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Neuronal and Astrocytic Primary Cilia in the Mature Brain

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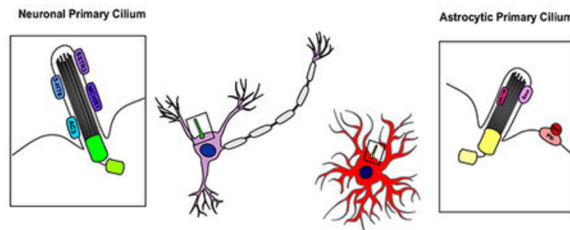
Abstract

Primary cilia are tiny microtubule-based signaling devices that regulate a variety of physiological functions, including metabolism and cell division. Defects in primary cilia lead to a myriad of diseases in humans such as obesity and cancers. In the mature brain, both neurons and astrocytes contain a single primary cilium. Although neuronal primary cilia are not directly involved in synaptic communication, their pathophysiological impacts on obesity and mental disorders are well recognized. In contrast, research on astrocytic primary cilia lags far behind. Currently, little is known about their functions and molecular pathways in the mature brain. Unlike neurons, postnatal astrocytes retain the capacity of cell division and can become reactive and proliferate in response to various brain insults such as epilepsy, ischemia, traumatic brain injury, and neurodegenerative β-amyloid plaques. Since primary cilia derive from the mother centrioles, astrocyte proliferation must occur in coordination with the dismantling and ciliogenesis of astrocyte cilia. In this regard, the functions, signal pathways, and structural dynamics of neuronal and astrocytic primary cilia are fundamentally different. Here we discuss and compare the current understanding of neuronal and astrocytic primary cilia.

Graphical Abstract



Keywords

Primary Cilia; Astrocytes; Type 3 Adenylyl Cyclase (AC3); ARL13B; Sonic Hedgehog

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Introduction

The primary cilium is a centriole-derived, membrane-ensheathed process present in most mammalian cells [1]. It relies on a highly conserved intraflagellar transport (IFT) system for trafficking select protein cargo into and out of the ciliary compartment [2]. There is a diffusion barrier at the base of the primary cilium, which restricts the free diffusion of unselected molecules into the compartment [3]. Hence, the microenvironment in the primary cilium is insulated from the main cytosolic compartment. Moreover, the volume of the primary cilium is very tiny compared to other cellular compartments, but it has a high ratio of plasma membrane to cytosol and can enrich a high density of membrane receptors. Second messengers such as cAMP can easily reach high concentrations in the narrow ciliary compartment. Given this, cilia are exquisitely sensitive to extracellular signals, including nutrients, neuropeptides, morphogens, and hormones [1, 4, 5]. As such, primary cilia are considered cellular “antennae” to detect extracellular signals [1, 4, 5].

The primary cilium emanates from the basal body residing underneath the plasma membrane, which is a special form of the mother centriole [6]. It maintains stability during interphase of the cell cycle, but dismantling the primary cilium is a prerequisite for the progression of mitosis [7]. Accordingly, primary cilia regulate cell division [7], development [4, 8, 9], and tissue regeneration [10]. Vigorous research in the past two decades has collected a great deal of evidence supporting the significance of primary cilia in both physiology and pathology [11, 12]. It is well recognized that malfunction of primary cilia causes numerous disorders, including polycystic kidney disease, obesity, cognitive impairment, developmental disorders [8], and certain cancers [11, 13, 14], which are collectively termed “ciliopathies” [11].

The brain is mainly comprised of neurons and glial cells. Glial cells in the brain include astrocytes, ependymal cells, microglia, and oligodendrocytes. It is known that neurons and astrocytes possess a single, non-motile primary cilium [8]. Ependymal cells, which line the ventricles of the brain and aid in the circulation of cerebrospinal fluid, have multiple motile cilia. Microglia, the resident macrophages in the central nervous system (CNS), do not display primary cilia [15] (see Figure 1). Primary cilia are observable in young oligodendrocyte precursor cells, but are lost as these cells differentiate [16]. Putatively, mature oligodendrocytes may also have primary cilia [17], but no direct evidence has been shown thus far. Additionally, adult neural stem cells (or astrocyte-like type 1 radial glial cells) in the subgranular zone (SGZ) in the dentate gyrus of the hippocampus and likely the subventricular zone (SVZ) of the lateral ventricles [18] possess primary cilia, which are essential for Sonic hedgehog (Shh) signaling and adult neurogenesis [19–21].

