Published in final edited form as:

Biol Psychiatry. 2018 December 01; 84(11): 778–786. doi:10.1016/j.biopsych.2017.10.029.

Exploring the Role of Astroglial Glutamate Release and Association with Synapses in Neuronal Function and Behavior

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Abstract

Astrocytes are stellate cells whose appearance can resemble a pointed star, especially when visualizing glial fibrillary acidic protein (GFAP), a canonical marker for astrocytes. Accordingly, there is a commonly made connection between the points of light that shine in the night sky and the diffuse and abundant cells that buffer ions and provide support for neurons. An exceptional amount of function has been attributed to, negated and potentially reaffirmed for these cells, especially regarding their ability to release neuroactive molecules and influence synaptic plasticity. This makes the precise role of astrocytes in tuning neural communication seem difficult to grasp. However, data from animal models of addiction demonstrate that a variety of drug-induced molecular adaptations responsible for relapse vulnerability take place in astrocyte systems that regulate glutamate uptake and release. These findings highlight astrocytes as a critical component of the neural systems responsible for addiction, serving as a key component of the plasticity responsible for relapse and drug-seeking. Here I assemble recent findings that utilize genetic tools to selectively manipulate or measure flux of internal calcium in astrocytes, focusing on GPCR-mediated mobilization of calcium and the induction of glutamate release. Further, I compile evidence regarding astrocyte glutamate release as well as astrocyte association with synapses with respect to the impact of these cellular phenomena in shaping synaptic transmission. I also place these findings in the context of the previous studies of Scofield et al which explore the role of astrocytes in the nucleus accumbens in the neural mechanisms underlying cocaine seeking.

Keywords

Astrocyte; Glutamate; GPCR; Calcium; Cocaine; Gliotransmission

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Disclosures

The author reports no biomedical financial interests or potential conflicts of interest.

Aiming for the Astrocyte

Astrocytes (etymologically formed from the combination of the Greek Astron, or star with Kytos, or cell) are specialized glial cells that vastly outnumber neurons in the human brain (1) and display brain-region specific specialization (2). Moreover, these cells contribute to the blood-brain barrier (3), maintain homeostatic ion balance and provide metabolic support for neurons (4). Astrocytes are also directly linked glutamatergic synaptic transmission as they serve as the primary means for uptake of synaptically released glutamate (5) and also tune or modulate synaptic plasticity, in large part by releasing glutamate (6, 7).

In recent years, the study of astrocytes has benefited greatly from the implementation of cell type-specific promoters (8–10) used to selectively express genetic constructs allowing for the manipulation of astroglial signaling (11). The most commonly used astrocyte-specific promoter is the GFAP promoter (8, 9, 12), a glial protein whose morphology is shown in Figure 1A. When considering which gene to express under direction of the GFAP promoter in order to manipulate astrocyte activity, both optogenetic and chemogenetic constructs are useful as astroglial activity can be manipulated via transient elevation of internal Ca^{2+} (13). Given that opsins allow for the flux of ions across the plasma membrane, it is no surprise that channelrhodopsin-based stimulation of astrocytes elevates internal Ca^{2+} levels (14). This approach has revealed roles for astrocytes in pupil dilation (15), neuronal response to visual stimuli (16, 17), regulation of cerebral blood flow (18) as well as sleep and wakefulness (19, 20). The finding that astrocytes play a role in the neural mechanisms regulating sleep is especially striking when considering that the monumental neuroanatomist Ramón y Cajal hypothesized the same role for these cells over 120 years ago. Cajal postulated that during sleep astrocytes becomes more closely associated with the synaptic machinery in order to inhibit firing, yet it seems that in the forebrain the opposite may be occurring, with astrocytes being more active and in closer proximity to the synaptic machinery during the waking hours (21, 22). The activity-dependent association of astrocyte processes with the synapse and the relationship between astroglial proximity to the synaptic machinery and plasticity is a topic I will explore in more depth below.

