A. (2019). Diversity and specificity of astrocyte-neuron communication. *Neuroscience* 396, 73–78. doi: 10.1016/j.neuroscience.2018.11.010

Hall, J. G. (1964). The cochlea and the cochlear nuclei in neonatal asphyxia. A histological study. *Acta Otolaryngol. Suppl.* 194, 1–93.

Huang, H. (2020). Pericyte-endothelial interactions in the retinal microvasculature. *Int. J. Mol. Sci.* 21:7413. doi: 10.3390/ijms21197413

Jiang, Z. D., Brosi, D. M., Chen, C., and Wilkinson, A. R. (2009). Impairment of perinatal hypoxia-ischemia to the preterm brainstem. *J. Neurol. Sci.* 287, 172–177. doi: 10.1016/j.jns.2009.07.029

Johnson, S. L., Eckrich, T., Kuhn, S., Zampini, V., Franz, C., Ranatunga, K. M., et al. (2011). Position-dependent patterning of spontaneous action potentials in immature cochlear inner hair cells. *Nat. Neurosci.* 14, 711–717. doi: 10.1038/nn.2803

Johnson, S. L., Kennedy, H. J., Holley, M. C., Fettiplace, R., and Marcotti, W. (2012). The resting transducer current drives spontaneous activity in prehearing mammalian cochlear inner hair cells. *J. Neurosci.* 32, 10479–10483. doi: 10.1523/JNEUROSCI.0803-12.2012

Kaga, K., Ichimura, K., Kitazumi, E., Kodak, K., and Tamai, F. (1996). Auditory brainstem responses in infants and children with anoxic brain damage due to near-suffocation or near-drowning. *Int. J. Pediatr. Otorhinolaryngol.* 36, 231–239. doi: 10.1016/0165-5876(96)01355-9

Kandler, K., Clause, A., and Noh, J. (2009). Tonotopic reorganization of developing auditory brainstem circuits. *Nat. Neurosci.* 12, 711–717. doi: 10.1038/nn.2332

Kaplan, L., Chow, B. W., and Gu, C. (2020). Neuronal regulation of the blood-brain barrier and neurovascular coupling. *Nat. Rev. Neurosci.* 21, 416–432. doi: 10.1038/s41583-020-0322-2

Kellner, V., Kersbergen, C. J., Li, S., Babola, T. A., Saher, G., and Bergles, D. E. (2021). Dual metabotropic glutamate receptor signaling enables coordination of astrocyte and neuron activity in developing sensory domains. *Neuron* 109, 2545–2555.e7. doi: 10.1016/j.neuron.2021.06.010

Knudsen, E. I., Zheng, W., and DeBello, W. M. (2000). Traces of learning in the auditory localization pathway. *Proc. Natl. Acad. Sci. U S A* 97, 11815–11820. doi: 10.1073/pnas.97.22.11815

Kozberg, M., and Hillman, E. (2016). Neurovascular coupling and energy metabolism in the developing brain. *Prog. Brain Res.* 225, 213–242. doi: 10.1016/bs.pbr.2016.02.002

Kozberg, M. G., Ma, Y., Shaik, M. A., Kim, S. H., and Hillman, E. M. C. (2016). Rapid postnatal expansion of neural networks occurs in an environment of altered neurovascular and neurometabolic coupling. *J. Neurosci.* 36, 6704–6717. doi: 10.1523/JNEUROSCI.2363-15.2016

Kurihara, T., Westenskow, P. D., and Fridlander, M. (2014). Hypoxia-inducible factor (HIF)/vascular endothelial growth factor (VEGF) signaling in the retina. *Adv. Med. Biol.* 801, 275–281. doi: 10.1007/978-1-4614-3209-8_35

Lacoste, B., Comin, C. H., Ben-Zvi, A., Kaeser, P. S., Xu, X., da Costa, L., et al. (2014). Sensory-related neural activity regulates the structure of vascular network in the cerebral cortex. *Neuron* 83, 1117–1130. doi: 10.1016/j.neuron.2014.07.034

Leu, T., Schützhold, V., Fandrey, J., and Ferenz, K. B. (2019). When the brain yearns for oxygen. *Neurosignals* 27, 50–61. doi: 10.33594/000000199

Lujan, B. J., Singh, M., Singh, A., and Renden, R. B. (2021). Developmental shift to mitochondrial respiration for energetic support of sustained transmission during maturation at the calyx of Held. *J. Neurophysiol.* 126, 976–996. doi: 10.1152/jn.00333.2021

Meister, M., Wong, R. O., Baylor, D. A., and Shatz, C. J. (1991). Synchronous bursts of action potentials in ganglion cells of the developing mammalian retina. *Science* 252, 939–943. doi: 10.1126/science.2035024

Mooney, R., Penn, A. A., Gallego, R., and Shatz, C. J. (1996). Thalamic relay of spontaneous retinal activity prior to vision. *Neuron* 17, 863–874. doi: 10.1016/s0896-6273(00)80218-4

Nasyrov, E., Nolan, K. A., Wenger, R. H., Marti, H. H., and Kunze, R. (2019). The neuronal-sensing pathway controls postnatal vascularization of the murine brain. *FASEB J.* 33, 12812–12824. doi: 10.1096/fj.201901385RR

Pham, N. S. (2017). The management of pediatric hearing loss caused by auditory neuropathy spectrum disorder. *Curr. Opin. Otolaryngol. Head Neck Surg.* 25, 396–399. doi: 10.1097/MOO.0000000000000390

Rabie, T., Kunze, R., and Marti, H. H. (2011). Impaired hypoxic response in senescent mouse brain. *Int. J. Dev. Neurosci.* 29, 655–661. doi: 10.1016/j.ijdevneu.2011.06.003

Rattner, A., Williams, J., and Nathans, J. (2019). Roles of HIFs and VEGF in angiogenesis in the retina and brain. *J. Clin. Invest.* 129, 3807–3820. doi: 10.1172/JCI126655

Rehn, A. E., Loeliger, M., Hardie, N. A., Rees, S. M., Dieni, S., and Shepherd, R. K. (2002). Chronic placental insufficiency has long-term effects on auditory function in the guinea pig. *Hear. Res.* 166, 159–165. doi: 10.1016/s0378-5955(02)00312-x

Rey, S., and Semenza, G. L. (2010). Hypoxia-inducible factor-1-dependent mechanisms of vasculaization and vascular remodeling. *Cardiovasc. Res.* 86, 236–242. doi: 10.1093/cvr/cvq045

Rubio, M. E. (2020). Auditory brainstem development and plasticity. *Curr. Opin. Physiol.* 18, 7–10. doi: 10.1016/j.cophys.2020.07.002

Sanes, D. H., and Bao, S. (2009). Tuning up the developing auditory CNS. *Curr. Opin. Neurobiol.* 19, 188–199. doi: 10.1016/j.conb.2009.05.014

Seabrook, T., Burbridge, T. J., Crair, M. C., and Huberman, A. D. (2017). Architecture, function and assembly of the mouse visual system. *Annu. Rev. Neurosci.* 40, 499–538. doi: 10.1146/annurev-neuro-071714-033842

Segura, I., Lange, C., Knevels, E., Moskalyuk, A., Pullizi, R., Eelen, G., et al. (2016). The oxygen sensor PHD2 controls dendritic spines and synapses via modification of filamin A. *Cell Rep.* 14, 2653–2667. doi: 10.1016/j.celrep.2016.02.047

Semenza, G. L. (2014). Oxygen sensing, hypoxia-inducibe factors and disease pathophysiology. *Annu. Rev. Pathol.* 9, 47–71. doi: 10.1146/annurev-pathol-012513-104720

Sendin, G., Bourien, J., Rassendren, F., Puel, J. L., and Nouvian, R. (2014). Spatiotemporal pattern of action potential firing in developing inner hair cells of the mouse cochlea. *Proc. Natl. Acad. Sci. U S A* 111, 1999–2004. doi: 10.1073/pnas.1319615111

Sonntag, M., Englitz, B., Kopp-Scheinpflug, C., and Rübsamen, R. (2009). Early postnatal development of spontaneous and acoustically evoked discharge activity of principal cells of the medial nucleus of the trapezoid body: an in vivo study in mice. *J. Neurosci.* 29, 9510–9520. doi: 10.1523/JNEUROSCI.1377-09.2009

Strata, F., Delpolyi, A. R., Bonham, B. H., Chang, E. F., Liu, R. C., Nakahara, H., et al. (2005). Perinatal anoxia degrades auditory system function in rats. *Proc. Natl. Acad. Sci. U S A* 102, 19156–19161. doi: 10.1073/pnas.0509520102

Strata, F., Stoianov, I. P., de Villers-Sidani, E., Bonham, B., Martone, T., Kenet, T., et al. (2010). Perinatal asphyxia affects rat auditory processing: implications for auditory perceptual impairments in neurodevelopment disorders. *PLoS One* 5:e15326. doi: 10.1371/journal.pone.0015326

Tekkök, S. B., Brown, A. M., and Ransom, B. R. (2003). Axon function persists during anoxia in mammalian white matter. *J. Cereb. Blood Flow Metab.* 23, 1340–1347. doi: 10.1097/01.WCB.0000091763.61714.B7

Tritsch, N. X., and Bergles, D. E. (2010). Developmental regulation of spontaneous activity in the mammalian cochlea. *J. Neurosci.* 30, 1539–1550. doi: 10.1523/JNEUROSCI.3875-09.2010

Tritsch, N. X., Rodríguez-Contreras, A., Crins, T. T., Wang, H. C., Borst, J. G. G., and Bergles, D. E. (2010). Calcium action potentials in hair cells pattern auditory neuron activity before hearing onset. *Nat. Neurosci.* 13, 1050–1052. doi: 10.1038/nn.2604

Tritsch, N. X., Yi, E., Gale, J. E., Glowatski, E., and Bergles, D. E. (2007). The origin of spontaneous activity in the developing auditory system. *Nature* 450, 50–55. doi: 10.1038/nature06233

Usui, Y., Westenskow, P. D., Kurihara, T., Aguilar, E., Sakimoto, S., Paris, L. P., et al. (2015). Neurovascular crosstalk between interneurons and capillaries is required for vision. *J. Clin. Invest.* 125, 2335–2346. doi: 10.1172/JCI80297

Wang, H. C., and Bergles, D. E. (2015). Spontaneous activity in the developing auditory system. *Cell Tissue Res.* 361, 65–75. doi: 10.1007/s00441-014-2007-5

Wang, Y., Sanghvi, M., Gribizis, A., Zhang, Y., Song, L., Morley, B., et al. (2021). Efferent feedback controls bilateral auditory spontaneous activity. *Nat. Commun.* 12:2449. doi: 10.1038/s41467-021-22796-8

Watchel, E. V., Verma, S., and Mally, P. V. (2019). Update on the current management of newborns with neonatal encephalopathy. *Curr. Probl. Pediatr. Adolesc. Health Care* 49:100636. doi: 10.1016/j.cppeds.2019.07.001

Weiner, G. A., Shah, S. H., Angelopoulos, C. M., Bartakova, A. B., Pulido, R. S., Murphy, A., et al. (2019). Cholinergic neural activity directs retinal layer-specific angiogenesis and blood retinal barrier formation. *Nat. Comm.* 10:2477. doi: 10.1038/s41467-019-10219-8

Whiteus, C., Freitas, C., and Grutzendler, J. (2014). Perturbed neural activity disrupts cerebral angiogenesis during a postnatal critical period. *Nature* 505, 407–411. doi: 10.1038/nature12821

Wong, R. O., Chernjavsky, A., Smith, S. J., and Shatz, C. J. (1995). Early functional neural networks in the developing retina. *Nature* 374, 716–718. doi: 10.1038/374716a0

Wong, R. O. L., Meister, M., and Shatz, C. J. (1993). Transient period of correlated bursting activity during development of the mammalian retina. *Neuron* 11, 923–938. doi: 10.1016/0896-6273(93)90122-8

Yu, X., Sanes, D. S., Aristizabal, O., Wadghiri, Y. Z., and Turnbull, D. H. (2007). Large-scale reorganization of the tonotopic map in mouse auditory midbrain revealed by MRI. *Proc. Natl. Acad. Sci. U S A* 104, 12193–12198. doi: 10.1073/pnas.0700960104

Yuen, T. J., Silbereis, J. C., Griveau, A., Chang, S. M., Daneman, R., Fancy, S. P. J., et al. (2014). Oligondendrocyte-encoded HIF function couples postnatal myelination and white matter angiogenesis. *Cell* 158, 383–396. doi: 10.1016/j.cell.2014.04.052

frontiers | Frontiers in Cellular Neuroscience

TYPE Review
PUBLISHED 19 January 2023
DOI 10.3389/fncel.2022.1037641

OPEN ACCESS

EDITED BY
Daniel Reyes-Haro,
Institute of Neurobiology, National Autonomous University of Mexico, Mexico

REVIEWED BY
Sergei Kirischuk,
Johannes Gutenberg University Mainz, Germany
Friederike Klempin,
Charité Universitätsmedizin Berlin, Germany

*CORRESPONDENCE
Ana María Estrada-Sánchez
ana.estrada@ipicyt.edu.mx

SPECIALTY SECTION
This article was submitted to Non-Neuronal Cells, a section of the journal Frontiers in Cellular Neuroscience

RECEIVED 06 September 2022
ACCEPTED 20 December 2022
PUBLISHED 19 January 2023

CITATION
Cuellar-Santoyo AO, Ruiz-Rodríguez VM, Mares-Barbosa TB, Patrón-Soberano A, Howe AG, Portales-Pérez DP, Miquelajáuregui Graf A and Estrada-Sánchez AM (2023) Revealing the contribution of astrocytes to glutamatergic neuronal transmission. Front. Cell. Neurosci. 16:1037641. doi: 10.3389/fncel.2022.1037641

Revealing the contribution of astrocytes to glutamatergic neuronal transmission

Ares Orlando Cuellar-Santoyo[1], Victor Manuel Ruiz-Rodríguez[1], Teresa Belem Mares-Barbosa[1,2], Araceli Patrón-Soberano[1], Andrew G. Howe[3], Diana Patricia Portales-Pérez[2], Amaya Miquelajáuregui Graf[4] and Ana María Estrada-Sánchez[1]*

[1]División de Biología Molecular, Laboratorio de Neurobiología, Instituto Potosino de Investigación Científica y Tecnológica (IPICYT), San Luis Potosí, Mexico, [2]Translational and Molecular Medicine Laboratory, Research Center for Health Sciences and Biomedicine, Autonomous University of San Luis Potosí, San Luis Potosí, Mexico, [3]Intelligent Systems Laboratory, HRL Laboratories, LLC, Malibu, CA, United States, [4]Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium

Research on glutamatergic neurotransmission has focused mainly on the function of presynaptic and postsynaptic neurons, leaving astrocytes with a secondary role only to ensure successful neurotransmission. However, recent evidence indicates that astrocytes contribute actively and even regulate neuronal transmission at different levels. This review establishes a framework by comparing glutamatergic components between neurons and astrocytes to examine how astrocytes modulate or otherwise influence neuronal transmission. We have included the most recent findings about the role of astrocytes in neurotransmission, allowing us to understand the complex network of neuron-astrocyte interactions. However, despite the knowledge of synaptic modulation by astrocytes, their contribution to specific physiological and pathological conditions remains to be elucidated. A full understanding of the astrocyte's role in neuronal processing could open fruitful new frontiers in the development of therapeutic applications.

KEYWORDS

calcium, gliotransmission, NMDA receptors, GLT-1, GLAST, VGLUT1, xCT

Introduction

The brain is one of the most complex organs in the human body; it consists of many distinct cell types, but most cells fall under the broad categories of neurons or glia with approximately even numbers in each category. The glial cells are divided into microglia, oligodendrocytes, and astrocytes; the latter comprise around 20% of the cells in the brain (Ventura and Harris, 1999; Salas et al., 2020). Astrocytes participate in many neurophysiological processes, including synaptogenesis (Allen and Eroglu, 2017), modulation of synaptic transmission, neuronal plasticity (Newman and Zahs, 1998; Araque et al., 1999), and regulation of blood flow in addition to the trafficking of small molecules and ions through their end-feet processes at the blood-brain barrier (Giaume et al., 1997; Simard et al., 2003). During physiological conditions, neurons and astrocytes have a coordinated functional relationship that ensures proper information flow, and each contributes to synaptic transmission by releasing neurotransmitters (by presynaptic neurons) or gliotransmitters (by astrocytes; see below).

Glutamate is the primary excitatory neurotransmitter in the mammalian brain, and it participates in diverse physiological processes such as learning, memory, and neuronal development (Yu et al., 1984; Behar et al., 1999; Hrabetova et al., 2000). However, glutamate can induce neuronal damage through excitotoxicity, which results from the over-activation of

glutamatergic receptors. Glutamate-mediated toxicity has been implicated in the pathogenesis of neurodegenerative diseases including Alzheimer's, Huntington's, and Parkinson's diseases (Koutsilieri and Riederer, 2007; Estrada-Sánchez et al., 2009; Ong et al., 2013), as well as psychiatric disorders such as schizophrenia (O'Donovan et al., 2017; Shah et al., 2020). Although glutamate toxicity contributes to these neuropathological conditions, the changes that lead to impaired glutamatergic neurotransmission are diverse, with different causes in each pathology. Nonetheless, an impaired relationship between neurons and astrocytes might be a common component. In the next section, we review the similarities and differences among the components of glutamatergic transmission in neurons and astrocytes.