Astrocytes comprise the largest number of glia in the mature brain and maintain a great level of heterogeneity in function and morphology [22, 23]. Astrocytes perform a wide variety of functions, including regulation of synaptic function by recycling neurotransmitters [22], supplying neurons with neurotransmitter precursors [23], and regulating synaptic pruning by marking neuronal junctions for degradation via complement proteins [24]. In the event of neuropathologies, astrocytes are altered phenotypically to become reactive. When this occurs, these cells act to repair the blood brain barrier [25], support neuronal survival, and

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restore homeostasis within the brain via creation of a glial scar followed by metabolite and chemical control [26].

While astrocytes and neurons derive from the same origin (neuroepithelial cells and radial glial cells) in the early stage of neurodevelopment [27], research on astrocytic cilia in the mature brain lags far behind that of neuronal cilia. Neuronal primary cilia in the adult brain have been the most extensively studied compared to other cilia [4, 8, 17, 28, 29]. They have been found to regulate metabolism [13, 30, 31], mood state [32], and cognitive function [33, 34]. To date, very little, except for a role in adult neurogenesis, is known regarding the function and molecular pathways of astrocytic primary cilia in the mature brain. Nevertheless, as postnatal astrocytes maintain the ability to proliferate throughout life [25, 26, 35–37], astrocyte cell division should occur in coordination with the resorption and ciliogenesis of primary cilia. Herein represents a fundamental difference between neuronal and astrocyte cilia. Neuronal primary cilia in the brain have been well described in several elegant review articles [4, 17, 28, 38]. Here in this review, we aim to describe some fundamentals related to primary cilia and compare neuronal and astrocytic primary cilia in the adult brain (Summarized in Table 1).

Cilia Markers and Transgenic Mouse Strains to Label Primary Cilia with Fluorescent Proteins or Calcium Sensors

The necessity for visualizing primary cilia and detecting ciliary molecules has grown as studies on primary cilia have rapidly expanded. To date, primary cilia in the brain can be visualized in immunofluorescence staining using commercial antibodies to target type 3 adenylyl cyclase (AC3), type 3 somatostatin receptor (SSTR3), ADP-ribosylation factor-like 13B (ARL13B) [15], type 6 serotonin receptor (5-HT6) [39], and melanin-concentrating hormone receptor 1 (MCHR1) [40]. However, it has been recognized that these cilia markers are not ubiquitously expressed, and their expression levels can vary. For example, 5-HT6 has a focal expression and is enriched in neuronal primary cilia in the striatum region [39]. MCHR1 expression is detected in neuronal primary cilia in the hippocampus, amygdala, piriform cortex, and hypothalamus [40, 41]. In a comparison of primary cilia markers, Sipos et al. showed regional differences in expression of AC3, ARL13B, and SSTR3 [15]. A high level of AC3 expression in the primary cilia of mature neurons was detected. In contrast, AC3 is only faintly expressed in a small number of glial fibrillary acidic protein (GFAP)-positive astrocytes and absent in adenomatous polyposis coli (APC)-positive oligodendrocytes and ionizing calcium-binding adaptor molecule 1 (IBA1)-labeled microglia [15] (confirmed by our observations, see Figure 1). SSTR3 was found to be expressed in mature neuronal primary cilia [15]. Several studies have demonstrated that SSTR3 is co-expressed with AC3 in neuronal primary cilia in the hippocampus [34] and the olfactory bulb [42]. It is noteworthy that AC3 is also distributed to other tissues and is highly expressed in olfactory sensory cilia, primary cilia of kidney epithelial cells [43], and primary cilia of brown and white adipose tissue [44]. Generally, AC3 is a commonly accepted neuronal primary cilia marker protein and ARL13B antibody is commonly used to label astrocytic primary cilia (Figure 1), but neither is universally localized in primary cilia. A study focusing on visualization of astrocytic primary cilia in mice showed differential

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expression based on age [45]. This study detected a high expression of AC3 in the primary cilia of both astrocytes and neurons ten days after birth, but by postnatal day 56, neuronal primary cilia highly expressed AC3 and astrocytic primary cilia showed high levels of ARL13B expression [45].

