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A ciliary synapse for "short-circuit" neuromodulation

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Nearly all neurons contain a primary cilium, but little is known about how this compartment contributes to neuromodulatory signaling. In a new study, Sheu et al. use cutting-edge electron microscopy and fluorescence imaging techniques to reveal a new type of synapse that enables chemical transmission between serotonergic axons and the primary cilia of hippocampal neurons.

In the mammalian nervous system, chemical synaptic transmission serves as the principal mode of inter-neuronal communication. The rich diversity of chemical synapses, which vary in the chemicals they release presynaptically and the spatiotemporal properties of their post-synaptic response, enables the formation of complex neural circuits, and the dynamic nature of synaptic plasticity allows circuits to be flexibly tuned. Chemical synapses between neurons are based on a stereotyped cleft formed between an axonal bouton—the enlarged swelling in the axon of the transmitting neuron that is filled with vesicles containing neurotransmitters or neuromodulators—and a post-synapse containing a highly organized array of receptors. The post-synapse can be on axons or the cell body but are most typically on dendrites of the target neuron. While such "axon-dendrite" synapses underlie a wide-range of brain functions and are at the core of many neurological and psychiatric disorders, in this issue of *Cell*, Sheu et al. (2022) report a new type of chemical synapse that forms between serotonergic axons and the primary cilium, revealing a previously unidentified mode of neuromodulation. The discovery of "axon-cilium" synapses (Figure 1) raises fundamental questions about the distinct roles and mechanisms employed by this novel inter-neuronal connection.

Nearly all eukaryotic cells, including neurons, contain a single primary cilium—a microtubule-rich organelle known to organize signal transduction pathways that control development and tissue homeostasis (Anvarian et al., 2019). This is exemplified by the hedgehog signaling pathway, which is initiated in cilia and plays central roles in embryonic development. Of note, mutations in ciliary genes are associated with a variety of disorders termed "ciliopathies." As most work has focused on developmental roles, little is known about the role of primary cilia in the adult brain. To understand the structural logic of primary cilia in mouse hippocampus, Sheu et al. (2022) used focused ion beam-scanning electron microscopy (FIB-SEM) to reveal that ~80% of cilia in the hippocampal CA1 region are closely opposed to apparent nerve terminals. The authors defined clear presynaptic specializations, including synaptic vesicles spaced within 20 nm of the plasma membrane and a 20–40 nm synaptic cleft. Immunohistochemistry and viral tracing revealed that a large portion of these synapses are formed from serotonergic axons originating in the dorsal raphe nucleus (DRN), a region far from the hippocampus in the midbrain.

The elucidation of the axon-cilium synapse structure raises many fascinating questions about its development. First and foremost, how does this synapse form? The authors recapitulated axon-cilium synapses between co-cultured CA1 and DRN neurons, suggesting that cilia express unique surface cues that are sufficient to attract and stabilize axons. This culture system should facilitate a thorough dissection of the molecular basis of the formation of axon-cilium synapses. Whether axon-cilium synapse formation is influenced by the activity of pre- or post-synaptic cells, and the relative timing and interdependence of this synaptogenesis with respect to afferent synapses formed onto CA1 dendrites or from CA1 axons onto target cells will also be important topics to address. Notably, a recent study found that ciliary disruption can impact axon guidance (Guo et al., 2019), suggesting that there may be an interplay between axon-cilium synapses and formation of circuits.