Glutamatergic neurotransmission

Glutamate synthesis

Glutamate plays a role in multiple biological processes in the brain, and yet it cannot cross the blood-brain barrier. Instead, the brain's glutamate is synthesized locally by astrocytes and neurons through one of several pathways. We provide a brief description of glutamate metabolism (this section and **Figure 1**; Hawkins, 2009; Fernstrom, 2018); further details can be found in the extensive review by Schousboe et al. (2014).

The main precursor of glutamate in the brain is glutamine, a key component of the glutamine-glutamate cycle that encompasses the exchange of glutamine and glutamate between astrocytes and neurons. The reuptake of glutamate by its transporters, glutamate transporter 1 (GLT-1) and glutamate aspartate transporter (GLAST) in astrocytes enables the synthesis of glutamine by the cytosolic enzyme glutamine synthetase, which is highly expressed in astrocytes. Glutamine synthetase promotes the conversion of glutamate to glutamine using the cofactors NADPH, ATP, and NH_4^+ (Lehre et al., 1995; Bergles and Jahr, 1997; Anlauf and Derouiche, 2013; Huyghe et al., 2014; Yamada et al., 2019). Glutamine is then transported to the synaptic cleft, where neurons take it up through specific glutamine transporters (i.e., members of the families SNAT, LAT, ASC, and $^+$LAT; Anlauf and Derouiche, 2013; Yamada et al., 2019). Once transported into neurons, the mitochondrial enzyme glutaminase (an amidohydrolase) generates glutamate from the glutamine provided by the astrocytes (**Figure 1A**). Glutamate synthesis occurs predominantly in neurons, although astrocytes can synthesize it through the same pathway (Hogstad et al., 1988). Neurons and astrocytes both preferentially express the mitochondrially-located, kidney-type glutaminase (GLS) isoenzyme type 1 (GLS1), although there is also the liver-type GLS2 that localizes to the mitochondrion and nucleus (Cardona et al., 2015). However, evidence suggests that neurons and astrocytes might express an isoform of glutaminase GLS1, which has not been characterized to date (Kvamme et al., 2001; Cardona et al., 2015).

Glutamate is a component of energy metabolism, which requires *de novo* synthesis to avoid an imbalance in glutamate concentrations. *De novo* glutamate synthesis occurs by the pyruvate carboxylase, which is located exclusively in astrocytic mitochondria; this enzyme metabolizes pyruvate into oxaloacetate, a precursor for α-ketoglutarate (Walker, 2014; Schousboe et al., 2019). Although neurons lack pyruvate carboxylase, they contribute to *de novo* glutamate synthesis by the pyruvate carboxylation to malate through the malic enzyme, which is found in the cytosol and mitochondria (McKenna et al., 1995; Hassel, 2001; Amaral et al., 2016).

Another enzyme that contributes to glutamate production is aspartate aminotransferase (found in the cytosol or mitochondria), which synthesizes glutamate by reversibly transferring the α-amino group from aspartate to 2-oxoglutarate, resulting in glutamate and oxaloacetate; this enzyme uses pyridoxal 5'-phosphate as a co-factor (**Figure 1B**; McKenna et al., 2006; Schousboe, 2017). Neurons and astrocytes express aspartate aminotransferase, and the enzyme appears to have the same function and activity in both cell types (McKenna et al., 2006).

Astrocytes and neurons contain alanine aminotransferase in the cytoplasm and mitochondria (Ruscak et al., 1982; Waagepetersen et al., 2000), which catalyzes the reversible interconversion of alanine and α-ketoglutarate into pyruvate and glutamate (**Figure 1C**). Low activity of this enzyme in neurons (Westergaard et al., 1993; Erecinska et al., 1994) suggests that, within this pathway, astrocytes exert a primary control (Schousboe et al., 2013).

Ammonia concentration in the brain is regulated by the mitochondrial glutamate dehydrogenase, which catalyzes the reversible conversion of glutamate to α-ketoglutarate and ammonia, using NADH or NADPH as a co-factor (Islam et al., 2010; Plaitakis et al., 2017). Glutamate dehydrogenase expression in astrocytes varies spatially by brain region, cellularly by astrocyte type, and temporally with the developmental stage (**Figure 1D**; Osterberg and Wattenberg, 1962). For example, astrocytes increase glutamate dehydrogenase expression during rat hippocampus maturation (Kugler and Schleyer, 2004). Interestingly, along with increased glutamate dehydrogenase activity, astrocytes also increase the expression of GLT-1, suggesting a deeper, interconnected regulatory system of glutamatergic dynamics (Kugler and Schleyer, 2004).

Glutamate packaging

In neurons, glutamate is packaged and stored in synaptic vesicles through specific vesicular glutamate transporters (VGLUT). Currently, three subtypes of VGLUTs (VGLUT1, 2, and 3) have been described, and their distribution differs among different brain structures. VGLUT1 is present in the cerebral cortex, cerebellum, hippocampus, and thalamus (Fujiyama et al., 2001; Herzog et al., 2004). VGLUT2 is expressed in the cortex, thalamus, diencephalon, and rhombencephalon (Fremeau et al., 2001; Herzog et al., 2004). VGLUT3 is less predominant than the other two transporters and is located in the striatum, neocortex, and hippocampus (Fremeau et al., 2002).

VGLUT function depends on the electrochemical proton gradient generated across the membrane by the activity of the vacuolar H^+-ATPase (Wolosker et al., 1996). This H^+-ATPase activity increases the H^+ concentration inside the vesicle leading to an acidic pH. The rate of glutamate transport by VGLUT correlates inversely with the concentration of chloride ions (Cl^-) such that a low extravesicular Cl^- concentration generates high glutamate uptake, whereas high Cl^- concentration leads to gradual inhibition of glutamate uptake (Wolosker et al., 1996; Juge et al., 2006).

There appears to be a proportional relationship between VGLUT levels and glutamatergic synapse response. A study of VGLUT1-knockout mice demonstrated that the knockout reduced the

10.3389/fncel.2022.1037641

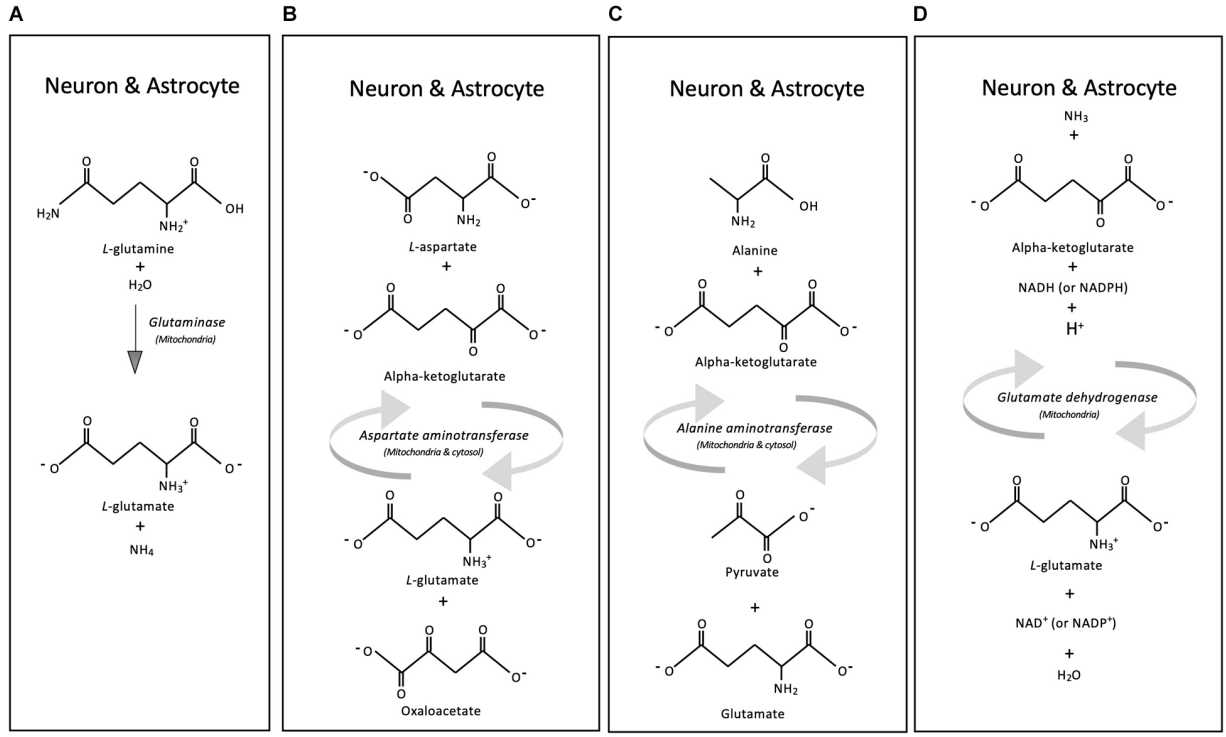


FIGURE 1
Glutamate synthesis. Schematic representation of four main metabolic reactions, synthesizing glutamate in neurons and astrocytes: Glutaminase **(A)**, Aspartate aminotransferase **(B)**, Alanine aminotransferase **(C)**, and Glutamate dehydrogenase **(D)**.

amplitude of miniature excitatory postsynaptic currents (mEPSCs), suggesting a smaller quantal size (Wojcik et al., 2004). In another study, vesicles containing a lower number of VGLUT1 showed a reduced release probability (Herman et al., 2014). Similar results have been described also in VGLUT3-knockout mice (Fasano et al., 2017).

The evidence for astrocyte expression of VGLUT is contradictory and requires further investigation to clarify the situation (for a review see Hamilton and Attwell, 2010). For example, Li et al. (2013a) describe the absence of VGLUT in mouse cortical, hippocampal, and cerebellar astrocytes. On the contrary, Ormel et al. (2012) identified VGLUT1 in astrocytic processes in the rat hippocampus, frontal cortex, and striatum. Likewise, astrocytes from postnatal rat brains express VGLUT1 and VGLUT2 (Montana et al., 2004), as well as cortical cultured astrocytes (Anlauf and Derouiche, 2005). VGLUT3 has been detected in astrocytes end-feet in microcultures of rat ventral tegmental area, substantia nigra pars compacta, and raphe nuclei (Fremeau et al., 2002). Despite the controversy about astrocytic VGLUTs, evidence indicates that astrocytes contain vesicular compartments and the molecular machinery to release glutamate in vesicular packages; this phenomenon—now known as gliotransmission—was later confirmed and contributes to neuronal information processing (see below; Bezzi et al., 2004).

Glutamate release

Once glutamate is packaged into the synaptic vesicles and stored in the synaptic bouton, it is ready to be released upon the arrival of an action potential, which will induce the opening of voltage-dependent calcium channels, increasing intracellular calcium (Ca^{2+}) concentration. The Ca^{2+} influx facilitates vesicle fusion with the plasma membrane, which releases the neurotransmitters into the synaptic cleft (**Figure 2**; de Wit et al., 2009). The soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family of proteins are key components for this process. These proteins are divided into two groups, the vesicular v-SNARE, highly abundant in the vesicles, and the target t-SNARE, highly expressed in the target zone in the cellular membrane (Han et al., 2017). A trans-SNARE complex -interaction between v and t SNAREs– must be formed to release the neurotransmitter; the main v-SNAREs are synaptobrevin, synaptotagmin, syntaxin, and the main t-SNARE is SNAP-25.

SNARE expression is not limited to neurons; SNAREs support gliotransmitter release in astrocytes (Crippa et al., 2006). Cultured astrocytes express synaptobrevin II and release glutamate that is reduced by the inhibitors of the neuronal exocytosis botulinum toxin-A and botulinum toxin-C, suggesting the expression of SNAP-25 and syntaxin in astrocytes (Jeftinija et al., 1997). The vesicular exocytosis process is similar between neurons and astrocytes (Crippa et al., 2006), with the principal difference arising in the initiation of exocytosis. Neuronal exocytosis is initiated by the arrival of an action potential at the synapse, which results in membrane depolarization in addition to an influx of Ca^{2+} through transmembrane channels involved in the action potential response; the Ca^{2+} wave initiates a cascade of signaling that results in vesicular release. Astrocytes cannot generate action potentials, but the astrocytic vesicular release also requires a transient increase in intracellular Ca^{2+} concentration. Astrocytes have multiple mechanisms to accomplish this Ca^{2+} increase, including activation of ionotropic or metabotropic receptors and the subsequent inositol

10.3389/fncel.2022.1037641

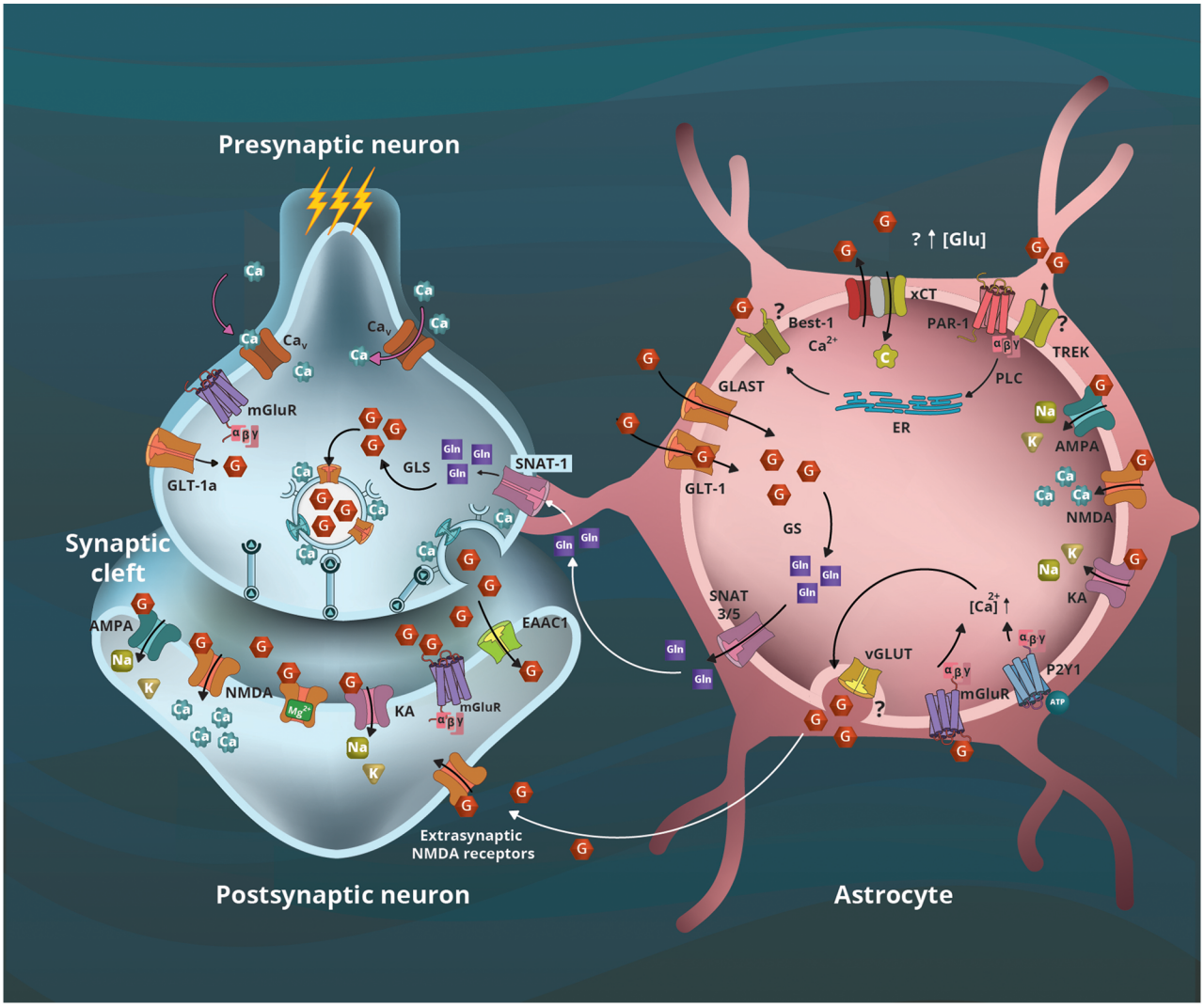


FIGURE 2
Astrocyte's contribution to glutamatergic neurotransmission. The arrival of action potential and calcium (Ca^{2+}) influx leads to presynaptic vesicular glutamate (G) release to the synaptic cleft, and the activation of its ionotropic (AMPA, NMDA, KA) and metabotropic (mGluR) receptors located in the postsynaptic terminal and astrocytes. The remaining neurotransmitter is captured by transporters located in neurons (EAAC and GLT-1a) and astrocytes (GLT-1 and GLAST). In the former, G is metabolized to glutamine (Gln) by the glutamine synthetase (GS), the initial step of the glutamate-glutamine cycle between astrocytes and neurons that use Gln as a precursor for G. Sodium Neutral Amino acid Transporter (SNAT) 3/5 transporters located in astrocytes will transport Gln to the synaptic space where the neuronal SNAT-1 transporter will internalize it to serve as a precursor for G by glutaminase (GLS) activity. The xCT transporters (SLC7A11) internalize cystine (C) cotransporting G outside the astrocytes; this is the first step of glutathione synthesis that involves astrocytes and neurons. Furthermore, a mechanism involving the interaction of protease-activated receptor 1 (PAR-1) with the potassium channel TREK-1 or Best-1, and the G transported by xCT could contribute to increased extracellular G concentrations. In astrocytes, the activation of mGLuR and purinergic P2Y1 receptors contribute to increased intracellular Ca^{2+} concentration, leading to vesicular G release that activates extrasynaptic NMDA receptors; however, the vesicular proteins involved remain controversial. Modified from Estrada-Sánchez and Rebec (2012).

