Astrocyte–Neuron Interactions in the Striatum: Insights on Identity, Form, and Function

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Abstract

The physiological functions of astrocytes within neural circuits remain incompletely understood. There has been progress in this regard from recent work on striatal astrocytes, where detailed studies are emerging. In this review, findings on striatal astrocyte identity, form, and function, are summarized with a focus on how astrocytes regulate striatal neurons, circuits, and behavior. Specific features of striatal astrocytes are highlighted to illustrate how they may be specialized to regulate medium spiny neurons (MSNs) by responding to, and altering, excitation and inhibition. Further experiments should reveal additional mechanisms for astrocyte–neuron interactions in the striatum and potentially reveal insights into the functions of astrocytes in neural circuits more generally.

Astrocytes and Neural Circuits

Documented over 150 years ago [1], astrocytes are part of the larger population of central nervous system (CNS) cells called glia, which includes oligodendrocytes, microglia, and other specialized cells [2]. Although neural circuits are often thought of as comprising only neurons, an anatomically exact definition of a neural microcircuit is that 'comprising neurons and associated cells such as glia, organized to carry out specific operations within a region of the nervous system' [3]. Astrocytes do not operate in isolation and their functions relate to interactions with many types of brain cell. In these settings, established and proposed roles for astrocytes include interactions with the vasculature, the blood–brain barrier, regulation of synapse formation and loss, ion homeostasis, neurotransmitter clearance, metabolic support, regulation of action potential waveforms, and synaptic plasticity [4].

The proportion of astrocytes varies considerably between brain areas [5] and species. Current estimates suggest that astrocytes represent ~19–40% of brain cells [6]. Numbers aside, astrocytes represent an important type of cell necessary for neural circuits to develop and function [2]. Furthermore, astrocytes are widely implicated in brain disorders [7–9].

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Broad advances in this field have renewed interest in the fundamental biology of astrocytes within fully developed brain areas. For instance, astrocyte regulation of neurons has been proposed to occur via: (i) vesicular gliotransmission; (ii) channel-mediated release of neuroactive substances; (iii) neurotransmitter homeostasis; (iv) ionic homeostasis; (v) movement of astrocyte processes towards or away from synapses; (vi) release of neurotoxic factors; (vii) regulation of extracellular diffusion; (viii) neurovascular coupling; (ix) metabolic coupling; and (x) astrocytes acting as intermediary mediators of neuromodulation [2,4,7,10–16]. However, there are few examples where astrocytes have been studied comprehensively in a single adult vertebrate brain area to uncover functions that may be tailored to that circuit (many of the aforementioned insights were from multiple brain areas). In this review, I summarize recent findings related to these topics in relation to astrocytes in the striatum, where detailed work is emerging. Other than two pioneering papers [17,18], little was known about striatal astrocytes until recently.

The Striatum: Exploring Astrocytes Systematically in a Defined Circuitry

The striatum is the largest input nucleus of the basal ganglia (BG), a group of interconnected subcortical nuclei [19]. In rodents, most (~95%) neurons within the striatum are γ-aminobutyric acid (GABA)ergic projection neurons called **medium spiny neurons (MSNs)** (see Glossary). The remainder are GABAergic and cholinergic interneurons. The striatum receives excitatory gluta-matergic input from the cortex and thalamus, which impinges on extensive dendritic spines of D1-dopamine receptor expressing and D2-dopamine receptor-expressing MSNs (Figure 1A). One essential function of the striatal circuit is to determine which subpopulations of MSNs fire during coordinated input, which alters the activity of downstream nuclei and, hence, BG-controlled behaviors [19–26]. In a simplified interpretation, activation of the D1 MSNs inhibits the firing of the BG output nuclei, the substantia nigra pars reticulata (SNr) and internal globus pallidus (GPi) via the 'direct' striatonigral projection, resulting in disinhibition of thalamocortical circuits. Conversely, activation of D2 MSNs enhances SNr activity via the striatopallidal (indirect pathway) circuit, with resultant inhibition of thalamocortical drive via activation of the striatopallidal circuit. While the relative activity level of each output pathway originating from the dorsolateral striatum was once thought of as having strictly opposing roles in movement facilitation and suppression (the rate model), recent work has indicated that it is a precise and concomitant pattern of complementary ensemble activity of these pathways, within spatially biased local clusters, that regulates successful and appropriate movement initiation, and that subsequently controls the smooth execution of action sequences or behavioral 'syllables' [27–29]. Thus, appropriate complementary activity patterns in D1 and D2 MSN pathways have important implications for pathological conditions that produce excessive repetitive behaviors, disrupted motor sequences, and/or excessive behavioral switching. An indication of the importance of the striatum derives from the fact that multiple neurological and psychiatric diseases involve striatal dysfunction, including Huntington's disease (HD), Parkinson's disease (PD), repetitive behaviors, attention-deficit hyperactivity disorders, obsessive-compulsive disorders, habits, rituals, and tics [20,22,30]. There are also peculiar pediatric throat infections that appear to alter striatal and BG function irreparably, leading to behavioral phenotypes such as obsessive-compulsive disorder (OCD) [31]. These involve

astrocytes and other glia [32,33], which form part of the immune response of the brain. In HD, there is progressive striatal degeneration in proportion to the severity of motor, psychiatric, and cognitive deficits [30] as well as evidence to support a role for astrocytes [9]. Hence, the striatum provides an opportunity to study astrocytes in a nucleus with not only well-characterized inputs and outputs (Figure 1A), but also the potential to inform about behavior and disease (Figure 1B,C). Furthermore, MSNs are relatively homogenous and can be studied in tissue slices and *in vivo*, although the striatum itself comprises distinct anatomical units that mediate separable functions. As discussed later, multiple MSNs interact anatomically with single astrocytes, providing a basis to explore signaling between these cell types. Finally, the striatum is cytoarchitecturally distinct with a sizeable volume in mice (~23 mm^3) and humans [34,35] that renders it amenable to a range of molecular, biochemical, and physiological approaches.

Identity: Striatal Astrocyte Molecular Signatures

Transcriptomics

Since cell isolation and culture conditions alter astrocytes from their *in vivo* state [36], an alternative method to assess astrocyte transcriptomes *in vivo* was needed. For this purpose, RNA sequencing (RNA-Seq) of adult (P63) astrocytes using *Aldh1/1*-**Cre/ERT2** × RiboTag mice was performed [37,38]. In these mice, the HA-tagged ribosomal subunit Rpl22HA [37] was expressed selectively in astrocytes [38]. Differential expression analysis revealed that 1180 genes were significantly enriched in striatal astrocytes relative to the hippocampus; that is, astrocytes were molecularly separable in the striatum relative to the hippocampus, a conclusion supported by other work [39–45].

