The Primary Cilium is a Microtubule-Driven Electrical Antenna. The Case of Renal Epithelial Cells

Horacio F Cantiello (✉ hcantiello@yahoo.com.ar)

Instituto Multidisciplinario de Salud, Tecnología y Desarrollo, (IMSaTeD, UNSE-CONICET)

Noelia Scarinci

Instituto Multidisciplinario de Salud, Tecnología y Desarrollo, (IMSaTeD, UNSE-CONICET)

Brenda C Gutierrez

Instituto Multidisciplinario de Salud, Tecnología y Desarrollo, (IMSaTeD, UNSE-CONICET)

Maria del Rocio Cantero

Instituto Multidisciplinario de Salud, Tecnología y Desarrollo, (IMSaTeD, UNSE-CONICET)

Research Article

Keywords:

Posted Date: February 23rd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1346209/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

The Primary Cilium is a Microtubule-Driven Electrical Antenna. The Case of Renal Epithelial Cells

Noelia Scarinci¹, Brenda C. Gutierrez¹, María del Rocío Cantero^{1*} and Horacio F. Cantiello^{1*}

¹Laboratorio de Canales Iónicos, Instituto Multidisciplinario de Salud, Tecnología y Desarrollo, IMSaTeD (UNSE-CONICET). Ruta Nacional N°9 km 1125 S/N, Villa El Zanjón (4206), Santiago del Estero, Argentina.

*Address correspondence to either Horacio F. Cantiello or María del Rocío Cantero, IMSaTeD (UNSE-CONICET), Laboratorios Centrales. Ruta Nacional N°9 km 1125 S/N, Villa El Zanjón, Santiago del Estero, Argentina 4206. E-mail: hcantiello@yahoo.com.ar or mdrcantero@gmail.com

The Primary Cilium is a Microtubule-Driven Electrical Antenna. The Case of Renal Epithelial Cells

Noelia Scarinci¹, Brenda C. Gutierrez¹, María del Rocío Cantero^{1*} and Horacio F. Cantiello^{1*}

Laboratorio de Canales Iónicos, Instituto Multidisciplinario de Salud, Tecnología y Desarrollo, IMSaTeD (UNSE-CONICET). Ruta Nacional N°9 km 1125 S/N, Villa El Zanjón (4206), Santiago del Estero, Argentina.

*Address correspondence to either Horacio F. Cantiello or María del Rocío Cantero, IMSaTeD (UNSE-CONICET), Laboratorios Centrales. Ruta Nacional N°9 km 1125 S/N, Villa El Zanjón, Santiago del Estero, Argentina 4206. E-mail: hcantiello@yahoo.com.ar or mdrcantero@gmail.com

Abstract

The primary cilium is a non-motile sensory organelle that acts as a transducer of environmental cues into cellular responses. It comprises an axoneme, which is a core of microtubules (MTs), coated by a specialized membrane populated by receptors and a high density of ion channels. Dysfunctional primary cilia generate several diseases known as ciliopathies. However, the nature of ciliary signaling remains largely unknown. Herein, we determined by the patch-clamp technique, the electrical activity of cytoplasmic and axonemal MTs from LLC-PK1 renal epithelial cells. We observed electrical oscillations with fundamental frequencies at ~39 Hz and ~93 Hz in sheets of cytoplasmic MTs. We also studied isolated and *in situ* intact and Triton X-permeabilized primary cilia, observing electrical oscillations with peak frequencies at either 29-49 (non-permeabilized) or ~40-49 Hz (permeabilized) and ~93 Hz (both). The axonemal electrical oscillations changed with maneuvers that modify ciliary length, including addition of external Ca^{2+} and Li^+ . We also applied Continuous Wavelet Transform (CWT) and cross-correlation analyses in the Time-Frequency domain to assess the coherence between cytoplasmic and axonemal MT electrical oscillations. The evidence indicates that the primary cilium is an electrical antenna that produces MT-based electrical oscillations regulated by ciliary channels and receptors.