1,4,5-trisphosphate (IP3) signaling cascade, activation of transient receptor potential (TRP) channels, or the release of Ca^{2+} by mitochondria (Guerra-Gomes et al., 2018); or selective activation of either purinergic receptors P2Y1 or protease-activated receptor 1 (PAR-1) that in astrocytes leads to increased intracellular Ca^{2+} concentration (Shigetomi et al., 2008).

Furthermore, recent evidence indicates that astrocytes can release glutamate by the interaction of metabotropic receptors PAR-1 with either the two-pore domain potassium channel (TREK-1) or the Bestrophin-1 (Best-1), a Ca^{2+}-activated chloride channel (**Figure 2**). Glutamate efflux from the intracellular space occurs when the TREK-1 intracellular domain interacts with PAR-1 allowing fast glutamate transient currents, whereas the interaction of PAR-1 and Best-1 leads to slow transient currents (Woo et al., 2012; Lalo et al., 2021). Taken together, the evidence supports the view that both neurons and astrocytes contribute to glutamatergic signaling.

Glutamate receptors

Glutamatergic receptors are widely distributed in the different regions of the central nervous system. Neurons and astrocytes express glutamate receptors, which split into two families. Ionotropic glutamate receptors (iGluRs; Kukley et al., 2001) and metabotropic glutamate receptors (mGluRs; Schools and Kimelberg, 1999; Fiacco and McCarthy, 2004; Perea and Araque, 2007; Cavaccini et al., 2020).

iGluRs

The iGluRs family, consisting of NMDA (N-methyl-D-aspartate) receptors, and the AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazol-propionate) and KA (kainic acid) receptors, which are responsible for the excitatory transmission in the central nervous system of vertebrates. These receptors are ligand-gated ion channels that allow the movement of cations such as Na^+, K^+, and Ca^{2+} across the cell membrane. Structurally, iGluRs are transmembrane proteins composed of four subunits that form a central ionic pore comprised of an extracellular amino-terminal domain, an extracellular ligand-binding domain, four transmembrane domains, and an intracellular carboxyl-terminal domain (Traynelis et al., 2010).

NMDA receptor

NMDA receptors are highly expressed in the brain, and their role in physiological and pathological mechanisms have been studied extensively (Lakhan et al., 2013; Zhou et al., 2013; Intson et al., 2022). The subunits that constitute these receptors are GluN1, GluN2 [A, B, C, D], GluN3A, and GluN3B. A functional receptor contains a tetrameric assembly with two possible configurations. All receptors must have two GluN1 subunits. The remaining two subunits can be a pair of GluN2 subunits, or one GluN2 subunit together with one GluN3 subunit (Schorge et al., 2005; Ulbrich and Isacoff, 2008; Traynelis et al., 2010; Hansen et al., 2021). Moreover, mRNA editing and alternative splicing of the different NMDA subunit genes confer more complex properties to the NMDA receptors' conformation, which have been extensively reviewed by Hansen and colleagues (Hansen et al., 2021).

Full activation of NMDA receptors in neurons requires membrane depolarization to displace the Mg^{2+} ion that blocks the ion channel with the simultaneous binding of glutamate and the co-agonist, glycine. When both conditions are met, NMDA receptor activation allows Na^+ and Ca^{2+} ion influx. In addition to glycine, D-serine is an NMDA receptor co-agonist, which is supplied by astrocytes (Henneberger et al., 2010). However, recent evidence indicated that neurons also contribute to the *de novo* synthesis of D-serine (Neame et al., 2019). D-serine metabolism depends on 3-phosphoglycerate dehydrogenase (Phgdh) enzyme activity and glycine concentration (Shibasaki et al., 2017; Neame et al., 2019). Since astrocytes require Phgdh to synthesize L-serine from glucose, astrocyte *de novo* synthesis of L-serine may be a previously unrecognized regulatory mechanism for the NMDA receptors co-agonism by D-serine (Masuoka et al., 2019; Neame et al., 2019).

On the other hand, astrocytes also express NMDA receptors, as evidenced by the presence of GluN1 and GluN2 subunit mRNA (Jimenez-Blasco et al., 2015). In astrocytes, the NMDA receptors are insensitive or weakly sensitive to the blockade of Mg^{2+} ion, and their activation might even occur at negative resting potential (Verkhratsky and Kirchhoff, 2007). Interestingly, the expression pattern of NMDA subunits in astrocytes varies depending on the brain region. For example, cortical astrocytes express GluN1, GluN2A/B, GluN2C, and GluN2D subunits (Conti et al., 1996; Palygin et al., 2011). GluN2B is more abundant in the Bergmann glia cells (Luque and Richards, 1995), whereas the GluN2C is predominantly expressed in the telencephalon glial cells (Alsaad et al., 2019). In hippocampal cultured astrocytes, GluN1 and GluN2 subunits are predominant (Araque et al., 1998a). *In vivo* evaluation of the GluN2C NMDA subunit localization showed that parvalbumin-positive neurons in the globus pallidus, ventral pallidum, and substantia nigra express this subunit, whereas GluN2C in the cortex, striatum, hippocampus, and amygdala colocalizes with astrocytes' markers (Ravikrishnan et al., 2018). The variety of NMDA subunit compositions confers different activation properties to the receptors, which, in combination with the regional expression patterns, results in differences in the contribution of NMDA receptors to neuronal and astrocyte activity (Palygin et al., 2011).

AMPA receptor

An AMPA receptor is composed of four subunits from the proteins GluA1, GluA2, GluA3, and GluA4 (Traynelis et al., 2010). AMPA receptors can either be homomers or heteromers. These subunit types are differentially expressed in the nucleus accumbens, dorsal striatum, prefrontal cortex, and hippocampus (Reimers et al., 2011). Activation of AMPA receptors allows Na^+ and K^+ influx, but if the receptor conformation lacks a GluA2 subunit or contains a post-transcriptionally modified GluA2 by RNA editing at the Q/R site, the channel will also be Ca^{2+} permeable (Traynelis et al., 2010; Hansen et al., 2021). AMPA subunits interact with the transmembrane AMPA receptor regulatory protein (TARP), which modulates channel opening (Hansen et al., 2021). Activation of AMPA receptors leads to membrane depolarization, leading to the displacement of the Mg^{2+} ion that blocks the NMDA ion channel allowing its activation.

Some studies suggest the presence of AMPA receptors in glial cells (Müller et al., 1992). These receptors have been described in cortical cultured astrocytes (David et al., 1996), and a subpopulation of hippocampal astrocytes express GluA1, GluA2, GluA3, and GluA4 subunits (Matthias et al., 2003). Regarding function, it has been shown that the AMPA receptors modulate the inward-rectifier potassium channels (also known as Kir) in hippocampal astrocytes, which induces gliotransmitter release and AMPA activation in neurons (Schröder et al., 2002; Fiacco and McCarthy, 2004). Of note, AMPA receptors containing at least one GluA3 or GluA4 subunit are permeable to Ca^{2+}, as described in hippocampal astrocytes (Seifert and Steinhauser, 1995), providing a direct mechanism to raise intracellular Ca^{2+} concentrations.

KA receptor

Among the iGluRs family proteins, KA receptors comprise the least studied class (Meyerson et al., 2016). The subunits that form the KA receptors are GluK1, GluK2, GluK3, GluK4, and GluK5. The KA channel is permeable to Na^+ and K^+. Some of the most important functions of these receptors are the regulation of synaptic activity (Fernandes et al., 2009) and neuronal plasticity (Lauri et al., 2006).

The expression of receptor subunits does indeed vary across species and brain regions. For example, the primate neocortex primarily expresses the GluK1-2-3 subunits (Huntley et al., 1993), whereas the rodent cortex expresses more GluK2 and GluK4 subunits (Herb et al., 1992). Hippocampal interneurons express KA receptors (Liu et al., 2004). Astrocytes in the hypothalamic arcuate nucleus express GluK1–3 subunits (Diano et al., 1998).

Hippocampal astrocytes express GluK2 (Matschke et al., 2015). Some KA subunits allow Ca^{2+} efflux, which in astrocytes can contribute to glutamate vesicular release or activate other Ca^{2+}-dependent signaling pathways (see Guerra-Gomes et al., 2018). Interestingly, after chemoconvulsive status epilepticus of temporal lobe epilepsy, CA1 hippocampal reactive astrocytes expressed GluK1, GluK2/3, GluK4, and GluK5 (Vargas et al., 2013). Of these, GluK1 and GluK5 expression in astrocytes persist during the presence of spontaneous seizures, suggesting that KA receptors in astrocytes might contribute to the pathophysiology of epilepsy (Vargas et al., 2013).

In general, iGluRs are a key component for synaptic activity and neuronal processing, however, more research is required to elucidate the contribution of each iGluRs in astrocytes and how it contributes to glutamatergic neurotransmission.

mGluRs

mGluRs are coupled to G-proteins and modulate slow synaptic transmission through second messengers. To date, eight mGluRs (mGluR1–8) have been described and divided into three groups designated I, II, and III according to similarities in their distinctive features: gene sequence, pharmacological properties, and intracellular signaling mechanisms (Sladeczek et al., 1985).

The group I receptors, mGluR1 and mGluR5, are associated with intracellular Ca^{2+} signaling, phospholipase C, and these receptors are mainly activated by 3,5-dihydroxyphenylglycine (DHPG). Group II includes mGluR2 and mGluR3, which are negatively coupled to adenylate cyclase and are selectively activated by LY379268. Finally, group III contains mGluR4, mGluR6, mGluR7, and mGluR8 receptors, which, like group II, are negatively coupled to the adenylate cyclase (Sugiyama et al., 1987; Masu et al., 1991).

Receptors from the Group I mGluRs are more widespread in the brain. They are expressed in neurons from the olfactory bulb, cerebral cortex, globus pallidus, lateral septum, cerebellar Purkinje cells, and thalamic nuclei (Crupi et al., 2019). Group II mGluRs are expressed in the olfactory bulb and cerebellar cortex (for more details see Crupi et al., 2019). In contrast, astrocytes showed a predominance of mGluR1, mGluR3, and mGluR5 receptors, which have been described thus far in the hippocampus and cerebral cortex (Schools and Kimelberg, 1999; Sun et al., 2013; Spampinato et al., 2018). Using electron microscopy and immunohistochemistry, mGluR2 and mGluR3 have been identified in astrocytes in the rat ventrobasal thalamus (Mineff and Valtschanoff, 1999).

The activation of mGluR3 and mGluR5 increases Ca^{2+} intracellular concentration, triggering vesicular glutamate release in neurons and astrocytes, which influences synaptic activity and plasticity (Fiacco and McCarthy, 2004; Perea and Araque, 2007; Cavaccini et al., 2020). Specific activation of metabotropic group II receptors in astrocyte cultures increases the expression of GLAST (Gegelashvili et al., 2000). Likewise, activating the mGluR5 receptor increases glutamate uptake through increased expression of GLT-1 and GLAST transporters (Vermeiren et al., 2005). However, contradicting results have been observed during the activation of group I mGluRs, which reduces the expression of GLAST (Gegelashvili et al., 2000). Therefore, more studies are needed to understand the specific role of each mGluR in astrocytic glutamate transporter expression and function.

Glutamate transporters

Glutamate transporters, also known as excitatory amino acid transporters (EAATs), maintain optimal extracellular glutamate concentration; these transporters belong to the solute carrier (SLC) family 1 (high-affinity glutamate transporters; He et al., 2009). These proteins are expressed in neurons and glial cells, especially astrocytes, and are responsible for the bulk of glutamate uptake (Rothstein et al., 1996) by the co-transport of glutamate, Na^+ (three molecules), H^+ (one molecule), and counter-transport of K^+ (one molecule; Levy et al., 1998).

Five EAATs have been identified in humans. In rodents, these transporters were named excitatory amino acid carrier 1 EAAC1/EAAT3, GLAST/EAAT1, GLT-1/EAAT2, excitatory amino acid transporter 4 (EAAT4), and excitatory amino acid transporter 5 (EAAT5; **Figure 2**; Kanai and Hediger, 1992; Pines et al., 1992; Storck et al., 1992; Fairman et al., 1995; Rothstein et al., 1996; Arriza et al., 1997).

Neurons in the rat cerebral cortex, hippocampus, cerebellum, and spinal cord express the EAAC1 transporters (Kanai et al., 1995; Shashidharan et al., 1997). Interestingly, this transporter is mainly involved in anion conductance and uptake of cysteine, a precursor of glutathione synthesis (Lee et al., 2020). EAAT4 is highly expressed in cerebellar Purkinje cells (Magi et al., 2019). However, it is also found in the fore- and mid-brain and the somatosensory cortex (Massie et al., 2008; de Vivo et al., 2010). EAAT5 is mainly expressed in the retina (Arriza et al., 1997).

GLAST and GLT-1 are highly expressed in astrocytes of the hippocampus, striatum, and cerebral cortex and oversee glutamate uptake at the synapse (Levy et al., 1993; Lehre et al., 1995; Bergles and Jahr, 1997; Mennerick et al., 1998). According to studies focused on evaluating the subcellular distribution of GLAST and GLT-1, both transporters are highly expressed in hippocampal astrocytes with a predominant presence of GLT-1 in the filopodium and perivascular end-feet, and GLAST is mostly present in the soma and processes (Schreiner et al., 2014; Radulescu et al., 2022). However, the GLT-1 isoforms (GLT-1a and b) are expressed in neurons from the hippocampus, cerebral cortex, striatum, thalamus, and midbrain (Chen et al., 2002, 2004; Berger et al., 2005). Astrocyte processes express more GLT-1a mRNA, whereas GLT-1b mRNA has been detected mainly in the cell body (Berger et al., 2005). In neurons, GLT-1a protein expression in axons, spines, and dendrites contributes to glutamate reuptake in the excitatory terminals (Chen et al., 2004). It has been suggested that GLT-1 in neurons provides glutamate as a substrate for energy metabolism and mitochondrial functionality (Petr et al., 2015; McNair et al., 2019).

Astrocytes are the main regulators of extracellular glutamate concentration through the GLT-1 and GLAST glutamate transporters; expression of these transporters is regulated by neuronal activity (Swanson et al., 1997; Perego et al., 2000). Interestingly, besides neurons, brain endothelial cells can also induce GLT-1 expression through Notch signaling (Lee et al., 2017).

In addition to EAATs, the SLC7A11/xCT transporter is a cystine/glutamate antiporter, which transports a cystine into the cell while exchanging for glutamate (1–1 ratio), in a sodium-independent fashion; therefore, contributing to astrocyte glutamate release (Bannai, 1986). It consists of two subunits, the light subunit (SLC7A11) and the heavy subunit (SLC3A2). Whereas the light subunit is responsible for the active transport of cystine and glutamate, the heavy subunit is necessary for intracellular trafficking

and proper membrane arrangement of the transporter (Nakamura et al., 1999; Shin et al., 2017). The SLC7A11/xCT transporter takes up cystine and, inside the cell, cystine will be converted to cysteine, the main precursor for the antioxidant glutathione (Conrad and Sato, 2012). SLC7A11/xCT is highly expressed in the human brain (Sato et al., 1999). In mice, SLC7A11/xCT is prominently expressed in the hippocampus, cortex, hypothalamus, and dentate gyrus (Sato et al., 2002). The SLC7A11/xCT expression occurs mainly in glial cells (Re et al., 2006), including astrocytes (Ottestad-Hansen et al., 2018). SLC7A11/xCT is essential to avoid oxidative damage (Lewerenz et al., 2012), probably due to its link with glutathione synthesis. It follows that blocking SLC7A11/xCT leads to an increase in oxidative stress and astrocyte death (Chen et al., 2000), a process known as oxidative glutamate toxicity (Schubert and Piasecki, 2001).