The set of genes highly expressed and not differentially expressed between striatum and hippocampus includes ones involved in core astrocyte functions, for instance *Slc1a2, Sparc, Kcnj10*, and *Slc6a11*. There was significant expression of nine K^+ channels and two auxiliary subunits in striatal astrocytes. Similarly, from known transmembrane Ca^{2+} flux pathways, significant gene expression for 23 proteins was found, consistent with the diversity of striatal astrocyte Ca^{2+} signals [46]. Furthermore, of the 249 known Ca^{2+} binding EF-hand containing proteins, 68 were expressed at appreciable levels [fragments per kilobase million (FPKM >10)] in striatal astrocytes, and 18 were differentially expressed in relation to the hippocampus. These data show that striatal astrocytes display richness in K^+ channels, Ca^{2+} flux pathways, and proteins likely to buffer and respond to Ca^{2+}. The RNA-Seq data are freely available (www.astrocyternaseq.org).

There were two surprises from RNA-Seq. The gene encoding **glial fibrillary acid protein** (*Gfap*) was not highly expressed in striatal astrocytes, but was highly expressed in hippocampal astrocytes. This is important, because it confirms that GFAP is not a robust marker for striatal astrocytes. This may have implications for the analysis of postmortem striatal tissue from neurodegenerative diseases, because such studies invariably use GFAP. Furthermore, the gene encoding **μ-crystallin** (*Crym*) was highly expressed in striatal, but not in hippocampal astrocytes. This suggests that *Crym* represents a striatum specific marker for astrocytes.

Proteomics

Proteomics was performed to validate RNA-Seq findings. The correlation between RNA and protein levels in striatum was strong, implying that mRNA levels reflected the abundance of most identified proteins. However, ~10% of the proteins showed high abundance, but low mRNA FPKM values (<10), which may indicate nonlinearity between protein lifetime and transcript levels. The top 20 proteins shared between hippocampus and striatum were: *Actb, Gapdh, Hist1h2bf, Slc1a3, Hist1h4a, Slc1a2, Eno1, Atp1a2, Atp5b, Glud1, Ppia, Eno2, Atp5a1, Phgdh, Dpysl2, Tubb4b, Prdx1, Cfl1, Asrgl1*, and *Ndrg2* (listed by their gene symbols). Although some are well studied, little is known about the functions of others. Furthermore, statistical analyses revealed that, out of 143 proteins reproducibly detected, 18 were markedly enriched in striatal astrocytes. These were: *Crym, Ppp1r1b, Tln1, Acaa2, Aldh5a1, Pdhb, Slc25a3, Epb4.1l2, Uqcrc2, Etfdh, Acadl, Acsf2, Acsbg1, Pcx, Aco2, Ldhb, Sept2*, and *Dld* (listed with their gene symbols in order of enrichment). The functions of most of these in striatal physiology are unknown. Concordant with the transcriptomic profiles, the most differentially expressed striatal and hippocampal astrocyte proteins were μ-crystallin and GFAP, respectively. Furthermore, μ-crystallin expression was restricted to striatal astrocytes and was not detected in astrocytes from several brain areas, although it did occur in a small population of striatal neurons [47]. μ-Crystallin is thought to bind thyroid hormone and function as a ketimine reductase [48], but its function in the striatum remains unknown.

Marker Expression

Although GFAP was a poor marker of striatal astrocytes, antibodies against Aldh1l1, S100β, and **GLT1** labeled most striatal astrocytes [38,46,49–53]. Kir4.1 expression identified most striatal astrocytes, but significant expression level differences existed between cells [49]. It is not clear whether this reflects true differences in Kir4.1 expression or exposure of the antibody epitope. By contrast, the astrocyte GABA transporter GAT-3 labeled ~30% of evenly distributed astrocytes [38,52]. Furthermore, μ-crystallin labeled ~85% of the astrocytes in the ventral striatum, but only ~30% in the dorsal regions, even though the density of astrocytes was equivalent (Figure 2). The significance of μ-crystallin expression within astrocytes along the dorsoventral axis may relate to the underlying neuronal circuity. It also suggests, but does not prove, local striatal astrocyte heterogeneity.

Form: Striatal Astrocyte Morphology and Interactions with Neurons

Morphology

Striatal astrocytes are beautifully complex cells to observe under the microscope (Figure 3A), and comprise a cell body, six or so major primary branches that emanate from the soma, multiple secondary and tertiary branches, one or more thicker aquaporin-4-laden blood vessel-associated end-feet, and myriad finer branchlets and leaflets [4]. The finest leaflets are too small to be resolved with light microscopy and are irregular, thin, and sheet-like with dimensions on the tens of nanometer scale and with high surface area:volume ratios. They lack easily observed organelles, are devoid of GFAP, but show localized calcium signaling. Thus, up to 95% of an astrocyte comprises such leaflets [54], which are a defining feature of healthy astrocytes throughout the CNS. Together, these anatomical features give

rise to the complex overall structure of astrocytes, which appear as 'bushy' cells with a clear soma when viewed with a cytosolic reporter, such as GFP, Lucifer yellow, or tdTomato [38,46,50,55] (Figure 3B). However, when viewed with a cell surface reporter, such as Lck-GFP, astrocytes appear as fluffy cloud-like cells [38], because the soma is not easily discernible.

Although astrocytes are morphologically complex (Figure 3A), they do not appear to be strongly polarized [54]. The only polarized astrocyte compartment appears to be one or more end foot-bearing branches that demarcate the vasculature. Other astrocyte structures, such as the major branches, branchlets, and leaflets, do not have currently known markers that define them as molecularly separable. For example, Kir4.1 and GLT1 appear to be highly and evenly expressed within striatal astrocytes and not restricted to specific subcellular structures that can be detected with light microscopy. This means either that synapses exist throughout entire astrocytes or that Kir4.1 and GLT1 may perform functions broadly in the neuropil. Furthermore, striatal astrocyte leaflets at excitatory synapses do not appear to contain structures, such as vesicles, to support vesicular gliotransmission [50], although this requires further study.