Introduction

The primary cilium is a sensory organelle that protrudes from the center of most eukaryotic cells, particularly renal epithelial cells [1]. Mobile cilia, also known as flagella, distinguish from primary cilia because of their ciliary microtubules (MTs) pattern. Mobile cilia and flagella are found in lower eukaryotes and specialized cell types such as sperm. The axoneme of mobile cilia is constituted by nine doublets of peripheral MTs surrounding two individual central MTs ($9 + 2$) [1]. Primary cilia have a $9 + 0$ pattern. Changes in shape and the loss of motility facilitated diversification of ciliary function into sensory properties. Primary cilia such as those found in the brain or olfactory epithelium are typically rod or whip-shaped. Other specialized cilia, such as those found in vertebrate rods and cone photoreceptors, have elaborate distal ciliary segments. The functional plasticity of primary cilia as sensory organelles has developed in both metazoans and vertebrates to include the transduction of many environmental signals. With few exceptions, such as lymphocytes and intercalar cells of the distal nephron, all cells express one or more primary cilia at some point in their cell cycle. Cilia are, therefore, relevant to eukaryotic cell homeostasis and tissue physiology and development, and a continuously-expanding number of human disorders classified as ciliopathies [2, 3, 4, 5, 6]. Primary cilia are surrounded by a continuous plasma membrane, the ciliary membrane, with a unique endowment of proteins, including ion channels [7, 8] and receptors [9, 10, 11, 12, 13]. The morphology of the primary cilium extends to the basal body, the base of axoneme MT doublets, and the centriole in a way that is coordinated with the cell cycle. The basal body is also anchored to the membrane by specialized MTs called transition fibers [14]. The core of the primary cilium is its MT-based axoneme, which is conserved in most extant protists and is present in most vertebrate cell types [15, 16].

MTs are long hollow cylinders assembled from $\alpha\beta$ -tubulin dimer subunits [17, 18, 19]. The MT surface forms different lattices by lateral apposition of protofilaments, generating two types of nanopores, at either $\alpha\beta$ interdimer or $\alpha\beta$ intradimer interfaces [20, 21]. Previous studies from our laboratory showed that different mammalian brain MT structures including 2D-sheets and bundles generate strong electrical oscillations [22, 23] that resemble those observed in isolated MTs [24]. This electrical behavior of MTs is mechanistically consistent with electrochemical transistors that support both amplification and self-sustained current- (and voltage-) oscillations [22, 23, 24, 25, 26]. From a structural viewpoint, the distinct electrical activity of the various brain MT structures [22, 23] is linked to the particular ensemble of MT subunits. Non-oscillating MT sheets display properties of memristive devices, possibly explaining the gating mechanism of the MT conductance [27]. The assemblies of MTs show remarkable electrical behavior and the capability of evolutionary computation similar to that observed in nanotube assemblies [28].

To gain insight into the universality of this electrical behavior of MT structures, herein, we explored the presence of electrical oscillations in MT sheets obtained from a non-excitable tissue, the renal epithelial cell line LLC-PK1. Isolated MT preparations from these cells readily formed MT sheets that generated spontaneous electrical oscillations. This electrical activity was also observed in the primary cilium of the cells, where the axoneme of the primary cilium behaved as an active oscillatory electrical antenna modulated by the ciliary membrane Ca^{2+} sensing receptor (CaSR) and

The Primary Cilium is an Electrical Antenna

local ion channels. Our findings indicate that the axoneme generates endogenous electrical oscillations different from those elicited by the cytoplasmic MTs, indicating that the primary cilium arranges its axonemal MTs to produce a frame of electrical oscillators that behave as an electrical antenna.

Results

Electrical signals from cytoplasmic MTs of LLC-PK1 renal epithelial cells. To test whether MTs from renal epithelial cells generated electrical signals, tubulin was isolated from the MT network present in confluent monolayers of LLC-PK1 cells (Fig. 1a). MTs were extracted following Fourest-Lieuvin [29] method, with several modifications (see Materials & Methods). MT sheets were readily observed under DIC microscopy and further immuno-labeled with anti acetylated α -tubulin antibody (Fig. 1b, Right). MT sheets were voltage-clamped under "symmetrical" conditions, in the presence of an intracellular-like, KCl-containing (140 mM) solution in both bath and patch pipette (Fig. 1c, d).

Fig. 1: Electrical activity of MT sheets obtained from confluent monolayers of LLC-PK1 cells. *a.* Representative image of confluent LLC-PK1 cells showing MT networks (Green, FITC) and counterstaining with DAPI to label cell nuclei. *b.* MT sheets of LLC-PK1 cells were isolated and observed in DIC (40x) and immuno-labeling with anti- α -tubulin antibody (40x). *c.* Configuration used to obtain electrical signals from MT sheets as described in [22]. *d.* MT sheet of LLC-PK1 cells with an attached patch pipette to get electrical signals. Horizontal bar represents 10 μm . *e.* Electrical oscillations of MT sheets follow the magnitude and polarity of the holding potentials, as indicated.