Utilizing cilia-specific proteins, strains of mice with fluorescent proteins restricted to primary cilia have been generated. These strains have proven effective in visualizing cilia or detecting calcium levels. For example, O'Connor et al. generated an inducible cilia-GFP model of mice to enable direct visualization primary cilia [46]. This strain linked GFP with the ciliary protein SSTR3. In observations of deeply anesthetized adult mice, primary cilia were found to be expressed in numerous cells within the mature brain, including neurons and in choroid plexus epithelial cells [46]. In 2016, Delling et al. developed an imaging tool to study calcium signaling in response to mechanical force. They engineered a strain of mouse with ARL13B marked with mCherry and genetically encoded calcium indicator 1.2 localized in primary cilia. This tool allows for detection of calcium influx into ciliary compartment responding to mechanical stimuli. They discovered that calcium signaling is not responsible for the mechano-sensation of renal cilia in both embryonic and adult murine cilia models [47]. Additionally, in 2015, Bangs et al. conducted a study using a strain of mice that had both primary cilia and centrosomes labeled with mCherry and GFP, respectively. This model, *ARL13B-mCerry;Centrin2-GFP*, allowed for visualization of primary cilia and centrioles throughout embryonic development. Using that strain, primary cilia were found to arise from epiblast cells at E6, and cells arising from the visceral endoderm and trophoectoderm maintained centrosomes, but lack cilia through development [48]. The *ARL13B-pmCerry;Centrin2-GFP* strain marks primary cilia of many epiblast-derived cells including neuroepithelial cells in the neural tube very clearly [48]. However, we observed that although ARL13-mCherry also labels astrocytes very well, but not well on neurons, in the mature brain. Thus, ARL13B is not a ubiquitous ciliary maker in mature. Presently, research tools to visualize primary cilia or detect ciliary molecules are far from adequate. Consequently, there remains the need to develop novel tools (or mouse strains) to detect key messenger molecules in cilia such as cAMP, as the explorations of primary cilia grow.

Ciliogenesis

Ciliogenesis is the process by which the microtubule-based cilium arises from the basal body within the cell [49]. Ciliogenesis is generally inhibited in actively dividing cells [50]. Initiation of ciliogenesis requires that a cell exit its mitotic cell cycle to allow the centriole to dock at the plasma membrane by fusing with a ciliary vesicle [49]. At this point, IFT begins to transport protein complexes in anterograde and retrograde directions to promote the growth of the cilium [51]. Due to the terminally differentiating nature of neurons and the consequential loss of mitotic ability [52], there is little need for neuronal primary cilia to destabilize and retract into the soma. This results in the absence of *de novo* ciliogenesis in mature neurons. Thus, studies exploring neuronal ciliogenesis are primarily limited to the early embryonic development stage or in cultured cells.

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The ciliogenesis of astrocytes occurs in the embryonic development stage as well as in the mature brain. Human astrocytes have five stages of ciliogenesis [53, 54]. The first stage can be identified by the localization and fusion of vesicles with the distal end of the basal body. During the second stage, the non-motile 9+0 axoneme begins to arise from the basal body. This structure continues to grow in the third stage and fuses with the plasma membrane. At this point, a ciliary bud can be detected on the surface of the cell. The fourth stage consists of continued growth and extension of the cilium past the surface of the cell due to IFT. In the fifth and final stage of astrocyte ciliogenesis, a fully formed primary cilium can be observed on the surface of the cell [54, 55]. Once fully mature, astrocyte-like neural precursors display a primary cilium with a length significantly shorter than that of a neuronal primary cilium [56] (see table 1). Moser et al. also explored the potential effects of disruption of the mammalian processing body, namely the GW/P body, on ciliogenesis of human astrocytes [57]. Greater than half of transcriptional silencing of mRNA occurs within GW/P bodies. The early stages of ciliogenesis were observed in astrocytes studied in this experiment, but GW/P small interfering RNA-transfected cells failed to display a matured cilium that extended properly out of the soma. This study demonstrates that inhibition of GW/P body components and the RNAi microprocessor disrupts ciliogenesis of astrocytes [57]. To date, there is limited understanding of the structural dynamics of astrocyte cilia. We postulate that elucidating the molecular mechanisms of ciliogenesis will help unravel the contributions of astrocyte cilia to astrocyte reactivity and pathology in the mature brain.