Interestingly, *Drosophila* xCT gene-knockout reduced the extracellular ambient glutamate concentration by 50%, suggesting that the xCT transporter is essential for extracellular glutamate regulation (Augustin et al., 2007). Also, the use of sulfasalazine, an xCT inhibitor, reduces the NMDA-induced current by 66.8% in mouse hippocampus slices, indicating that glutamate release through xCT contributes to neuronal activation (Koh et al., 2022). In addition, xCT deletion in mice induces an age-dependent anxiety-like behavior (Bentea et al., 2015). In a related experiment, exposing the astrocytoma-derived cell line (1321N1) to peroxide increased both ambient glutamate concentrations and the population of xCT transporters, suggesting that xCT activity contributes to glutamate release and accumulation (Kazama et al., 2020). Further experiments are necessary to clarify whether the release of glutamate by the xCT transporter contributes to the activation of iGluRs and mGluRs in neurons or astrocytes and if it contributes to pathological processes *in vivo*.

Role of astrocytes in glutamatergic neurotransmission during physiological conditions

Astrocyte function was initially thought to support neuronal activity or protect neurons from excitotoxicity. However, later studies suggested that astrocytes can directly or indirectly modulate synaptic neuronal activity (**Figure 2**; Nedergaard, 1994; Beppu et al., 2021) and influence behavior (Lyon and Allen, 2022). The first level of regulation is glutamate uptake by astrocytic transporters as they regulate the neurotransmitter levels in the synaptic cleft; these transporters indirectly modulate neuronal transmission (Jabaudon et al., 1999), neuronal activity (Estrada-Sánchez et al., 2019) and survival (Estrada-Sánchez et al., 2007, 2019). Glutamate uptake can also regulate the availability of glutamine to synthesize glutamate. As mentioned in an earlier section, once astrocytes take up glutamate, it can be metabolized into α-ketoglutarate by glutamate dehydrogenase or into glutamine through amidation of glutamate by the glutamine synthetase (as part of the glutamate/glutamine cycle; Laake et al., 1999; Islam et al., 2010). As this enzyme is highly expressed in astrocytes, they are considered the major glutamine reservoir and an important source of precursor for the metabolism of glutamate and gamma-aminobutyric acid (GABA; Hamberger et al., 1979; Norenberg and Martinez-Hernandez, 1979). Therefore, astrocytes also might regulate glutamatergic neuronal dynamics by the amount of glutamine released into the synaptic cleft.

Astrocytes can also regulate neuronal activity by releasing gliotransmitters such as glutamate, ATP, D-serine, or GABA, also known as gliotransmission. Once the gliotransmitter is released, it activates its target receptors and, consequently, generates responses in the same astrocyte (autocrine response) or nearby cells, including neurons (Lapato and Tiwari-Woodruff, 2018; Savtchouk and Volterra, 2018; Beppu et al., 2021; Sherwood et al., 2021a). In addition to gliotransmission, astrocytes contribute to neuronal activity by regulating the availability of NMDA receptor co-agonists (glycine and D-serine; Sherwood et al., 2021a). However, more studies are needed to better understand this process's physiological and pathological implications. For a more detailed description of this topic, see Sherwood et al. (2021a). Also, astrocytes can release active molecules through hemichannels (Lee et al., 2011; Montero and Orellana, 2015; Lalo et al., 2021). This topic is beyond the scope of this review, but for more information refer to Sahlender et al. (2014), Montero and Orellana (2015), and Caudal et al. (2020).

Experiments using electrophysiology, optical imaging, and molecular biology demonstrated that astrocytes respond to neurotransmitters. Activation of mGluR initiates a cellular signaling cascade that increases intracellular Ca^{2+} concentration on a timescale of about 50–200 ms (Batchelor and Garthwaite, 1997; Marcaggi et al., 2009), in contrast to the comparatively fast ionotropic receptors that take approximately 1–10 ms in neurons to initiate the same response (Traynelis et al., 2010; Reiner and Levitz, 2018). iGluRs are fast-acting because extracellular Ca^{2+} directly enters the cell through the open channel, although a significant Ca^{2+} concentration rise requires a substantial number of simultaneously open iGluR channels. The activation of mGluR initiates a cellular signaling cascade that amplifies the input signal, albeit at the cost of response time; the activated mGluR activates the phospholipase C/IP3 pathway, which then generates the release of Ca^{2+} from the endoplasmic reticulum (Decrock et al., 2013; Rodriguez-Prados et al., 2020). This intracellular source of Ca^{2+} induces gliotransmitter release by Ca^{2+}-dependent exocytosis (Bezzi et al., 1998, 2004; Zhang et al., 2004; Mothet et al., 2005; Crippa et al., 2006; Woo et al., 2012; Li et al., 2013b; Navarrete et al., 2013; Heller et al., 2020; Takata-Tsuji et al., 2021). Gliotransmitter release in turn affects neuronal functioning, forming a feedback loop.

Although astrocytes express both iGluRs and mGluRs, intracellular Ca^{2+} concentration rises mainly due to the activation of mGluRs rather than iGluRs (Conti et al., 1996; Schools and Kimelberg, 1999). However, we cannot dismiss the contribution of iGluRs in astrocytes for two reasons. First, the temporal dynamics of iGluRs are approximately an order of magnitude faster than mGluRs. Second, the presence of certain subunits confers different Ca^{2+} permeability to iGluRs (Seifert et al., 1997; Brand-Schieber and Werner, 2003; Brand-Schieber et al., 2004; Palygin et al., 2010). The subunits that increase Ca^{2+} permeability are GluN3 subunits for NMDA (Cull-Candy et al., 2001; Kvist et al., 2013), GluA2 for AMPA (Traynelis et al., 2010), and GluK3-4 for KA (Burnashev et al., 1995). The ratio of iGluRs with and without increased Ca^{2+} permeability in astrocytes may constitute a regulatory mechanism to modulate Ca^{2+} influx into the cell, affecting Ca^{2+}-dependent pathways.

The release of glutamate from astrocytes contributes to NMDA-related long-term depression. This mechanism is initiated

by cannabinoid receptor type 1 (CB1) activation of astrocytes, which increases Ca^{2+} concentration and produces astrocytic glutamate release. Increased extracellular glutamate activates NMDA receptors, promoting internalization of AMPA receptors (Han et al., 2012; Min and Nevian, 2012) in the cortex and hippocampus. In the cortex, the activation of astrocytic CB1 receptors by exogenous cannabinoids impairs spatial working memory (Han et al., 2012; Min and Nevian, 2012).

In the striatum, two subpopulations of astrocytes may selectively regulate the response for dopamine D1 vs. D2 medium spiny neuron (MSN) subpopulations. Astrocytic CB1 activation elicits astrocytic glutamate release that specifically induces activity from either D1 or D2 MSNs, but not both (Martín et al., 2015). This finding suggests nuanced organization and interaction of neurons and astrocytes in the striatum.

On the other hand, Beppu et al. (2021) demonstrated that Bergmann glial cells amplify excitatory neuronal signals in the cerebellar cortex by releasing glutamate through a mechanism involving bicarbonate efflux and resultant intracellular acidification, a mechanism sensitive to the inhibition of volume-regulated ion channels. Although this is a novel and exciting mechanism by which astrocytes can regulate the neuronal activity, more experiments are needed to elucidate its specific molecular components and their contribution during physiological conditions *in vivo*. Also, this opens the question of whether this is a mechanism restricted to the cerebellar cortex or if it also occurs in other brain areas.

Interneurons also play an important role in astrocyte modulation of neuronal activity. Interneurons are locally projecting neurons that regulate neuronal activity levels through inhibitory signaling that counteracts excitatory (e.g., glutamatergic) signaling, and helps to prevent runaway excitatory cascades. For example, in the hippocampus, stimulation of inhibitory GABAergic interneurons activates $GABA_B$ receptors in astrocytes, which subsequently triggers increased Ca^{2+} waves in the surrounding astrocytes, potentiating pyramidal inhibition. This effect is blocked by glutamatergic antagonist CNQX [cyanquixaline (6-cyano-7-nitroquinoxaline-2,3-dione)] and AP5 (2-amino-5-phosphopentanoic acid), suggesting that interneuron-astrocyte-mediated potentiated inhibition of pyramidal neurons depends on astrocyte-mediated glutamate release (Kang et al., 1998). Also, in the hippocampus, Perea et al. (2016) described that, besides activating $GABA_B$ receptors in astrocytes, GABA release by interneurons also activates $GABA_A$ receptors in presynaptic neurons, which inhibited synaptic activity. However, when the interneuron leads to astrocyte-mediated glutamate release, presynaptic activation of mGluR 1/5 receptors contributes to synaptic potentiation (Kang et al., 1998; Perea et al., 2016). In the mouse cerebellar cortex, activation of purinergic P2Y1 receptors and AMPA receptors in Bergmann glial cells leads to glutamate-vesicular release that activates NMDA receptors in the interneurons, enhancing the inhibitory synaptic input to Purkinje cells (Rudolph et al., 2016).

Shen et al. (2017) described that autocrine activation of P2Y1 purinergic receptors in astrocytes modulates the release of glutamate mediated by the Ca^{2+}-dependent chloride channel Best-1 and the subsequent activation of extra-synaptic NMDA receptors in neurons. Related studies evaluated the effect of astrocytic Ca^{2+}-dependent glutamate release on the activity of neuronal extra-synaptic NMDA receptors (Le Meur et al., 2007; Shen et al., 2017; Koh et al., 2021). These receptors contain the GluN2B subunit and their activation produce a slow inward current with an amplitude of 18–477 pA, with a rise time of 13–332 ms, and decay times of 72–1,630 ms. The presence of extra-synaptic NMDA currents directly depends on astrocytes' intracellular Ca^{2+} concentrations; they decrease when Ca^{2+} signaling is abolished and increase when intracellular Ca^{2+} concentration rises (Araque et al., 1998b; Pirttimaki et al., 2011; Perea et al., 2014); these neuronal currents are generated by astrocyte activity in the hippocampus, cortex (Gomez-Gonzalo et al., 2017, 2018), and nucleus accumbens (Corkrum et al., 2019).

Moreover, Santello et al. (2011) showed that the P2Y1 activation and consequent Ca^{2+}-dependent glutamate release in astrocytes from the dentate gyrus also involve the tumor necrosis factor-alpha (TNFα), which at physiological concentrations (pM range), favors the adequate exocytosis of astrocytic glutamatergic vesicles. Interestingly, glutamate released activates presynaptic NMDA receptors, particularly expressing the GluN3A subunit with a low voltage-dependent Mg^{2+} block (Savtchouk et al., 2019). In an excellent review, Di Castro and Volterra (2022) describe how this mechanism might be relevant within the entorhinal cortex-dentate gyrus circuit involved in memory processing.

Additional evidence beyond the hippocampus indicates that astrocytes have a functional role in behavior regulation. For example, astrocytes in the suprachiasmatic nucleus increase Ca^{2+} signaling at night. This signaling is highly related to circadian behavior regulated by glutamate release (Brancaccio et al., 2019). Blum et al. (2021) demonstrated in *Drosophila melanogaster* through *in vivo* two-photon experiments that increased astrocyte Ca^{2+} activity correlates with sleep needs. Another behavior modulated by astrocytes is feeding (Sweeney et al., 2016; Varela et al., 2021). Specific stimulation of astrocytes from mice's medial basal hypothalamus suppresses food intake (Sweeney et al., 2016). Finally, mice lacking astrocyte glucocorticoid receptors in the amygdala show attenuated anxiety behaviors in the open field behavioral test and fear memory (Wiktorowska et al., 2021), demonstrating a direct involvement of astrocytes in fear memory and anxiety.

The extensive evidence reflects the key role of Ca^{2+}-mediated signaling in astrocytes, which requires stimulation of IP3 receptors (IP_3R). There are three subtypes of IP_3R in mammals (IP_3R1, IP_3R2, and IP_3R3) and among them, IP_3R2 was widely accepted as the only functional subtype in astrocytes (Sherwood et al., 2021b). However, recent evidence showed that IP_3R1 and IP_3R3 are also present in astrocytes and participate in Ca^{2+}-mediated signaling, especially in astrocytic processes (Sherwood et al., 2017, 2021b). This new finding raises questions about the functional role and subcellular localization of the different IP_3R subtypes in astrocytes during gliotransmission and its relevance during physiological processes.

As a whole, this evidence indicates that gliotransmission modulates the activity of astrocytes and neuronal circuits (projecting and interneurons) and appears to be a widespread mechanism in the brain. Besides glutamate, ATP purinergic receptors emerge as a key component that triggers astrocytes' modulation of neuronal circuits. Although the current evidence indicates that gliotransmission can influence behavior, more research is needed to dissect the different roles of astrocytes in shaping animal behavior.

Role of astrocytes in glutamatergic neurotransmission during pathological conditions

Alterations in the glutamate transporters are related to neurodegenerative diseases (Pajarillo et al., 2019). *Postmortem* brains of Alzheimer's disease patients showed a reduction in EAAT1 and EAAT2 (Masliah et al., 2000). Epilepsy is associated with decreased EAAT2 (Tanaka et al., 1997). In the intrahippocampal kainic acid model of temporal lobe epilepsy, GLT-1 and GLAST expression increase early after the treatment, suggesting that dysregulation in the expression of astrocyte glutamate transporters could contribute to the development of epilepsy. However, the accuracy of this hypothesis has not yet been determined (Peterson and Binder, 2019). In Huntington's disease, an inherited neurodegenerative disorder, reduced expression of EAAT2 was observed in *postmortem* brain samples (Faideau et al., 2010). In the R6/2 Huntington's disease transgenic mouse model, decreased content of GLT-1 and GLAST correlates with increased vulnerability to glutamate-induced toxicity (Estrada-Sánchez et al., 2009, 2010). Likewise, cortical pyramidal neurons in the R6/2 mice are more vulnerable to glutamate-mediated paroxysmal activity during the inhibition of both GLT-1 and GLAST transporters (Estrada-Sánchez et al., 2019).

To date, exploration of how gliotransmission contributes to neural information processing and behavior focused mostly on physiological conditions. Less is known about how changes in gliotransmission contribute to the pathology of neurological, neuropsychiatric, or neurodegenerative conditions. However, data from physiological studies point out that astrocytes in the striatum can modulate differentially D1 or D2 MSNs (see above), and its dysfunction may contribute to diseases like Parkinson's and Huntington's disease. D1 and D2 MSNs comprise key components of the brain circuits that control movement and, at the behavioral level, both diseases involve substantial alterations in movement control. At the cellular level, D1/D2 MSN impairment contributes to neuropathology, and a distinct line of evidence demonstrates altered astrocyte functioning (for a review, see Estrada-Sánchez et al., 2017). The results from Martín et al. (2015) suggest that these two lines of evidence from disease pathology are connected to healthy tissue function (Martín et al., 2015); further understanding of how this system works may yield promising pathways for future therapeutic interventions for these diseases.

Another possible contribution of gliotransmission to neuropathology might be through the activation of extra-synaptic NMDA receptors since the NMDA-mediated response in neurons depends on its subcellular localization. Whereas activation of synaptic NMDA receptors leads to survival pathways, activation of extra-synaptic NMDA receptors initiates neuronal death cascades (Kaufman et al., 2012). In fact, it has been documented that activation of extra-synaptic NMDA receptors contributes to the neurodegenerative processes described in Huntington's disease and ischemia (Hardingham and Bading, 2010; Milnerwood et al., 2012). Because activation of extra-synaptic NMDA receptors indicates astrocytic Ca^{2+}-dependent glutamate release, it is important to evaluate the possible role of astrocytes in the balance/imbalance between synaptic and extra-synaptic glutamatergic receptors activation and whether this contributes to survival or neuronal death pathways.

The description of three functional IP_3R subtypes in astrocytes raises new questions about their role in pathological states. Recently, it was shown that the IP_3R1 subtype has a key role in chronic itching (Shiratori-Hayashi et al., 2021) and the absence of IP_3R2 in astrocytes generates autism spectrum disorder-like behaviors (Wang et al., 2021).

More studies are needed to fill the knowledge gaps about the contribution of gliotransmission during the pathological process of neurodegenerative, psychiatric, and neuropathological conditions.

Perspectives

The evidence reviewed strongly suggests a complex functional interaction between neurons and astrocytes. The extent to which astrocytes modulate the synapse could vary depending on the brain region, influencing its information processing and behavioral output. To date, most of the information on gliotransmission has been centered in the hippocampus, and less is known about gliotransmission in other areas of the brain. Similarly, most studies have focused on physiological conditions, and although there is evidence that astrocytes contribute to the neuropathological process, the precise role of astrocytes during neuropathological processing is still to be determined. The review also suggests extensive opportunities for further research, including the specific contribution of each gliotransmitter described to date and perhaps the identification of new gliotransmitters, their synthesis, and release systems. It is also important to better understand the effects of gliotransmitters on neighboring cells, including the same astrocyte or afferent, efferent neurons, interneurons, and microglia cells. Finally, additional studies will clarify the functional interconnection among different signaling pathways in a tripartite synapse, such as glutamatergic, purinergic, and GABAergic, during physiological and pathological conditions. Current evidence is limited to *in vitro* and brain slice experiments, which limits our understanding of the functional role of all these components *in vivo*.