Spontaneous electrical oscillations were generated at different holding potentials except for zero mV, as expected for the absence of an electrochemical driving force (Fig. 1e & 2a Left). Frequency-domain analysis of the recordings showed a distinct pattern of discrete frequencies representing the most prevalent oscillatory modes, with peaks at ~ 39 Hz and ~ 93 Hz (Fig. 2a, Right). Most evident were monophasic limit cycles observed in 3D phase space Poincaré portraits at 80 mV and -80 mV, but not at zero mV (Fig. 2b), as expected. The mean current-to-voltage relationship was fitted with the GHK equation as a linear function of a 19.1 ± 1.1 nS ($n = 6$, Fig. 2c) conductance in the

The Primary Cilium is an Electrical Antenna

presence of symmetrical KCl, which is consistent with its electrodiffusional movement through the wall of the MT assembly.

Fig. 2. Electrical oscillations of MT sheets from LLC-PK1 cells. **a.** Left. Electrical oscillations of MT sheets follow the magnitude of the holding potential. Recordings are shown at different applied voltages from the patch pipette. Oscillations are absent at 0 mV under symmetrical KCl. Right. Fourier spectrum shows fundamental frequencies of 39 Hz and 93 Hz. **b.** 3D phase space portrait showing limit cycles at 40 mV (Red), 0 mV (Blue), and -40 mV (Black), respectively. Arrow indicates the plot at zero potential. **c.** Mean current-to-voltage relationships fitted with the GHK equation in the presence of symmetrical K^+ conditions. Mean values representative of $n = 6$ experiments.

Electrical activity of primary cilia from LLC-PK1 Cells. To evaluate the electrical activity from the axoneme of primary cilia from LLC-PK1 renal epithelial cells, the organelle was isolated from confluent monolayers, as previously described [8]. Primary cilia were observed under DIC and immunolabeled with an anti-acetylated- α -tubulin antibody to visualize the axoneme (Figs. 3a, b & e). Primary cilia were then patch-clamped with the loose patch clamp configuration [23], under symmetrical ionic conditions as described in Materials and Methods (Fig. 3c-e). As previously

reported, spontaneous single-channel activity was observed in $n = 15/25$ experiments (Fig. 3f & g) [8].

Fig. 3: Patch clamping of isolated primary cilia from LLC-PK1 cells. **a.** Primary cilia obtained as described in Materials and Methods was observed by microscopy (x40). **b.** Isolated primary cilium was observed under DIC and patched clamped under the loose patch configuration (**c.**), as previously reported [23]. **d.** Primary cilia were identified by immunolabeling with anti-acetylated - α - tubulin exposing the MTs axoneme (40x). **e.** Schematics of loose patch configuration of the primary cilium. **f.** (**Left**), Representative tracing of electrical activity shows ion channels in the presence of symmetrical KCl. **g.** Amplitude histogram of single-channel tracings, frequency domain spectra for tracings in **f.** All tracings were obtained in symmetrical KCl.

To gain access to the axoneme, primary cilia were membrane-permeabilized by addition of Triton X-100 (0.1%, Fig. 4) to the bath solution. Interestingly, the open state noise of the recordings showed periodic oscillations more clearly defined in the expanded tracings and then confirmed by frequency domain analysis with Fourier transformation of the time series (Fig. 4). The oscillatory signals had a dominant frequency at ~ 39 Hz. The addition of Triton X-100 eliminated the spontaneous single-channel activity, but the expanded tracings disclosed an oscillatory pattern, with

the same peak frequency at ~39 Hz. The results suggested that the oscillations were associated with the ciliary MTs transmitting electrical information to the ciliary membrane. The addition of the MT stabilizer Paclitaxel (2.5 mM) eliminated the oscillations, which is in agreement with its inhibitory effect on brain MT 2D-sheets and bundles [22, 23].

Fig. 4: Electrical activity of isolated primary cilia from LLC-PK1 cells. **a.** Representative single-channel currents of membrane-attached patches under control (Top), and after subsequent addition of the detergent Triton X-100 (Middle) and the MT stabilizer Paclitaxel (Bottom) in symmetrical KCl solution. The recording's open state noise displayed oscillatory behavior ($n = 6$). **b.** Fourier spectra of the tracings in (a.) show a fundamental frequency in 39 Hz (Top and Middle). Paclitaxel inhibited traces, only show two minor peaks at 50 Hz and 100 Hz from line contamination following frequency domain analysis.

