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Challenges and opportunities of advanced gliomodulation technologies for excitation-inhibition balance of brain networks

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Recent neuroscience studies have highlighted the critical role of glial cells in information processing. This has increased the demand for technologies that selectively modulate glial cells that regulate the excitation-inhibition balance of neural network function. Engineered technologies that modulate glial activity may be necessary for precise tuning of neural network activity in higher-order brain function. This perspective summarizes how glial cells regulate excitation and inhibition of neural circuits, highlights available technologies for glial modulation, and discusses current challenges and potential opportunities for glial engineering technologies.

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Introduction

While traditionally neurons have been considered solely responsible for maintaining balance between excitation and inhibition in the nervous system [1,2], recent evidence highlights the crucial role of glia in regulating neural network activity and neuronal health within the brain [3]. Originally, glia were only considered to be the 'glue' that hold neurons in place in the brain [4]. However, glia are functionally integrated into the network to precisely monitor and modulate neuronal excitability in brain circuits [5]. Emerging discoveries indicate that glial cells offer a dynamic role across an array of information processing for high-order functions [6•,7–9]. Therefore, there is an increasing demand of cutting-edge tools specifically target glia-neuron, gliovascular-neuron, and even glial subtype interactions to drive novel excitatory and inhibitory neural circuit activity in neuroscience research and clinical applications [10••,11]. This perspective briefly summarizes the state-of-the-art knowledge about glia-neuron interactions, how these interactions can be modulated by advanced glial technologies, and current engineering challenges and future development opportunities in this new multidisciplinary field of *gliomodulation* technologies.

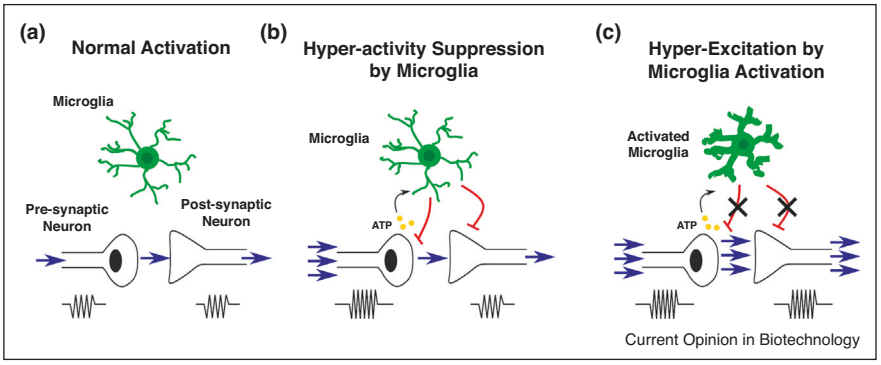
Glial modulation of brain network

Microglia can sense and regulate neuronal hyperexcitability

a) Microglia normally surveil surrounding environment and prune synaptic structures, which in turn change connectivity patterns among neurons during normal activation. b) When networks receive hyper-excitable input, microglia can sense extracellular ATP levels through microglial purinergic receptor, P2Y12R, which in turns triggers adenosine production through CD39 and CD73 on microglial surface. The microglial-dependent adenosine binds to neuronal Gi/o-protein coupled adenosine A1 receptors (A1Rs), which suppresses hyper-excitatory neuronal responses through A1R-mediated protein kinase A (PKA) inhibition [6•,12,13••,14••]. Alternatively, they can extend their processes to physically block presynaptic GABAergic neurons causing stabilization of network activity [15,16]. c) Pro-inflammatory microglia activation and retraction of microglial processes can disrupt their suppression of neuronal over excitability, resulting in functional hyperexcitability [17].

Microglia are understood to regulate synaptic maturation through elimination of synapses; however, they have also received attention due to their critical influence on inhibitory signal transmission (Figure 1) [13••]. Ablation of microglia disrupts inhibitory synaptic transmission critical for neural development [18,19]. Additionally, because microglia are highly motile cells, they can extend processes and regulate synaptic transmission by physically blocking or displacing synapses. For example, microglial P2Y12 receptors detect extracellular ATP/adenosine, indicative of excessive excitation, and displace

Figure 1



Microglia modulation of network communication.

presynaptic GABAergic neurons terminating on glutamatergic soma to stabilize network activity [15,16] This evidence [15,20] supports the idea that microglia can fine tune neuronal activity through regulation of GABAergic transmission.

Recent evidence has also shown that microglia detect and secrete neurotransmitters to maintain network communication balance and prevent hyperexcitability, such as during seizures [12,13••,14••]. In healthy subjects, microglia monitor the extracellular ATP released by synapses. When microglia detect elevated excitatory input, they extend processes toward active neuronal compartments via ATP-dependent purinergic signaling and release adenosine, reducing synaptic activity of nearby neurons [13••,14••]. Furthermore, release of inflammatory TNF-α by microglia increases type 1 metabotropic glutamate receptor (mGluR) signaling, thereby potentiating neuronal intrinsic excitability [17,21], demonstrating that microglia can also modulate the excitation of nearby neuronal activity.

Alterations in microglia activity can have consequential effects on behavior. Activation of pro-inflammatory microglia can lead to the retraction or redirection of processes away from neurons, such as towards injured or inflamed vascular structures [22]. In turn, changes in microglial morphology could result in a loss of inhibition of neurons by microglial processes and increase the hyperexcitability of local neurons [22]. As expected, pro-inflammatory activation of microglia has been shown to increase the excitability of nearby neurons, including increasing the frequency of action potentials as well as the amplitude and frequency of excitatory postsynaptic currents (EPSPs) [17]. Behaviorally, microglia activation can result in depression-like phenotypes or increase innate fear responses [12,17] and suppressing microglia activity by inhibiting colony-stimulating factor 1 receptor can rescue neuronal hyperexcitation and behavioral abnormalities [17,21]. Together, these findings suggest that modulation of microglia activity can regulate neural network function and behavior (Figure 2).