Chemogenetic strategies that activate Gq-coupled GPCR signaling also transiently enhance Ca^{2+} levels in astrocytes (23–25). Unlike optogenetics, chemogenetic astrocyte manipulation can be used on an organism wide basis (26, 27), or can be targeted to specific regions of interest (24, 28, 29). Activation of Gq-GPCR signaling in all astrocytes provides evidence for astroglial involvement in the regulation of autonomic nervous system function (27), while chemogenetic manipulation targeted to specific regions demonstrates roles for astrocytes in gut contraction (29), memory function (30), feeding (28) motivation for ethanol seeking (24) and cued cocaine seeking (31). Interestingly, in the NAc core, activation of Gq-DREADD increased intracranial self-stimulation, suggesting that NAc core astrocytes can modulate rewarding stimulation (24). Taken together, the body of work resulting from the selective manipulation of astrocytes supports the hypothesis that these cells are involved in a variety of complex neurological processes.

Astrocyte GPCR signaling

In the previous 2015 study, Scofield et al (31) hypothesized that if engaging Ca^{2+} oscillation via activation of GPCR signaling selectively in astrocytes promotes glutamate release (32), this chemogenetic manipulation could serve as means for normalization of glutamate levels in the NAcore following cocaine exposure (31, 33) similar to what has been observed with N-acetylcysteine (34). This strategy was considered in light of the cocaine-induced decrease in cystine-glutamate exchange (35, 36) which contributes to a decrease in extracellular glutamate in the NAcore following cocaine exposure (33). Drug-induced decreases in the level of extrasynaptic glutamate in the NAcore results in reduced glutamate tone on release-regulating presynaptic mGluR2/3 receptors on cortical terminals (36), creating a synaptic potentiation. This synaptic potentiation, when paired with reduced glial glutamate uptake (37), also caused by cocaine exposure (38), sets the stage for relapse precipitated by glutamate overflow in response to conditioned cue exposure. For review see (33, 38). Accordingly, Scofield et al designed their DREADD study to engage glial glutamate release and restore tone on mGluR2/3 via normalization of extrasynaptic glutamate levels, with the overall goal being to inhibit relapse in response to conditioned cue exposure (31).

Given that Ca^{2+} oscillation represents a primary modality for astrocyte excitability, G-protein coupled (GPCR) receptors and the intracellular signaling cascades they engage, [for review see (11)] are an attractive putative mechanism for astrocytes to send and receive signals from neurons. However, specific concerns regarding the physiological relevance of Gq-GPCR signaling in astrocytes, and the resulting Ca^{2+} oscillation, arose when the effects on autonomic nervous system function observed in the Gq-DREADD transgenic mice discussed above appeared not to be mediated by inositol 1,4,5-triphosphate (IP_3), the second messenger required to induce endoplasmic reticulum-mediated release of internal Ca^{2+} stores (27). Moreover, genetic deletion of IP_3 receptor subtype 2 ($\text{IP}_3\text{R}2$), the subtype predominantly expressed in astrocytes when compared to $\text{IP}_3\text{R}1$ or 3, also appeared to block GPCR-mediated Ca^{2+} increases as visualized with the calcium indicator Oregon Green (39). Further, deletion of $\text{IP}_3\text{R}2$ had no impact on neighboring neuronal activity in hippocampus, indicating no real importance for Gq-GPCR signaling and the mobilization of internal Ca^{2+} stores in astrocytes regarding modulation of synaptic plasticity (27). Finally, global $\text{IP}_3\text{R}2$ conditional knock out mice, showed no discernable phenotypes when assayed with a variety of behavioral tasks, displaying intact motor and sensory function, no change in anxiety-like behaviors and unaltered learning and memory (39). These results indicate a lack of importance of Gq GPCR-mediated oscillations of internal Ca^{2+} in astrocytes and by extension the lack of impact of any resulting gliotransmission on neuronal activity linked to the neurological processes that govern complex behaviors.

Interestingly, subsequent reports using the highly sensitive (40) and rapidly expanding class of genetically encoded calcium indicators (GECIs) demonstrate Ca^{2+} dynamics in animals that lack $\text{IP}_3\text{R}2$ (41–44), leading to the hypothesis that multiple IP_3 receptor subtypes are expressed in astrocytes. This hypothesis was supported by the finding that complete pharmacological blockade of all IP_3 receptor subtypes ($\text{IP}_3\text{R}1$, 2 and 3) impairs high frequency stimulation-induced Ca^{2+} responses in astrocytes beyond what was observed with the genetic removal of both $\text{IP}_3\text{R}2$ and 3, providing evidence for the expression of all three