Electrical activity of in situ primary cilia. To confirm the electrical oscillatory activity of the axoneme, permeabilized primary cilia were also patched under *in situ* conditions. For these experiments, confluent monolayers of LLC-PK1 cells were incubated in an intracellular-type solution containing high KCl (140 mM, see Materials and Methods), supplemented with both Triton X-100 (0.1%) to permeabilize the cell membranes, and a complex comprising an anti-acetylated α -tubulin antibody, and a FITC fluorescent secondary antibody to label the axoneme. We used combined DIC-immunofluorescent imaging to identify primary cilium-expressing single cells (Fig. 5a), which were then patch clamped under the loose patch configuration [23], (Fig. 5b). Electrical oscillations were readily observed from the *in situ* permeabilized primary cilium (Fig. 5c). However, a frequency domain analysis of the oscillatory tracings indicated that the fundamental frequency

differed from the cellular MT sheets. Namely, a most prominent ~93 Hz fundamental frequency and frequencies in the range of ~40-49 Hz were observed. Interestingly, the 93 Hz frequency not apparent in mammalian brain MT preparations [22, 23] is present in honeybee brain MT preparations [30].

Electrical activity of permeabilized isolated primary cilia. To better access the ciliary axoneme, the isolated organelle was incubated for up to 18 hours with Triton X-100 (0.1%) to guarantee complete membrane permeabilization. In the presence of symmetrical KCl (140 mM), we did not observe spontaneous single-channel activity; but instead, electrical oscillations were readily present (Fig. 5d & f). A frequency-domain analysis (Fig. 5e) showed a pattern of oscillations with distinct frequencies, including a most prominent ~93 Hz signal and a pattern in the range of 29-49 Hz. However, the amplitude of the oscillations followed a linear response with the magnitude and polarity of the holding potential in the range of ± 180 mV (Fig. 5f), indicating their electrodiffusional nature.

Fig. 5: Patch clamping of in situ primary cilia. **a.** Representative images of LLC-PK1 cells incubated in an intracellular-type solution containing high KCl and FITC-anti acetylated tubulin antibody complexes to identify the primary cilium. **b.** (Top) Configuration used to obtain electrical signals from MT sheets as described in [22]. (Bottom) Combined DIC-immunofluorescence imaging of an in situ primary cilia approached by the patch pipette. **c.** Electrical oscillations of in situ patched permeabilized primary

cilium. Expanded tracings are shown at Bottom Left. Frequency domain analysis of tracings is shown on Bottom Right. d. Electrical activity of permeabilized isolated primary cilia. Overnight incubation, primary cilia were permeabilized in a high KCl solution containing Triton X-100 (0.1%). Top, Representative tracing showing electrical oscillations obtained under symmetrical 140 mM KCl solution conditions. Data representative of $n = 6$. Bottom, the expanded tracing shows the pattern of electrical oscillations. e. The frequency spectrum shows a prominent peak at ~ 93 Hz and several minor peaks between 29 and 49 Hz. Data obtained at 120 mV. f. Representative tracing during staircase holding potentials between -80 and 80 mV indicates a linear response of the amplitude parameter of the oscillations ($n = 4$).

Presence and activation of CaSR in the electrical activity of non-permeabilized primary cilia. To explore the role the ciliary membrane might have on the electrical properties of the axoneme, non-permeabilized primary cilia were first labeled for the calcium-sensing receptor (CaSR), which was recently reported in various cell compartments of LLC-PK1 cells [31]. We recorded the electrical activity of primary cilia before and after adding Ca^{2+} and the calcimimetic agonist of the receptor R-568 (Fig. 6a). Co-immunolabeling of the axoneme and the CaSR were confirmed with anti-acetylated- α -tubulin and CaSR-specific antibodies, respectively (Fig. 6b). The CaSR is segregated to the entire length of the primary cilium. The non-permeabilized primary cilia were isolated and patch-clamped in the presence of either a standard extracellular solution containing high NaCl (135 mM) in both the bath and patch pipette or asymmetric conditions in the presence of a high KCl (140 mM) in the pipette and 135 mM NaCl in the bath. We observed spontaneous single-channel activity at different holding potentials between ± 100 mV ($n = 11$) (Fig. 6a, Control, 40 mV). Spontaneous single-channel activity (Ctrl) was then challenged by subsequent additions of external Ca^{2+} (10 mM) and the CaSR agonist R-568 (50 μM). Relative mean currents were higher after Ca^{2+} addition and further increased after R568, respectively (Fig. 6c). Both additions were statistically different between and from control (Fig. 6d, $p < 0.05$). The data confirmed the integrity of the ciliary membrane and the response to Ca^{2+} by the CaSR. An expansion of the tracings unmasked the presence of underlying electrical oscillations (Fig. 6c, left) similar to those observed with cytoplasmic MT sheets, with a fundamental frequency of ~ 39 Hz (Fig. 6c, right). Although increased mean current amplitude was observed after both, Ca^{2+} and R568 maneuvers, the main observation was a dramatic shift in the frequency spectrum of the oscillations. Ca^{2+} addition elicited a spread in frequencies of $\sim 38, 43, 57, 93, 99$, and 144 Hz, while, the R-568 addition changed the spectrum of frequencies to 7, 29, 38, 42, 57, 90, 101, and 144 Hz (Fig. 6c, right). Thus, CaSR stimulation and corresponding ion channel activation in the primary cilium regulated the oscillatory behavior of the ciliary axoneme, as shown in the Poincaré plots of the respective oscillatory regimes (Fig. 6e).