Conclusions

The development of new experimental tools has widened our understanding of the synapse, where astrocytes emerge as a complex contributor. The evidence indicates that astrocyte release of gliotransmitters such as glutamate, ATP, D-serine, and GABA can activate, potentiate, or inhibit the activity of projection neurons, interneurons, or other astrocytes. Furthermore, astrocytes can influence neuronal synaptic modification through effects on long-term depression and potentiation. It is likely that more than one gliotransmitter coexists within the same astrocyte, providing a new degree of complexity to astrocyte modulatory activity. These mechanisms suggest additional layers of information-processing capability, enabled by astrocytes that extend beyond the traditional focus on neurons as the information-processing cells of the brain. More studies are needed to understand how astrocytes modulate neuron-astrocyte network activity across the brain during

physiological and pathological conditions. Finally, deepening the understanding of the functional dynamics between gliotransmitter signaling in neurons and astrocytes will widen the therapeutic targets for neuropathological conditions and neurodegenerative diseases, including ischemia, stroke, and Huntington's disease.

Author contributions

AOC-S and AME-S conceived the review topic. AOC-S, VMR-R, TBM-B, AP-S, AGH, DPP-P, AMG, and AME-S prepared the first draft and figures. All authors contributed to the article and approved the submitted version.

Funding

This work received support from the Consejo Nacional de Ciencia y Tecnología (CONACYT) FOSEC SEP-INVESTIGACIÓN BÁSICA A1-S-26479, and the International Society for Neurochemistry-CATEGORY 1C: Return Home grant to AME-S. CONACYT also awarded a master's fellowship to AOC-S (753050), and doctoral fellowship to AOC-S (800824), TBM-B (714872), and a postdoctoral fellowship to VMR-R (626561). AMG was funded by PR-INBRE (NIH-NIGMS P20GM103475).

Acknowledgments

We would like to thank Manuel Estrada for his help during figure preparation and the IPICYT for providing access to academic journals for the review.

Conflict of interest

AGH was employed by the company Intelligent Systems Laboratory, HRL Laboratories, LLC.

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

Publisher's note

References

Allen, N. J., and Eroglu, C. (2017). Cell biology of astrocyte-synapse interactions. *Neuron* 96, 697–708. doi: 10.1016/j.neuron.2017.09.056

Alsaad, H. A., DeKorver, N. W., Mao, Z., Dravid, S. M., Arikkath, J., and Monaghan, D. T. (2019). In the telencephalon, GluN2C NMDA receptor subunit mRNA is predominately expressed in glial cells and GluN2D mRNA in interneurons. *Neurochem. Res.* 44, 61–77. doi: 10.1007/s11064-018-2526-7

Amaral, A. I., Hadera, M. G., Tavares, J. M., Kotter, M. R., and Sonnewald, U. (2016). Characterization of glucose-related metabolic pathways in differentiated rat oligodendrocyte lineage cells. *Glia* 64, 21–34. doi: 10.1002/glia.22900

Anlauf, E., and Derouiche, A. (2005). Astrocytic exocytosis vesicles and glutamate: a high-resolution immunofluorescence study. *Glia* 49, 96–106. doi: 10.1002/glia.20094

Anlauf, E., and Derouiche, A. (2013). Glutamine synthetase as an astrocytic marker: its cell type and vesicle localization. *Front. Endocrinol. (Lausanne)* 4:144. doi: 10.3389/fendo.2013.00144

Araque, A., Sanzgiri, R. P., Parpura, V., and Haydon, P. G. (1998a). Calcium elevation in astrocytes causes an NMDA receptor-dependent increase in the frequency of miniature synaptic currents in cultured hippocampal neurons. *J. Neurosci.* 18, 6822–6829. doi: 10.1523/JNEUROSCI.18-17-06822.1998

Araque, A., Parpura, V., Sanzgiri, R. P., and Haydon, P. G. (1998b). Glutamate-dependent astrocyte modulation of synaptic transmission between cultured hippocampal neurons. *Eur. J. Neurosci.* 10, 2129–2142. doi: 10.1046/j.1460-9568.1998.00221.x

Araque, A., Parpura, V., Sanzgiri, R. P., and Haydon, P. G. (1999). Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci.* 22, 208–215. doi: 10.1016/s0166-2236(98)01349-6

Arriza, J. L., Eliasof, S., Kavanaugh, M. P., and Amara, S. G. (1997). Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. *Proc. Natl. Acad. Sci. U S A* 94, 4155–4160. doi: 10.1073/pnas.94.8.4155

Augustin, H., Grosjean, Y., Chen, K., Sheng, Q., and Featherstone, D. E. (2007). Nonvesicular release of glutamate by glial xCT transporters suppresses glutamate receptor clustering *in vivo*. *J. Neurosci.* 27, 111–123. doi: 10.1523/JNEUROSCI.4770-06.2007

Bannai, S. (1986). Exchange of cystine and glutamate across plasma membrane of human fibroblasts. *J. Biol. Chem.* 261, 2256–2263. doi: 10.1016/S0021-9258(17)35926-4

Batchelor, A. M., and Garthwaite, J. (1997). Frequency detection and temporally dispersed synaptic signal association through a metabotropic glutamate receptor pathway. *Nature* 385, 74–77. doi: 10.1038/385074a0

Behar, T. N., Scott, C. A., Greene, C. L., Wen, X., Smith, S. V., Maric, D., et al. (1999). Glutamate acting at NMDA receptors stimulates embryonic cortical neuronal migration. *J. Neurosci.* 19, 4449–4461. doi: 10.1523/JNEUROSCI.19-11-04449.1999

Bentea, E., Demuyser, T., Van Liefferinge, J., Albertini, G., Deneyer, L., Nys, J., et al. (2015). Absence of system xc- in mice decreases anxiety and depressive-like behavior without affecting sensorimotor function or spatial vision. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 59, 49–58. doi: 10.1523/JNEUROSCI.1672-22.2022

Beppu, K., Kubo, N., and Matsui, K. (2021). Glial amplification of synaptic signals. *J. Physiol.* 599, 2085–2102. doi: 10.1113/JP280857

Berger, U. V., DeSilva, T. M., Chen, W., and Rosenberg, P. A. (2005). Cellular and subcellular mRNA localization of glutamate transporter isoforms GLT1a and GLT1b in rat brain by in situ hybridization. *J. Comp. Neurol.* 492, 78–89. doi: 10.1002/cne.20737

Bergles, D. E., and Jahr, C. E. (1997). Synaptic activation of glutamate transporters in hippocampal astrocytes. *Neuron* 19, 1297–1308. doi: 10.1016/s0896-6273(00)80420-1

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

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

Blum, I. D., Keleş, M. F., Baz, E. S., Han, E., Park, K., Luu, S., et al. (2021). Astroglial calcium signaling encodes sleep need in *Drosophila*. *Curr. Biol.* 31, 150–162.e7. doi: 10.1016/j.cub.2020.10.012

Brancaccio, M., Edwards, M. D., Patton, A. P., Smyllie, N. J., Chesham, J. E., Maywood, E. S., et al. (2019). Cell-autonomous clock of astrocytes drives circadian behavior in mammals. *Science* 363, 187–192. doi: 10.1126/science.aat4104

Brand-Schieber, E., Lowery, S. L., and Werner, P. (2004). Select ionotropic glutamate AMPA/kainate receptors are expressed at the astrocyte-vessel interface. *Brain Res.* 1007, 178–182. doi: 10.1016/j.brainres.2003.12.051

Brand-Schieber, E., and Werner, P. (2003). AMPA/kainate receptors in mouse spinal cord cell-specific display of receptor subunits by oligodendrocytes and

astrocytes and at the nodes of Ranvier. *Glia* 42, 12–24. doi: 10.1002/glia.10136

Burnashev, N., Zhou, Z., Neher, E., and Sakmann, B. (1995). Fractional calcium currents through recombinant GluR channels of the NMDA, AMPA and kainate receptor subtypes. *J. Physiol.* 485, 403–418. doi: 10.1113/jphysiol.1995.sp020738

Cardona, C., Sanchez-Mejias, E., Davila, J. C., Martin-Rufian, M., Campos-Sandoval, J. A., Vitorica, J., et al. (2015). Expression of Gls and Gls2 glutaminase isoforms in astrocytes. *Glia* 63, 365–382. doi: 10.1002/glia.22758

Caudal, L. C., Gobbo, D., Scheller, A., and Kirchhoff, F. (2020). The paradox of astroglial Ca^{2+} signals at the interface of excitation and inhibition. *Front. Cell. Neurosci.* 14:609947. doi: 10.3389/fncel.2020.609947

Cavaccini, A., Durkee, C., Kofuji, P., Tonini, R., and Araque, A. (2020). Astrocyte signaling gates long-term depression at corticostriatal synapses of the direct pathway. *J. Neurosci.* 40, 5757–5768. doi: 10.1523/JNEUROSCI.2369-19.2020

Chen, W., Aoki, C., Mahadomrongkul, V., Gruber, C. E., Wang, G. J., Blitzblau, R., et al. (2002). Expression of a variant form of the glutamate transporter GLT1 in neuronal cultures and in neurons and astrocytes in the rat brain. *J. Neurosci.* 22, 2142–2152. doi: 10.1523/JNEUROSCI.22-06-02142.2002

Chen, C. J., Liao, S. L., and Kuo, J. S. (2000). Gliotoxic action of glutamate on cultured astrocytes. *J. Neurochem.* 75, 1557–1565. doi: 10.1046/j.1471-4159.2000.0751557.x

Chen, W., Mahadomrongkul, V., Berger, U. V., Bassan, M., DeSilva, T., Tanaka, K., et al. (2004). The glutamate transporter GLT1a is expressed in excitatory axon terminals of mature hippocampal neurons. *J. Neurosci.* 24, 1136–1148. doi: 10.1523/JNEUROSCI.1586-03.2004

Conrad, M., and Sato, H. (2012). The oxidative stress-inducible cystine/glutamate antiporter, system x (c) (-) : cystine supplier and beyond. *Amino Acids* 42, 231–246. doi: 10.1007/s00726-011-0867-5

Conti, F., DeBiasi, S., Minelli, A., and Melone, M. (1996). Expression of NR1 and NR2A/B subunits of the NMDA receptor in cortical astrocytes. *Glia* 17, 254–258. doi: 10.1002/(SICI)1098-1136(199607)17:3<254::AID-GLIA7>3.0.CO;2-0

Corkrum, M., Rothwell, P. E., Thomas, M. J., Kofuji, P., and Araque, A. (2019). Opioid-mediated astrocyte-neuron signaling in the nucleus accumbens. *Cells* 8:586. doi: 10.3390/cells8060586

Crippa, D., Schenk, U., Francolini, M., Rosa, P., Verderio, C., Zonta, M., et al. (2006). Synaptobrevin2-expressing vesicles in rat astrocytes: insights into molecular characterization, dynamics and exocytosis. *J. Physiol.* 570, 567–582. doi: 10.1113/jphysiol.2005.094052

Crupi, R., Impellizzeri, D., and Cuzzocrea, S. (2019). Role of metabotropic glutamate receptors in neurological disorders. *Front. Mol. Neurosci.* 12:20. doi: 10.3389/fnmol.2019.00020

Cull-Candy, S., Brickley, S., and Farrant, M. (2001). NMDA receptor subunits: diversity, development and disease. *Curr. Opin. Neurobiol.* 11, 327–335. doi: 10.1016/s0959-4388(00)00215-4

David, J. C., Yamada, K. A., Bagwe, M. R., and Goldberg, M. P. (1996). AMPA receptor activation is rapidly toxic to cortical astrocytes when desensitization is blocked. *J. Neurosci.* 16, 200–209. doi: 10.1523/JNEUROSCI.16-01-00200.1996

de Vivo, L., Melone, M., Bucci, G., Rothstein, J. D., and Conti, F. (2010). Quantitative analysis of EAAT4 promoter activity in neurons and astrocytes of mouse somatic sensory cortex. *Neurosci. Lett.* 474, 42–45. doi: 10.1016/j.neulet.2010.03.003

de Wit, H., Walter, A. M., Milosevic, I., Gulyas-Kovacs, A., Riedel, D., Sorensen, J. B., et al. (2009). Synaptotagmin-1 docks secretory vesicles to syntaxin-1/SNAP-25 acceptor complexes. *Cell* 138, 935–946. doi: 10.1016/j.cell.2009.07.027

Decrock, E., De Bock, M., Wang, N., Gadicherla, A. K., Bol, M., Delvaeye, T., et al. (2013). IP3, a small molecule with a powerful message. *Biochim. Biophys. Acta* 1833, 1772–1786. doi: 10.1016/j.bbamcr.2012.12.016

Di Castro, M. A., and Volterra, A. (2022). Astrocyte control of the entorhinal cortex-dentate gyrus circuit: relevance to cognitive processing and impairment in pathology. *Glia* 70, 1536–1553. doi: 10.1002/glia.24128

Diano, S., Naftolin, F., and Horvath, T. L. (1998). Kainate glutamate receptors (GluR5–7) in the rat arcuate nucleus: relationship to tanycytes, astrocytes, neurons and gonadal steroid receptors. *J. Neuroendocrinol.* 10, 239–247. doi: 10.1046/j.1365-2826.1998.00195.x

Erecinska, M., Nelson, D., Nissim, I., Daikhin, Y., and Yudkoff, M. (1994). Cerebral alanine transport and alanine aminotransferase reaction: alanine as a source of neuronal glutamate. *J. Neurochem.* 62, 1953–1964. doi: 10.1046/j.1471-4159.1994.62051953.x

Estrada-Sánchez, A. M., Camacho, A., Montiel, T., and Massieu, L. (2007). Cerebellar granule neurons are more vulnerable to transient transport-mediated glutamate release than to glutamate uptake blockade. correlation with excitatory amino acids levels. *Neurochem. Res.* 32, 423–432. doi: 10.1007/s11064-006-9243-3

Estrada-Sánchez, A. M., Castro, D., Portillo-Ortiz, K., Jang, K., Nedjat-Haiem, M., Levine, M. S., et al. (2019). Complete but not partial inhibition of glutamate transporters exacerbates cortical excitability in the R6/2 mouse model of Huntington's disease. *CNS Neurosci. Ther.* 25, 509–518. doi: 10.1111/cns.13070

Estrada-Sánchez, A. M., Levine, M. S., and Cepeda, C. (2017). "Chapter 71 - epilepsy in other neurodegenerative disorders: Huntington's and Parkinson's diseases," in *Models of Seizures and Epilepsy (Second Edition)*, eds A. Pitkänen, P. S. Buckmaster, A. S. Galanopoulou, and S. L. Moshé (Cambridge, MA: Academic Press), 1043–1058.