subtypes in astrocytes (41). Moreover, blockade of all IP₃ receptor subtypes in astrocytes inhibits hippocampal LTP, supporting a role for this signaling moiety and Gq GPCR-mediated gliotransmission in the regulation of synaptic plasticity (41). Further, studies utilizing GECIs have demonstrated that Ca²⁺ fluctuation occurs in IP₃R2 knock out animals and that each IP₃ receptor subtype is likely responsible for unique modalities of spatially and temporally regulated Ca²⁺ responses. Specifically, IP₃R2 mediates slower somatic responses and the other subtypes are required for the more frequent and larger amplitude oscillations in fine processes. Speaking to this distinction, in the hippocampus fine astrocyte processes exhibit high frequency spontaneous Ca²⁺ responses whereas the somatic compartment rarely shows spontaneous activity (45). Responses in the finest processes persist in IP₃R2 knock out animals (45), and appear to require both influx of extracellular Ca²⁺ and Ca²⁺ from internal stores (46). Moreover, stimulation of Schaffer collaterals in hippocampal slices produces distinct Ca²⁺ responses in both the somata and fine processes; similar to the data described above, Ca²⁺ responses in the fine processes are faster and of higher magnitude (47). Interestingly, responses in both compartments can be evoked with application of group I and II/III mGluR agonists in combination with ATP, providing a potential mechanism for signaling molecules released from neurons or nearby astrocytes in eliciting these types of Ca²⁺ responses. Additional experimentation will be required to gain a more complete understanding of how each IP₃ receptor subtype contributes to the spatial and temporal aspects of Ca²⁺ signaling and how this in turn impacts astrocyte-neuron communication.

Astroglial Glutamate Release

A considerable amount of evidence exists for the inducible release of glutamate from astrocytes using the techniques described above (10). Optogenetic-based stimulation of astrocytes demonstrates light-induced glutamate release from immortalized astrocyte cell lines (48) as well as from cortical (14, 16, 49) and cerebellar astrocytes (15, 50). Interestingly, activation of ArchT (an inhibitory opsin that functions as a proton pump) also causes increased Ca²⁺ levels in cortical astrocytes in-vivo, leading to an enhancement in extracellular glutamate levels that are potentially of glial origin (51). Moreover, in addition to ChR2-based stimulation, activation of the light-gated Ca²⁺-permeable ionotropic GluR6 glutamate receptor (LiGluR) as well as the Ca²⁺-translocating variant of channelrhodopsin (CatCh) both promote glutamate release in cultured cortical astrocytes, which occurs via anion channels and not vesicular exocytosis (49). Unpublished data from the Kalivas lab are in agreement with the studies discussed above, using the same electrochemical detection strategy described in the 2015 study from Scofield et al (31). These data demonstrate light induced enhancement of glutamate levels following activation of channelrhodopsin in NAcro astrocytes (Figure 2). In this section, I discuss modalities of GPCR-mediated glutamate release from astrocytes presenting evidence that support or refute each mechanism. I also place these findings in context of the previous study of Scofield et al utilizing Gq-DREADD activation in NAcro astrocytes (31).

Vesicular Release

Early on, activation of mGluR5 and the subsequent elevation of internal Ca²⁺ in astrocytes was thought to promote vesicular exocytosis of glutamate (52, 53). This model was

comprised in light of electron microscopy studies demonstrating the existence of small synaptic-like microvesicles in astrocytes (54), which appeared to contain glutamate (55). However, this model has been heavily refuted given the apparent reduction of mGluR5 expression in adult astrocytes when compared to cultured cells or slices derived from embryonic tissue (46, 56). Moreover, there has been difficulty in detecting expression of the vesicular glutamate transporter (57) and synaptic vesicle proteins (58). These data, combined with reported off-target neuronal expression in a dominant negative SNARE (dnSNARE) transgenic animal model used to demonstrate the importance of vesicular exocytosis in astrocytes (59, 60), has caused a shift in favor away from the vesicular exocytosis as a model for glutamate release, yet results from multiple current studies support this mechanism (61–63). Scofield et al used this dnSNARE transgenic mouse model in the 2015 study employing NAcro astrocyte Gq-DREADD activation (31). In these experiments, a reduction of Gq-DREADD-evoked glutamate release in dnSNARE mice was observed compared to wild type littermates (31), causing the authors to hypothesize that glutamate release evoked by DREADD activation in astrocytes could be vesicular. Interestingly the dnSNARE animal, which exhibits a global disruption of the vesicular exocytosis in all astrocytes, displayed lack of cue-induced reinstatement of cocaine seeking and reduced cocaine-induced reinstatement of conditioned place preference (64). Recently, this model has been used to demonstrate that astrocytes control the maturation, survival and dendritic spine formation in adult born neurons of the hippocampus via vesicular release of D-serine (65). While no off-target expression of the dnSNARE protein in neurons was reported in this study, microdialysis in the dentate gyrus yielded only differences in extracellular levels of D-serine and no other putative gliotransmitters like glycine, glutamate or GABA (65). These data indicate that while D-serine is potentially released by astrocytes via vesicular exocytosis glutamate is not, at least in the dentate gyrus.