Primary Cilia are Required for Sonic Hedgehog Signal Transduction in Vertebrate Cells

Shh signal transduction is essential for proper embryonic development and morphogenesis of vertebrates. The components within this pathway are required for patterning and organization of the notochord, floorplate, and cells within the zone of polarizing activity [58]. This patterning is needed for embryonic organization of tissue that is required not simply for symmetry, but also appropriate organogenesis. Hence, the Shh pathway is necessary for proper CNS development. Conceivably, malfunction of Shh pathway is also implicated in certain forms of cancer [59]. Shh mediates cell signaling and also regulates cell survival. The best-known components in the Shh pathway are the Shh ligand, Smoothened (Smo), and Patched1 (Ptch1) [59]. Binding of Shh to Ptch1 leads to translocation of Smo from the plasma membrane into primary ciliary compartment and activation of the Shh pathway, promoting the Gli transcription factors to translocate to the nucleus, where they can stimulate cell proliferation and encourage cell survival [60].

In vertebrate cells, primary cilia constitute key modulators for Shh transduction, which is essential for embryonic development of neural cells [61]. In the developing notochord, neural progenitor cells are guided in proliferation and differentiation by the Shh signaling [61]. Many key components of the Shh pathway localize in the primary cilium and can shift regionally upon receipt of the Shh ligand [62]. Patched1 localizes in the primary cilium in the absence of Shh and regulates the activity of Smoothened [63]. *Suppressor of Fused*, a downstream regulator of this pathway, localizes in the tip of the primary cilia of cells in the neural tube [61]. The G-protein-coupled receptor GPR161 is expressed on the primary

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cilium in the neural tube and can suppress Shh signaling and inhibit the growth of medulloblastomas [64].

Extensive evidence has underscored the importance of primary cilia in Shh signaling. Transgenic mice lacking cilia in astrocyte-like neural precursors have abnormal development and disruption in Shh signaling [56]. Disturbance of ARL13B in primary cilia can result in lessened Shh signaling in mouse medulloblastoma cultures [65]. Ablation of ciliary genes or Smoothened results in interrupted development of radial astrocytes (the postnatal progenitors in the dentate gyrus), while constitutive expression of these genes results in an enlargement of the dentate gyrus [66]. SAG, a Shh agonist, can act in a protective fashion via ciliary signaling for astrocytes under starving conditions [67]. However, most research focuses on the aspect of neurodevelopment or neurogenesis (and adult neurogenesis). To date, there are few reports directly addressing the Shh pathway in neuronal and astrocytic primary cilia in the mature brain. This is possibly because the Shh pathway in neuronal primary cilia is more prominent in neurodevelopment, not so much in adulthood, and astrocyte cilia in the mature brain is generally under-studied.

Potential Function of Astrocytic Primary Cilia in the Mature Brain

Many studies have emphasized the importance of primary cilia in cell division [4, 8, 9]. Evidence is emerging to associate astrocyte cilia with brain pathophysiology, particularly brain tumorigenesis. As primary cilia must first destabilize and retract into the cell to allow for the movement of the centriole and cell division, inability to stabilize the primary cilium can be related with abnormal proliferation of cells. Indeed, dysfunction of the primary cilium is recognized in the development of some types of tumors [12, 64, 68]. For example, glioblastomas, brain tumors arising from abnormal proliferation of astrocytes, are highly related to loss of or malformed primary cilia [68]. In an experiment characterizing the tissue of seven human glioblastoma samples from mature brains [53], defects in the early stages of ciliogenesis of astrocytes have been identified. These abnormalities included absent vesicle pairs, non-mature axonemes, and other morphological differences that impeded ciliary maturation and normal function. Lisophosphatidic acid receptor 1 (LPAR1), a G-protein coupled receptor, localizes on the primary cilia of astrocytes, but when the primary cilium is absent, LPAR1 transitions into the plasma membrane. This regionalization is accompanied by an increase of association with $G\alpha_{12}$ and $G\alpha q$ [68], which have been reported to be associated with cancer proliferation [69]. Consequently, glioblastoma proliferation is enhanced [68].