While stereotypical presynaptic boutons onto cilia were seen via FIB-SEM, the

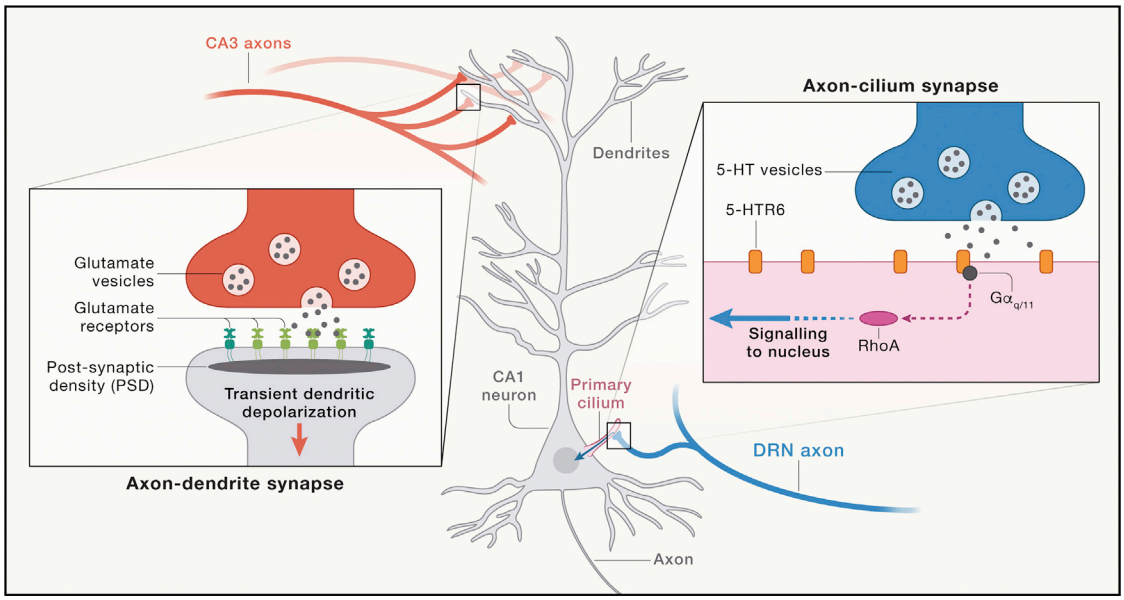

Figure 1. Axon-cilium synapses enable an alternative, neuromodulatory mode of inter-neuronal communication to complement traditional chemical synapses

CA1 pyramidal neurons typically receive extensive (>10,000) synaptic inputs from CA3 axons onto their dendrites. These axon-dendrite synapses (left inset) are characterized by presynaptic, neurotransmitter-filled vesicles and a highly organized post-synaptic machinery. Sheu et al. (2022) reveal that CA1 neurons in the mouse hippocampus also receive inputs onto their primary cilium from serotonergic axons originating in the dorsal raphe nucleus (DRN). These axon-cilium synapses (right inset) contain presynaptic serotonin vesicles, which can be released to activate 5-HTR6 receptors on the cilium. 5-HTR6 coupling to $G\alpha_q$ G proteins leads to activation of RhoA GTPases and, ultimately, drives changes in chromatin accessibility in the nucleus of the CA1 neuron.

authors did not observe a post-synaptic density, a hallmark of the post-synaptic response machinery of many axon-dendrite synapses. This implies that a different mode of transduction is being employed—one that does not depend on rapid ion channel-based electrical signaling. Indeed, CA1 primary cilia were found to be enriched for the 5-HTR6 G protein-coupled receptor (GPCR), suggesting that this synapse is neuromodulatory. GPCR signaling would provide a means of linking synaptic transmission directly to biochemical ciliary signaling cascades. Prior work has established that primary cilia are enriched in GPCRs and contain much of the critical G protein signaling machinery (Hilgendorf et al., 2016). To test this model, the authors generated a novel cilia-targeted biosensor using a GPCR-activation-based ("GRAB") strategy (Ravotto et al., 2020). Since this "GRAB-HTR6" sensor is based on 5-HTR6 itself, it likely maintains similar localization and ligand-sensing abilities compared to the native receptor, a critical feature for optical tools to study neuromodulation. Following stimulation of DRN afferents, the authors observed a small but clear increase in GRAB-HTR6 fluorescence. Notably, these responses were observed following intense stimulation of many axons and produced slow responses on the minute timescale, raising the need for future studies of the spatiotemporal properties of 5-HTR6 activation following different presynaptic stimulation patterns.