Non-vesicular Release

Selective activation Gq-GPCR signaling via the protease activated receptor 1 (PAR1) in astrocytes induced glutamate release that was detected using sniffer. Release occurring through this mechanism exists in two distinct modalities, fast release with a peak concentration of ~100 μ M and slow release with a peak concentration at ~1 μ M. Fast release is mediated by the glutamate permeable potassium channel TREK-1 whereas the slow release is mediated by the glutamate permeable Ca^{2+} -activated anion channel Best1 (66). Interestingly, the TREK-1 channel is activated by $\text{G}\beta\gamma$ and is located primarily at the cell body and processes, whereas the Best1 channel appears to be localized closer to the synaptic cleft. Further, activation of PAR1 in hippocampal astrocytes enhances glutamate release onto Schaffer collateral – CA1 synapses and activates GluN2A-containing NMDA receptors, lowering the threshold for induction of NMDAR-dependent hippocampal LTP (67). These data demonstrate a clear role for Gq-GPCR activation and subsequent Ca^{2+} -mediated glutamate release from astrocytes in the modulation of hippocampal plasticity. It is certainly possible that in the case of the Scofield et al study (31), that activation of Gq-DREADD in NAcro astrocytes may have evoked glutamate release through this mechanism as well (Figure 3).

Endocannabinoid-mediated Release

endocannabinoid (eCB) receptors are expressed on both neurons and astrocytes and serve as a means for the modulation of synaptic plasticity on both cell types, albeit with different mechanisms of action. With respect to astrocytes, the cannabinoid receptor type one (CB1R) is a particularly interesting mediator of synaptic communication. Canonically, these receptors are considered to be Gi/o coupled expressed on presynaptic terminals, where their activation leads to suppression of synaptic transmission (68). However, CB1Rs expressed on astrocytes are reportedly Gq-coupled (69), and when activated produce an enhancement of internal Ca^{2+} levels (70, 71). The ability of eCBs to modulate neural communication was thought to take place predominately as a result presynaptic inhibition engaged by neuronal eCB receptors. However, the localization of CB1R expression in astrocytes gives rise to a novel method for the mobilization of internal Ca^{2+} and the putative induction of glutamate release. This modality of neuron-astrocyte bidirectional communication is particularly interesting given that removal of CB1Rs specifically in astrocytes impairs spatial working memory as well as LTD induced at hippocampal synapses following the application of delta-9-tetrahydrocannabinol, an agonist of the CB1R and the psychoactive component of marijuana (72). A particularly striking example of eCB-mediated astrocytic glutamate release in the regulation of medium spiny neuron (MSNs) activity was recently described. In the striatum, D1 dopamine receptor-expressing MSNs are part of the striatomesencephalic “direct” pathway whereas D2 dopamine receptor-expressing MSNs are part of the striatopallidal “indirect” pathway. In this region, when stimulating a D1 neuron, there was the expected eCB-mediated inhibition of activity through the presynaptic inhibition of synaptic transmission, yet there was also enhanced excitatory inputs onto neighboring neurons of the same pathway. The authors illustrate that eCB-mediated stimulation of astrocytes results in an enhanced internal Ca^{2+} , leading to the release of glutamate and potentiation of neurons that exist in the same striatomesencephalic or striatopallidal pathway, enhancing activity via activation of presynaptic mGluR1/5 receptors (73, 74). Not only does this finding underscore the functional relevance of GPCR-mediated astrocyte glutamate release in modulating striatal synaptic communication, it also puts forth the novel concept that there exists cell type-specificity in the association of astroglial processes with particular neuronal subtypes. In concert with the diametrically opposed D1 and D2 medium spiny neurons, it appears that astrocytic perisynaptic processes associate specifically with D1 or D2 expressing MSNs in the striatum. Additional experimentation may determine that these two classes of astrocytes themselves are functionally distinct, or simply that the system just requires astrocytes not to get their “wires crossed”.