Estrada-Sánchez, A. M., Montiel, T., and Massieu, L. (2010). Glycolysis inhibition decreases the levels of glutamate transporters and enhances glutamate neurotoxicity in the R6/2 Huntington's disease mice. *Neurochem. Res.* 35, 1156–1163. doi: 10.1007/s11064-010-0168-5

Estrada-Sánchez, A. M., Montiel, T., Segovia, J., and Massieu, L. (2009). Glutamate toxicity in the striatum of the R6/2 Huntington's disease transgenic mice is age-dependent and correlates with decreased levels of glutamate transporters. *Neurobiol. Dis.* 34, 78–86. doi: 10.1016/j.nbd.2008.12.017

Estrada-Sánchez, A. M., and Rebec, G. V. (2012). Corticostriatal dysfunction and glutamate transporter 1 (GLT1) in Huntington's disease: interactions between neurons and astrocytes. *Basal Ganglia* 2, 57–66. doi: 10.1016/j.baga.2012.04.029

Faideau, M., Kim, J., Cormier, K., Gilmore, R., Welch, M., Auregan, G., et al. (2010). *In vivo* expression of polyglutamine-expanded huntingtin by mouse striatal astrocytes impairs glutamate transport: a correlation with Huntington's disease subjects. *Hum. Mol. Genet.* 19, 3053–3067. doi: 10.1093/hmg/ddq212

Fairman, W. A., Vandenberg, R. J., Arriza, J. L., Kavanaugh, M. P., and Amara, S. G. (1995). An excitatory amino-acid transporter with properties of a ligand-gated chloride channel. *Nature* 375, 599–603. doi: 10.1038/375599a0

Fasano, C., Rocchetti, J., Pietrajtis, K., Zander, J. F., Manseau, F., Sakae, D. Y., et al. (2017). Regulation of the hippocampal network by VGLUT3-positive CCK-GABAergic basket cells. *Front. Cell. Neurosci.* 11:140. doi: 10.3389/fncel.2017.00140

Fernandes, H. B., Catches, J. S., Petralia, R. S., Copits, B. A., Xu, J., Russell, T. A., et al. (2009). High-affinity kainate receptor subunits are necessary for ionotropic but not metabotropic signaling. *Neuron* 63, 818–829. doi: 10.1016/j.neuron.2009.08.010

Fernstrom, J. D. (2018). Monosodium glutamate in the diet does not raise brain glutamate concentrations or disrupt brain functions. *Ann. Nutr. Metab.* 73, 43–52. doi: 10.1159/000494782

Fiacco, T. A., and McCarthy, K. D. (2004). Intracellular astrocyte calcium waves *in situ* increase the frequency of spontaneous AMPA receptor currents in CA1 pyramidal neurons. *J. Neurosci.* 24, 722–732. doi: 10.1523/JNEUROSCI.2859-03.2004

Fremeau, R. T., Jr., Burman, J., Qureshi, T., Tran, C. H., Proctor, J., Johnson, J., et al. (2002). The identification of vesicular glutamate transporter 3 suggests novel modes of signaling by glutamate. *Proc. Natl. Acad. Sci. U S A* 99, 14488–14493. doi: 10.1073/pnas.222546799

Fremeau, R. T., Jr., Troyer, M. D., Pahner, I., Nygaard, G. O., Tran, C. H., Reimer, R. J., et al. (2001). The expression of vesicular glutamate transporters defines two classes of excitatory synapse. *Neuron* 31, 247–260. doi: 10.1016/s0896-6273(01)00344-0

Fujiyama, F., Furuta, T., and Kaneko, T. (2001). Immunocytochemical localization of candidates for vesicular glutamate transporters in the rat cerebral cortex. *J. Comp. Neurol.* 435, 379–387. doi: 10.1002/cne.1037

Gegelashvili, G., Dehnes, Y., Danbolt, N. C., and Schousboe, A. (2000). The high-affinity glutamate transporters GLT1, GLAST and EAAT4 are regulated via different signalling mechanisms. *Neurochem. Int.* 37, 163–170. doi: 10.1016/s0197-0186(00)00019-x

Giaume, C., Tabernero, A., and Medina, J. M. (1997). Metabolic trafficking through astrocytic gap junctions. *Glia* 21, 114–123. doi: 10.1002/(SICI)1098-1136(199709)21:1%3C114::AID-GLIA13%3E3.0.CO;2-V

Gomez-Gonzalo, M., Martin-Fernandez, M., Martinez-Murillo, R., Mederos, S., Hernandez-Vivanco, A., Jamison, S., et al. (2017). Neuron-astrocyte signaling is preserved in the aging brain. *Glia* 65, 569–580. doi: 10.1002/glia.23112

Gomez-Gonzalo, M., Zehnder, T., Requie, L. M., Bezzi, P., and Carmignoto, G. (2018). Insights into the release mechanism of astrocytic glutamate evoking in neurons NMDA receptor-mediated slow depolarizing inward currents. *Glia* 66, 2188–2199. doi: 10.1002/glia.23473

Guerra-Gomes, S., Sousa, N., Pinto, L., and Oliveira, J. F. (2018). Functional roles of astrocyte calcium elevations: from synapses to behavior. *Front. Cell. Neurosci.* 11:427. doi: 10.3389/fncel.2017.00427

Hamberger, A. C., Chiang, G. H., Nylen, E. S., Scheff, S. W., and Cotman, C. W. (1979). Glutamate as a CNS transmitter. I. Evaluation of glucose and glutamine as precursors for the synthesis of preferentially released glutamate. *Brain Res.* 168, 513–530. doi: 10.1016/0006-8993(79)90306-8

Hamilton, N. B., and Attwell, D. (2010). Do astrocytes really exocytose neurotransmitters? *Nat. Rev. Neurosci.* 11, 227–238. doi: 10.1038/nrn2803

Han, J., Kesner, P., Metna-Laurent, M., Duan, T., Xu, L., Georges, F., et al. (2012). Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. *Cell* 148, 1039–1050. doi: 10.1016/j.cell.2012.01.037

Han, J., Pluhackova, K., and Bockmann, R. A. (2017). The multifaceted role of SNARE proteins in membrane fusion. *Front. Physiol.* 8:5. doi: 10.3389/fphys.2017.00005

Hansen, K. B., Wollmuth, L. P., Bowie, D., Furukawa, H., Menniti, F. S., Sobolevsky, A. I., et al. (2021). Structure, function and pharmacology of glutamate receptor ion channels. *Pharmacol. Rev.* 73, 298–487. doi: 10.1124/pharmrev.120.000131

Hardingham, G. E., and Bading, H. (2010). Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat. Rev. Neurosci.* 11, 682–696. doi: 10.1038/nrn2911

Hassel, B. (2001). Pyruvate carboxylation in neurons. *J. Neurosci. Res.* 66, 755–762. doi: 10.1002/jnr.10044

Hawkins, R. A. (2009). The blood-brain barrier and glutamate. *Am. J. Clin. Nutr.* 90, 867S–874S. doi: 10.3945/ajcn.2009.27462BB

He, L., Vasiliou, K., and Nebert, D. W. (2009). Analysis and update of the human solute carrier (SLC) gene superfamily. *Hum. Genomics* 3, 195–206. doi: 10.1186/1479-7364-3-2-195

Heller, J. P., Odii, T., Zheng, K., and Rusakov, D. A. (2020). Imaging tripartite synapses using super-resolution microscopy. *Methods* 174, 81–90. doi: 10.1016/j.ymeth.2019.05.024

Henneberger, C., Papouin, T., Oliet, S. H., and Rusakov, D. A. (2010). Long-term potentiation depends on release of D-serine from astrocytes. *Nature* 463, 232–236. doi: 10.1038/nature08673

Herb, A., Burnashev, N., Werner, P., Sakmann, B., Wisden, W., and Seeburg, P. H. (1992). The KA-2 subunit of excitatory amino acid receptors shows widespread expression in brain and forms ion channels with distantly related subunits. *Neuron* 8, 775–785. doi: 10.1016/0896-6273(92)90098-x

Herman, M. A., Ackermann, F., Trimbuch, T., and Rosenmund, C. (2014). Vesicular glutamate transporter expression level affects synaptic vesicle release probability at hippocampal synapses in culture. *J. Neurosci.* 34, 11781–11791. doi: 10.1523/JNEUROSCI.1444-14.2014

Herzog, E., Gilchrist, J., Gras, C., Muzerelle, A., Ravassard, P., Giros, B., et al. (2004). Localization of VGLUT3, the vesicular glutamate transporter type 3, in the rat brain. *Neuroscience* 123, 983–1002. doi: 10.1016/j.neuroscience.2003.10.039

Hogstad, S., Svenneby, G., Torgner, I. A., Kvamme, E., Hertz, L., and Schousboe, A. (1988). Glutaminase in neurons and astrocytes cultured from mouse brain: kinetic properties and effects of phosphate, glutamate and ammonia. *Neurochem. Res.* 13, 383–388. doi: 10.1007/BF00972489

Hrabetova, S., Serrano, P., Blace, N., Tse, H. W., Skifter, D. A., Jane, D. E., et al. (2000). Distinct NMDA receptor subpopulations contribute to long-term potentiation and long-term depression induction. *J. Neurosci.* 20:RC81. doi: 10.1523/JNEUROSCI.20-12-j0002.2000

Huntley, G. W., Rogers, S. W., Moran, T., Janssen, W., Archin, N., Vickers, J. C., et al. (1993). Selective distribution of kainate receptor subunit immunoreactivity in monkey neocortex revealed by a monoclonal antibody that recognizes glutamate receptor subunits GluR5/6/7. *J. Neurosci.* 13, 2965–2981. doi: 10.1523/JNEUROSCI.13-07-02965.1993

Huyghe, D., Nakamura, Y., Terunuma, M., Faideau, M., Haydon, P., Pangalos, M. N., et al. (2014). Glutamine synthetase stability and subcellular distribution in astrocytes are regulated by γ-aminobutyric type B receptors. *J. Biol. Chem.* 289, 28808–28815. doi: 10.1074/jbc.M114.583534

Intson, K., Geissah, S., McCullumsmith, R. E., and Ramsey, A. J. (2022). A role for endothelial NMDA receptors in the pathophysiology of schizophrenia. *Schizophr. Res.* 249, 63–73. doi: 10.1016/j.schres.2020.10.004

Islam, M. M., Nautiyal, M., Wynn, R. M., Mobley, J. A., Chuang, D. T., and Hutson, S. M. (2010). Branched-chain amino acid metabolon: interaction of glutamate dehydrogenase with the mitochondrial branched-chain aminotransferase (BCATm). *J. Biol. Chem.* 285, 265–276. doi: 10.1074/jbc.M109.048777

Jabaudon, D., Shimamoto, K., Yasuda-Kamatani, Y., Scanziani, M., Gahwiler, B. H., and Gerber, U. (1999). Inhibition of uptake unmasks rapid extracellular turnover of glutamate of nonvesicular origin. *Proc. Natl. Acad. Sci. U S A* 96, 8733–8738. doi: 10.1073/pnas.96.15.8733

Jeftinija, S. D., Jeftinija, K. V., and Stefanovic, G. (1997). Cultured astrocytes express proteins involved in vesicular glutamate release. *Brain Res.* 750, 41–47. doi: 10.1016/s0006-8993(96)00610-5

Jimenez-Blasco, D., Santofimia-Castano, P., Gonzalez, A., Almeida, A., and Bolanos, J. P. (2015). Astrocyte NMDA receptors' activity sustains neuronal survival through a Cdk5-Nrf2 pathway. *Cell Death Differ.* 22, 1877–1889. doi: 10.1038/cdd.2015.49

Juge, N., Yoshida, Y., Yatsushiro, S., Omote, H., and Moriyama, Y. (2006). Vesicular glutamate transporter contains two independent transport machineries. *J. Biol. Chem.* 281, 39499–39506. doi: 10.1074/jbc.M607670200

Kanai, Y., Bhide, P. G., DiFiglia, M., and Hediger, M. A. (1995). Neuronal high-affinity glutamate transport in the rat central nervous system. *Neuroreport* 6, 2357–2362. doi: 10.1097/00001756-199511270-00020

Kanai, Y., and Hediger, M. A. (1992). Primary structure and functional characterization of a high-affinity glutamate transporter. *Nature* 360, 467–471. doi: 10.1038/360467a0

Kang, J., Jiang, L., Goldman, S. A., and Nedergaard, M. (1998). Astrocyte-mediated potentiation of inhibitory synaptic transmission. *Nat. Neurosci.* 1, 683–692. doi: 10.1038/3684

Kaufman, A. M., Milnerwood, A. J., Sepers, M. D., Coquinco, A., She, K., Wang, L., et al. (2012). Opposing roles of synaptic and extrasynaptic NMDA receptor signaling in cocultured striatal and cortical neurons. *J. Neurosci.* 32, 3992–4003. doi: 10.1523/JNEUROSCI.4129-11.2012

Kazama, M., Kato, Y., Kakita, A., Noguchi, N., Urano, Y., Masui, K., et al. (2020). Astrocytes release glutamate via cystine/glutamate antiporter upregulated in response to increased oxidative stress related to sporadic amyotrophic lateral sclerosis. *Neuropathology* 40, 587–598. doi: 10.1111/neup.12716

Koh, W., Park, M., Chun, Y. E., Lee, J., Shim, H. S., Park, M. G., et al. (2021). Astrocytes render memory flexible. *bioRxiv* [Preprint]. doi: 10.1101/2021.03.25.436945

Koh, W., Park, M., Chun, Y. E., Lee, J., Shim, H. S., Park, M. G., et al. (2022). Astrocytes render memory flexible by releasing D-serine and regulating NMDA receptor tone in the hippocampus. *Biol. Psychiatry* 91, 740–752. doi: 10.1016/j.biopsych.2021.10.012

Koutsilieri, E., and Riederer, P. (2007). Excitotoxicity and new antiglutamatergic strategies in Parkinson's disease and Alzheimer's disease. *Parkinsonism Relat. Disord.* 13, S329–331. doi: 10.1016/S1353-8020(08)70025-7

Kugler, P., and Schleyer, V. (2004). Developmental expression of glutamate transporters and glutamate dehydrogenase in astrocytes of the postnatal rat hippocampus. *Hippocampus* 14, 975–985. doi: 10.1002/hipo.20015

Kukley, M., Barden, J. A., Steinhauser, C., and Jabs, R. (2001). Distribution of P2X receptors on astrocytes in juvenile rat hippocampus. *Glia* 36, 11–21. doi: 10.1002/glia.1091

Kvamme, E., Torgner, I. A., and Roberg, B. (2001). Kinetics and localization of brain phosphate activated glutaminase. *J. Neurosci. Res.* 66, 951–958. doi: 10.1002/jnr.10041

Kvist, T., Steffensen, T. B., Greenwood, J. R., Mehrzad Tabrizi, F., Hansen, K. B., Gajhede, M., et al. (2013). Crystal structure and pharmacological characterization of a novel N-methyl-D-aspartate (NMDA) receptor antagonist at the GluN1 glycine binding site. *J. Biol. Chem.* 288, 33124–33135. doi: 10.1074/jbc.M113.480210

Laake, J. H., Takumi, Y., Eidet, J., Torgner, I. A., Roberg, B., Kvamme, E., et al. (1999). Postembedding immunogold labelling reveals subcellular localization and pathway-specific enrichment of phosphate activated glutaminase in rat cerebellum. *Neuroscience* 88, 1137–1151. doi: 10.1016/s0306-4522(98)00298-x

Lakhan, S. E., Caro, M., and Hadzimichalis, N. (2013). NMDA receptor activity in neuropsychiatric disorders. *Front. Psychiatry* 4:52. doi: 10.3389/fpsyt.2013.00052

Lalo, U., Rasooli-Nejad, S., Bogdanov, A., More, L., Koh, W., Muller, J., et al. (2021). Synergy between vesicular and non-vesicular gliotransmission regulates synaptic plasticity and working memory. *bioRxiv* [Preprint]. doi: 10.1101/2021.03.25.437028

Lapato, A. S., and Tiwari-Woodruff, S. K. (2018). Connexins and pannexins: at the junction of neuro-glial homeostasis & disease. *J. Neurosci. Res.* 96, 31–44. doi: 10.1002/jnr.24088

Lauri, S. E., Vesikansa, A., Segerstrale, M., Collingridge, G. L., Isaac, J. T., and Taira, T. (2006). Functional maturation of CA1 synapses involves activity-dependent loss of tonic kainate receptor-mediated inhibition of glutamate release. *Neuron* 50, 415–429. doi: 10.1016/j.neuron.2006.03.020

Le Meur, K., Galante, M., Angulo, M. C., and Audinat, E. (2007). Tonic activation of NMDA receptors by ambient glutamate of non-synaptic origin in the rat hippocampus. *J. Physiol.* 580, 373–383. doi: 10.1113/jphysiol.2006.123570

Lee, M., Ko, D. G., Hong, D. K., Lim, M. S., Choi, B. Y., and Suh, S. W. (2020). Role of excitatory amino acid carrier 1 (EAAC1) in neuronal death and neurogenesis after ischemic stroke. *Int. J. Mol. Sci.* 21:5676. doi: 10.3390/ijms21165676

Lee, M. L., Martinez-Lozada, Z., Krizman, E. N., and Robinson, M. B. (2017). Brain endothelial cells induce astrocytic expression of the glutamate transporter GLT-1 by a Notch-dependent mechanism. *J. Neurochem.* 143, 489–506. doi: 10.1111/jnc.14135

Lee, M., McGeer, E. G., and McGeer, P. L. (2011). Mechanisms of GABA release from human astrocytes. *Glia* 59, 1600–1611. doi: 10.1002/glia.21202

Lehre, K. P., Levy, L. M., Ottersen, O. P., Storm-Mathisen, J., and Danbolt, N. C. (1995). Differential expression of two glial glutamate transporters in the rat brain: quantitative and immunocytochemical observations. *J. Neurosci.* 15, 1835–1853. doi: 10.1523/JNEUROSCI.15-03-01835.1995

Levy, L. M., Lehre, K. P., Rolstad, B., and Danbolt, N. C. (1993). A monoclonal antibody raised against an [Na^+K^+]-coupled L-glutamate transporter purified from rat brain confirms glial cell localization. *FEBS Lett.* 317, 79–84. doi: 10.1016/0014-5793(93)81495-l

Levy, L. M., Warr, O., and Attwell, D. (1998). Stoichiometry of the glial glutamate transporter GLT-1 expressed inducibly in a Chinese hamster ovary cell line selected for low endogenous Na^+-dependent glutamate uptake. *J. Neurosci.* 18, 9620–9628. doi: 10.1523/JNEUROSCI.18-23-09620.1998

Lewerenz, J., Maher, P., and Methner, A. (2012). Regulation of xCT expression and system x(c) (-) function in neuronal cells. *Amino Acids* 42, 171–179. doi: 10.1007/s00726-011-0862-x

Li, D., Herault, K., Silm, K., Evrard, A., Wojcik, S., Oheim, M., et al. (2013a). Lack of evidence for vesicular glutamate transporter expression in mouse astrocytes. *J. Neurosci.* 33, 4434–4455. doi: 10.1523/JNEUROSCI.3667-12.2013