Remarkably, adult neurogenesis is well recognized as occurring in the mature brain [18]. Generation of new neurons arises from adult neural stem cells in the subventricular zone and dentate gyrus, rather than from neurons [18]. Adult neural stem cells exhibit glial nature [27] and astrocyte-like adult neural stem cells are maintained in the subgranular layer (SGL) of the dentate gyrus (DG) and in the subventricular zone (SGZ) in the mammalian brain, and they give rise to new neurons in the adult mammalian brain throughout life [18, 70]. The primary cilium and Shh signaling of adult neural stem cells were found to be required for adult neurogenesis and regulate the proliferation of progenitors in the adult hippocampus, implicating in learning and memory [19]. Moreover, the dendritic refinement and synaptic

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integration of adult-born neurons in the dentate gyrus's subgranular zone was reported to be modulated by primary cilia [71].

In contrast to neurons, which terminally differentiate and lose proliferative capacity upon maturation, astrocytes throughout the brain maintain the capacity of cell division following differentiation and new astrocytes are continuously generated during postnatal development. Astrocytes have the ability to become reactive and a proportion of reactive astrocytes proliferate in response to various neuropathological conditions such as ischemia, traumatic injury, and epilepsy, as well as neurodegenerative amyloid plaques [25, 35, 72, 73]. During the process of astrocyte proliferation, astrocyte primary cilia need to be resorbed to liberate the centriole and allow the centrosomes to form the mitotic spindle. Thus, astrocyte cilia are conceivably subjected to a dynamic change in conjunction with astrocyte proliferation. However, to date, how astrocytic primary cilia modulate astrocyte reactivity *in vivo* and how astrocytic primary cilia dynamically change their structure in accordance with astrocyte proliferation remain to be elusive. We postulate that investigation into molecular fundamentals of astrocyte cilia in the context of reactive astrogliosis will advance our understanding of how the "antenna" of astrocytes senses pathological milieu and how astrocyte cilia regulate the initiation and termination of astrocytes.

ARL13B, a Ciliary Protein Essential for Cilia Structure, is Implicated in Joubert Syndrome and Developmental Abnormalities

ADP-ribosylation factor-like 13B (ARL13B) is a member of the monomeric small GTPase superfamily [74, 75] and selectively expressed in many primary cilia including astrocytic primary cilia [45]. ARL13B is required for ciliogenesis in certain organs [76]. Studies have shown that ARL13B modulates ciliary protein trafficking and cilia length [62, 77], and supports connectivity between neurons [78]. ARL13B is required for radial glial polarity, disruption of which causes abnormal formation of the cerebral cortex [79]. Notably, ARL13B also regulates ciliary targeting of inositol polyphosphate-5-phosphatase E (INPP5E) [80]. INPP5E is a ciliary protein which prevents actin polymerization in primary cilia [81]. Prior to ciliary destabilization, INPP5E is depleted in cilia, allowing for the re-localization of phosphatidylinositol 4,5-bisphosphate into the primary cilium, which triggers actin polymerization and cilia decapitation, and consequently drives the cell cycle [81].

Joubert syndrome is an autosomal recessive ciliopathy featured by the "molar tooth sign" [82] and characterized by dysfunction in different viscera, eyes, digits, and the brain [74, 83]. ARL13B mutations cause Joubert syndrome, which was first identified in two families [74, 84]. Joubert syndrome patients demonstrate cerebral disorder, mental retardation and developmental delay as well as other variable common-shared ciliopathy manifestations such as cystic kidney, blindness, and polydactyly. Many mutations in ARL13B have been identified in Joubert syndrome patients, all having impeded neural development to some degree. A homozygous missense variant c.[223G>A] (p.(Gly75Arg) identified in the ARL13B gene cause intellectual disability, ataxia, ocular defects, and epilepsy in Jourbert syndrome patients [85]. Interestingly, ARL13B-c.[223G>A] (p.(Gly75Arg) displayed a marked loss of ARL13B guanine nucleotide-exchange factor activity, with retention of its

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GTPase activities [85]. A crystal structure of *Chlamydomonas rheinhardtii* ARL13B shows that R79Q as well as R200C, two missense ARL13B mutations identified in Joubert syndrome patients, are involved in stabilizing important intramolecular interactions [86].

ARL13B also plays a critical role in supporting the activity of Shh signaling and acts to regulate the trafficking of its signaling components into the primary cilium [87]. It can induce cell proliferation and survival [87]. Given the localization of Shh components to primary cilia [16, 62], disruption of ARL13B results in abrogated Shh signaling [62]. Consequently, ablation of ARL13B leads to medulloblastoma formation, due to the correlated deletion of cilia and Shh components [65].