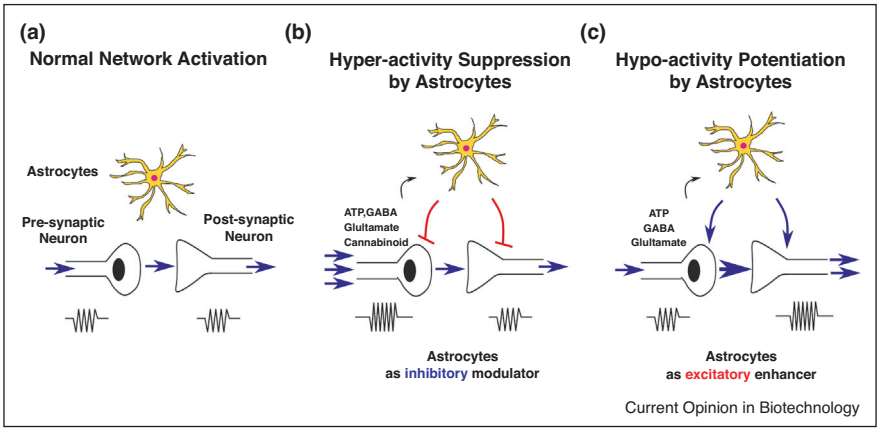
Astrocytes play a key role in maintaining network excitation-inhibition balance

a) An astrocyte, and a pre-synaptic and post-synaptic neuron form the tripartite synapse allowing glial modulation of neuronal synaptic activity. b–c) Astrocytes respond to many of the same neurotransmitters in diverse ways, promoting inhibition (b) or excitation (c). (b) In particular, astrocytes can release gliotransmitters, which can depress presynaptic activity (ATP), or inhibit post-synaptic activity (GABA). c) Astrocytes can also promote excitation by releasing glutamate to increase presynaptic excitatory transmission or promote synaptic activity by acting on the post-synaptic neuron. Importantly, these actions are specific to brain regions, circuits, and the pattern of neural activity.

Recently astrocytes have been recognized as critical mediators of excitation-inhibition balance in higher-order brain functions such as learning and memory [23••,24–26], attention [27], compulsive behaviors [28••], sensory acuity [29••], and plasticity [30••,31•,32–34]. In fact, the idea that astrocytes are significantly involved in the integration of neural activity and excitatory/inhibitory modulation and the influence on behavior has been extensively discussed in recent excellent reviews [35–37]. Here, we emphasize a few recent examples that highlight the diversity of astrocyte involvement in the regulation of neural activity and behavior.

Astrocytes sense GABAergic transmission through the expression of GABA receptors ($GABA_A$, $GABA_B$), regulate synaptic GABA uptake through the expression of GABA transporters (GAT-1, GAT-3) [38], and modulate inhibitory activity via secretion of gliotransmitters [32]. Inhibiting astrocyte GAT-1 and GAT-3 results in an

Figure 2



Astrocytes play a dual role in network computations.

increase in GABAergic transmission, demonstrating that astrocyte regulation of synaptic GABA can decrease GABAergic transmission [39]. Additionally, inhibitory activity was demonstrated to elicit $GABA_B$-mediated activation of astrocyte calcium activity and release of glutamate onto presynaptic inhibitory neurons to amplify GABAergic inhibition of pyramidal neurons [40]. Thus, astrocyte activity can modulate inhibitory transmission in diverse ways ultimately influencing behavior.

Astrocytes also modulate excitatory transmission through similar mechanisms such as glutamate transporters and exocytosis of other neuroactive substances. Importantly, release of glutamate from astrocytes can act on either pre-synaptic or post-synaptic neurons to increase glutamatergic signaling and excitability [34,41–43]. On the other hand, GAT-3 activity increases astrocytic calcium through the Na/Ca2+ exchanger inducing a release of ATP/adenosine, which acts on presynaptic neurons to reduce glutamate release and downregulate excitatory transmission [44]. Therefore, astrocyte activity can both increase and decrease excitatory transmission through different mechanisms.

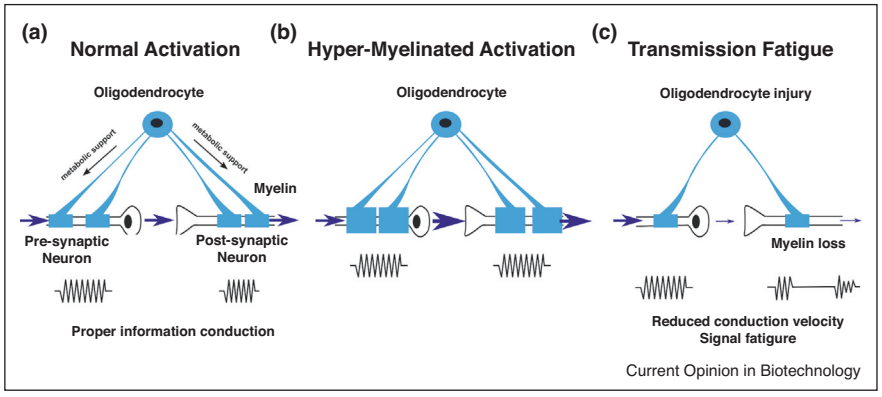
Astrocytic modulation of inhibitory and excitatory activity has diverse influences on behavior. For example, obsessive compulsive behaviors increased in response to increased activity of astrocytic GAT-3. Specifically, decreasing calcium activity increased the expression of GAT-3 and caused excessive grooming, demonstrating a critical role for astrocyte-mediated GABA uptake in regulating behavior [28••]. Alternatively, sensory discrimination was impaired when astrocytic production of GABA or release through Best-1 channels was blocked, suggesting that astrocyte modulation of GABAergic activity is critical for sensory processing [29••]. Additionally, astrocytes can regulate learning and memory through their modulation of excitatory transmission. Specifically, in fear-conditioning paradigms, astrocytes release ATP/adenosine in response to endocannabinoids, which depress excitatory synapses via presynaptic adenosine (A1) receptors and reduce fear expression [25]. Moreover, disruption of astrocyte GABAb receptor expression in the prefrontal cortex was demonstrated to impair goal-directed behavior through deficits in working memory [45••]. Modulating astrocyte activity with advanced biotechnologies has been proven to uncover important roles for astrocytes in modulating behavior [10••,35,37], therefore similar discoveries could be made regarding oligodendrocyte lineage cells that have not been fully investigated.