Li, D., Agulhon, C., Schmidt, E., Oheim, M., and Ropert, N. (2013b). New tools for investigating astrocyte-to-neuron communication. *Front. Cell. Neurosci.* 7:193. doi: 10.3389/fncel.2013.00193

Liu, Q. S., Xu, Q., Arcuino, G., Kang, J., and Nedergaard, M. (2004). Astrocyte-mediated activation of neuronal kainate receptors. *Proc. Natl. Acad. Sci. U S A* 101, 3172–3177. doi: 10.1073/pnas.0306731101

Luque, J. M., and Richards, J. G. (1995). Expression of NMDA 2B receptor subunit mRNA in Bergmann glia. *Glia* 13, 228–232. doi: 10.1002/glia.440130309

Lyon, K. A., and Allen, N. J. (2022). From synapses to circuits, astrocytes regulate behavior. *Front. Neural Circuits* 15:786293. doi: 10.3389/fncir.2021.786293

Magi, S., Piccirillo, S., and Amoroso, S. (2019). The dual face of glutamate: from a neurotoxin to a potential survival factor-metabolic implications in health and disease. *Cell. Mol. Life Sci.* 76, 1473–1488. doi: 10.1007/s00018-018-3002-x

Marcaggi, P., Mutoh, H., Dimitrov, D., Beato, M., and Knopfel, T. (2009). Optical measurement of mGluR1 conformational changes reveals fast activation, slow deactivation and sensitization. *Proc. Natl. Acad. Sci. U S A* 106, 11388–11393. doi: 10.1073/pnas.0901290106

Martín, R., Bajo-Graneras, R., Moratalla, R., Perea, G., and Araque, A. (2015). Circuit-specific signaling in astrocyte-neuron networks in basal ganglia pathways. *Science* 349, 730–734. doi: 10.1126/science.aaa7945

Masliah, E., Alford, M., Mallory, M., Rockenstein, E., Moechars, D., and Van Leuven, F. (2000). Abnormal glutamate transport function in mutant amyloid precursor protein transgenic mice. *Exp. Neurol.* 163, 381–387. doi: 10.1006/exnr.2000.7386

Massie, A., Cnops, L., Smolders, I., McCullumsmith, R., Kooijman, R., Kwak, S., et al. (2008). High-affinity Na^+/K^+-dependent glutamate transporter EAAT4 is expressed throughout the rat fore- and midbrain. *J. Comp. Neurol.* 511, 155–172. doi: 10.1002/cne.21823

Masu, M., Tanabe, Y., Tsuchida, K., Shigemoto, R., and Nakanishi, S. (1991). Sequence and expression of a metabotropic glutamate receptor. *Nature* 349, 760–765. doi: 10.1038/349760a0

Masuoka, T., Ikeda, R., and Konishi, S. (2019). Persistent activation of histamine H1 receptors in the hippocampal CA1 region enhances NMDA receptor-mediated synaptic excitation and long-term potentiation in astrocyte- and D-serine-dependent manner. *Neuropharmacology* 151, 64–73. doi: 10.1016/j.neuropharm.2019.03.036

Matschke, V., Theiss, C., Hollmann, M., Schulze-Bahr, E., Lang, F., Seebohm, G., et al. (2015). NDRG2 phosphorylation provides negative feedback for SGK1-dependent regulation of a kainate receptor in astrocytes. *Front. Cell. Neurosci.* 9:387. doi: 10.3389/fncel.2015.00387

Matthias, K., Kirchhoff, F., Seifert, G., Huttmann, K., Matyash, M., Kettenmann, H., et al. (2003). Segregated expression of AMPA-type glutamate receptors and glutamate transporters defines distinct astrocyte populations in the mouse hippocampus. *J. Neurosci.* 23, 1750–1758. doi: 10.1523/JNEUROSCI.23-05-01750.2003

McKenna, M. C., Hopkins, I. B., Lindauer, S. L., and Bamford, P. (2006). Aspartate aminotransferase in synaptic and nonsynaptic mitochondria: differential effect of compounds that influence transient hetero-enzyme complex (metabolon) formation. *Neurochem. Int.* 48, 629–636. doi: 10.1016/j.neuint.2005.11.018

McKenna, M. C., Tildon, J. T., Stevenson, J. H., Huang, X., and Kingwell, K. G. (1995). Regulation of mitochondrial and cytosolic malic enzymes from cultured rat brain astrocytes. *Neurochem. Res.* 20, 1491–1501. doi: 10.1007/BF00970599

McNair, L. F., Andersen, J. V., Aldana, B. I., Hohnholt, M. C., Nissen, J. D., Sun, Y., et al. (2019). Deletion of neuronal GLT-1 in mice reveals its role in synaptic glutamate homeostasis and mitochondrial function. *J. Neurosci.* 39, 4847–4863. doi: 10.1523/JNEUROSCI.0894-18.2019

Mennerick, S., Dhond, R. P., Benz, A., Xu, W., Rothstein, J. D., Danbolt, N. C., et al. (1998). Neuronal expression of the glutamate transporter GLT-1 in hippocampal microcultures. *J. Neurosci.* 18, 4490–4499. doi: 10.1523/JNEUROSCI.18-12-04490.1998

Meyerson, J. R., Chittori, S., Merk, A., Rao, P., Han, T. H., Serpe, M., et al. (2016). Structural basis of kainate subtype glutamate receptor desensitization. *Nature* 537, 567–571. doi: 10.1038/nature19352

Milnerwood, A. J., Kaufman, A. M., Sepers, M. D., Gladding, C. M., Zhang, L., Wang, L., et al. (2012). Mitigation of augmented extrasynaptic NMDAR signaling and apoptosis in cortico-striatal co-cultures from Huntington's disease mice. *Neurobiol. Dis.* 48, 40–51. doi: 10.1016/j.nbd.2012.05.013

Min, R., and Nevian, T. (2012). Astrocyte signaling controls spike timing-dependent depression at neocortical synapses. *Nat. Neurosci.* 15, 746–753. doi: 10.1038/nn.3075

Mineff, E., and Valtschanoff, J. (1999). Metabotropic glutamate receptors 2 and 3 expressed by astrocytes in rat ventrobasal thalamus. *Neurosci. Lett.* 270, 95–98. doi: 10.1016/s0304-3940(99)00484-x

Montana, V., Ni, Y., Sunjara, V., Hua, X., and Parpura, V. (2004). Vesicular glutamate transporter-dependent glutamate release from astrocytes. *J. Neurosci.* 24, 2633–2642. doi: 10.1523/JNEUROSCI.3770-03.2004

Montero, T. D., and Orellana, J. A. (2015). Hemichannels: new pathways for gliotransmitter release. *Neuroscience* 286, 45–59. doi: 10.1016/j.neuroscience.2014.11.048

Mothet, J. P., Pollegioni, L., Ouanounou, G., Martineau, M., Fossier, P., and Baux, G. (2005). Glutamate receptor activation triggers a calcium-dependent and SNARE protein-dependent release of the gliotransmitter D-serine. *Proc. Natl. Acad. Sci. U S A* 102, 5606–5611. doi: 10.1073/pnas.0408483102

Müller, T., Möller, T., Berger, T., Schnitzer, J., and Kettenmann, H. (1992). Calcium entry through kainate receptors and resulting potassium-channel blockade in Bergmann glial cells. *Science* 256, 1563–1566. doi: 10.1126/science.1317969

Nakamura, E., Sato, M., Yang, H., Miyagawa, F., Harasaki, M., Tomita, K., et al. (1999). 4F2 (CD98) heavy chain is associated covalently with an amino acid transporter and controls intracellular trafficking and membrane topology of 4F2 heterodimer. *J. Biol. Chem.* 274, 3009–3016. doi: 10.1074/jbc.274.5.3009

Navarrete, M., Perea, G., Maglio, L., Pastor, J., Garcia de Sola, R., and Araque, A. (2013). Astrocyte calcium signal and gliotransmission in human brain tissue. *Cereb. Cortex* 23, 1240–1246. doi: 10.1093/cercor/bhs122

Neame, S., Safory, H., Radzishevsky, I., Touitou, A., Marchesani, F., Marchetti, M., et al. (2019). The NMDA receptor activation by d-serine and glycine is controlled by an astrocytic Phgdh-dependent serine shuttle. *Proc. Natl. Acad. Sci. U S A* 116, 20736–20742. doi: 10.1073/pnas.1909458116

Nedergaard, M. (1994). Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science* 263, 1768–1771. doi: 10.1126/science.8134839

Newman, E. A., and Zahs, K. R. (1998). Modulation of neuronal activity by glial cells in the retina. *J. Neurosci.* 18, 4022–4028. doi: 10.1523/JNEUROSCI.18-11-04022.1998

Norenberg, M. D., and Martinez-Hernandez, A. (1979). Fine structural localization of glutamine synthetase in astrocytes of rat brain. *Brain Res.* 161, 303–310. doi: 10.1016/0006-8993(79)90071-4

O'Donovan, S. M., Sullivan, C. R., and McCullumsmith, R. E. (2017). The role of glutamate transporters in the pathophysiology of neuropsychiatric disorders. *NPJ Schizophr.* 3:32. doi: 10.1038/s41537-017-0037-1

Ong, W. Y., Tanaka, K., Dawe, G. S., Ittner, L. M., and Farooqui, A. A. (2013). Slow excitotoxicity in Alzheimer's disease. *J. Alzheimers Dis.* 35, 643–668. doi: 10.3233/JAD-121990

Ormel, L., Stensrud, M. J., Bergersen, L. H., and Gundersen, V. (2012). VGLUT1 is localized in astrocytic processes in several brain regions. *Glia* 60, 229–238. doi: 10.1002/glia.21258

Osterberg, K. A., and Wattenberg, L. W. (1962). Oxidative histochemistry of reactive astrocytes. *Arch. Neurol.* 7, 211–218. doi: 10.1001/archneur.1962.04210030049007

Ottestad-Hansen, S., Hu, Q. X., Follin-Arbelet, V. V., Bentea, E., Sato, H., Massie, A., et al. (2018). The cystine-glutamate exchanger (xCT, Slc7a11) is expressed in significant concentrations in a subpopulation of astrocytes in the mouse brain. *Glia* 66, 951–970. doi: 10.1002/glia.23294

Pajarillo, E., Rizor, A., Lee, J., Aschner, M., and Lee, E. (2019). The role of astrocytic glutamate transporters GLT-1 and GLAST in neurological disorders: potential targets for neurotherapeutics. *Neuropharmacology* 161:107559. doi: 10.1016/j.neuropharm.2019.03.002

Palygin, O., Lalo, U., and Pankratov, Y. (2011). Distinct pharmacological and functional properties of NMDA receptors in mouse cortical astrocytes. *Br. J. Pharmacol.* 163, 1755–1766. doi: 10.1111/j.1476-5381.2011.01374.x

Palygin, O., Lalo, U., Verkhratsky, A., and Pankratov, Y. (2010). Ionotropic NMDA and P2X1/5 receptors mediate synaptically induced Ca^{2+} signalling in cortical astrocytes. *Cell Calcium* 48, 225–231. doi: 10.1016/j.ceca.2010.09.004

Perea, G., and Araque, A. (2007). Astrocytes potentiate transmitter release at single hippocampal synapses. *Science* 317, 1083–1086. doi: 10.1126/science.1144640

Perea, G., Gomez, R., Mederos, S., Covelo, A., Ballesteros, J. J., Schlosser, L., et al. (2016). Activity-dependent switch of GABAergic inhibition into glutamatergic excitation in astrocyte-neuron networks. *eLife* 5:e20362. doi: 10.7554/eLife.20362

Perea, G., Yang, A., Boyden, E. S., and Sur, M. (2014). Optogenetic astrocyte activation modulates response selectivity of visual cortex neurons *in vivo*. *Nat. Commun.* 5:3262. doi: 10.1038/ncomms4262

Perego, C., Vanoni, C., Bossi, M., Massari, S., Basudev, H., Longhi, R., et al. (2000). The GLT-1 and GLAST glutamate transporters are expressed on morphologically distinct astrocytes and regulated by neuronal activity in primary hippocampal cocultures. *J. Neurochem.* 75, 1076–1084. doi: 10.1046/j.1471-4159.2000.0751076.x

Peterson, A. R., and Binder, D. K. (2019). Regulation of synaptosomal GLT-1 and GLAST during epileptogenesis. *Neuroscience* 411, 185–201. doi: 10.1016/j.neuroscience.2019.05.048

Petr, G. T., Sun, Y., Frederick, N. M., Zhou, Y., Dhamne, S. C., Hameed, M. Q., et al. (2015). Conditional deletion of the glutamate transporter GLT-1 reveals that astrocytic GLT-1 protects against fatal epilepsy while neuronal GLT-1 contributes significantly to glutamate uptake into synaptosomes. *J. Neurosci.* 35, 5187–5201. doi: 10.1523/JNEUROSCI.4255-14.2015

Pines, G., Danbolt, N. C., Bjoras, M., Zhang, Y., Bendahan, A., Eide, L., et al. (1992). Cloning and expression of a rat brain L-glutamate transporter. *Nature* 360, 464–467. doi: 10.1038/360464a0

Pirttimaki, T. M., Hall, S. D., and Parri, H. R. (2011). Sustained neuronal activity generated by glial plasticity. *J. Neurosci.* 31, 7637–7647. doi: 10.1523/JNEUROSCI.5783-10.2011

Plaitakis, A., Kalef-Ezra, E., Kotzamani, D., Zaganas, I., and Spanaki, C. (2017). The glutamate dehydrogenase pathway and its roles in cell and tissue biology in health and disease. *Biology (Basel)* 6:11. doi: 10.3390/biology6010011

Radulescu, A. R., Todd, G. C., Williams, C. L., Bennink, B. A., Lemus, A. A., Chesbro, H. E., et al. (2022). Estimating the glutamate transporter surface density in distinct sub-cellular compartments of mouse hippocampal astrocytes. *PLoS Comput. Biol.* 18:e1009845. doi: 10.1371/journal.pcbi.1009845

Ravikrishnan, A., Gandhi, P. J., Shelkar, G. P., Liu, J., Pavuluri, R., and Dravid, S. M. (2018). Region-specific expression of NMDA receptor GluN2C subunit in parvalbumin-positive neurons and astrocytes: analysis of GluN2C expression using a novel reporter model. *Neuroscience* 380, 49–62. doi: 10.1016/j.neuroscience.2018.03.011

Re, D. B., Nafia, I., Melon, C., Shimamoto, K., Kerkerian-Le Goff, L., and Had-Aissouni, L. (2006). Glutamate leakage from a compartmentalized intracellular metabolic pool and activation of the lipoxygenase pathway mediate oxidative astrocyte death by reversed glutamate transport. *Glia* 54, 47–57. doi: 10.1002/glia.20353

Reimers, J. M., Milovanovic, M., and Wolf, M. E. (2011). Quantitative analysis of AMPA receptor subunit composition in addiction-related brain regions. *Brain Res.* 1367, 223–233. doi: 10.1016/j.brainres.2010.10.016

Reiner, A., and Levitz, J. (2018). Glutamatergic signaling in the central nervous system: ionotropic and metabotropic receptors in concert. *Neuron* 98, 1080–1098. doi: 10.1016/j.neuron.2018.05.018

Rodriguez-Prados, M., Rojo-Ruiz, J., Garcia-Sancho, J., and Alonso, M. T. (2020). Direct monitoring of ER Ca^{2+} dynamics reveals that Ca^{2+} entry induces ER-Ca^{2+} release in astrocytes. *Pflugers Arch.* 472, 439–448. doi: 10.1007/s00424-020-02364-7

Rothstein, J. D., Dykes-Hoberg, M., Pardo, C. A., Bristol, L. A., Jin, L., Kuncl, R. W., et al. (1996). Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron* 16, 675–686. doi: 10.1016/s0896-6273(00)80086-0

Rudolph, R., Jahn, H. M., Courjaret, R., Messemer, N., Kirchhoff, F., and Deitmer, J. W. (2016). The inhibitory input to mouse cerebellar Purkinje cells is reciprocally modulated by Bergmann glial P2Y1 and AMPA receptor signaling. *Glia* 64, 1265–1280. doi: 10.1002/glia.22999

Ruscak, M., Orlicky, J., Zubor, V., and Hager, H. (1982). Alanine aminotransferase in bovine brain: purification and properties. *J. Neurochem.* 39, 210–216. doi: 10.1111/j.1471-4159.1982.tb04720.x

Sahlender, D. A., Savtchouk, I., and Volterra, A. (2014). What do we know about gliotransmitter release from astrocytes? *Philos. Trans. R Soc. Lond B Biol. Sci.* 369:20130592. doi: 10.1098/rstb.2013.0592

Salas, I. H., Burgado, J., and Allen, N. J. (2020). Glia: victims or villains of the aging brain? *Neurobiol. Dis.* 143:105008. doi: 10.1016/j.nbd.2020.105008