Spatial Interactions with Neurons

Striatal astrocytes exist at a density of ~8 in the volume of a cube with 100-μm sides (~10^6 μm^3) in Swiss-Webster mice [50]. A dye-filled striatal astrocyte has a volume of ~13 000 μm and the volume occupied by the smallest object around it (i.e., the volume that the astrocyte occupies) is ~59 000 μm^3. These numbers are likely to be different for other mouse strains and vertebrate species, as is the volume of the striatum [35]. In a large area of dorsolateral striatum of several hundred square micrometers, there are about six times more neurons than astrocytes [50]. In accord, single fluorescently labeled striatal astrocytes contact multiple MSN somata (Figure 3C), with numbers that differ depending on whether MSN somata are counted only when they are entirely contained within an astrocyte territory (~11), or are also counted if they touch at the edges of the astrocyte (~20) [50,51]. The former represents a more conservative and consistent estimate, since the edge of an astrocyte is hard to define. Among the ~11 MSMs (on average) contacting each astrocyte, approximately six are D1 MSNs and five D2 MSNs [51]; this ratio is nearly identical to the relative densities of D1 and D2 MSN within the dorsolateral striatum [51]. Furthermore, the subpopulation of striatal astrocytes demarcated by *Crym* did not interact preferentially with D1 or D2 MSN somata [51]. The dendrites of a single MSN arborize locally to an area ~400 μm diameter in a plane [56], which would thus include ~170 astrocytes based on a flattened area of ~3000 μm^2 per astrocyte [51]. The finding that striatal astrocytes are proximate to many MSN somata suggests that they regulate the extracellular milieu, as suggested by modeling in the lateral habenula [57] and by in-tissue K$^+$ measurements in the striatum [49].

Spatial Interactions with Synapses

The density of excitatory synapses in the striatum [58] is ~0.9 per μm^3, implying that single striatal astrocyte territories encompass ~50 700 excitatory synapses. The spatial interactions and approximate distances of astrocyte leaflets with such synapses has been assessed [51] with a fluorescence resonance energy transfer (FRET)-based method called the neuron

astrocyte proximity assay (NAPA) and with serial block face scanning electron microscopy (SBF-SEM), although neither method accurately reflects the extracellular space. The proximity between striatal astrocyte leaflets and presynaptic terminals of asymmetric excitatory synapses that receive input from cortex and thalamus was evaluated [59]. The average distance from the pre- or postsynaptic membrane to the membrane of the nearest astrocyte leaflet was ~50 nm (Figure 3D). The distance between astrocyte leaflets and presynaptic terminals was variable: ~53% of the closest contacts were <10 nm away and likely measurable by NAPA. The other ~47% were between 10 nm and 400 nm away (i.e., at a distance longer than that measured with NAPA, but resolvable with colocalization).

The average distance from a tyrosine hydroxylase (TH)-identified putative dopaminergic pre- or postsynaptic membrane to the membrane of the nearest astrocyte leaflet was ~250 nm. Approximately 11% of the interactions were <10 nm away, but ~89% were between 10 nm and 1100 nm away (i.e., at a distance longer than that detected by NAPA). Taken together, these data show that excitatory and TH-positive synapses in the striatum are not ensheathed by astrocyte processes. Instead, astrocyte processes make variable finger-like associations with synapses [60,61]. NAPA was also used to assess where in an astrocyte different inputs occurred: the density of inputs tracked with the complexity of the astrocyte, implying that distinct inputs do not sample observable astrocyte subdomains, at least at the resolution discernible with light microscopy. Furthermore, stimulation of cortical inputs did not alter NAPA-based FRET between astrocyte leaflets and excitatory synapses, whereas strong ischemia in brain slices did [51]. Contact between astrocyte processes and excitatory synapses was altered in mouse models of HD [51]. The simplest explanation may be that the finest astrocyte processes withdraw from corticostriatal synapses in HD model mice at early stages of pathology.

Functional Properties

In rats, three electrophysiological signatures for striatal astrocytes have been reported, each with subtly different current-voltage relations and rectification [17,18]. However, in the dorsolateral region of the mouse striatum, the current-voltage relations were essentially linear [49–52] (Figure 3E). Striatal astrocytes displayed a negative resting membrane potential (V_m) of ~ −85 mV, a low membrane resistance of <10 MOhm and a passive Ohmic current-voltage relationship [46,49–52]. They expressed Kir4.1 channels, the blockade of which increased membrane resistance and depolarized the membrane by a few millivolts, implying that additional open K^+ channels must draw the V_m towards the K^+ equilibrium potential [49,50]. If ionic conductances with positive equilibrium potentials exist for Na^+, Ca^{2+}, or Cl^-, then their contribution to V_m is dominated by K^+ even when Kir4.1 is blocked. Thus, striatal astrocytes function like Nernstian K^+ electrodes [49].

Gap-junctional coupling between astrocytes in the striatum is extensive: dialyzing a single astrocyte with dye led to spread to ~100 astrocytes within ~20 min [50], 98% of which were S100β-positive astrocytes [51]. With longer recording periods, this could be as high as 500 coupled cells [17,18]. The extent of gap-junctional coupling *in vivo* is unknown, but could alter how astrocytes regulate hundreds of MSNs within 3D volumes of coupled cells. The spread of dye between astrocytes was strongly inhibited by blocking gap junctions, and

striatal astrocytes expressed significant mRNA for connexin 43 (encoded by *Gja1*) [50,51]. The PARIS method could be used to study regulation of gap junctional coupling between astrocytes in real time [62]. There are no available data on SWELL1 channel functions in striatal astrocytes [63], although they do occur there [50].

Function: Astrocyte–Neuron Interactions in Circuits and Behavior

After astrocyte Ca^{2+} signaling was reported to be regulated by neurotransmitters [64–66], Smith hypothesized that astrocyte Ca^{2+} signals may affect the function of neural circuits [67,68]. Testing such ideas has proven challenging. The exploration of astrocyte functions within the striatum is at a nascent stage, but progress has been made. Here, I summarize studies that have explored astrocytic regulation of MSNs with clear circuit, behavioral, or disease relevance. However, channelrhodopsin is not appropriate for stimulating striatal astrocytes, because it results in transient elevations in extracellular K^+ [69]. Melanopsin may work, but has not been tried in striatum [70].

Striatal Astrocyte Ca^{2+} Signals

Striatal astrocytes display intracellular Ca^{2+} elevations, which are driven by Ca^{2+} entry across the plasma membrane and by Ca^{2+} release from intracellular stores [38,46,50,52,55]. At least three types of spontaneous Ca^{2+} signal are known. Global waves encompass the soma and large parts of astrocyte branches. Local waves are restricted and cover smaller parts of branches. Microdomains are frequent and cover only micrometer-scale areas of branches and somata. Fast signals lasting less than a few seconds in duration were not detected in striatal astrocytes [46]. Furthermore, striatal astrocytes responded poorly to corticostriatal axonal stimulation, but responded with intracellular Ca^{2+} elevations to applications of GABA, cannabinoids, ATP, phenylephrine, glutamate, and dopamine [38,46,50,52,53,55,71]. Mitochondrial Ca^{2+} signals were not evaluated, but have been reported elsewhere [72,73].