Myelinating oligodendrocytes act as a positive modulator of networks

a) Oligodendrocytes have large, direct contact with neuronal compartments by myelin sheath, enabling saltatory propagation of action potentials. Myelinating oligodendrocyte thus modulate spike conduction velocity and support network computations. Additionally, activity-dependent neuronal activation increases metabolite transportation from myelin processes to axons through myelinic channel and monocarboxylate transporters to maintain transmission efficacy [46,47]. b) Hypermyelination is likely to preserve signal conduction efficacy [48]. c) Oligodendrocyte injury and/or myelin loss impair signal conduction quality in functional network with reduced transmission velocity and fast signal fatigue [49••].

Oligodendrocytes are responsible for propagating information transmission by insulating axons with myelin in white matter and cortex (Figure 3) [50•]. Recently, some oligodendrocytes have been shown to preferentially myelinate inhibitory neurons [51•] and thus modulate information processing of inhibitory networks [52•]. Additionally, oligodendrocyte precursor cells, which are

Figure 3



Oligodendrocytes support relay of information in brain networks.

responsible for maintaining oligodendrocyte densities, can be situated in close proximity to neuronal soma forming specialized OPC-neuron interactions and can receive direct synaptic communication from inhibitory neurons. Importantly, potentiating $GABA_B$ receptors with baclofen, or blocking $GABA_A$ receptors with picrotoxin, increased the number of OPC-neuron contacts using stereological quantification [53–56]. These results demonstrate the myelinating glia can provide support on inhibitory network computation.

Oligodendrocytes maintain significant myelin coverage over excitatory axons and therefore are critical for modulation of excitatory networks [57]. The conduction velocity of action potentials propagating along an axon is increased in myelinated excitatory axons [57]. The diversity of myelination profiles such as length, thickness, spacing between myelin sheaths, contributes to the complexity of network excitation [57]. Also, oligodendrocytes transport metabolites or energy to nearby neurons via myelin processes and is essential to sustain elevated firing activity and detect subtle differences in sensory input, especially during periods of elevated excitation [49••]. Additionally, oligodendrocytes can regulate neuronal excitation by synthesizing glutamate [50•], the major excitatory input to network communication.

Damage to oligodendrocytes and myelin have been associated with dysfunction of processing sensory input that discriminate sensory stimuli [49••]. Demyelination due to oligodendrocyte loss or injury can result in impairments in signal transmission such as increased signal decay rates, impaired firing rates, and reduced sustainability of neuronal firing [49••,50•,58,59]. Depleting oligodendrocyte-specific metabolic support to neurons by mutating monocarboxylate transporter 1 receptor has demonstrated similar but moderate signal firing rate and sustainability impairment [44]. Overall, modulation of oligodendrocytes with available neural technologies may regulate excitatory and inhibitory networks that contribute to both gross and fine-tuning of behaviors.

Advanced glial engineering technologies

Although the concept of glial modulation and gliomodulation have existed for decades [36,45••,60–63], technological limitations have impeded advancements in scientific knowledge. In contrast to neural activity which can be easily detected or evoked with an electrode, glial activity has been much more difficult to detect and manipulate [11]. The development of tools that can provide deeper insight into the physiology of glial cells will help to accelerate us towards a comprehensive understanding of their influence over neural activity and ultimately aid targeted therapeutics. Recent advancements in technologies to image, manipulate, and electrically record-specific cellular activity have made tremendous strides in shifting the perspective of astrocytes as homeostatic regulators to important components of information processing [35–37,64]. Applying these concepts to microglia, oligodendrocytes, OPCs, and even pericytes or endothelial cells is likely to open important avenues for scientific advancement and therapeutic development.

Molecular scale modulation technologies

Although it resulted in substantial and long-lasting debate [65], the identification of astrocyte calcium signaling in response to neural activity led to exciting opportunities and interest in the investigation of their dynamic integration and modulation of neural activity [66–72]. While the topic has been extensively reviewed [37,65,70], improvements with *in vivo* calcium imaging, such as with two-photon microscopy [70,73], has provided insight into the diverse calcium signals throughout the specialized functional domains of astrocytes. In particular, these tools enabled the identification of astrocyte calcium signaling as an important effector in many physiological processes

involving communication with neurons and influencing behavior [35,38,69,71,74•,75]. Additionally, genetic and molecular tools that can induce targeted increases in astrocyte calcium through light activated ion channels (e.g. channelrhodopsin [75]), or g-protein coupled receptors (e.g. melanopsin [45••,64]), referred to as optogenetics [76], has revealed neuronal subtype-specific communication [75,77,78], as well as an influential role in goal-directed behaviors [23••]. Furthermore, the application of designer receptors exclusively activated by designer drugs (DREADDs, chemogenetics [79]), which induce slower and longer-lasting amplification of astrocyte calcium through g-protein coupled signaling, has also highlighted astrocyte roles in sensory evoked oscillatory activity in the gamma range [74•] and fear expression [24] (See Yu *et al.* [37] for deeper discussion). On the other hand, decreasing astrocyte calcium by disrupting intracellular calcium processes (IP3 pathway [38,80]) or expressing proteins that act to extrude calcium [28••,81•] further implicated astrocytes in modulating inhibitory activity as well as obsessive-compulsive phenotypes in mice. Because of the increasing number of studies employing these tools to investigate astrocyte activity, parallel experiments in other non-neuronal cells could lead to comparable break throughs [82].