Fig. 6. Effect of Ca^{2+} and the CaSR agonist R-568 on non-permeabilized isolated primary cilia. **a.** Representative electrical tracing showing channel activity obtained from an isolated, non-permeabilized primary cilium in the presence of 140 mM KCl in the pipette and 135 mM NaCl in the bath ($n = 8$), before (Ctrl) and after subsequent addition of Ca^{2+} (10 mM) and R-568 (50 μM) **b.** Co-immunolabeling of the primary cilium axoneme and the CaSR, where acetylated- α -tubulin labeling is shown in Green and CaSR in Red (see Materials and Methods for details). **c.** Top Left, Representative tracings at 80 mV, indicating Control (Top), after Ca^{2+} (middle), and R568 (bottom) conditions, respectively. **d.** The bar graph shows the changes in the relative mean current before and after Ca^{2+} and R568, respectively. The asterisk represents statistical difference between experimental conditions and the control ($p < 0.05$), and the triangle reflects statistical difference between experimental groups. **e.** Poincaré plot for the

oscillatory regimes under Control (Black), after Ca^{2+} (Red), and after R568 (Green) conditions, respectively.

Effect of Li^+ on the electrical properties of the primary cilium. Lithium increases ciliary length in neurons and other cells [32], including LLC-PK1 renal epithelial cells [33], and has a strong inhibitory effect on the PC2 channel [34], which is present in renal epithelial primary cilia [8, 35]. The effect of Li^+ on the electrical oscillations of cytoplasmic MTs was evaluated in MT sheets (Fig. 7a, Top panel). We observed that Li^+ addition (280 mM) produced a significant 243% increase in the amplitude of the oscillations (Mean conductance 7.23 ± 1.21 nS, $n = 3$, vs. 17.06 ± 4.86 nS, $n = 3$, $p < 0.05$), and also produced a novel pattern of oscillatory frequencies that displayed a more prominent peak at ~ 93 Hz (Fig 7b). A 3D Poincaré plot evidenced the chaotic behavior in the presence of Li^+ compared to control condition (Fig 7c). We further explored the contribution of ion transport to the electrical properties of the primary cilia. The isolated organelle was patch clamped in the presence of added Li^+ (20 mM) and Triton-X100 (0.1 %) to permeabilize primary cilia (Fig. 7d). Electrical recordings showed that in the presence of Li^+ electrical oscillations were in their maximal amplitude (see Top Fig 7d). Addition of Paclitaxel (1.25 mM) reduced the amplitude of the oscillations, and further addition (2.5 mM) eliminated completely the oscillatory behavior (1.94 ± 0.62 nS vs 0.11 ± 0.04 nS, $n = 3$ $p < 0.001$, Fig. 7e).

Fig. 7. Effect of Lithium on electrical oscillations of primary cilia. Top panel. **a.** Representative tracing in symmetrical KCl (140 mM, Ctrl) and after addition of Li^+ (280 mM). Representative of $n = 4$. **b.** Fourier spectra of tracings on the Left show an increase in the 39 Hz and 93 Hz peaks. **c.** A three-dimensional phase-space portrait showing limit cycles under control (Black lines) and after the addition of LiCl (Red lines), respectively. The time delay for the first and second derivatives was 10 ms. **d.** Representative oscillations from an isolated permeabilized primary cilium were obtained from LLC-PK1 cells. Tracings were obtained in the presence of KCl 140 mM, LiCl 20 mM, and Triton-X 0.1% (Top, $n =$

4) and after the addition of Paclitaxel 1.25 mM and 2.5 mM, as indicated. Paclitaxel eliminated the oscillatory currents. d. Fourier spectra of tracings on the Left show a wider range of frequency peaks entirely inhibited by the addition of paclitaxel (2.5 mM).