Circuit-Specific Signaling by Striatal Astrocytes

A recent study concluded that astrocytes in the dorsal striatum formed functional interactions with groups of D1 and D2 MSNs, suggesting that astrocyte subpopulations operate in a circuit-specific manner [71]. When the authors recorded from two D1 MSNs and strongly depolarized one to 0 mV for 5 s, they recorded the consequences of postsynaptic endocannabinoid release from the first MSN as an increase in astrocyte Ca^{2+} signaling and as a decrease in neurotransmitter release onto the first recorded neuron. The responses were mediated by astrocytic and presynaptic cannabinoid receptors (CB1Rs), respectively. However, in the second recorded D1 MSN, the authors measured an increase in glutamate release probability that was downstream of astrocyte CB1 receptor activation and the subsequent Ca^{2+}-dependent release of astrocytic glutamate, which activated presynaptic metabotropic mGlur1/5 glutamate receptors. This only happened when they recorded from pairs of D1–D1 or D2–D2 MSNs (i.e., homotypic pairs) [71]. This is interesting, because the D1 and D2 MSNs are intermingled with no anatomical specificity in the dorsal striatum [74]. This led the authors to propose that astrocytes form subnetworks with specific populations of MSNs. This suggestion could have important implications in the striatum, but

it should be interpreted with caution. At this point, there is no evidence to ascertain what the functional significance of such interactions could be for behavior or for *in vivo* circuit function [71]. The experiments were also reliant on 12–18-day-old mice (i.e., before gliogenesis is complete) [75]. Furthermore, striatal MSNs are well known to display prolonged postnatal development that extends to the end of Week 3 for the emergence of mature excitatory synapses and intrinsic membrane properties [76]. Although astrocytes were suggested to form subnetworks, intracellular dialysis results in coupling with many, perhaps all, astrocytes nearby [18,50]. In relation to mGlur1/5, in striatal astrocytes from adult mice, RNA-Seq and imaging instead show mGlur2/3 [38,46,50,77,78]. Astrocyte Ca^{2+}-dependent glutamate gliotransmission occurs under some settings [71], but not in others [50], in the striatum and elsewhere [79,80].

Attenuation of Striatal Astrocyte Ca^{2+}-Dependent Signaling

Intracellular Ca^{2+} signals have been explored as a possible basis for astrocyte–neuron interactions [81]. However, deletion of IP3 type 2 receptors produced few detectable behavioral effects [82–84] even though astrocyte Ca^{2+} signals were attenuated, including in the striatum [46]. To try to resolve this disconnect, another method to attenuate astrocyte Ca^{2+} signals was needed. A recent study used overexpression of a plasma membrane pump, called PMCA, and found evidence to support a role for astrocyte Ca^{2+} signaling in the regulation of neural circuit function and mouse behavior via a mechanism involving GAT-3. These findings are illustrated in Figure 4 with a current working model. Perhaps most unexpectedly, the attenuation of astrocyte Ca^{2+} signaling caused excessive self-grooming, which is related to OCD-like behaviors in humans [21]. The data showed that astrocytes regulate striatal MSNs and a specific behavior via ambient GABA-mediated neuromodulation of large volumes of brain tissue. However, further studies are needed, because the method used to attenuate astrocyte Ca^{2+} signaling lacks precise temporal resolution. Furthermore, the hPMCA2w/b approach worked well for striatum and hippocampus, but needs to be tested in other brain areas. Interpreted as a 'loss-of-function' experiment, these studies nonetheless provide evidence that astrocyte Ca^{2+} signaling is consequential for striatal function in adult mice [52].

Stimulation of Striatal Astrocyte–Neuron Interactions

Striatal astrocytes responded to Gi-coupled G-protein-coupled receptor (GPCR) activation with elevations in intracellular Ca^{2+} levels [50]. This is consistent with data for astrocytes elsewhere in the brain [78,85,86] and likely occurred by activation of phospholipase C by $G_{\beta\gamma}$ subunits and the subsequent generation of IP3. This type of response is not a peculiarity of astrocytes, and has been well documented [87]. However, the functional significance of Gi-mediated responses in the striatum were unknown. Recent progress and key findings are summarized in Figure 5. The working model suggests that MSNs communicate with astrocytes via the release of GABA during heightened activity, such as upstate transitions [53]. Such functional interactions are favored by the proximity of MSNs within single striatal astrocyte territories. The data are consistent with the hypothesis that GABA is released from MSN dendrites and activates Gi-coupled $GABA_B$ GPCRs, which are highly expressed in astrocytes [50,53]. $GABA_B$ receptor activation in turn results in the elevation of intracellular Ca^{2+} levels via release from stores. Selective activation of the Gi pathway in

astrocytes using chemogenetics (**hM4Di**) resulted in elevated *Thbs1* expression, and the resultant TSP-1 actions (a known synaptogenic molecule) increased excitatory synapse formation, enhanced MSN firing *in vivo*, and were causal for the behavioral hyperactivity and disturbed attention phenotypes triggered by the striatal astrocyte Gi pathway. The data also show that physiological activity of neurons triggers astrocyte signaling, and that signaling from astrocytes to neurons is sufficient to alter circuits and behavior in adults. This could have relevance for brain plasticity and neural network dynamics on timescales beyond fast neuronal activity alone. More conceptually, therefore, the findings suggested that complex behavioral phenotypes that currently lack mechanistic understanding in adults have an astrocytic component. However, the findings should not be overinterpreted because the responses could only be assessed 30–120 min after chemogenetic activation of the astrocyte Gi pathway [53]. It remains to be determined whether additional responses occur on faster timescales of seconds or minutes. Furthermore, the available data only assessed the dorsal striatum, and the functional relevance for other parts of the striatum are unknown.

Striatal Astrocyte–Neuron Interactions in HD

HD is a progressive neurodegenerative disorder characterized by motor, cognitive, and psychiatric symptoms. It is caused by a single genetic defect, a polyglutamine expansion in Huntingtin (HTT) that results in the expression of a mutant protein (mHTT) [30]. Although HTT and mHTT are expressed throughout the body, the striatum is especially vulnerable and undergoes marked atrophy. Striatal astrocytes express mHTT in HD mouse models and in postmortem human tissue [88,89].

Selective expression of mHTT in astrocytes led to phenotypes related to HD in mice [88,90], and mHTT deletion from astrocytes in a HD mouse model slowed progression of some disease-related symptoms [91]. Normal and mHTT-expressing human glia delivered to the striatum of HD model and control mice, respectively, ameliorated and caused HD striatum-dependent cellular and behavioral phenotypes in mice [92], including alterations in MSNs. In terms of molecular mechanisms, the expression of two astrocyte-enriched proteins (Kir4.1 and GLT1) are reduced in HD mouse models [49,93–96]. Striatal astrocytes in HD model mice also display smaller territory sizes, reduced Ca^{2+} signaling, reduced cholesterol metabolism, and altered GABA transporter function [46,51,52,97–100]. Systems biology approaches are needed to shed light on underlying astrocyte mechanisms in HD and to evaluate how they contribute to pathophysiology. The function of HTT in astrocytes and the possible role of neurotoxic astrocytes in HD are both unknown [101]. Answering these questions may provide opportunities to exploit the basic biology of striatal astrocytes and to use HD as an exemplar neurodegenerative disease (see Outstanding Questions).