While microglia usually have less spontaneous calcium activity, evidence suggests that these signals regulate critical functions such as motility, phenotype polarization, cytokine/chemokines releases, receptor trafficking/diffusion, neural circuit plasticity, neurological disorders, and brain injuries [83]. In contrast, investigation of calcium signaling in oligodendrocytes and OPCs has only recently begun [84]. With various receptors and channels that allow calcium flux, oligodendrocyte lineage cells could experience intracellular calcium elevation in response to neuronal activity [84], leading to activity-driven myelination that alters information conduction in functional networks. While some microglial and oligodendroglia calcium activity has been investigated *in vitro* and *in situ* [83,84], there is limited number of *in vivo* studies that utilize the advanced intracellular modulation technologies to explore the roles of microglia and oligodendrocyte lineage cells in modulation of excitation-inhibition balance of functional networks. With pioneering applications of optogenetics and chemogenetics that enable selective manipulation of microglia [85•,86••], OPC [87] and pericytes [88] and the growing catalogue of genetically encoded fluorescent indicators [89], these technologies could be further used to study microglia, oligodendrocytes, OPC, and even gliovascular or mural cell involvement in circuit modulation in normal and pathological conditions [82,90].

Cellular scale modulation technologies

While tools that aim to modulate astrocyte calcium activity have provided a greater appreciation beyond their homeostatic roles, advanced technologies that allow finer interrogation of electrical and chemical signals will provide a more comprehensive understanding of glial communication with neurons [10••,91••]. In particular, the use of advanced, soft and ultrasmall electrode arrays such as gold-coated silicon nanowires [92••], or graphene interfaces [93••] have been proposed as a strategy for uncovering astrocyte-neuron coupling, but this has previously been limited by technological constraints [10••]. Importantly, these newly developed technologies also allow simultaneous imaging and electrophysiology, thus providing an opportunity for a multi-modal paradigm that can establish a more comprehensive understanding of these underappreciated cells [94–96]. The knowledge gained from these 'glial interfaces' could help understand the critical role of not only of astrocytes, but also of many other non-neuronal cells in guiding therapeutic outcomes for neuromodulation [10••,96,97••].

The knowledge gained would also contribute to next-generation designs of stimulation paradigms that regulate glia-neuron interactions to achieve excitatory and/or inhibitory neural network modulation. Electrical brain stimulation can drive cellular activity through activation of voltage-gated receptors and channels, which are also expressed on glia [38,98]. For example, Monai *et al.* [97••], recently demonstrated that a non-invasive brain stimulation paradigm elicited astrocyte activity with limited influence on immediate neural activation. Importantly, the authors suggested that this intervention modulated the cortical metaplasticity through astrocytes, ultimately improving depression phenotypes in mice [97••,99]. Thus, therapeutic electrical neuromodulation paradigms could be improved by utilizing these newly developed micro-scale and nano-scale tools to characterize the effect of stimulation amplitude, frequency, duration, and temporal pattern on electrical and calcium signals in glia.

Opportunities for glial engineering technologies and glial modulation

Current limitations of glial engineering tools presented here have been widely discussed elsewhere [37]. In particular, these tools can be limited by poor spatial or temporal resolution as well as selectivity for subcellular structures or cellular subtypes. For example, DREADDs are activated through systemic administration of a specific drug that takes minutes to take effect and can last for hours [37,79]. Additionally, there is functional diversity within glial cells with phenotypes that have overlapping genetic profiles [37,38]. Thus, broad modulation of glial subtypes serving different functions is likely to add complex influence over neural network activity [10••]. Moreover, electrical stimulation is well-understood to be non-specific, influencing many cells within the region of activation making it difficult to disentangle contributions to the therapeutic benefits of neuromodulation. However, these limitations present great opportunities for

engineers and technologists. Some of these limitations could be addressed with recent advancements in materials and genetic tools [10••,92••]. For example, Maiolo *et al.* discuss how smaller glial engineering technologies could help target recording or stimulation of localized structures while reducing the inflammatory reactivity of cells through improved biocompatibility [10••].

While emerging glial technologies have provided deeper insight into the role of glia-neuron communication, many unanswered questions remain. These unanswered questions present an expansive research frontier ripe for scientists and engineering researchers to explore mechanisms for modulating glial activity and regulating glial-neural interactions. Because of the fact that glial cells are important mediators of neuronal network activity and have large variability in different brain regions disorders [15,100–103,104••], it is important to identify how disruptions in circuit-specific glial function influence neuronal network communication and contribute to neurological dysfunctions. For example, there is remarkable diversity within astrocyte RNA expression across brain areas, and conditional deletion of a single transcriptional factor can impair hippocampal plasticity as well as learning and memory [30••,104••]. Glial engineering tools that identify and target specialized glial functions across different circuits would begin to close the frontier of pathologies of neurological disorders and potentially aid the development of novel therapeutic paradigms and next-generation glial technologies. In other words, discoveries at the forefront of glial modulation of network excitation and inhibition will generate new opportunities not only for scientists to explore glial functions but also for engineers to develop advanced tools to more preciously control glial function in brain circuits.

In turn, on the translational side, basic science discoveries made by invasive gliomodulation technologies will provide the scientific foundations for the development of non-invasive or minimally invasive therapeutic treatment. For example, Iaccarino *et al.* initially utilized optogenetics to understand microglia-neuron interactions [105]. Based on their discoveries, they applied a non-invasive 40 Hz visual stimulation paradigm to rebalance network excitation/inhibition, reduce inflammatory activation of microglia and restore fast-spiking inhibitory neuron activity [105]. Thus, investigating glia interactions with neuronal network excitation/inhibition has begun to provide considerable insight necessary to engineer novel glial intervention strategies to address neurological disorders and brain injuries.