Time-Frequency domain analysis of the electrical oscillations. The frequency-domain analysis by Fourier transformation of the electrical recordings showed the oscillatory behavior of the tracings and disclosed the prevalent frequency peaks in each preparation. However, high precision in the frequency domain gives no information about the correlation in time. Thus, we applied dual Time-Frequency algorithms to compare the oscillatory currents of the MT sheets (cytoplasmic MTs) and primary cilia (axonemal MTs). We applied Continuous Wavelet Transform (CWT) and Cross-Spectral Density (CSD) analyses to the tracings that provided dual Time-Frequency (TF) information from the wavelet coefficients after decomposition into elementary waveforms (see Materials & Methods, [36]). The results disclosed frequency variations through time in the samples. We compared the CWT spectra of averaged signals ($n = 3$), from MTs sheets and permeabilized primary cilia (Fig. 8) (see Materials & Methods). The CWT of the primary ciliary data showed an 87-99 Hz frequency band, while the MTs sheets showed a 35-44 Hz band and only a smoother one at 87-99 Hz. We further explored the relationship between the signals by cross-spectral density function (CSDF) as the Fourier spectrum of the mutual coherence function [37] that defined the correlation between amplitudes of the spectral frequency components. The CSDF of the axoneme and cytoplasmic MTs showed coherence between their fundamental frequencies. A notable coherence was observed at ~93 Hz, while coherence between signals at 39 Hz was much lower but significant. Thus, the different MT assemblies shared frequency peaks but in different proportions, suggesting a higher degree of coherence or synchrony in the primary cilium, which may correlate with its function in the cell.

Fig. 8: Time-Frequency analysis of electrical oscillations from cytoplasmic and axonemal MTs. **a.** CWT of electrical oscillations from LLC-PK1 MTs sheets (Left) and permeabilized primary cilia (Right). Signals at 40 mV were averaged ($n = 3$ for each sample). White dashed lines draw the cone of influence showing (Gray) areas in the scalograms potentially affected by boundary effects. **b.** Fourier Power Spectra of LLC-PK1 MTs sheets currents (Top Left) and primary cilia currents (Bottom left). CPSD between both signals is shown (Right). Arrows indicate coherence in predominant frequencies at 39 and 93 Hz.

Discussion

Primary cilia are MT-based transducers of environmental signals that regulate cell proliferation, differentiation, transcription, migration, polarity, and survival [38]. Dysfunctional primary cilia are implicated in human diseases known as ciliopathies. However, the connection between ciliary function and MTs remains ill-defined. Cystin [39] and Polaris, located to the ciliary basal body and axoneme [40] and the channel/receptor complex proteins polycystins 1 (PC1) and 2 (PC2) that cause autosomal dominant polycystic kidney disease (ADPKD), are functionally associated with MTs [39, 40, 41, 42], and various animal models of cystic disease also showed a connection between primary cilia and MTs [43]. *Orpk* mice with dysfunctional Polaris have, in addition to polycystic kidneys, shortened cilia, left-right symmetry defects [41], and a more substantial location of PC2 in primary cilia [42]. Homozygous *cpk/cpk* mice with cystic kidney disease moderate its progression by treatment with the microtubular stabilizer paclitaxel [44]. Morphologically, the

axoneme-regulated ciliary structure has been linked with maneuvers that either shorten or lengthen the primary cilium [33, 45]. The present study provides evidence that the axonemal MTs produce electrical oscillations that serve as a mechanism for transmitting information between the organelle and the rest of the cell.

Electrical properties of renal epithelial microtubules. We observed that cytoplasmic MTs from LLC-PK1 renal epithelial cells, produce robust electrical oscillations that resemble the oscillatory behavior of mammalian brain MTs [22, 23]. Voltage-clamped bundles of brain MTs display electrical oscillations with a prominent fundamental frequency at ~39 Hz that progress through various periodic regimes. Membrane-permeabilized neurites of cultured mouse hippocampal neurons also generate electrical oscillations conducted along their length [23, 25, 26]. Thus, the electrical activity of MTs remains evolutionarily conserved, despite functional heterogeneity of the different tubulin genes, encoding isoforms in the various tissues, and diverse posttranslational modifications of the tubulins [46, 47, 48]. The evolutionary importance of this behavior is further suggested by the electrical oscillations observed in MTs from insect brains [30], and preliminary evidence indicating their presence in the bacterial ancestor of MTs, FtsZ [49].