Concluding Remarks

Water-breathing lamprey, the oldest group of vertebrates that diverged from mammals ~560 million years ago, appear to have putative GFAP-positive astrocytes [102] and a BG circuitry [103]. Thus, it is feasible that striatal neurons and astrocytes were in existence at about the same time as the dawn of vertebrate evolution [104] and coevolved to function in a synergistic manner. In accord, recent studies summarized herein provide evidence for striatal

astrocyte–neuron bidirectional interactions with relevance to neural circuit function, behavior, and disease. Based on these insights, I suggest that astrocytes would be amenable to local pharmacological or genetic manipulation to produce desirable effects in the circuits that drive complex disease-related behaviors. This may be particularly relevant in complex psychiatric traits, including, but not limited to, those with a neuroinflammatory component. It is likely that such ideas will be tested in the years ahead and lead us into areas of physiology hitherto not considered and, potentially, into novel therapeutics for human disorders.

Acknowledgments

Glossary

μ-Crystallin

a protein thought to bind thyroid hormone and function as a ketimine reductase.

Cre/ERT2

a tamoxifen-inducible Cre recombinase that allows controlled gene expression.

Crym

gene encoding μ-crystallin that is highly expressed in striatal astrocytes.

Glial fibrillary acid protein (GFAP)

an intermediate filament protein that is highly expressed in astrocytes in several brain regions (e.g., hippocampus) but is not well expressed in striatal astrocytes.

GLT1

a plasma membrane glutamate transporter strongly enriched in astrocytes.

hM4Di

a chemogenetic receptor used to stimulate the Gi pathway in striatal astrocytes *in vivo.* This has been used to study striatal astrocyte functions *in vivo.*

Medium spiny neuron (MSN)

frequently also called striatal projection neurons or spiny projection neurons. These represent most neurons in the striatum and are GABAergic.

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Highlights

The striatum has been leveraged for studying astrocytes systematically in a defined circuitry, and for examining how their interactions with neurons relate to specific behaviors.

Morphologically complex 'bushy' astrocytes are integral to neural circuits and evenly tile the entire striatum, the largest nucleus of the basal ganglia.

Striatal astrocytes have several specific molecular features, such as high μ-crystallin expression, low GFAP expression, and striatal astrocyte-specific gene expression.

Striatal astrocytes make extensive contacts with striatal medium spiny neuron somata and additional finger-like connections with most excitatory synapses.

Striatal astrocytes modulate medium spiny neuron synapses to alter circuit function and mouse behavior, such as causing OCD-like excessive self-grooming, via slow homeostatic functions involving ambient GABA, K^+, and synaptogenic cues.

Outstanding Questions

Are astrocytes locally heterogeneous in the striatum at molecular and functional levels?

Are specific striatal astrocytes organized anatomically in relation to either striosome or matrix compartments?

Which proteins are expressed within the plasma membrane of striatal astrocytes as well as within other physiological compartments, such as end-feet? What functions do such proteins serve?

Do subcompartment specific markers exist within branches, branchlets, and leaflets?

How do striatal astrocytes interact with striatal neurons, the vasculature, and microglia?

How do astrocytes and microglia interact to modify neuronal function during inflammatory cues?

How are striatal astrocytes affected by slow neuromodulators, such as dopamine?

Can striatal astrocytes be exploited as endogenous neuromodulators to treat disorders that involve striatal dysfunction?
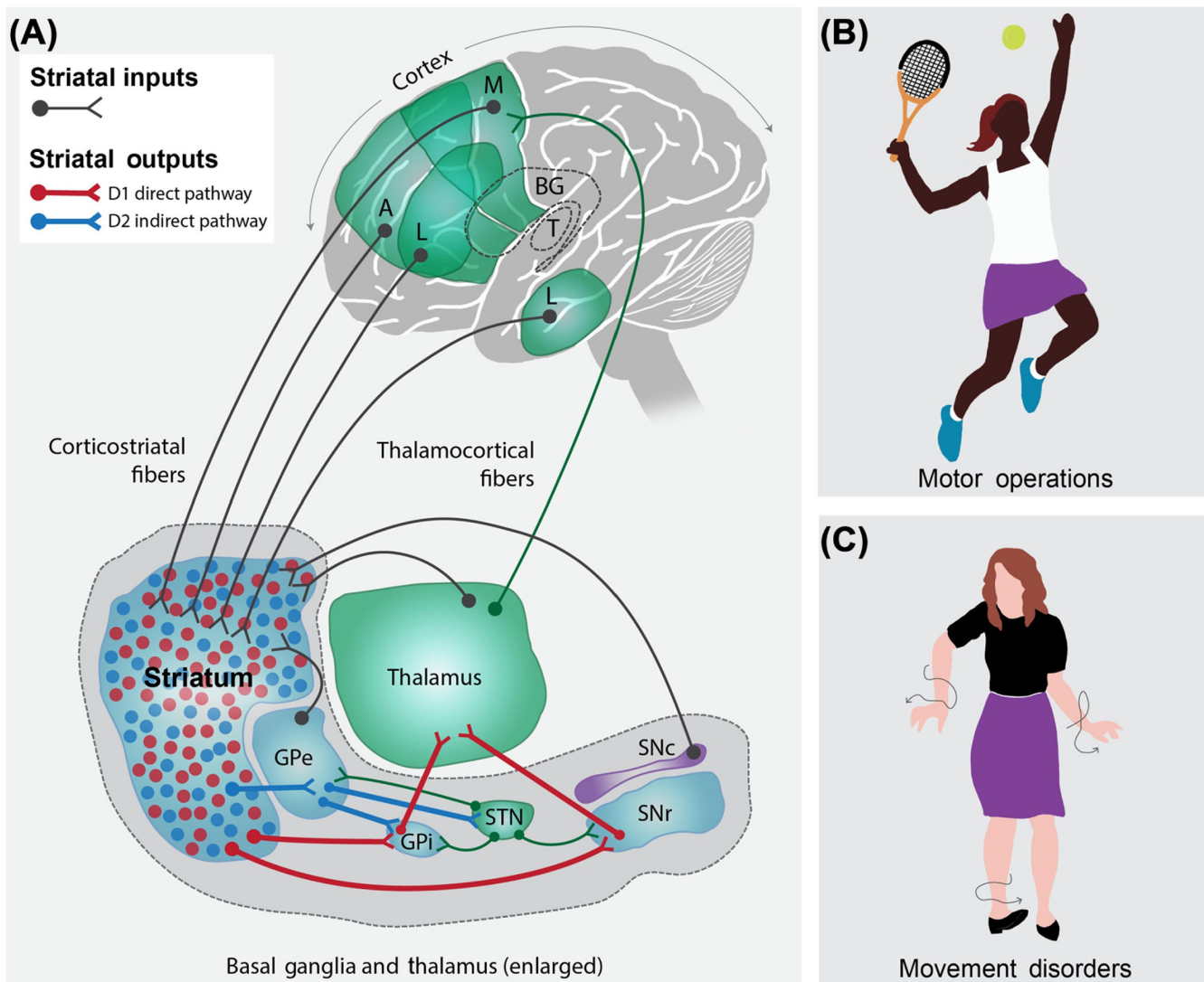
Figure 1. Simplified View of the Striatum in Relation to the Basal Ganglia Circuit and Its Known Roles in Behavior.