Conclusion

Despite herculean efforts by neuroscientists and neural engineers to fully understand the brain and the neural basis of cognition, inexplicable and sometimes seemingly contradictory results continue to generate more questions. There is mounting evidence that glial physiology modulates excitatory and inhibitory neuronal activity challenging the neuron-centric view of animal behavior and hinting that these are at least several of the missing keys for opening an unexplored avenue for modulating neural network function. Exploring this frontier at the intersection of neurons, glia, and technologies will require an enormous collaboration of a tremendous number of pioneering scientists and engineers from vast multidisciplinary backgrounds. Emerging gliomodulation technologies such as optogenetics, DREADDS, and electrical stimulation and recording technologies, such as genetically encoded fluorescent indicators, viral vectors, and *in vivo* multiphoton microscopy, graphene-glial interfaces allow for revolutionary access to research on the complexity and depth of glia-neuron interactions and engineering of the next-generation glial technologies and therapies. Taken together, these technologies present an opportunity to uncover foundational basic science discoveries related to glial modulation of neural activity and to innovate novel investigative tools and therapeutic technologies for treating neurological diseases and cognitive dysfunctions.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Sherrington CS: **Ferrier lecture—some functional problems attaching to convergence**. *Proc R Soc Lond B Biol Sci* 1929, **105**:332-362.

2. Calvin WH: **Normal repetitive firing and its pathophysiology**. *Epilepsy: A Window to Brain Mechanisms*. New York: Raven Press; 1980, 97-121.

3. Perea G, Sur M, Araque A: **Neuron-glia networks: integral gear of brain function**. *Front Cell Neurosci* 2014, **8**:378.

4. Zuchero JB, Barres BA: **Glia in mammalian development and disease**. *Development (Cambridge, England)* 2015, **142**:3805-3809.

5. Kozai TD *et al.*: **Brain tissue responses to neural implants impact signal sensitivity and intervention strategies**. *ACS Chem Neurosci* 2015, **6**:48-67.

6. • Merlini M *et al.*: **Microglial Gi-dependent dynamics regulate brain network hyperexcitability**. *Nat Neurosci* 2021, **24**:19-23
Authors demonstrate a causal role of microglial Gi signaling in preventing neuronal hyperexcitation and network hypersynchronization through neuron-microglia cross-talks.

7. Wolf SA, Boddeke HWGM, Kettenmann H: **Microglia in physiology and disease**. *Ann Rev Physiol* 2017, **79**:619-643.

8. Liddelow SA, Barres BA: **Reactive astrocytes: production, function, and therapeutic potential**. *Immunity* 2017, **46**:957-967.

9. Raabe FJ *et al.*: **Oligodendrocytes as a new therapeutic target in schizophrenia: from histopathological findings to neuron-oligodendrocyte interaction**. *Cells* 2019, **8**:1496.

10. •• Maiolo L *et al.*: **Glial interfaces: advanced materials and devices to uncover the role of astroglial cells in brain function and dysfunction**. *Adv Healthc Mater* 2021, **10**:2001268
Authors provide an in depth challenge to the neuron centric view of neuroscience and provide detailed dicsussion of newly developed technologies that will aid the advancement of our understanting of astrocyte and glial physiology previously underappreciated due to technological limitations.

11. Salatino JW *et al.*: **Glial responses to implanted electrodes in the brain**. *Nat Biomed Eng* 2017, **1**:862-877.

12. Peng J *et al.*: **Microglial P2Y12 receptor regulates ventral hippocampal CA1 neuronal excitability and innate fear in mice**. *Mol Brain* 2019, **12**:71.

13. •• Badimon A *et al.*: **Negative feedback control of neuronal activity by microglia**. *Nature* 2020, **586**:417-423
Authors reveal the detailed mechanism of microglial suppression on neuronal hyperactivity, suggesting microglia-driven negative feedback is essential neuroprotection from pathological hyperexcitation.

14. •• Cserép C *et al.*: **Microglia monitor and protect neuronal function through specialized somatic purinergic junctions**. *Science* 2020, **367**:528
Authors identify a novel mechanism of neuron-microglia communication through specialized purinergic signaling on neuronal soma, and that disruption of this communication exacerbates brain damage from stroke, suggesting that this purinergic junction contributes to neuroprotection following injury.

15. Wan Y *et al.*: **Microglial displacement of GABAergic synapses is a protective event during complex febrile seizures**. *Cell Rep* 2020, **33**:108346.

16. Chen Z *et al.*: **Microglial displacement of inhibitory synapses provides neuroprotection in the adult brain**. *Nat Commun* 2014, **5**:4486.

17. Yamamoto M *et al.*: **Microglia-triggered plasticity of intrinsic excitability modulates psychomotor behaviors in acute cerebellar inflammation**. *Cell Rep* 2019, **28**:2923-2938.e8.

18. Nakayama H *et al.*: **Microglia permit climbing fiber elimination by promoting GABAergic inhibition in the developing cerebellum**. *Nat Commun* 2018, **9**:2830.

19. Ma X *et al.*: **Depletion of microglia in developing cortical circuits reveals its critical role in glutamatergic synapse development, functional connectivity, and critical period plasticity**. *J Neurosci Res* 2020, **98**:1968-1986.

20. Lee M, Schwab C, McGeer PL: **Astrocytes are GABAergic cells that modulate microglial activity**. *Glia* 2011, **59**:152-165.

21. Shim HG *et al.*: **TNF-α increases the intrinsic excitability of cerebellar Purkinje cells through elevating glutamate release in Bergmann Glia**. *Sci Rep* 2018, **8**:11589.

22. Arcuri C *et al.*: **The pathophysiological role of microglia in dynamic surveillance, phagocytosis and structural remodeling of the developing CNS**. *Front Mol Neurosci* 2017, **10**:191.

23. •• Mederos S *et al.*: **Melanopsin for precise optogenetic activation of astrocyte-neuron networks**. *Glia* 2019, **67**:915-934
Authors optogenetic variant utilizing melanopsin, a g-protein coupled photopigment to target more physiological calcium activity in astrocytes through IP3-mediated mechanisms and demonstrate a its utility through identification of photostimulation-induced astrocyte modulation of syanptic transmission.