Electrical activity of axonemal microtubules. MTs behave as bio-electrochemical transistors [50, 51] acting as nonlinear transmission lines capable of transmitting electrical signals at a distance. This electrical behavior was identified as spontaneous oscillations and bursts of electrical activity similar to that of action potentials [22, 23]. The electrical oscillations of MTs represent, a highly synchronized, time-dependent, change in ionic conductance through the wall of the MTs that is triggered by their gating mechanism as memristive devices [27]. The electrical activity of MTs provides a robust signaling mechanism based on frequency modulation that may couple to, or regulate the activity of membrane events. The electrical behavior of the axoneme MTs was similar to that of cytoplasmic MTs while connected to the ciliary membrane and soon after permeabilization but shifted their pattern of fundamental frequencies after overnight permeabilization and incubation in an intracellular-type solution. Thus, the particular arrangement of axonemal MTs may control their electrical activity based on the ionic composition of the organelle. A physical connection of this cytoskeleton provides continuity to the basal body, consisting of nine radially-oriented MT triplets. The base of the primary cilium is positioned close to the trans-Golgi apparatus and the nucleus, providing immediate access to intracellular signaling machinery [52]. Electrical information by axonemal MTs link ciliary signals to cell function. This electrical signaling is modulated by the ionic changes taking place at the ciliary membrane.

Ciliary membrane regulation of axonemal MTs. The primary cilium membrane is endowed with a unique complement of proteins, including ion channels [7, 8] and receptors [10, 11, 12, 13, 53]. Several ciliary channels, particularly PC2, are implicated in the sensory properties of the organelle in renal epithelial cells [7]. The PC1/PC2 channel complex involved in Ca^{2+} entry and cell activation [54, 55], interacts with Cystin, Polaris, and the kinesin-2 components KIF3A and KIF3B [56] to contribute to MT organization and the function of this organelle. Bending of the primary cilium in polarized renal epithelial cells causes an increase in intracellular Ca^{2+} [54, 57]. However, the issue as to how the primary cilium transmits environmental signals into the cell, has remained hitherto largely unknown. It has been speculated that the response would require external Ca^{2+} and

ciliary proteins such as PC1, PC2, and TRPV4 [7, 54, 58]. However, more recent observations posed that no Ca^{2+} influx is observed in primary cilia in a time frame consistent with mechanosensitive channel activation [59]. It was suggested that if mechanosensory abilities for cilia did indeed exist, they could induce mediators but not initiate cell Ca^{2+} signaling. Other studies demonstrated that, during deflection, Ca^{2+} levels increased within the cilium [60, 61]. The present findings suggest that regardless of whether Ca^{2+} signals can indeed be observed in the primary cilium, delivery of the ion into the ciliary compartment may signal events by modulating the oscillatory frequency of the electrical signals of the axoneme. The converse is also true since the axoneme machinery regulates ciliary PC2 function [56]. Because PC2 permeates Ca^{2+} , which is a robust MT depolymerizing agent [62], a likely scenario would include the regulation by PC2 of the axoneme machinery, which is reflected in the changes in oscillatory behavior elicited by high external Ca^{2+} and CaSR agonists. The present study provides evidence for electrical oscillations observed in addition to ion channel activity in the primary cilium, with amplitude and frequencies similar to those previously observed in brain MTs *in vitro* [22, 23]. This finding forwards the possibility that the primary cilium works strictly as an electrical antenna.

To further test the ability of Ca^{2+} signals to modulate axonemal MT oscillatory activity, the present study further identified the presence of the G protein-coupled Ca^{2+} receptor, CaSR that detects Ca^{2+} extracellular levels [63, 64, 65] in the primary cilium of the LLC-PK1 renal epithelial cells. High external Ca^{2+} and the calcimimetic compound R-568 increased ciliary channel activity and modulated the axoneme's oscillatory frequencies. Because PC2 in the primary cilium is regulated by the local production of cAMP induced by activation of a ciliary V2 vasopressin receptor [8, 12], Ca^{2+} transport by PC2 regulates ciliary length in a cAMP-dependent manner [33, 45]. This morphological parameter of the primary cilium also changes in consonance with the expected properties of an antenna, which may explain a critical aspect of PC2 regulation of cellular function, which could not by itself explain the increase in cell Ca^{2+} concentration [7] and activation of cell signaling, despite external Ca^{2+} signaling events [31].

The primary cilium is an electrical antenna. There is strong evidence for electrical signaling in the renal primary cilium. Previous studies obtained direct electrical information of the ciliary membrane, both isolated [8] and more recently *in situ* [66, 67, 68]. Several spontaneously active non-selective cation currents were identified in the ciliary membrane of different cell types [67]. However, how ciliary channel activity translates into cell signaling and activation remains an open question. Our present findings incorporate the first cytoskeletal structure to contribute to the electrical activity of the organelle (Fig. 9), and demonstrate that the ciliary axoneme behaves as an active antenna capable of oscillatory amplification and frequency modulation, shifting the frequency peaks to provide the basis for a novel electrical intracellular signaling mechanism that targets the cellular compartment. The process is consistent with the coherent synchronization of oscillators and random antennas [69, 70, 71].