(A) Highly schematized cartoon illustrating the striatum in humans in relation to its synaptic inputs [thalamus (T), cortex – associative (A), motor (M), and limbic (L), substantia nigra pars compacta(SNc), and external segment of the globus pallidus(GPe)] and outputs via the basal ganglia (BG) circuitry [GPe, internal segment of the globus pallidus (GPi), and substantia nigra pars reticulate (SNr)]. The striatum receives glutamatergic excitation from multiple regions of the cortex, which impinges on D1 and D2 medium spiny neurons [MSNs (also called striatal projection neurons in some studies); colored red and blue, respectively]. These inputs project to downstream nuclei via the direct and indirect pathway formed by D1 and D2 MSNs, respectively. (B) Cartoon of a tennis player to illustrate that the striatum is intimately involved in movement, action selection, and motor operations. (C) Illustration of the chorea and abnormal movements observed in a patient with advanced-stage Huntington's disease (HD), whereby movements are uncontrolled and abnormal. Other disorders thought to involve the BG and the striatum are mentioned in the main text. For simplicity, the

striatum is shown as a single structure, although in humans it comprises the caudate nucleus, putamen, and nucleus accumbens. Abbreviation: STN, subthalamic nucleus.
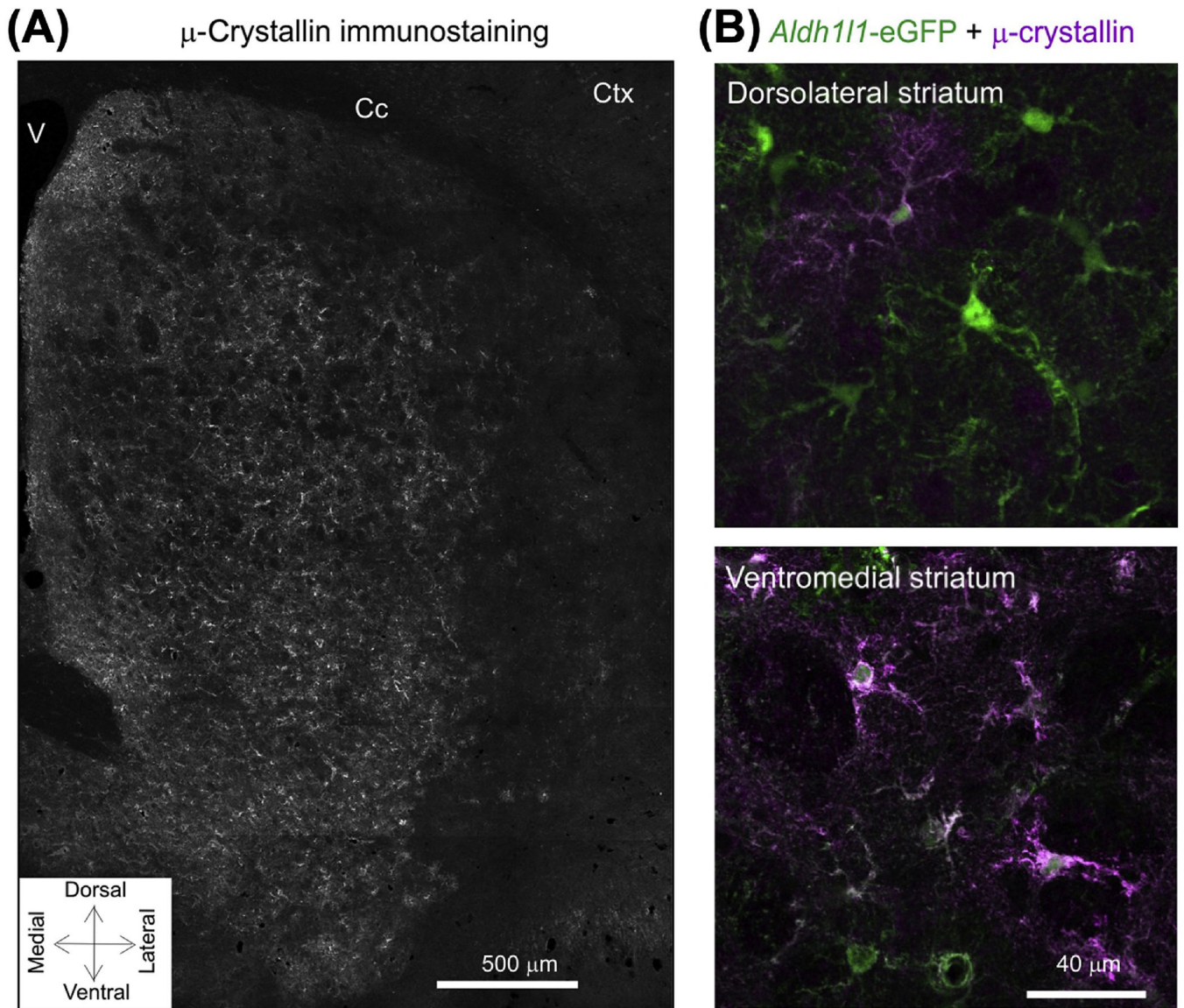
Figure 2. μ-Crystallin Displays a Gradient of Expression within Striatal Astrocytes.
(A) μ-crystallin immunostaining in striatum showing its spatial gradient. There are higher levels of expression in the ventral striatum compared with dorsal areas. (B) Representative images for μ-crystallin immunostaining in dorsolateral and ventromedial parts of the striatum in brain sections from *Aldh1l1*-eGFP mice. In the dorsolateral area, ~30% of astrocytes were μ-crystallin positive, whereas, in the ventromedial area, this was ~90%. Adapted from [50]. Abbreviations: Cc, corpus callosum; Ctx, cortex; V, ventricle.