24. Adamsky A *et al.*: **Astrocytic activation generates de novo neuronal potentiation and memory enhancement**. *Cell* 2018, **174**:59-71.e14.

25. Martin-Fernandez M *et al.*: **Synapse-specific astrocyte gating of amygdala-related behavior**. *Nat Neurosci* 2017, **20**:1540-1548.

26. Robin LM *et al.*: **Astroglial CB1 receptors determine synaptic d-serine availability to enable recognition memory**. *Neuron* 2018, **98**:935-944.e5.

27. Nagai J *et al.*: **Hyperactivity with disrupted attention by activation of an astrocyte synaptogenic cue**. *Cell* 2019, **177**:1280-1292.e20.

28. •• Yu X *et al.*: **Reducing astrocyte calcium signaling in vivo alters striatal microcircuits and causes repetitive behavior**. *Neuron* 2018, **99**:1170-1187.e9
Authors challenge the view that astrocytes do not regulate behavior by demonstrating that reducing striatal astrocyte calcium activity with AAV-mediated astrocyte expression of a plasma membrane calcium pump causes excessive grooming by reducing inhibition through increased expression of the gaba transporter GAT-3.

29. •• Kwak H *et al.*: **Astrocytes control sensory acuity via tonic inhibition in the thalamus**. *Neuron* 2020
Authors reveal that astrocytic release of GABA contributes to fine-tuning sensory discrimination by demonstrating that blocking astrocyte GABA release by attenuating GABA synthesis or release from Best1 channels impairs sensory discrimination.

30. •• Huang AY-S *et al.*: **Region-specific transcriptional control of astrocyte function oversees local circuit activities**. *Neuron* 2020, **106**:992-1008. e9
Authors provide functional evidence that regional astrocyte heterogeneity is important for neural circuit function by demonstrating that disruption of a single astrocyte trascription factor substantially influenced hippocampal physiology and impaired learning and memory behaviors.

31. • Cavaccini A *et al.*: **Astrocyte signaling gates long-term depression at corticostriatal synapses of the direct pathway**. *J Neurosci* 2020, **40**:5757
Authors demonstrate that astrocyte activation during high frequency stimulation is necessary for synaptic plasticity in the form of long-term depression through astrocyte-derived adenosine.

32. Covelo A, Araque A: **Neuronal activity determines distinct gliotransmitter release from a single astrocyte**. *eLife* 2018, **7**: e32237.

33. Martin-Fernandez M *et al.*: **Synapse-specific astrocyte gating of amygdala-related behavior**. *Nat Neurosci* 2017, **20**:1540.

34. Perea G *et al.*: **Activity-dependent switch of GABAergic inhibition into glutamatergic excitation in astrocyte-neuron networks**. *eLife* 2016, **5**:e20362.

35. Nagai J *et al.*: **Behaviorally consequential astrocytic regulation of neural circuits**. *Neuron* 2021, **109**:576-596.

36. Mederos S, Perea G: **GABAergic-astrocyte signaling: a refinement of inhibitory brain networks**. *Glia* 2019, **67**:1842-1851.

37. Yu X, Nagai J, Khakh BS: **Improved tools to study astrocytes**. *Nat Rev Neurosci* 2020, **21**:121-138.

38. Verkhratsky A, Nedergaard M: **Physiology of astroglia**. *Physiol Rev* 2018, **98**:239-389.

39. Moldavan M, Cravetchi O, Allen CN: **GABA transporters regulate tonic and synaptic GABAA receptor-mediated currents in the suprachiasmatic nucleus neurons**. *J Neurophysiol* 2017, **118**:3092-3106.

40. Kang J *et al.*: **Astrocyte-mediated potentiation of inhibitory synaptic transmission**. *Nat Neurosci* 1998, **1**:683-692.

41. Araque A *et al.*: **Glutamate-dependent astrocyte modulation of synaptic transmission between cultured hippocampal neurons**. *Eur J Neurosci* 1998, **10**:2129-2142.

42. Araque A *et al.*: **Calcium elevation in astrocytes causes an NMDA receptor-dependent increase in the frequency of miniature synaptic currents in cultured hippocampal neurons**. *J Neurosci* 1998, **18**:6822-6829.

43. Jourdain P *et al.*: **Glutamate exocytosis from astrocytes controls synaptic strength**. *Nat Neurosci* 2007, **10**:331-339.

44. Boddum K, Astrocytic GABA *et al.*: **transporter activity modulates excitatory neurotransmission**. *Nat Commun* 2016, **7**:13572.

45. •• Mederos S *et al.*: **GABAergic signaling to astrocytes in the prefrontal cortex sustains goal-directed behaviors**. *Nat Neurosci* 2021, **24**:82-92
Authors highlight the importance of astrocyte Gabaergic signaling by demonstrating that the elimination of astrocyte GABAb receptors impairs goal directed behaviors, which can be recovered through selective photostimulation of a g-protein coupled optogenetic protein.

46. Philips T, Rothstein JD: **Oligodendroglia: metabolic supporters of neurons**. *J Clin Invest* 2017, **127**:3271-3280.

47. Morrison BM, Lee Y, Rothstein JD: **Oligodendroglia: metabolic supporters of axons**. *Trends Cell Biol* 2013, **23**:644-651.

48. Fields RD: **A new mechanism of nervous system plasticity: activity-dependent myelination**. *Nat Rev Neurosci* 2015, **16**:756-767.

49. •• Moore S *et al.*: **A role of oligodendrocytes in information processing**. *Nat Commun* 2020, **11**:5497
Authors reveal that myelination contributes to auditory information processing by demonstrating severity of myelin loss correlated with deficits in sustained axon activity and temporal acuity.