Summary

In light of early cell-culture based experiments (75–78), astrocytes were considered to be incredibly robust regulators of synaptic plasticity sending and receiving signals to orchestrate synaptic communication, yet observations potentially limiting the importance of IP_3 receptor-mediated signaling and the resulting of Ca^{2+} -transients in these cells (39) among others (46), suggested they may act as more passive members of the neuropil providing structural support and perhaps not releasing neuroactive molecules (79, 80). Recent data suggest that these cells regulate and shape neuronal communication through both support and active release mechanisms (46, 81). A recent study performed in the

suprachiasmatic nucleus (SCN) demonstrates a crucial role for Ca^{2+} -mediated glial glutamate release in dampening neuronal activity during circadian night (81). Long term calcium imaging of organotypic SCN slices reveals that neurons are active during the circadian day displaying higher internal Ca^{2+} levels, while astrocytes display antiphasic activity, with higher Ca^{2+} levels during circadian night. Levels of extracellular glutamate were higher during circadian night, corresponding with bouts of higher astrocyte Ca^{2+} levels. The authors demonstrate that the glutamate required to inhibit neuronal activity during the circadian night was of glial origin. Elongation of the regulated oscillating astrocyte glutamate release produced a corresponding elongation in the circadian period of locomotor activity, demonstrating a functional relationship between the astrocytic glutamate release and circadian timekeeping in the SCN (81, 82). At least in the case of the SCN, it seems that Cajal's intuition was correct as SCN astrocytes keep SCN neurons inhibited during periods of circadian night, however this is achieved by glutamate release and inhibition of synaptic transmission as opposed to physical invasion of the synaptic cleft.

Reaching out

Across the evolutionary timeline, a marked increase in the number, diversity and complexity of astrocytes is observed when compared to neurons (83), indicating that the evolution of the human brain gives rise to an increase in the complexity and function of astrocytes (84). In a remarkable set of experiments, human glial progenitor cells were grafted into mice in order to produce chimeric mice with functional human astrocytes. After implantation into neonatal immunodeficient mice, the progenitor cells fully integrated into the murine astroglial syncytium. These hominid-derived astrocytes formed gap junctions with the host astrocytes and interfaced with the host vasculature, yet retained their larger and more complex hominid-specific morphological phenotype and propagated Ca^{2+} signals faster than the host astrocytes (85). Remarkably, hippocampal LTP was enhanced in the chimeric mice and these animals displayed enhanced performance in auditory and contextual fear conditioning tasks as well as enhanced performance in the Barnes maze and object location recognition memory tasks when compared to controls that were grafted with murine glial progenitor cells (85). These experiments not only demonstrate that there is an evolutionary shift in the morphological and functional role of astrocytes in the brain but also support the hypothesis that astrocytes are capable of modulating synaptic communication and plasticity to an extent that can impact the information processing underlying complex behaviors.

Apart from contacting the vasculature, astroglial peripheral or perisynaptic processes are closely associated with dendritic spines (86), embracing or ensheathing them (87), forming structures that resemble spoons or cradles (Figure 1E Arrow, also see Supplemental Movie). This intimate association controls the flow of neurotransmitter insulating individual synaptic units to prevent synaptic overflow and to efficiently remove glutamate following release (88). Using live imaging, the association of astrocytic processes with dendritic spines can be studied in detail. Remarkably, the juxtaposition of astrocyte processes with synapses, specifically with postsynaptic dendritic spines, is extraordinarily dynamic with the extension and retraction of processes observed on the scale of minutes with near continuous remodeling (89). Given that the GFAP filament protein does not extend all the way into the finest processes (Figure 1D, F) closest to dendritic spines, the rapid modification of