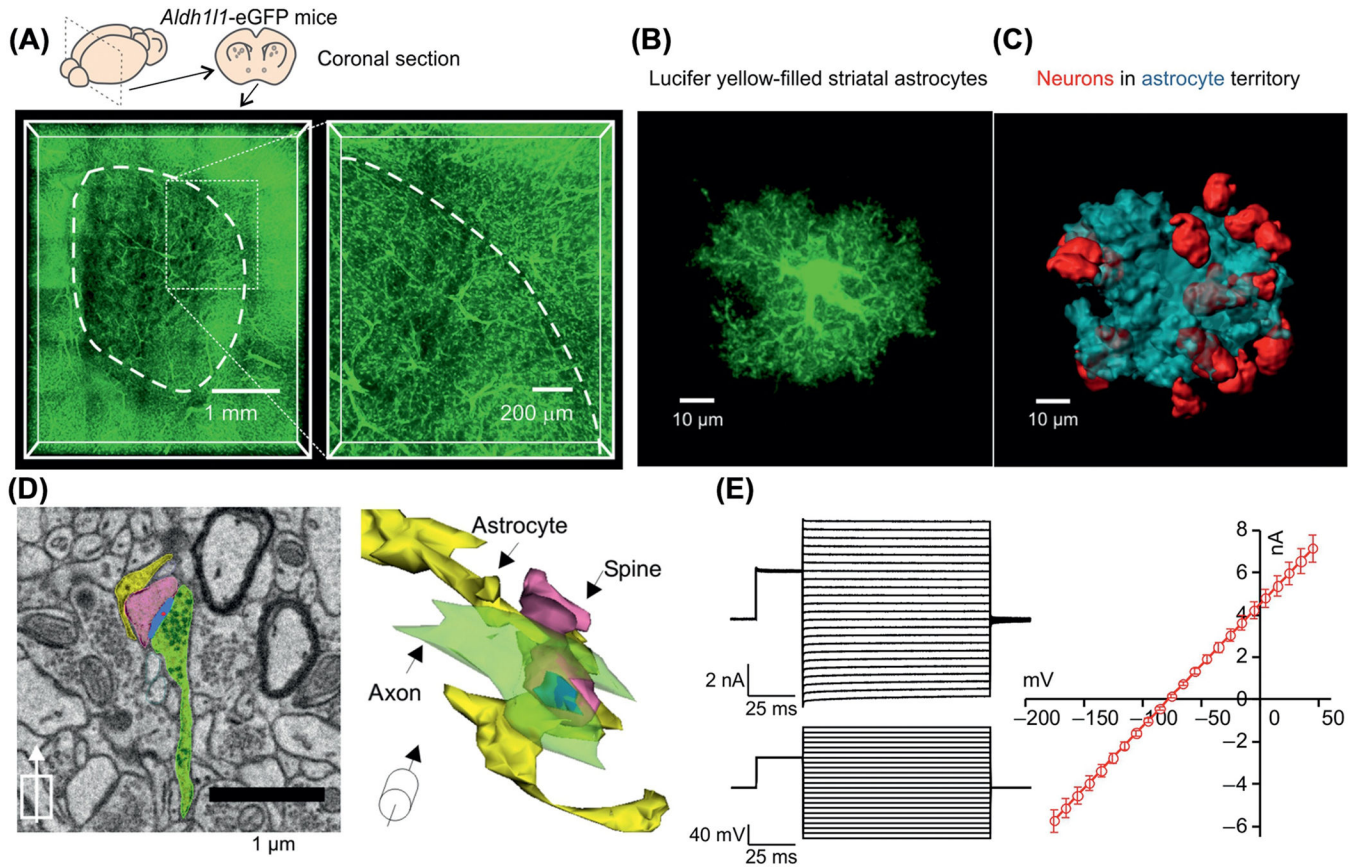
Figure 3. Properties of Striatal Astrocytes.
(A) Coronal sections of *Aldh1l1*-eGFP mouse brains cleared using the Sca/eS method and imaged using confocal microscopy to show abundant astrocytes within the striatum. The magnified section on the right shows the dorsolateral region of the striatum. Blood vessels are demarcated by their associations with GFP-expressing astrocyte end-feet. (B) Confocal volumes of a Lucifer yellow-filled striatal astrocyte. (C) 3D reconstructions of volumes enclosed by striatal astrocyte territories (blue) and NeuN (red). (D) Example of scanning electron microscopy (SEM) image from the striatum with corresponding 3D rendering displayed at an angle. The synaptic structures and closest astrocyte processes are colored as follows: yellow, astrocytes; blue, postsynaptic densities (PSDs); green, axons; and pink, spines. The center of the PSD is denoted by a red dot. (E) Representative current waveforms for a striatal astrocyte in response to stepwise changes membrane potential, along with an average current voltage relation from multiple cells. Reproduced from [50] (A–D); data in (E) from [51].
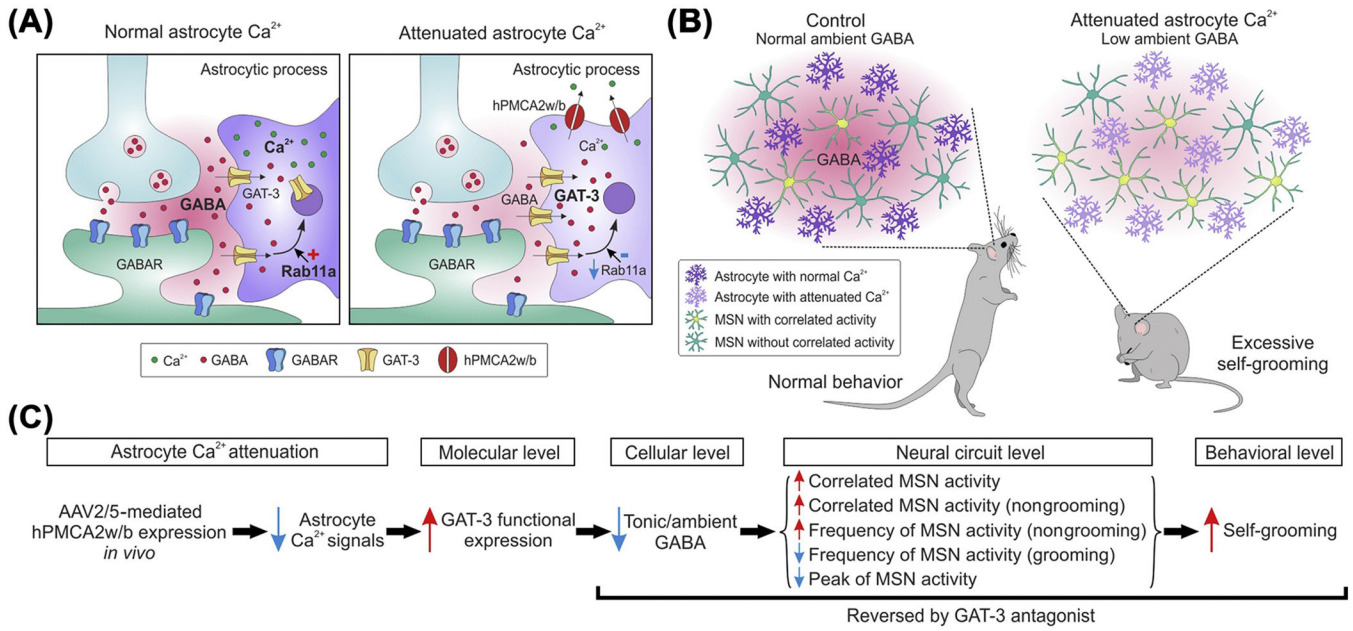
Figure 4. Working Model for How Attenuating Striatal Astrocyte Ca^{2+}-Dependent Signaling *In Vivo* Altered Striatal Neural Circuit Function with Behavioral Consequences.