AC3, a Key Enzyme Mediating the cAMP Signaling in Neuronal Cilia, is Associated with Obesity and Psychiatric Diseases

Neuronal primary cilia are commonly fortified with G protein-coupled receptors (GPCRs) [40] including Smoothened, SSTR3, 5-HT6, MCHR1, and many others [88]. Some types of GPCRs, such as Smoothened, do not continuously reside in primary cilia and can transition into and out of the cilium [88]. Interestingly, at this time all ciliary GPCRs are found to be either Gαs- or Gαi-protein coupled receptors, which depend on adenylyl cyclases (ACs) to generate cAMP and transduce a signal into the cell. AC3 is widely recognized as a key enzyme mediating the cAMP signaling in neuronal cilia [89]. AC3 was first established as an obligate protein in olfactory cilia mediating olfactory signal transduction in the main olfactory epithelium [90–92]. In 2007, Bishop et al. reported that AC3 predominantly localizes to neuronal primary cilia throughout the mature mouse brain [93], expanding the AC3's fame originally as the olfactory adenylyl cyclase to a well-known neuronal cilia marker in the CNS.

The most prominent function of AC3 in the CNS is to regulate metabolism [94]. Many lines of evidence support the pathological role of AC3 in obesity. First, numerous human genetic analyses have clearly defined *ADCY3* as a gene associated with obesity [95–101]. Second, obesity is also one of the most common symptoms for ciliopathies including Bardet-Biedl Syndrome [9, 102], and Joubert Syndrome [83]. Third, both conventional and tamoxifen-induced conditional AC3 KO mice exhibit adult-onset obesity [44, 103]. Conversely, a gain-of-function mutation of AC3 in mice can protect the animals from diet-induced obesity [104]. Hence, AC3 plays a critical role in regulating energy balance, and defects in the ciliary cAMP pathway lead to obesity [89, 94]. This is in line with the evidence demonstrating that neuronal primary cilia play a critical role in regulating energy balance. Specifically, ablations of many ciliary proteins including KIF3a [105], Bbip10 [106], IFT88 [107], Tubby [108], BBS1, and BBS4 [107, 109] all lead to obesity in mouse models [89].

However, it is not well understood how cAMP in neuronal primary cilia regulates energy balance. It has been postulated that AC3 functionally couples to melanocortin 4 receptor (MC4R) in the hypothalamus [89, 103], because activation of adenylyl cyclase by alpha-melanocyte stimulating hormone (α-MSH) downstream in the leptin pathway is required for the anorectic activity of leptin[110, 111]. Siljee at al. have recently shown that MC4R co-localizes with *ADCY3* at the primary cilia of a subset of neurons of the paraventricular

nucleus (PVN) of the hypothalamus [31]. They also discovered that obesity-associated MC4R mutations impair ciliary localization and that suppression of cAMP production using GPR88, a constitutively active version of the cilia-specific Gαi-protein-coupled receptor [112], at the primary cilia in these neurons increases body weight. The findings of Siljee et al. suggest that MC4R may couple to AC3 and positively regulate cAMP generation in neuronal primary cilia in a subset of neurons in the PVN, and impaired cAMP signaling in the primary cilia of MC4R-positive neurons lead to obesity [31]. However, further research is warranted to clearly address how leptin, α-MSH, MC4R and ciliary cAMP signaling in different sublocations of the hypothalamus hook up to regulate feeding and energy balance.

Another pathological role of AC3 relates to mental disorders. A genome-wide association study based on over five thousand patients with major depressive disorder (MDD) and healthy subjects identified AC3 (*ADCY3)* as a top-ranked gene for MDD [113]. In animal model, we discovered that constitutive AC3 knockout (KO) mice exhibit strong depression-like phenotypes in several behavioral assays [32]. Disturbances of sleep including alterations in sleep architecture and increased REM sleep are one of the core symptoms associated with MDD [114]. Our sleep analysis based on electroencephalogram-electromyogram showed that AC3 KO mice have altered sleep patterns characterized by an increased percentage of rapid eye movement sleep [32]. Moreover, MDD also is associated with neuronal atrophy [115]. We further found that basal synaptic activity at CA3-CA1 synapses was significantly lower in AC3 KO mice, and they also exhibited attenuated long-term potentiation as well as deficits in spatial navigation [32]. Conditional knockout mice with AC3 ablated in the mature brain also exhibit depression-like phenotypes [32]. In addition to MDD, human genetic studies have also associated AC3 with autism [116, 117] and intellectual disability [118], Although the underlying molecular mechanisms remain to be elucidated. In summary, AC3 in the mature brain mediates olfactory signal transduction, regulates energy balance and mood state, and contributes to psychiatric diseases.

Conclusion

Neurons are terminally differentiated excitable cells that lose mitotic ability in maturity [52]. Neuronal primary cilia are relatively stable and apparently lack de novo ciliogenesis. Moreover, no synaptic structures, ionotropic glutamate receptors, or $GABA_A$ receptors have been identified in neuronal primary cilia thus far. But many types of GPCRs have been found in neuronal primary cilia [88] (Table 1). Therefore, neuronal primary cilia mostly depend on these metabotropic receptors and downstream effector proteins to send a signal to regulate neuronal activity [89]. Hence, AC3 represents a key enzyme to mediate the cAMP signaling in neuronal primary cilia in the mature brain and regulate energy balance, mood state, and probably cognitive function.

In contrast, astrocytes are non-excitable cells and do not electrically wire to one another via chemical synapses. They are not terminally differentiated cells and maintain proliferative ability throughout life (Table 1). Presently, little is known about the function, signaling pathways, and structural dynamics of astrocytic primary cilia in the mature brain, although astrocytes fulfill a wide range of functions including providing trophic support, maintaining homeostasis, and protecting neurons from acute insults or brain injury [36]. Since astrocytes

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can proliferate under certain pathological conditions [26], astrocytic primary cilia are not static but subject to dynamic changes. Hence, it is not surprising that ARL13B, a protein regulating cilia protein trafficking, Shh pathway and cell division, prevails over AC3 in astrocytic cilia as a protein marker.

In summary, the function, molecular markers, signaling pathways, and structural dynamics of neuronal primary cilia and astrocytic cilia are fundamentally distinct (Table 1). Conceivably, ciliary GPCR- and AC3-mediated cAMP signaling in neurons provide excellent targets to design therapeutics to combat obesity, depression and cognitive disorders. In the long term, research on astrocytic primary cilia will provide useful clues to intervene in astrocyte-proliferation and reactive astrogliosis to combat various neuropathologies such as ischemia and brain injury.

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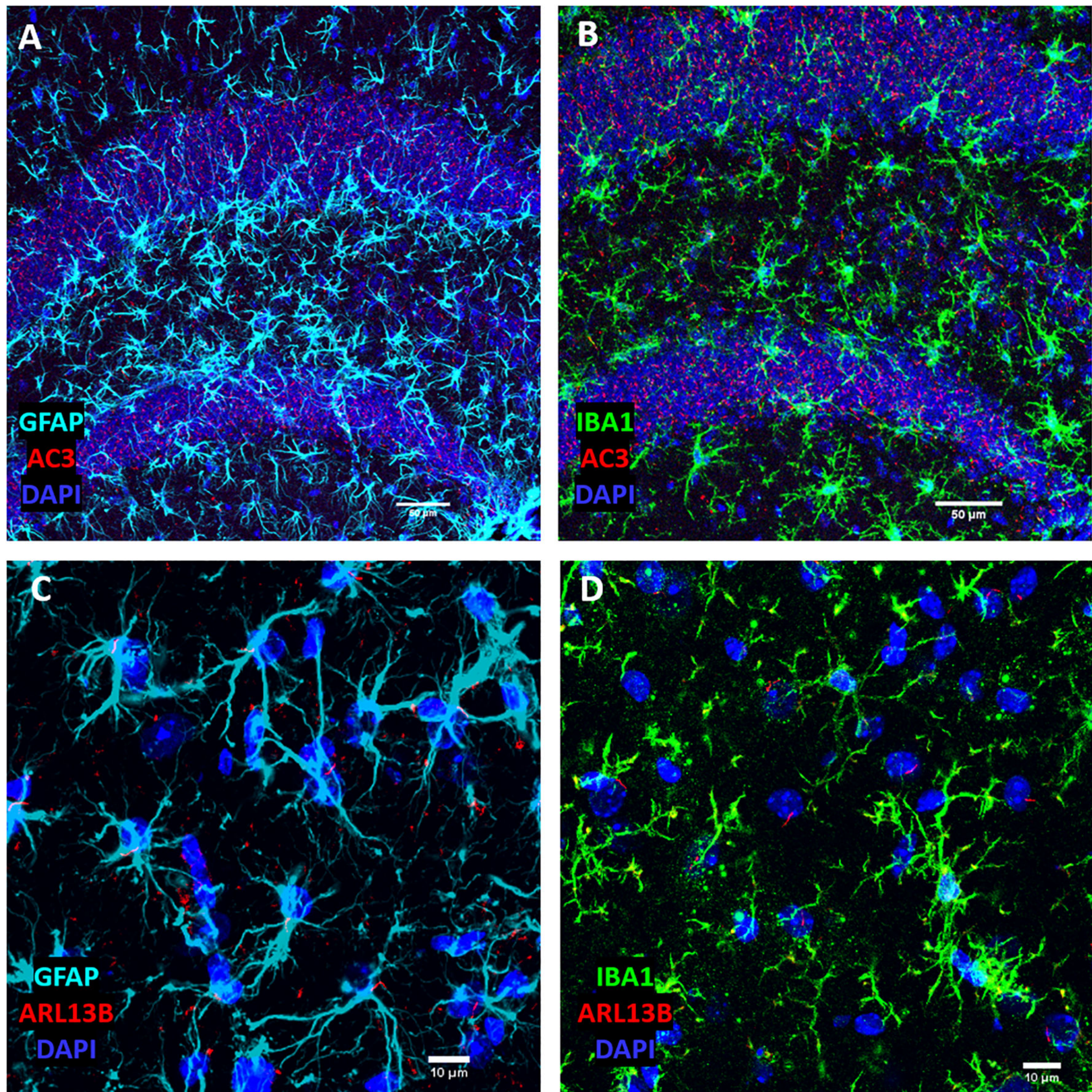

Figure 1.

Neuronal and astrocytic primary cilia are marked by AC3 and ARL13B, respectively, while microglia do not posess primary cilia. **(A)** AC3 is highly expressed in neuronal primary cilia, but not well expressed GFAP-labeled astrocyte cilia. **(B)** IBA1-marked microglia lack AC3-stained primary cilia. **(C)** ARL13B is highly expressed in astrocyte primary cilia. **(D)** IBA1-marked microglia lack ARL13B-stained primary cilia. All images were taken from the mouse hippocampus.

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Table 1.

Comparision of Neuronal and Astrocytic Primary Cilia in the Mature Brain

Features	Neurons	Astrocytes
Origination	Ectoderm [119] Neuroepithelial Cells and Radial Glial Cells [27]	Ectoderm [119] Neuroepithelial Cells and Radial Glial Cells[27]
Excitability	Excitable	Non-excitable
Connectivity	Highly connected via synapses	Not wired to one another via chemical synapses; albeit gap junctions found [120, 121]
Differentiation	Terminally differentiate upon maturation	Regional differentiation & become reactive in response to insults
Proliferative Capacity	Lose mitotic ability in maturation [52]	Maintain proliferative capacity throughout life [26]
Primary Cilia Markers	AC3 (Figure 1), SSTR3, 5-HT6, & MCHR1 [15]	Arl13B (Figure 1) [15]
AC3	Highly expressed in neuronal primary cilia [45]	Faintly expressed in immature astrocyte cilia, few in adult astrocyte cilia [45]
ARL13B	Faintly expressed in immature neurons, not prominent in mature neurons [45]	Highly expressed in astrocyte primary cilia in the mature brain [45] (Figure 1)
Cilia Length (Hippocampus)	5.0–5.91 μm (AC3-positive) [15]	2.8–3.2 μm (ARL13B-positive) [15]
Shh Components	Smoothened [17], Patched1 [63], GPR161 [64,56] and Gli transcription factors [63] detected in primary cilia in the neural tube. Presence in primary cilia of mature neurons not shown.	Smoothened and Patched1 detected in primary cilia of astrocytes in the postnatal brain [122]
Structural Dynamics	Relatively stable	Subject to dynamic change during astrocyte proliferation
Ciliogenesis	Research confined to embryonic neuronal ciliogenesis; Lack of *de novo* ciliogenesis in mature neurons	5 known stages of ciliogenesis in adult astrocytes [53, 54]
Ciliary Disease Implications	Obesity, cognitive impairment & mental disorders [89]	Astrocytoma/glioblastoma [53, 54]