50. • Xin W *et al.*: **Oligodendrocytes support neuronal glutamatergic transmission via expression of glutamine synthetase**. *Cell Rep* 2019, **27**:2262-2271.e5
Authors reveal an important role of oligodendrocytes in modulating excitatory transmission by demonstrating dysruption of oligodendrocyte glutamine synthetase impairs glutamatergic mediated behaviors, thus challenging the view of oligodendrocytes as simply support cells.

51. • Marisca R *et al.*: **Functionally distinct subgroups of oligodendrocyte precursor cells integrate neural activity and execute myelin formation**. *Nat Neurosci* 2020, **23**:363-374
Authors demonstrate that functional heterogeneity of oligodendrocyte precursor cells in morphology, dynamics, interactions with neurons, and following proliferation in zebrafish spinal cord.

52. • Benamer N *et al.*: **Myelination of parvalbumin interneurons shapes the function of cortical sensory inhibitory circuits**. *Nat Commun* 2020, **11**:5151
Authors shows that deficts in interneurons myelination impairs firing activity, inhibitory transmission, and discrimination behaviors.

53. Barres BA, Raff MC: **Proliferation of oligodendrocyte precursor cells depends on electrical activity in axons**. *Nature* 1993, **361**:258-260.

54. Gibson EM *et al.*: **Neuronal activity promotes oligodendrogenesis and adaptive myelination in the mammalian brain**. *Science* 2014, **344**:1252304.

55. Boulanger JJ, Messier C: **Oligodendrocyte progenitor cells are paired with GABA neurons in the mouse dorsal cortex: Unbiased stereological analysis**. *Neuroscience* 2017, **362**:127-140.

56. Kirchhoff F, Kettenmann H: **GABA triggers a [Ca2+] i increase in murine precursor cells of the oligodendrocyte lineage**. *Eur J Neurosci* 1992, **4**:1049-1058.

57. Bechler ME, Byrne L, Ffrench-Constant C: **CNS myelin sheath lengths are an intrinsic property of oligodendrocytes**. *Curr Biol* 2015, **25**:2411-2416.

58. Pepper RE *et al.*: **How do cells of the oligodendrocyte lineage affect neuronal circuits to influence motor function, memory and mood?** *Front Cell Neurosci* 2018, **12**:399.

59. Wellman SM *et al.*: **Cuprizone-induced oligodendrocyte loss and demyelination impairs recording performance of chronically implanted neural interfaces**. *Biomaterials* 2020, **239**:119842.

60. DeGeorge JJ, Morell P, McCarthy KD, Lapetina EG: **Adrenergic and cholinergic stimulation of arachidonate and phosphatidate metabolism in cultured astroglial cells**. *Neurochem Res* 1986, **11**:1061-1071.

61. Ransom BR: **Glial modulation of neural excitability mediated by extracellular pH: a hypothesis**. *Prog Brain Res* 2000, **125**:217-228.

62. Deitmer JW, Brockhaus J, Casel D: **Modulation of synaptic activity in Purkinje neurons by ATP**. *Cerebellum* 2006, **5**:49.

63. Jackson FR, Haydon PG: **Glial cell regulation of neurotransmission and behavior in Drosophila**. *Neuron Glia Biol* 2008, **4**:11-17.

64. Mederos S *et al.*: **Melanopsin for precise optogenetic activation of astrocyte-neuron networks**. *Glia* 2019, **67**:915-934.

65. Bazargani N, Attwell D: **Astrocyte calcium signaling: the third wave**. *Nat Neurosci* 2016, **19**:182-189.

66. Cornell-Bell AH *et al.*: **Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling**. *Science* 1990, **247**:470-473.

67. Pearce B *et al.*: **Astrocyte glutamate receptor activation promotes inositol phospholipid turnover and calcium flux**. *Neurosci Lett* 1986, **72**:335-340.

68. Dani JW, Chernjavsky A, Smith SJ: **Neuronal activity triggers calcium waves in hippocampal astrocyte networks**. *Neuron* 1992, **8**:429-440.

69. Santello M, Toni N, Volterra A: **Astrocyte function from information processing to cognition and cognitive impairment**. *Nat Neurosci* 2019, **22**:154-166.

70. Shigetomi E, Patel S, Khakh BS: **Probing the complexities of astrocyte calcium signaling**. *Trends Cell Biol* 2016, **26**:300-312.

71. Semyanov A, Henneberger C, Agarwal A: **Making sense of astrocytic calcium signals — from acquisition to interpretation**. *Nat Rev Neurosci* 2020, **21**:551-564.

72. Accorsi-Mendonça D *et al.*: **Glial cells modulate the synaptic transmission of NTS neurons sending projections to ventral medulla of Wistar rats**. *Physiol Rep* 2013, **1**.

73. Losi G *et al.*: **New tools to study astrocyte Ca(2+) signal dynamics in brain networks in vivo**. *Front Cell Neurosci* 2017, **11**:134.

74. • Lines J *et al.*: **Astrocytes modulate sensory-evoked neuronal network activity**. *Nat Commun* 2020, **11**:3689
Authors utilize chemogenetic and transgenic tools to highlight an important role of astrocyte activity in modulating sensory processing and neural network oscillatory activity.

75. Perea G *et al.*: **Optogenetic astrocyte activation modulates response selectivity of visual cortex neurons in vivo**. *Nat Commun* 2014, **5**:3262.

76. Deisseroth K: **Optogenetics**. *Nat Methods* 2011, **8**:26-29.

77. Sasaki T, Beppu K, Tanaka K, Fukazawa Y, Shigemoto R, Matsui K: **Application of an optogenetic byway for perturbing neuronal activity via glial photostimulation**. *PNAS* 2012, **109**:20720-20725.

78. Mariotti L *et al.*: **Interneuron-specific signaling evokes distinctive somatostatin-mediated responses in adult cortical astrocytes**. *Nat Commun* 2018, **9**:82.

79. Roth BL: **DREADDs for neuroscientists**. *Neuron* 2016, **89**:683-694.

80. Srinivasan R *et al.*: **Ca(2+) signaling in astrocytes from Ip3r2(-/-) mice in brain slices and during startle responses in vivo**. *Nat Neurosci* 2015, **18**:708-717.

81. • Yu X, Moye SL, Khakh BS: **Local and CNS-wide astrocyte intracellular calcium signaling attenuation in vivo with CalExflox mice**. *J Neurosci* 2021, **41**:4556-4574
Authors engineer a transgenic mouse line that permits selective attenuation of astrocyte calcium activity which represents a novel tool that will help to futher explore the roles of astrocytes in neural network modulation.

82. Hirbec H, Rassendren F, Audinat E: **Microglia reactivity: heterogeneous pathological phenotypes**. *Microglia* 2019:41-55.

83. Umpierre AD, Wu L-J: **How microglia sense and regulate neuronal activity**. *Glia* 2021, **69**:1637-1653.

84. Paez PM, Lyons DA: **Calcium signaling in the oligodendrocyte lineage: regulators and consequences**. *Ann Rev Neurosci* 2020, **43**:163-186.

85. Yi M-H *et al.*: **Optogenetic activation of spinal microglia**
• **triggers chronic pain in mice**. *PLoS Biol* 2021, **19**:e3001154
Authors use optogenetic approach to artificially depolarize microglial membrane potential, inducing pro-inflammatory activation and chronic pain hypersensitivity.

86. Yi M-H *et al.*: **Chemogenetic manipulation of microglia inhibits**
•• **neuroinflammation and neuropathic pain in mice**. *Brain Behav Immun* 2021, **92**:78-89
Authors demonstrate the utility of chemogenetic tools to investigate the role of micorglia in neurological disorders, specifically chronic pain, providing more insight into tratments of chronic pain.

87. Giraldo E *et al.*: **Optogenetic modulation of neural progenitor cells improves neuroregenerative potential**. *Int J Mol Sci* 2021, **22**.

88. Hartmann DA *et al.*: **Brain capillary pericytes exert a substantial but slow influence on blood flow**. *Nat Neurosci* 2021, **24**:633-645.

89. Shen Y *et al.*: **Engineering genetically encoded fluorescent indicators for imaging of neuronal activity: Progress and prospects**. *Neurosci Res* 2020, **152**:3-14.

90. Holland PR *et al.*: **Gliovascular disruption and cognitive deficits in a mouse model with features of small vessel disease**. *J Cereb Blood Flow Metab* 2015, **35**:1005-1014.

91. Mestre ALG *et al.*: **Ultrasensitive gold micro-structured**
•• **electrodes enabling the detection of extra-cellular long-lasting potentials in astrocytes populations**. *Sci Rep* 2017, **7**:14284
Authors engineer novel gold microstructured electrodes that have improved electrical characteristics that provide new opportunities for understanding electrical signaling within astrocyte populaitons.

92. Saracino E *et al.*: **A Glial-silicon nanowire electrode junction**
•• **enabling differentiation and noninvasive recording of slow oscillations from primary astrocytes**. *Adv Biosyst* 2020, **4**: e1900264
Authors engineer a novel nanoscale electrode technology and demonstrated its utility to investigate electrical signaling of astrocytes in an *in vitro* model of epilepsy.

93. Fabbri R *et al.*: **Graphene glial-interfaces: challenges and**
•• **perspectives**. *Nanoscale* 2021, **13**:4390-4407
Authors describe important advancements in graphene-based technologies and their important utility for studying glial physiology.

94. Michelson NJ *et al.*: **Multi-scale, multi-modal analysis uncovers complex relationship at the brain tissue-implant neural interface: new emphasis on the biological interface**. *J Neural Eng* 2018, **15**:033001.

95. Kuzum D *et al.*: **Transparent and flexible low noise graphene electrodes for simultaneous electrophysiology and neuroimaging**. *Nat Commun* 2014, **5**:1-10.

96. Borrachero-Conejo AI *et al.*: **Electrical stimulation by an organic transistor architecture induces calcium signaling in nonexcitable brain cells**. *Adv Healthc Mater* 2019, **8**:e1801139.

97. Monai H *et al.*: **Calcium imaging reveals glial involvement in**
•• **transcranial direct current stimulation-induced plasticity in mouse brain**. *Nat Commun* 2016, **7**:11100
Authors engineer a novel technology that provides important utility in imaging and modulating astrocyte activity, thus opening new doors to study astrocyte physiology and their role in neuromodulation.

98. Kettenmann H *et al.*: **Physiology of microglia**. *Physiol Rev* 2011, **91**:461-553.

99. Monai H, Hirase H: **Astrocytes as a target of transcranial direct current stimulation (tDCS) to treat depression**. *Neurosci Res* 2018, **126**:15-21.

100. Dong Q *et al.*: **An astrocytic influence on impaired tonic inhibition in hippocampal CA1 pyramidal neurons in a mouse model of Rett syndrome**. *J Neurosci* 2020, **40**:6250.

101. Jo S *et al.*: **GABA from reactive astrocytes impairs memory in mouse models of Alzheimer's disease**. *Nat Med* 2014, **20**:886-896.

102. Werner CT *et al.*: **Circuit mechanisms of neurodegenerative diseases: a new frontier with miniature fluorescence microscopy**. *Front Neurosci* 2019, **13**:1174.

103. Park G-H *et al.*: **Activated microglia cause metabolic disruptions in developmental cortical interneurons that persist in interneurons from individuals with schizophrenia**. *Nat Neurosci* 2020, **23**:1352-1364.

104. Batiuk MY *et al.*: **Identification of region-specific astrocyte**
•• **subtypes at single cell resolution**. *Nat Commun* 2020, **11**:1-15
Authors identify five distinct astrocyte subtypes based on transcriptomically and spatially heterogenity in mouse cortex and hippocampus, suggesting the functional diversity in astrocytes across different brain microcircuits.

105. Iaccarino HF *et al.*: **Gamma frequency entrainment attenuates amyloid load and modifies microglia**. *Nature* 2016, **540**:230-235.