The cartoon summary is of the main findings at synaptic (A) and *in vivo* levels (B). (C) Description of the proposed sequence of events. In brief, attenuation of striatal astrocyte Ca^{2+} signals reduces Rab11a, which results in increased GAT-3 functional expression. This reduces ambient γ-aminobutyric acid (GABA) levels in the extracellular space and tonic inhibition. The data are consistent with a model in which reduced tonic inhibition alters medium spiny neurons (MSN) firing and downstream circuits to cause excessive self-grooming. In accord, tonic inhibition and self-grooming were rescued by a GAT-3 antagonist. Reproduced from [52].
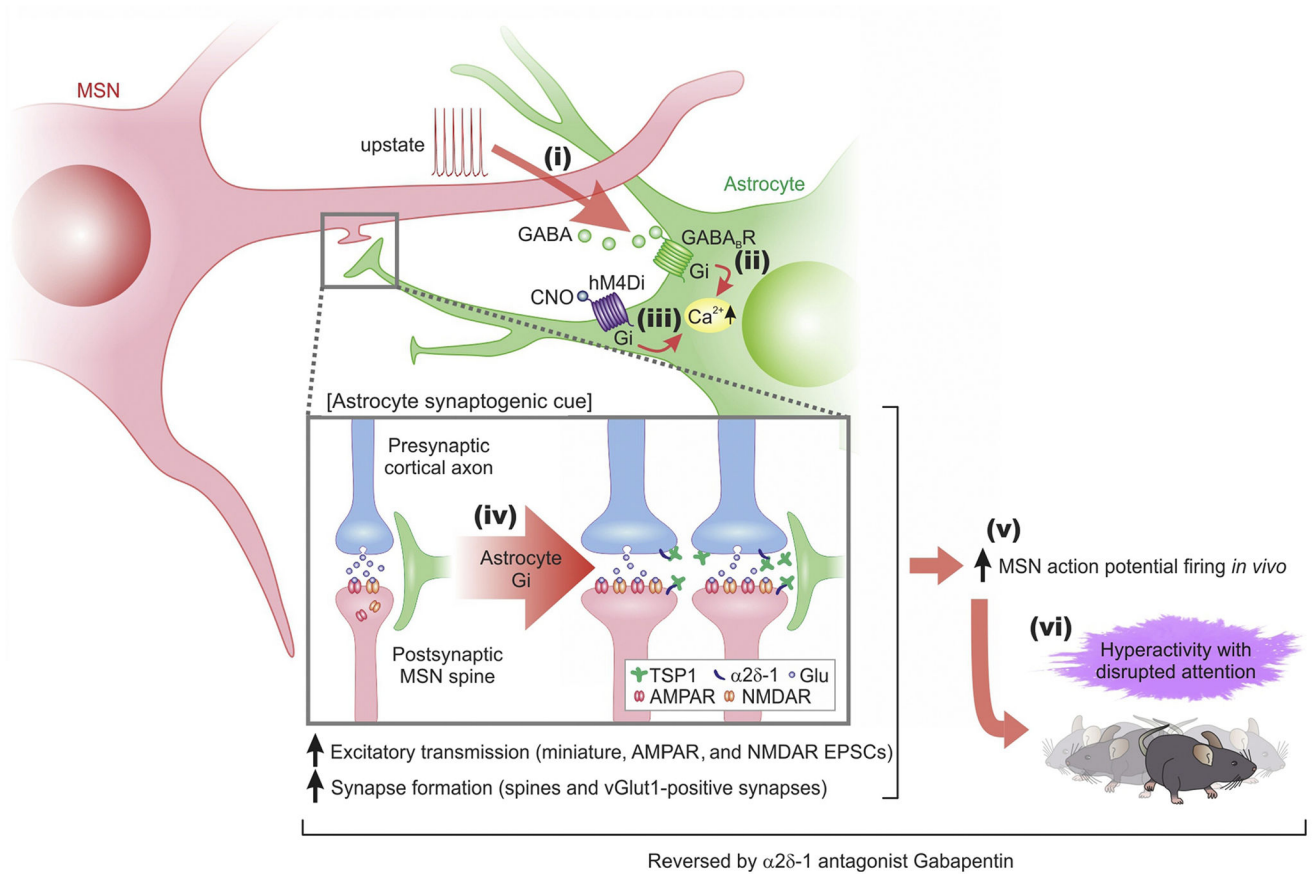
Figure 5. A Schematized Working Model for how Gi G-Protein-Coupled Receptor (GPCR)-Mediated Medium Spiny Neuron (MSN)–Astrocyte Bidirectional Interactions Affect Behavior.
When MSNs were depolarized to levels associated with upstates, they released γ-aminobutyric acid (GABA) (step i), which activated Gi-protein coupled GABA$_B$ G-protein-coupled receptors (GPCRs) on striatal astrocytes, leading to an increase in intracellular Ca^{2+} signals (step ii). Selectively stimulating the Gi pathway with hM4Di and clozapine-n-oxide (CNO) evoked Ca^{2+} signals in striatal astrocytes (step iii), upregulated the astrocyte synaptogenic molecule TSP-1, boosted excitatory synapse formation and fast excitatory synaptic transmission (step iv) and increased firing of MSNs (step v), which together resulted in hyperactivity with disrupted attention phenotypes in mice (step vi). The synaptic, circuit, and behavioral effects resulting from Gi pathway activation *in vivo* (steps iv–vi) were reduced or reversed by blocking TSP-1 actions on neuronal $\alpha 2\delta$–1 receptors with gabapentin. Reproduced from [53]. Abbreviation: EPSC, excitatory postsynaptic current.