perisynaptic astrocyte processes is thought to take place via actin cycling (89). In the hippocampus, astrocyte retraction from spines can be induced following high frequency stimulation requiring GPCR-mediated Ca^{2+} elevations (90). Interestingly, Scofield et al observed a decrease in colocalization of the plasma membrane of astrocytes, labeled with GFAP promoter-driven LCK GFP viral vector (Figure 1B), with a synaptic marker in the NAc core following cocaine self-administration and extinction (91). This colocalization of synapsin and the astrocyte membrane, was used as an index of synaptic contact (91). In a recent set of unpublished data from the Kalivas lab, retraction of astrocytes from accumbens core synapses is also observed following heroin self-administration and extinction training. Interestingly, if animals enter into 15 minutes of a cued relapse session, levels of synaptic association return to levels observed in saline controls. This finding is consistent with data from others demonstrating that astrocytes sense bouts of high activity and approach synapses to ensure appropriate removal of transmitter, ensheathing active synapses in order to stabilize excitatory synapse (92). In the hippocampus, the extensive invasion of astroglial processes into the synaptic cleft sets synaptic strength by enhancing glutamate clearance and decreasing AMPAR-mediated synaptic transmission, a process that is mediated by the gap junction protein connexin 30 (Cx30) (93). Together, these data support the hypothesis that the proximity of astrocyte processes to the synapse not only impacts glutamate clearance but also glutamatergic neuronal communication. The association of the astroglial plasma membrane with glutamatergic synapses likely functions as an inverted “U”. Too little association leads to inefficient communication, overflow of transmitter beyond individual synapses and lack of rapid uptake of glutamate (94) and potentially impaired LTP and LTD (95, 96). At optimal levels of association there is an ample amount of insulation, proper synaptic cradling, efficient communication and rapid removal of glutamate (87, 92). However, at maximum association, astrocytes begin to impinge on the cleft and inhibition of glutamate receptor activation can take place (93), echoing Cajal’s intuition (21). It is possible that astrocytes can rapidly shrink or expand via mechanisms beyond actin cycling, such as using gap junction pore proteins like connexins or water channels like Aquaporin 4 (97, 98). In cultured astrocytes, application of a cyclic adenosine monophosphate analogue induces dramatic shrinkage (80), whereas application of glutamate induces swelling mediated by Aquaporin 4. The swelling effect could also be obtained via pharmacological activation of mGluR5 yet was not sensitive to blockade of GLT-1 (98). The swelling of astrocytes in response to GPCR activation may have also contributed to the inhibition of cocaine seeking observed by Scofield et al (31), potentially limiting cue-induced glutamate overflow linked to relapse vulnerability (38) (Figure 3). The dynamic association of astrocyte perisynaptic processes with neurons likely serves as a method for the tuning of synaptic plasticity, the bidirectional communication required for this to occur and what drug or disease states derail this process is an interesting topic for future research.

Conclusions and Future Directions

Considering the diversity of functions that can be impacted by altering activity of astrocytes across different brain regions discussed above, the existence of a large degree of region-specific functions of astroglial cells appears an attractive hypothesis, especially given recent observations regarding differences morphology, electrophysiological properties and the

program of astrocyte RNA translation across brain regions (99). However, it may be difficult to determine if astrocytes themselves are solely responsible for any such effect, given that manipulation of astrocytes will potentially impact underlying neuronal activity. As the function and role of astrocytes is becoming more intensely studied, it seems apparent that both astrocytes and neurons should be investigated as a conjoined system, when studying a particular neurological system or complex behavior (4).

Similar to what has been reported for neurons (100), local translation of transcripts in the peripheral processes of astrocytes has recently been described (101). These data support the hypothesis that a functional distinction exists between Ca^{2+} fluctuation observed in the somatic versus peripheral compartments. It follows then that there may also be region specific gliotransmitter release along the astroglial process and neuronal arbor, occurring at functionally distinct locations in proximity particularly active or inactive synapses. As the techniques surrounding the high magnification analysis of astroglial Ca^{2+} dynamics are refined in concert with endoscope-based freely moving recordings, the stage is truly set for an enhanced understanding of how Ca^{2+} signaling in astrocytes relates to potential gliotransmitter release or altered association with synapses and how this in turn impacts neuronal activity during a panoply of interesting behaviors.