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

Sato, H., Tamba, M., Ishii, T., and Bannai, S. (1999). Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. *J. Biol. Chem.* 274, 11455–11458. doi: 10.1074/jbc.274.17.11455

Sato, H., Tamba, M., Okuno, S., Sato, K., Keino-Masu, K., Masu, M., et al. (2002). Distribution of cystine/glutamate exchange transporter, system x(c)-, in the mouse brain. *J. Neurosci.* 22, 8028–8033. doi: 10.1523/JNEUROSCI.22-18-08028.2002

Savtchouk, I., Di Castro, M. A., Ali, R., Stubbe, H., Lujan, R., and Volterra, A. (2019). Circuit-specific control of the medial entorhinal inputs to the dentate gyrus by atypical presynaptic NMDARs activated by astrocytes. *Proc. Natl. Acad. Sci. U S A* 116, 13602–13610. doi: 10.1073/pnas.1816013116

Savtchouk, I., and Volterra, A. (2018). Gliotransmission: beyond black-and-white. *J. Neurosci.* 38, 14–25. doi: 10.1523/JNEUROSCI.0017-17.2017

Schools, G. P., and Kimelberg, H. K. (1999). mGluR3 and mGluR5 are the predominant metabotropic glutamate receptor mRNAs expressed in hippocampal astrocytes acutely isolated from young rats. *J. Neurosci. Res.* 58, 533–543. doi: 10.1002/(sici)1097-4547(19991115)58:4<533::aid-jnr6>3.0.co;2-g

Schorge, S., Elenes, S., and Colquhoun, D. (2005). Maximum likelihood fitting of single channel NMDA activity with a mechanism composed of independent dimers of subunits. *J. Physiol.* 569, 395–418. doi: 10.1113/jphysiol.2005.095349

Schousboe, A. (2017). A tribute to Mary C. McKenna: glutamate as energy substrate and neurotransmitter-functional interaction between neurons and astrocytes. *Neurochem. Res.* 42, 4–9. doi: 10.1007/s11064-015-1813-9

Schousboe, A., Bak, L. K., and Waagepetersen, H. S. (2013). Astrocytic control of biosynthesis and turnover of the neurotransmitters glutamate and GABA. *Front. Endocrinol. (Lausanne)* 4:102. doi: 10.3389/fendo.2013.00102

Schousboe, A., Scafidi, S., Bak, L. K., Waagepetersen, H. S., and McKenna, M. C. (2014). Glutamate metabolism in the brain focusing on astrocytes. *Adv. Neurobiol.* 11, 13–30. doi: 10.1007/978-3-319-08894-5_2

Schousboe, A., Waagepetersen, H. S., and Sonnewald, U. (2019). Astrocytic pyruvate carboxylation: status after 35 years. *J. Neurosci. Res.* 97, 890–896. doi: 10.1002/jnr.24402

Schreiner, A. E., Durry, S., Aida, T., Stock, M. C., Ruther, U., Tanaka, K., et al. (2014). Laminar and subcellular heterogeneity of GLAST and GLT-1 immunoreactivity in the developing postnatal mouse hippocampus. *J. Comp. Neurol.* 522, 204–224. doi: 10.1002/cne.23450

Schröder, W., Seifert, G., Huttmann, K., Hinterkeuser, S., and Steinhauser, C. (2002). AMPA receptor-mediated modulation of inward rectifier K^+ channels in astrocytes of mouse hippocampus. *Mol. Cell. Neurosci.* 19, 447–458. doi: 10.1006/mcne.2001.1080

Schubert, D., and Piasecki, D. (2001). Oxidative glutamate toxicity can be a component of the excitotoxicity cascade. *J. Neurosci.* 21, 7455–7462. doi: 10.1523/JNEUROSCI.21-19-07455.2001

Seifert, G., Rehn, L., Weber, M., and Steinhäuser, C. (1997). AMPA receptor subunits expressed by single astrocytes in the juvenile mouse hippocampus. *Brain Res. Mol. Brain Res.* 47, 286–294. doi: 10.1016/s0169-328x(97)00059-4

Seifert, G., and Steinhauser, C. (1995). Glial cells in the mouse hippocampus express AMPA receptors with an intermediate Ca^{2+} permeability. *Eur. J. Neurosci.* 7, 1872–1881. doi: 10.1111/j.1460-9568.1995.tb00708.x

Shah, P., Plitman, E., Iwata, Y., Kim, J., Nakajima, S., Chan, N., et al. (2020). Glutamatergic neurometabolites and cortical thickness in treatment-resistant schizophrenia: implications for glutamate-mediated excitotoxicity. *J. Psychiatr. Res.* 124, 151–158. doi: 10.1016/j.jpsychires.2020.02.032

Shashidharan, P., Huntley, G. W., Murray, J. M., Buku, A., Moran, T., Walsh, M. J., et al. (1997). Immunohistochemical localization of the neuron-specific glutamate transporter EAAC1 (EAAT3) in rat brain and spinal cord revealed by a novel monoclonal antibody. *Brain Res.* 773, 139–148. doi: 10.1016/s0006-8993(97)00921-9

Shen, W., Nikolic, L., Meunier, C., Pfrieger, F., and Audinat, E. (2017). An autocrine purinergic signaling controls astrocyte-induced neuronal excitation. *Sci. Rep.* 7:11280. doi: 10.1038/s41598-017-11793-x

Sherwood, M. W., Arizono, M., Hisatsune, C., Bannai, H., Ebisui, E., Sherwood, J. L., et al. (2017). Astrocytic IP_3 Rs: Contribution to Ca^{2+} signalling and hippocampal LTP. *Glia* 65, 502–513. doi: 10.1002/glia.23107

Sherwood, M. W., Oliet, S. H. R., and Panatier, A. (2021a). NMDARs, coincidence detectors of astrocytic and neuronal activities. *Int. J. Mol. Sci.* 22:7258. doi: 10.3390/ijms22147258

Sherwood, M. W., Arizono, M., Panatier, A., Mikoshiba, K., and Oliet, S.H.R. (2021b). Astrocytic IP_3Rs: beyond IP_3R2. *Front. Cell. Neurosci.* 15:695817. doi: 10.3389/fncel.2021.695817

Shibasaki, K., Hosoi, N., Kaneko, R., Tominaga, M., and Yamada, K. (2017). Glycine release from astrocytes via functional reversal of GlyT1. *J. Neurochem.* 140, 395–403. doi: 10.1111/jnc.13741

Shigetomi, E., Bowser, D. N., Sofroniew, M. V., and Khakh, B. S. (2008). Two forms of astrocyte calcium excitability have distinct effects on NMDA receptor-mediated slow inward currents in pyramidal neurons. *J. Neurosci.* 28, 6659–6663. doi: 10.1523/JNEUROSCI.1717-08.2008

Shin, C.-S., Mishra, P., Watrous, J. D., Carelli, V., D'Aurelio, M., Jain, M., et al. (2017). The glutamate/cystine xCT antiporter antagonizes glutamine metabolism and reduces nutrient flexibility. *Nat. Commun.* 8:15074. doi: 10.1038/ncomms15074

Shiratori-Hayashi, M., Yamaguchi, C., Eguchi, K., Shiraishi, Y., Kohno, K., Mikoshiba, K., et al. (2021). Astrocytic STAT3 activation and chronic itch require IP_3R1/TRPC-dependent Ca^{2+} signals in mice. *J. Allergy Clin. Immunol.* 147, 1341–1353. doi: 10.1016/j.jaci.2020.06.039

Simard, M., Arcuino, G., Takano, T., Liu, Q. S., and Nedergaard, M. (2003). Signaling at the gliovascular interface. *J. Neurosci.* 23, 9254–9262. doi: 10.1523/JNEUROSCI.23-27-09254.2003

Sladeczek, F., Pin, J. P., Recasens, M., Bockaert, J., and Weiss, S. (1985). Glutamate stimulates inositol phosphate formation in striatal neurones. *Nature* 317, 717–719. doi: 10.1038/317717a0

Spampinato, S. F., Copani, A., Nicoletti, F., Sortino, M. A., and Caraci, F. (2018). Metabotropic glutamate receptors in glial cells: a new potential target for neuroprotection? *Front. Mol. Neurosci.* 11:414. doi: 10.3389/fnmol.2018.00414

Storck, T., Schulte, S., Hofmann, K., and Stoffel, W. (1992). Structure, expression and functional analysis of a Na^+-dependent glutamate/aspartate transporter from rat brain. *Proc. Natl. Acad. Sci. U S A* 89, 10955–10959. doi: 10.1073/pnas.89.22.10955

Sugiyama, H., Ito, I., and Hirono, C. (1987). A new type of glutamate receptor linked to inositol phospholipid metabolism. *Nature* 325, 531–533. doi: 10.1038/325531a0

Sun, W., McConnell, E., Pare, J. F., Xu, Q., Chen, M., Peng, W., et al. (2013). Glutamate-dependent neuroglial calcium signaling differs between young and adult brain. *Science* 339, 197–200. doi: 10.1126/science.1226740

Swanson, R. A., Liu, J., Miller, J. W., Rothstein, J. D., Farrell, K., Stein, B. A., et al. (1997). Neuronal regulation of glutamate transporter subtype expression in astrocytes. *J. Neurosci.* 17, 932–940. doi: 10.1523/JNEUROSCI.17-03-00932.1997

Sweeney, P., Qi, Y., Xu, Z., and Yang, Y. (2016). Activation of hypothalamic astrocytes suppresses feeding without altering emotional states. *Glia* 64, 2263–2273. doi: 10.1002/glia.23073

Takata-Tsuji, F., Chounlamountri, N., Do, L. D., Philippot, C., Novion Ducassou, J., Coute, Y., et al. (2021). Microglia modulate gliotransmission through the regulation of VAMP2 proteins in astrocytes. *Glia* 69, 61–72. doi: 10.1002/glia.23884

Tanaka, K., Watase, K., Manabe, T., Yamada, K., Watanabe, M., Takahashi, K., et al. (1997). Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science* 276, 1699–1702. doi: 10.1126/science.276.5319.1699

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., et al. (2010). Glutamate receptor ion channels: structure, regulation and function. *Pharmacol. Rev.* 62, 405–496. doi: 10.1124/pr.109.002451

Ulbrich, M. H., and Isacoff, E. Y. (2008). Rules of engagement for NMDA receptor subunits. *Proc. Natl. Acad. Sci. U S A* 105, 14163–14168. doi: 10.1073/pnas.0802075105

Varela, L., Stutz, B., Song, J. E., Kim, J. G., Liu, Z. W., Gao, X. B., et al. (2021). Hunger-promoting AgRP neurons trigger an astrocyte-mediated feed-forward autoactivation loop in mice. *J. Clin. Invest.* 131:e144239. doi: 10.1172/JCI144239

Vargas, J. R., Takahashi, D. K., Thomson, K. E., and Wilcox, K. S. (2013). The expression of kainate receptor subunits in hippocampal astrocytes after experimentally induced status epilepticus. *J. Neuropathol. Exp. Neurol.* 72, 919–932. doi: 10.1097/NEN.0b013e3182a4b266

Ventura, R., and Harris, K. M. (1999). Three-dimensional relationships between hippocampal synapses and astrocytes. *J. Neurosci.* 19, 6897–6906. doi: 10.1523/JNEUROSCI.19-16-06897.1999

Verkhratsky, A., and Kirchhoff, F. (2007). NMDA receptors in glia. *Neuroscientist* 13, 28–37. doi: 10.1177/1073858406294270

Vermeiren, C., Najimi, M., Vanhoutte, N., Tilleux, S., de Hemptinne, I., Maloteaux, J. M., et al. (2005). Acute up-regulation of glutamate uptake mediated by mGluR5a in reactive astrocytes. *J. Neurochem.* 94, 405–416. doi: 10.1111/j.1471-4159.2005.03216.x

Waagepetersen, H. S., Sonnewald, U., Larsson, O. M., and Schousboe, A. (2000). A possible role of alanine for ammonia transfer between astrocytes and glutamatergic neurons. *J. Neurochem.* 75, 471–479. doi: 10.1046/j.1471-4159.2000.0750471.x

Walker, V. (2014). Ammonia metabolism and hyperammonemic disorders. *Adv. Clin. Chem.* 67, 73–150. doi: 10.1016/bs.acc.2014.09.002

Wang, Q., Kong, Y., Wu, D. Y., Liu, J. H., Jie, W., You, Q. L., et al. (2021). Impaired calcium signaling in astrocytes modulates autism spectrum disorder-like behaviors in mice. *Nat. Commun.* 12:3321. doi: 10.1038/s41467-021-23843-0

Westergaard, N., Varming, T., Peng, L., Sonnewald, U., Hertz, L., and Schousboe, A. (1993). Uptake, release and metabolism of alanine in neurons and astrocytes in primary cultures. *J. Neurosci. Res.* 35, 540–545. doi: 10.1002/jnr.490350510

Wiktorowska, L., Bilecki, W., Tertil, M., Kudla, L., Szumiec, L., Mackowiak, M., et al. (2021). Knockdown of the astrocytic glucocorticoid receptor in the central nucleus of the amygdala diminishes conditioned fear expression and anxiety. *Behav. Brain Res.* 402:113095. doi: 10.1016/j.bbr.2020.113095

Wojcik, S. M., Rhee, J. S., Herzog, E., Sigler, A., Jahn, R., Takamori, S., et al. (2004). An essential role for vesicular glutamate transporter 1 (VGLUT1) in postnatal development and control of quantal size. *Proc. Natl. Acad. Sci. U S A* 101, 7158–7163. doi: 10.1073/pnas.0401764101

Wolosker, H., de Souza, D. O., and de Meis, L. (1996). Regulation of glutamate transport into synaptic vesicles by chloride and proton gradient. *J. Biol. Chem.* 271, 11726–11731. doi: 10.1074/jbc.271.20.11726

Woo, D. H., Han, K. S., Shim, J. W., Yoon, B. E., Kim, E., Bae, J. Y., et al. (2012). TREK-1 and Best1 channels mediate fast and slow glutamate release in astrocytes upon GPCR activation. *Cell* 151, 25–40. doi: 10.1016/j.cell.2012.09.005

Yamada, D., Kawabe, K., Tosa, I., Tsukamoto, S., Nakazato, R., Kou, M., et al. (2019). Inhibition of the glutamine transporter SNAT1 confers neuroprotection in mice by modulating the mTOR-autophagy system. *Commun. Biol.* 2:346. doi: 10.1038/s42003-019-0582-4

Yu, A. C., Hertz, E., and Hertz, L. (1984). Alterations in uptake and release rates for GABA, glutamate and glutamine during biochemical maturation of highly purified cultures of cerebral cortical neurons, a GABAergic preparation. *J. Neurochem.* 42, 951–960. doi: 10.1111/j.1471-4159.1984.tb12696.x

Zhang, Q., Fukuda, M., Van Bockstaele, E., Pascual, O., and Haydon, P. G. (2004). Synaptotagmin IV regulates glial glutamate release. *Proc. Natl. Acad. Sci. U S A* 101, 9441–9446. doi: 10.1073/pnas.0401960101

Zhou, X., Hollern, D., Liao, J., Andrechek, E., and Wang, H. (2013). NMDA receptor-mediated excitotoxicity depends on the coactivation of synaptic and extrasynaptic receptors. *Cell Death Dis.* 4:e560. doi: 10.1038/cddis.2013.82

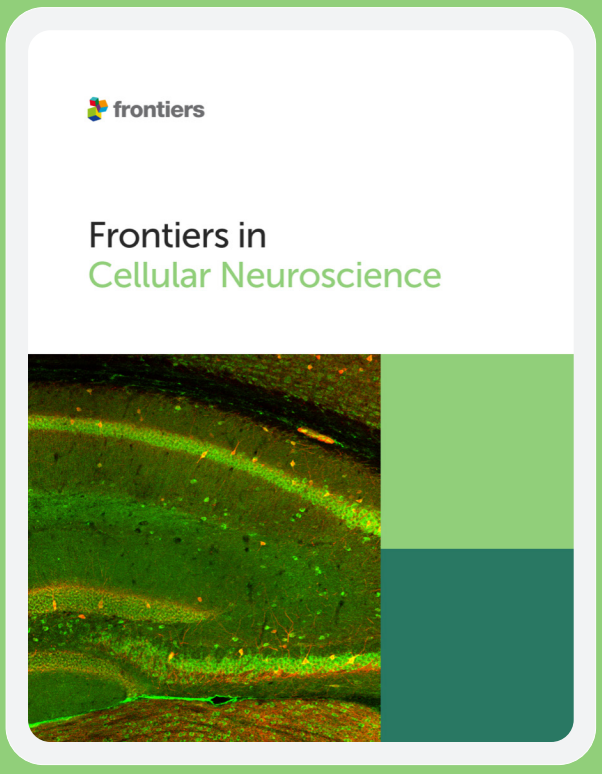
Frontiers in
Cellular Neuroscience

Leading research in cellular mechanisms underlying brain function and development

Part of the world's most cited neuroscience journal series that advances our understanding of the cellular mechanisms underlying cell function in the nervous system across all species.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact