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Primary cilia in the postnatal brain: Subcellular compartments for organizing neuromodulatory signaling

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Abstract

Primary cilia have well characterized roles in early brain development, relaying signals critical for neurogenesis and brain formation during embryonic stages. Less understood are the contributions of cilia-mediated signaling to postnatal brain function. Several cilia-localized receptors that bind neuropeptides and neurotransmitters endogenous to the brain have been identified in adult neurons, but the functional significance of signaling through these cilia-localized receptors is largely unexplored. Ciliopathic disorders in humans often manifest with neurodevelopmental abnormalities and cognitive deficits. Intriguingly, recent research has also linked several neuropsychiatric disorders and neurodegenerative diseases to ciliary dysfunction. This review summarizes recent evidence suggesting that cilia signaling may dynamically regulate postnatal neuronal physiology and connectivity, and highlights possible links among cilia, neuronal circuitry, neuron survival, and neurological disorders.

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Introduction

With a few exceptions, microtubule-based primary cilia are found as solitary organelles projecting from the surface of nearly every mammalian cell type including neurons and glia [1]. These immotile cilia are compact signaling structures that concentrate a variety of signaling machinery and are well-suited to transduce extracellular and environmental stimuli. Indeed, a key breakthrough in establishing the importance of these structures in broadly regulating cellular development and homeostasis in mammals was the finding that cilia are essential for transduction of the developmental morphogen Sonic hedgehog (Shh) [2], and the subsequent implication of aberrant ciliary signaling in human pathophysiology [3]. Although the presence of cilia in the brain had been described as early as the 1950s, initial efforts focused primarily on the roles of cilia in transducing external stimuli in sensory neurons, and the roles of these organelles in other neuron types and glia remained largely unexplored for decades.

Subsequent work has now described critical developmental roles for ciliary signaling in brain patterning, neurogenesis, neuronal migration and maturation, neurite outgrowth, and circuit integration both during embryonic development and in adult neurogenic niches [1,4,5]. However, less appreciated is the fact that cilia are not only retained on postmitotic neurons and astrocytes at all postnatal stages through adulthood, but continue to serve critical neuromodulatory functions throughout an animal's lifespan. Characteristic features of many disorders arising from disrupted cilia function (collectively termed ciliopathies) include neurodevelopmental defects, cognitive deficits, obesity, and neurodegeneration [6,7]. Recently, several neuropsychiatric disorders have also been linked to ciliary dysfunction [7–9]. Whether altered ciliary signaling in the postnatal brain contributes to these disorders is not yet established but represents an exciting area of current and future research.

In this brief review, we highlight a subset of recent work that describes unexpected emerging roles for ciliary signaling in modulating and maintaining neuron and circuit functions in the postnatal brain. We refer the reader to several excellent reviews on the roles of primary cilia in neuronal development and sensory functions [1,4]; these topics are not further discussed here.

Cilia in the postnatal brain

Cilia are compartmentalized organelles that actively concentrate receptors and signaling molecules while excluding non-ciliary proteins [10,11]. The

identification of ciliary molecules has now permitted the characterization of cilia on cell types across different mammalian brain regions. The majority of mature neurons and astrocytes in the central nervous system extend a single primary cilium (Figure 1), although curiously, neither mature microglia nor oligodendrocytes appear to contain cilia [12,13]. Cilia typically emanate from the soma and can be oriented in different directions depending on the brain region and neuron type [14], [15**]. Neurons with defined apical dendrites tend to have apically oriented cilia, whereas subtypes lacking apical dendrites display a wider range of orientations [14], [15**], [16].

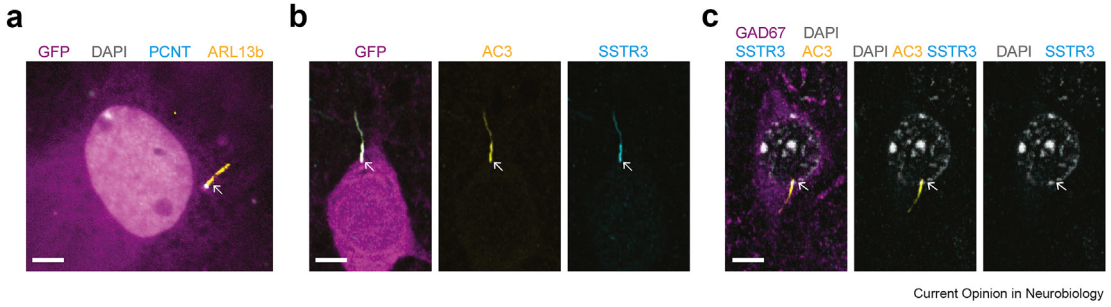
In the mouse cortex, neuronal cilia elongate during postnatal development, reaching their maximal lengths by P60–P90 [16]. Both their rates of elongation and maximal lengths vary across brain region and cortical layer [12,16,17]. Further complicating matters, cilia length and morphologies are also regulated by ligand binding and the complement of signaling proteins expressed within the ciliary membrane [18–21]. Cilia are retained as neurons age, although their protein composition is altered across the lifespan, and may contribute to age-related neurological dysfunction [22].

Postnatal neuronal cilia contain G protein-coupled receptors

Neuronal cilia house a variety of signaling molecules including G protein-coupled receptors (GPCRs), receptor tyrosine kinases (RTKs), ion channels and downstream effectors [23,24]. The complement of ciliary proteins is dynamically regulated across developmental stages and environmental conditions. In the postnatal brain, the best characterized ciliary receptors are GPCRs for amine neurotransmitters and neuropeptides, many of which have been well-established to play wide-ranging and critical roles in modulating brain physiology. Ciliary GPCR expression varies across brain region and cell type and includes receptors for somatostatin (SSTR3), kisspeptin (KISS1R), serotonin (HT6R), melanin concentrating hormone (MCHR1), dopamine (DRD1, DRD2, DRD5), and neuropeptide Y (NPY2R) among others [24–26]. These receptors appear to be enriched in cilia with low to negligible levels in the cell's plasma membrane (Figure 1b), suggesting that signaling through the cilium plays a distinct role in regulating neuronal functions. Sequestering signaling components within the cilium may allow neurons to achieve diverse physiological effects through shared signaling pathways. For instance, recent work has shown that ciliary GPCRs and cAMP, but not plasma membrane-localized GPCRs or cytoplasmically produced cAMP, regulate sonic Hedgehog signaling in part via activation of cilia-localized protein kinase A [27]. Moreover, the larger surface to volume ratio in cilia likely enables robust physiological responses to small but nevertheless relevant environmental signals in part via generation of high local concentrations of effectors [27,28].

Although the expression of a subset of ciliary receptors has been examined in some detail, and proteomic and transcriptomic efforts are now establishing the complete ciliary compendium in many cell types [29,30], these approaches have yet to be widely applied to defined neuronal subtypes. Consequently, the degree to which the ciliary signaling machinery is fine-tuned for specific neuronal functions is still unknown. In addition to exhibiting cell and region specificity, the ciliary complement of GPCRs is subject to dynamic modulation. Localization of a subset of receptors is ligand-dependent [31–33], and neuropeptide GPCRs such as SSTR3 and NPY2R can be actively removed from the ciliary membrane upon ligand addition via internalization, ciliary

Figure 1



Cilia are present on postnatal neurons and astrocytes. **(a)** Primary cilium of a GFP-expressing DIV20 astrocyte cultured from newborn rat cortex. Antibodies against ARL13b and PCNT label the length and base of the cilium respectively. **(b)** Primary cilium of a GFP-expressing pyramidal neuron from P22 rat cortex immunolabeled with antibodies against AC3 and SSTR3. **(c)** Primary cilium of a GAD67-expressing neuron from P22 rat cortex stained with DAPI and immunolabeled with antibodies against AC3 and SSTR3. Note absence of SSTR3 from interneuron cilium. Arrows point to cilia in all images. Scale bars: 5 μm. **b,c** adapted from [65**].

scission, or ectosome shedding [32,34–36]. As some of these experiments were carried out in non-neuronal cell types or *in vitro*, the degree to which similar principles operate in neurons *in vivo* is not yet clear.

The best characterized effector of neuronal ciliary GPCRs is adenylate cyclase 3 (AC3) which converts ATP to cAMP to initiate downstream signaling [37]. Originally identified in olfactory sensory neurons, AC3 is nearly ubiquitously present in neuronal cilia in the brain (Figure 1b and c), with rare expression in astrocytic cilia [12,38,39]. Additional effectors downstream of ciliary GPCRs include PI3 kinases which generate PIP3 from PIP2; PIP3 in turn activates the AKT kinase to regulate multiple aspects of cellular functions [40]. Chemo- or optogenetic activation of cilia-localized AC3, PI3 kinase or AKT was shown to be sufficient to modulate growth cone dynamics and neurite outgrowth in developing neurons [41,42]. These signaling pathways are known to influence a wide range of neuronal functions in postnatal and adult neurons, including synapse formation, turnover, and plasticity [43,44]. Ciliary signaling is thus poised to influence microcircuit wiring by efficiently coupling neuropeptide or aminergic activation of GPCRs to intracellular second messenger pathways.

Hypothalamic neuronal cilia regulate metabolic homeostasis circuits

A particularly important and well-characterized role for cilia-dependent neuromodulation in the postnatal brain is in the regulation of satiety. Obesity is characteristic of many human ciliopathies, pointing to a critical role of cilia function in the hypothalamus [45–47]. While congenital knockouts of many ciliary genes are linked to obesity [47], whether these effects are due to developmental or post-developmental roles for cilia in the hypothalamus are unclear. Recent work indicates that ciliary signaling continues to be essential during postnatal stages to maintain metabolic homeostasis. Multiple studies have now shown that disruption of cilia via conditional knockouts of ciliary genes throughout the central nervous system or from specific hypothalamic neurons in adult mice leads to hyperphagia and obesity within a few weeks [48*], [49–51].

Subtypes of hypothalamic neurons differentially express several ciliary neuropeptide receptors linked to weight and food intake [52,53**]. Recently, the melanocortin 4 receptor (MC4R), known to regulate appetite, was shown to localize to the cilia of a subset of neurons in the paraventricular nucleus (PVN) in the hypothalamus. Mutations in MC4R linked to obesity in humans disrupted this ciliary localization [53**]. While addition of MC4R agonists resulted in weight loss, this effect was abolished upon cilia ablation in adult mice over a period of a few weeks [49]. In addition, conditional inhibition of ciliary AC3 signaling in PVN neurons in 20-week old mice resulted in weight gain within 3–6 weeks [49,53**]. It is interesting to note that cilia disruption in hypothalamic arcuate nucleus neurons that produce the MC4R agonist α-melanocyte stimulating hormone (α-MSH), results in obesity only when manipulated during early postnatal development (P1–P14) but not at later stages [17]. Ciliary signaling may be required during distinct temporal stages in different regions of hypothalamus to regulate energy metabolism. Given the remarkable complexity of hypothalamic networks underlying weight and appetite control, it is also possible that neuron subtype-specific ciliary disruption in different hypothalamic nuclei can be compensated for only at specific post-developmental stages.

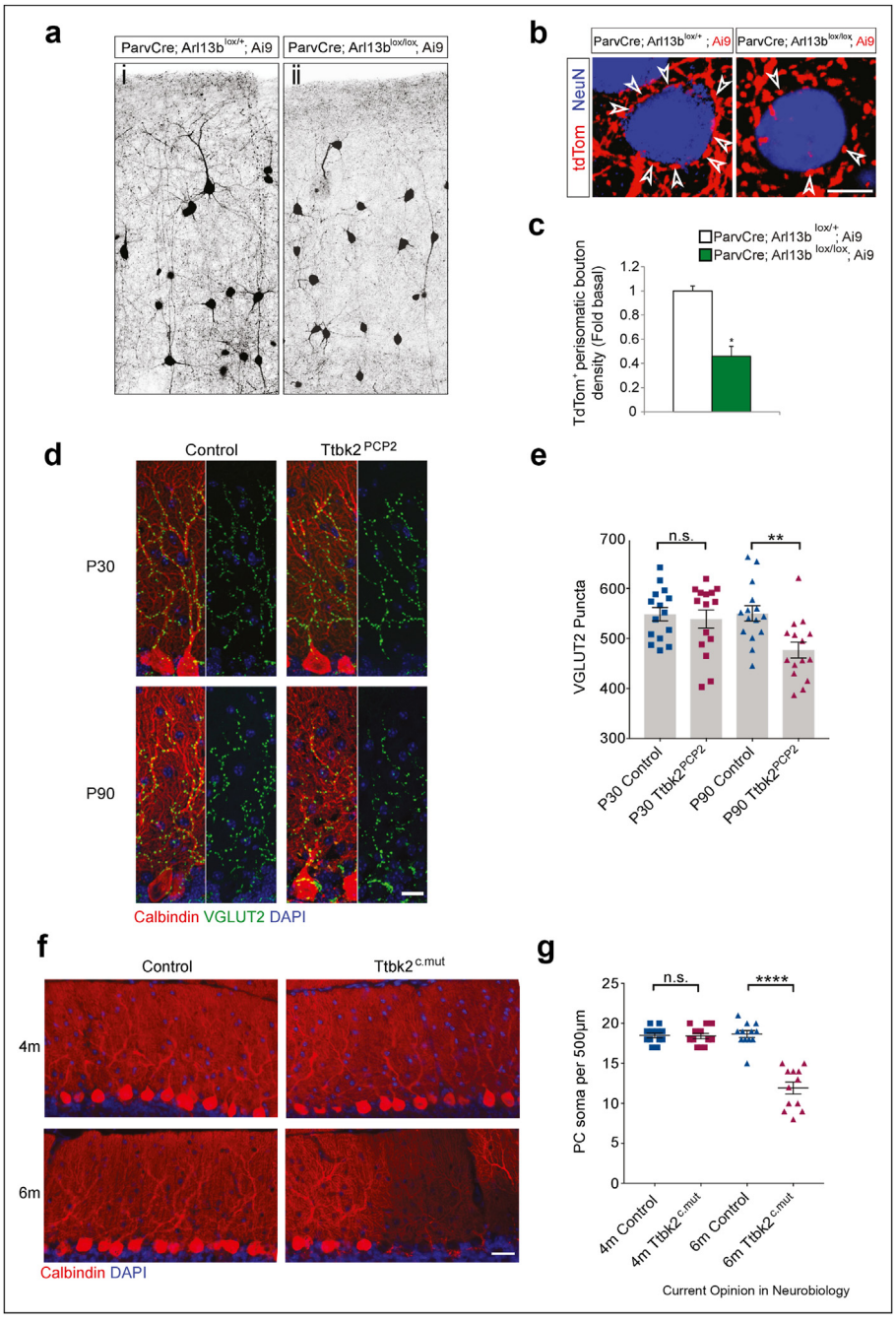
Cilia are required for the maintenance of postnatal neuronal circuits and neuron survival

There is exciting recent evidence for an ongoing role for cilia in maintaining circuit connections and promoting neuronal survival in the postnatal brain. Conditional knockout of the ciliary small GTPase Arl13b from parvalbumin-expressing neurons during early postnatal development had no impact at P30, but by P60, interneurons in the striatum, cortex and hippocampus exhibited markedly reduced neurite length and synapse density [42] (Figure 2a–c). This study suggests that ciliary signaling is important over long timescales for maintaining inhibitory synaptic connectivity.

In the cerebellum, disruption of neuronal cilia led first to loss of excitatory synapses, and then to neurodegeneration [54**]. Mutations in tau tubulin kinase 2 (TTBK2), a regulator of ciliogenesis, cause spinocerebellar ataxia type 11 (SCA11) in humans, a neurodegenerative disorder characterized by Purkinje cell loss [55]. Cell-autonomous knockout of TTBK2 or disruption of cilia structure led to markedly reduced excitatory synapses from climbing fibers onto Purkinje neurons after three months [54**] (Figure 2d and e). When analyses were extended to 5–6 months, a progressive degenerative phenotype was observed, characterized by Purkinje neuron cell death [54**] (Figure 2f and g). Moreover, these mice exhibited motor coordination phenotypes characteristic of SCA11 [54**]. Whether neuronal degeneration ultimately occurs in all brain regions following prolonged cilia loss, or if this is specific to the cerebellum, is currently unknown.

Recent studies have also uncovered a possible mechanistic link between cilia dysfunction and the loss of nigrostriatal dopaminergic neurons characteristic of Parkinson's disease. Pathogenic mutations in Leucine-rich repeat kinase 2 (LRRK2) were found to disrupt ciliogenesis in cells *in vitro* and *in vivo* [56,57*], and led to defects in ciliation and cilia length in striatal astrocytes [58*]. Reciprocal trophic factor signaling between neurons in the nigrostriatal pathway is critical for the

Figure 2



Cilia maintain synaptic connectivity and promote neuronal survival. **(a)** Cortical interneurons expressing tdTomato from control (i) and ciliary GTPase *Arl13b* conditional knockout (ii) mouse brains at P60. Cre was expressed in interneurons starting at P14 using the Parvalbumin (Parv) promoter. Note marked loss of dendritic arbors upon knockout of *Arl13b*. Adapted from Ref. [42]. **(b–c)** Representative images **(b)** and quantification **(c)** of perisomatic synaptic bouton density of tdTomato-expressing cortical interneurons contacting NeuN+ projection neurons upon conditional knockout of *Arl13b*and in control animals. Arrowheads indicate synaptic boutons in **b**. Scale bar: 20 μm. Adapted from Ref. [42]. **(d–e)** Representative images **(d)** and quantification of VGLUT2 puncta **(e)** on Purkinje cell dendrites in cerebellar tissue from control and *Ttbk2* conditional mutant mouse brains at P30 or P90. *Ttbk2* loss was induced specifically in Purkinje cells using *Cre* under the *Pcp2* promoter that drives expression starting at P6. In **d**, Purkinje cells are immunolabeled with antibodies against Calbindin, glutamatergic synapses from climbing fibers are immunolabeled with antibodies against VGLUT2, nuclei are stained with DAPI. Each data point in **e** represents one analyzed field, five fields per animal, n = 3 animals each. Errors are SEM. P = 0.0098 (one-way ANOVA and Tukey correction). Scale bar: 20 μm. Note loss of glutamatergic synapses at P90 but not at P30. **(f–g)** Representative images **(f)** and quantification **(g)** of Calbindin+ Purkinje cell soma upon conditional loss of Ttbk2 at 4 or 6 months along with controls. Note loss of Purkinje cells in Ttbk2 mutants at 6 months. Scale bar: 50 μm. Errors are SEM. P < 0.0001 (Student's unpaired t-test). **d–g** adapted from [54**].

survival of the circuit. Mesencephalic dopaminergic neurons signal to striatal cholinergic and fast spiking interneurons via Shh to regulate expression of the neurotrophic factor GDNF. Conversely, trophic GDNF signaling from striatal cholinergic neurons regulates Shh expression in dopaminergic neurons [59]. Loss of Shh signaling results in the progressive degeneration of both mesencephalic dopaminergic and striatal interneurons. Since Shh signaling requires cilia, this suggests that ciliary signaling is neuroprotective for dopaminergic neurons and is critical for maintenance of the nigrostriatal circuit [58*].

There are intriguing hints that cilia may also influence other neurodegenerative diseases and protect against environmental insults although the mechanistic links are not fully understood [60]. Signaling via the cilia-localized insulin-like growth factor receptor and AKT in the cerebral cortex has been suggested to be neuroprotective against stressors such as alcohol and anesthetics during early postnatal stages [61]. In mouse models of Alzheimer's and *in vitro*, the presence of pathogenic amyloid β correlates with reduced cilia length [62]. Intriguingly, amyloid precursor protein localizes to cilia, and inhibition of Shh signaling reduces its cleavage into pathogenic amyloid β [63]. Similarly, cilia length in the striatum but not cortex is also affected in a mouse model of Huntington's disease [64]. Whether disrupted cilia structure is a cause or effect of these diseases, and whether disruption of ciliary signaling contributes to disease progression is not yet known. Established animal models for neurodegenerative disorders may provide unique opportunities to reveal how cilia contribute to circuit maintenance and neuron survival in aging animals.

Ciliary signaling dynamically regulates postnatal neuronal properties

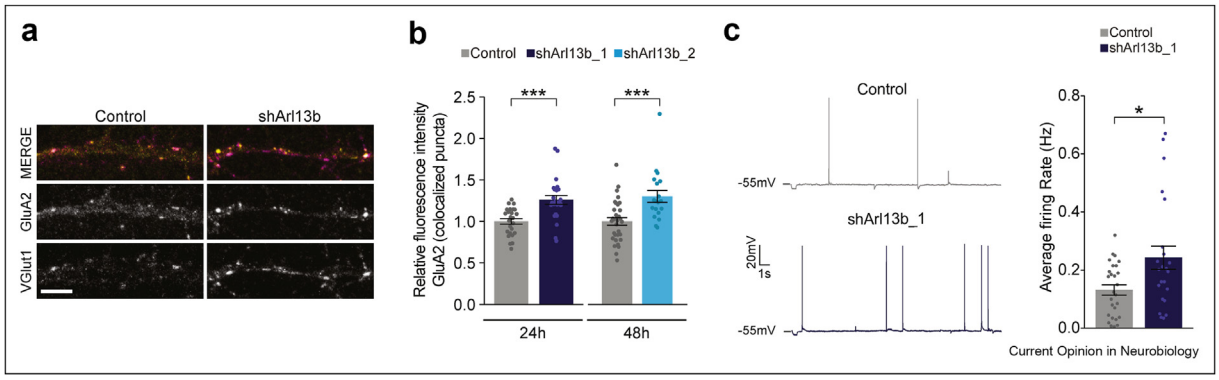
The examples above illustrate how cilia contribute to the long-term maintenance of neurons and circuits in the mature brain, but do not address whether ciliary signaling might play a more dynamic role in brain function. Given the concentration of peptide and hormone receptors in the cilium, neuromodulation via cilia provides an intriguing mechanism by which neurons might continuously monitor ongoing network activity and rapidly adjust their synaptic and intrinsic properties in response. Evidence in support of acute cilia-dependent neuromodulation has now been provided by three recent studies described below.

In the first, acute disruption of cilia structure and/or signaling in postnatal neocortical cultures for as little as 24–48h induced significant strengthening of excitatory synapses onto excitatory pyramidal neurons and increased network excitability [65**] (Figure 3). Interestingly, these manipulations had no effect on inhibitory synapses onto pyramidal neurons. Additionally, excitatory but not inhibitory neuronal cilia in the visual cortices of P22 rats were found to contain SSTR3 [65**] (Figure 1b). The neuropeptide somatostatin is produced by a subset of interneurons in the cortex although its function is largely uncharacterized. Acute (24–48h) pharmacological antagonism and agonism of SSTR3 led to bidirectional modulation of excitatory synaptic strength of pyramidal neurons, such that agonism weakened, and antagonism strengthened, excitatory synapses [65**]. This work suggests that ciliary signaling may continuously adjust neuronal properties in response to incoming stimuli to maintain the balance of excitation and inhibition within a homeostatic range. Whether synapses of inhibitory neurons are similarly modulated and if so, via which ciliary signaling pathway(s), is unknown.

In a second study, overexpression of ciliary 5-HT6R in hippocampal neurons cultured from newborn mice was shown to result in decreased excitability [66]; neurons overexpressing 5-HT6R fired fewer action potentials in response to current injection than control neurons. This decreased excitability correlated with changes in cilia and axon initial segment (AIS) morphologies. In agreement with a previous report [19], overexpression of 5-HT6R caused lengthening and aberrant branching of cilia, and disrupted the localization of endogenous ciliary proteins including AC3 and SSTR3. Surprisingly, a subset of AIS components such as AnkG and the voltage-gated sodium channel $Nav_{1.2}$ were mislocalized to the ciliary compartment in neurons overexpressing 5-HT6R [66]. Expression of 5-HT6R was also found to regulate properties of the AIS; overexpression of the receptor increased AIS length and decreased its distance from the soma, and conversely, siRNA-mediated knockdown of 5HTR6 decreased AIS length [66]. Structural changes in the AIS are associated with homeostatic adaptations in intrinsic excitability [67], suggesting an intriguing possible mechanism by which ciliary signaling can modulate neuronal excitability in the postnatal brain.

The role of ciliary HT6R in hippocampal neurons was further explored in a third study. Using focused ion beam-scanning electron microscopy (FIB-SEM), a subset of adult hippocampal pyramidal neuron cilia containing 5-HT6R was found to be in close apposition to axonal varicosities with physical features of synapses; these axons were proposed to be from serotonergic neurons of the midbrain [15**]. Activation of serotonergic signaling in hippocampal neurons was associated with transcriptional changes, suggesting that acute ciliary signaling may regulate neuronal excitability and synaptic properties via transcriptional mechanisms [15**]. The identification of axo-ciliary synapses in dense brain neuropils raises a number of interesting questions regarding how these synapses are established and maintained, and how these synapses contribute to modulating neuronal functions. Together, a general

Figure 3



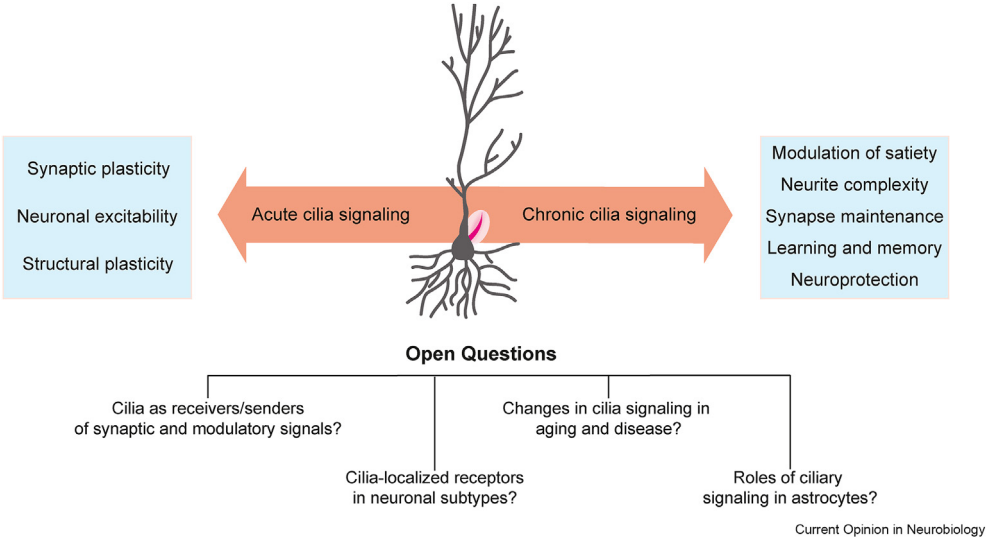
Ciliary signaling acutely modulates neuronal properties. **(a)** Dendrites of control and shArl13b-transfected DIV11 cortical pyramidal neurons cultured from newborn rats. Excitatory synapses are immunolabeled with anti-VGlut1 and anti-GluA2 antibodies. Scale bar: 5 μm. **(b)** Quantification of relative fluorescence intensity of immunolabeled GluA2 at excitatory synapses 24h or 48h following transfection of control or two independent shArl13b-containing plasmids. Each dot is the average summed fluorescence intensity of all measured synapses from a single neuron. Errors are SEM. $P < 0.001$ (Wilcoxon rank-sum test). **(c)** Increased spontaneous activity in pyramidal neurons expressing the shArl13b plasmid as compared to neurons transfected with a control plasmid. $P < 0.05$ (Wilcoxon rank-sum test). **a–c** adapted from [65**].

theme arising from these initial studies is that signaling from the cilium provides ongoing input to individual neurons to dynamically adjust their excitability through both synaptic and intrinsic mechanisms.

The function of primary cilia on mature astrocytes

In contrast to neurons, the role of cilia on mature astrocytes is poorly understood. Astrocytic cilia do not appear to be enriched for neuropeptide receptors or AC3 found on neuronal cilia, although they do contain Arl13b and receptors (LPARs) for the mitogen lysophosphatidic acid (LPA) [12,13]. Excitotoxic injury can result in the formation of reactive astrocytes which can be neuroprotective or neurotoxic, and unlike postmitotic neurons, can proliferate [68]. Seizures have been shown to decrease astrocytic cilia length [69], and congenital or conditional loss of the ciliary trafficking protein BBS8 at early postnatal stages results in increased expression of subsets of markers for reactive astrocytes [70]. Moreover, signaling through the astrocytic cilium has been linked to astrocyte proliferation and glioblastomas [71–73]. Whether ciliary signaling in astrocytes modulates synapse

Figure 4



Known and unknown roles and mechanisms of ciliary signaling in the postnatal brain. Shown are known functions of acute and prolonged ciliary signaling in the postnatal brain. Indicated below are a subset of the questions that remains to be addressed in this area.

remodeling and neurotransmitter recycling is unknown, but astrocytic cilia defects may for instance underlie a subset of the striatal circuit dysfunction associated with pathogenic mutations in LRRK2 [58*]. The role of cilia in astrocytes remains an unexplored but exciting frontier for future research.

Conclusions

We now know just enough to recognize that there are many unknowns about the role of cilia in the postnatal brain (Figure 4). How neuropeptides and amines reach and activate ciliary GPCRs is one critical question; we do not currently know whether the primary mode of activation is via diffusion from distant sources, or whether cilia are commonly juxtaposed to synaptic structures in the dense neuropils within which they are embedded. In addition to receiving extracellular signals, cilia are now understood to also send signals via the production of extracellular vesicles [74,75]. Whether this occurs *in vivo* in the mammalian brain is not known but may represent another form of cilia-mediated communication in the adult brain. Providing a complete description of cilia morphological positions, and defining the origin and release mechanisms of the neuropeptides and amines that trigger ciliary signaling in the postnatal brain, are critical immediate challenges.

The signaling protein content of neuronal and glial cilia also needs to be defined not only by cell type and developmental stage but also in different cellular conditions. For instance, cilia transcriptomes in the primate brain appear to be subject to circadian control and age, pointing to additional complexities in deciphering the ciliary protein complement [22,76]. Specific manipulation of cilia signaling pathways across postnatal stages, and determination of their effects on neuronal and circuit properties, are additional important priorities.

Given the emerging roles of ciliary signaling in the postnatal brain, it is also now imperative to understand how aberrant ciliary signaling at adult stages contributes to multiple neurocognitive and neurodegenerative disorders [7—9,60,77—79]. Gene therapy approaches to restore cilia function in adult olfactory neurons and photoreceptors have been shown to partially reverse sensory phenotypes such as anosmia and blindness [50,80,81]. Perhaps pharmacological targeting of cilia-specific receptors in adult brains could similarly ameliorate some of the symptoms associated with cilia-related diseases. We expect that renewed appreciation of this highly conserved structure will continue to foster collaborations between cilia biologists and neuroscientists, and will lead to new and unexpected insights into the contributions of this ancient organelle to the dynamic regulation of the developing and mature brain.

Topic selection

Discussed articles were selected following a PubMed search for the terms ‘cilia’ and ‘brain’ and were largely restricted to those from 2016-current. A subset of relevant articles from bioRxiv is also highlighted.

Conflict of interest statement

Nothing declared.

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** of outstanding interest

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Using focused ion beam scanning electron microscopy, the authors describe 'axo-ciliary synapses' between cilia of hippocampal CA1 pyramidal neurons and midbrain serotonergic axons. Optogenetics-mediated release of serotonin triggers signaling via cilia-localized HTR6 serotonin receptors and results in altered transcription.

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