Given the expansion of our understanding of the how the morphological properties of dendritic spines relate to synaptic plasticity (102–106), and their physiological relevance in drug addiction (33) it seems that there is a parallel expansion of our understanding of astroglial morphological plasticity on the horizon. With the continual advances in deconvolution (107) and super-resolution microscopy (108–111) and the advent of expansion microscopy (112, 113), it seems that we may be moving closer to using light microscopy to image the very small astrocyte perisynaptic processes and their association with synapses (114). This will likely lead to a greater understanding of the structure function dynamics in astrocytes with respect to established functional changes in dendritic spines and hopefully lead to an elucidation functional relationship between the two. A more complete understanding of the interconnection of both astrocyte and neuronal function will no doubt lead to a better understanding of the brain and potentially to improved treatments for neurological disorders and addiction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by R00DA040004.

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Figure 1.

High Resolution Image of a Single Astrocyte in the Rat Nucleus Accumbens Core. **A)** Immunostain for the glial fibrillary acid protein (GFAP) shown in blue. This structural filament protein provides support for the astrocyte and a means for trafficking proteins. The nucleus of the astrocyte cell can be visualized as a small circular region in the center relatively devoid of labeling. **B)** Usage of GFP protein expression directed to the plasma membrane using an AAV5-GFAP promoter driven LCK-GFP vector developed by the Khakh group allows for full visualization of the astrocyte plasma membrane. A commonly stated fact can be clearly visualized here where the GFAP filaments do not extend into the finest terminal aspects of the astrocyte plasma membrane. In this panel, the white square depicts the inset panel shown below in D–F. **C)** the merged image shows the relationship of the skeletal GFAP protein and the shape of the plasma membrane architecture. The “holes” or spheroid gaps in the astrocyte signal are areas where the cell bends around the somas of neurons or blood vessels. In A–C, the scale bar represents 10 microns. **D)** High magnification of the GFAP filament protein. **E)** High magnification of the LCK-GFP signal reveals morphological aspects of the astrocyte plasma membrane. The white arrow shows a “cradle” shape that ensheathes the synaptic machinery. At very high resolution and very small optical section thickness, (0.3 – 0.1 Microns) multitudes of these spoon like shapes can be seen. **F)** the merged image depicts the relationship between the finest terminal processes of the astrocyte membrane with the GFAP structural filament. In D–F, the scale bar represents 2 microns.

Figure 2.

Activation of GFAP Promoter Driven ChR2 in the NAc Core Astrocytes Promotes Glutamate Release. **A)** Schematic of electrochemical probe construction with optical fiber positioned over the microelectrode to deliver light over the glutamate oxidase coated sites. **B)** Glutamate trace showing evoked release that coincides with delivery of laser light. **C)** IHC detection of ChR2 and GFAP filament protein in astrocytes. These data demonstrate astrocyte-specific expression of the ChR2 protein in the NAc core akin to what was observed in our previously published report (31). The scale bar represents 25 microns

Figure 3.

Updated model for engaging glutamate gliotransmission and its impact on NAc core plasticity during cued cocaine seeking. Cocaine exposure and extinction of cocaine seeking causes two glial modifications linked to relapse vulnerability, decreased basal glutamate levels (via decreased expression of Xc- shown above as a blue sphere with red and grey arrows) and a decrease in expression of GLT-1 (shown as a Green transporter) (33). Engaging Gq-DREADD in astrocytes (shown in red and blue on the astrocyte) with CNO (purple circles), as described in the Scofield et al study (31) evokes a Ca^{2+} response, which we observed leads to glutamate release. Glial glutamate release normalizes a cocaine induced decrease in basal glutamate and inhibits reinstatement by engaging mGluR2/3 (located presynaptically shown in blue). We hypothesized that activation of Gq-DREADD in astrocytes could evoke a synaptic glutamate release (purple vesicles). However, it is also possible that this could engage non-vesicular glutamate release through Best1 (grey) and TRPV-1 (brown). In addition to reversing the cocaine induced decrease in basal glutamate, Gq-GPCR activation via DREADD stimulation in astrocytes may also enhance GLT-1 uptake (115) and astrocyte swelling (98). This could also have contributed to the anti-reinstatement effects of Gq-DREADD stimulation in astrocytes observed in the Scofield et al DREADD study (31).