What are the downstream effects of ciliary 5-HTR6 activation? 5-HTR6 is typically a $G\alpha_s$-coupled receptor (Avet et al., 2022). However, Sheu et al. (2022) find that ciliary 5-HTR6 signals via $G\alpha_q$ to stimulate the small GTPase RhoA. In a key experiment, following chemogenetic DRN axon stimulation, the authors observed RhoA activation only in cilia that apposed serotonergic axons. Given that primary cilia contain $G\alpha_s$ (Hilgendorf et al., 2016), this proposed pathway raises the question of how ciliary context changes the G protein coupling preference of 5-HTR6.

Many other questions remain about the signaling of axon-cilium synapses, for which the large body of work on axon-dendrite synapses can guide future work. For example, does vesicular serotonin release onto cilia occur through the same exocytic mechanisms? Does retrograde signaling from the cilium to the axon occur? Do autoreceptors on the axon provide feedback regulation? Can serotonergic axons synapse on both dendrites and the primary cilium of the same target cell? Perhaps most important for deciphering the physiological roles of axon-cilium synapses is to determine if there are forms of functional or structural plasticity that regulate the fidelity of their transmission.

A typical CA1 neuron receives more than 10,000 traditional synaptic inputs but has only one cilium. What may be the functional significance of axon-cilium synapses given that they are far outnumbered by classical chemical synapses? Based on their proximity to the nucleus, the authors speculate that axon-cilium synapses may function to "short-circuit" the transcriptional landscape of target neurons without the need for complex dendritic processing. Indeed, the authors show that stimulation of serotonergic DRN afferents modifies chromatin accessibility in CA1 neurons. Such a serotonergic synapse-to-nucleus shortcut could serve as a critical link that allows neurons to efficiently translate

transient neuromodulatory inputs into long-lasting global changes in neuronal function at both the cellular and circuit levels. This is in line with a wide range of studies showing that serotonin plays an evolutionarily conserved role regulating circuit function and global brain state transitions (Flavell et al., 2013; Haddad and Marder, 2018). Notably, the authors find that many cilia in CA1 receive inputs from regions other than DRN, indicating that other neuromodulatory systems may use this mode of transmission. Consistent with this, a wide range of neuromodulatory GPCRs, including dopamine receptors and neuropeptide receptors (Hilgendorf et al., 2016), localize to primary cilia.

One final implication of this work is that the pathophysiology of ciliopathies or other neurological or psychiatric disorders may be due to altered neuromodulation as a consequence of deficits in axon-cilium synapse assembly or function. Intriguingly, Sheu et al. (2022) point out that 5-HTR6 recently emerged from a large-scale genome-wide association study as a potential contributor to bipolar disorder (Mullins et al., 2021). Overall, the discovery of the axon-cilium synapse should motivate broad interest in deciphering this new neuromodulatory axis from the molecular to circuit level in health and disease.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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1000 Genomes Project phase 4: The gift that keeps on giving

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In this issue of *Cell*, Bryska-Bishop et al. report the release of the expanded, high-depth sequencing data that characterize the fourth phase of the 1000 Genomes Project. Using extensive comparisons and benchmarks, they demonstrate how this dataset is positioned to serve as a more comprehensive and accurate resource for global genomics.

For the past decade, the 1000 Genomes Project's catalog of human genetic variation has stood as the "go to" reference for global genetic variation (Auton et al., 2015). With data derived from >2,500 individuals from 26 populations, spanning 4 continents, the combination of high depth-of-coverage sequencing of coding regions (exomes) and moderate depth-of-coverage sequencing of the remaining ~98% of the genome (Figure 1), allowed "1000 genomes" to have pride of place in providing a diverse, well-curated register of genetic variation across human populations. Crucially, the project's data