The Primary Cilium is an Electrical Antenna

Fig. 9: Model of electrical signal propagation by axoneme MTs in the primary cilium of renal epithelial cells. (Left) The primary cilium is composed of a ciliary membrane with various receptors and ion channels, which surrounds the ciliary cytoskeleton, the axoneme, with a 9 + 0 complex of MT doublets. The present study indicates that the axoneme produces spontaneous electrical oscillations. (Right) These oscillations are regulated by ionic signals generated by receptor-regulated ion channel activity in the ciliary membrane and PC2-permeable ions such as Li^+ . The primary cilium is an active electrical antenna whose signaling to the cell is frequency-modulated.

Conclusions

Despite current interest and extensive literature on the properties and functions of MTs, practically nothing is yet known about how different MT structures contribute electrically to cell function. Distinct electrical properties will depend on their specific MT assembly and geometry. Cilia and flagella have prevailed during evolution beyond their original role as motility organelles; they have transformed into sensory electrical devices. Each MT is an entirely functional electrical oscillator [24], such that the geometry of nine doublets observed in the axoneme is richer and more complex. This geometry would also change as the axoneme reaches the basal body, and again different assemblies will endow the cytoplasmic MT structures with distinct electrical properties. Electrical stimulation of the MTs in the axoneme is regulated by receptor-mediated channel activity at the ciliary membrane that allows electrical amplification and synchronization of the axially transferred signals. The findings in the present study, suggest that frequency shifts in the electrical oscillations may represent a novel signaling mechanism.

The present study provides evidence that the axoneme of primary cilia from renal epithelial cells is an electrical antenna that generates and conducts electrical oscillations that is regulated by interaction with ciliary ion channels and ciliary receptors, and implicates an essential relationship between Ca^{2+} signals, ciliary channels, and ciliary channels cytoskeleton (Fig. 9). As a sophisticated electrical amplifier, the axoneme is an integral component of an electrodynamic network coupled to current generators (i.e., MT-regulated ion channels [56]) and MT-coupled intracellular transmission lines (F-actin) [72, 73]. Electrical amplification and frequency modulation by primary cilium MTs may generate and conduct electrical information to the intracellular compartment. A general

mechanism may also be envisioned in which MT-ion channel interactions regulate cell functions such as synaptic plasticity where spatially oriented intracellular electrical signals connect with actin filaments also able to transmit electrical information [26]. Electrical amplification by axonemal MTs may be central to the sensing of environmental signals by the primary cilium. Future studies will further explore the resonance and chaotic properties of the oscillating frequencies [74] and how they affect cell function.

In conclusion, we provide evidence for a novel signaling mechanism associated to the sensory function of primary cilia, which is consistent with a universal connection between ciliary channels, receptors, and the electrical activity of MTs that may aid in our understanding of ciliopathies as ADPKD.

Materials and Methods

Cell culture. The present studies on primary cilia from LLC-PK1 renal epithelial cells (ATTC CL-101) were conducted with previously reported techniques [8, 22, 31]. Briefly, cells were maintained in DMEM medium supplemented with 3% FBS and grown to confluence in the presence of a humid atmosphere of 5% CO₂ and 37°C, renewing the culture medium once a week.

Tubulin purification from LLC-PK1 cells. As previously reported, Tubulin was obtained from LLC-PK1 cells [29, 30] with modifications. Briefly, confluent monolayers grown on Petri dishes in Dulbecco's modified Eagle's medium (DMEM) were rinsed with PBS, trypsinized at 37°C, and supplemented with 3% fetal bovine serum (FBS). The sample was centrifugated at 320g for 3 min at 37°C. The pellet obtained was washed with PEM (100 mM PIPES pH 6.7, 1 mM EGTA, 1 mM MgCl₂) at 37°C, and centrifugated at 320g for 3 min. The pellet was resuspended for cell lysis in 40 ml of OPT Buffer at 37°C (80 mM PIPES, pH 6.7, 1 mM EGTA, 1 mM MgCl₂, 0.5% Triton-X100, 10% Glycerol, 1 μ M pepstatin, 400 μ M PMSF). The lysed cells were centrifuged at 320g for 3 min at 37°C, and the supernatant was carefully discarded. The pellet obtained was resuspended in 2 ml of OPT at 4°C and incubated for 15 min on ice. At the end of this incubation, it was ultracentrifuged at 200,000g for 10 min at 4°C. The supernatant, called HOPT extract, was collected. Subsequently, 9 ml of HOPT extracts were supplemented with 5 mM MgCl₂, 1 mM GTP and 5% DMSO. The solution was then incubated for 30 min at 35°C to allow polymerization of the MTs. The sample of polymerized MTs was placed on a PEM cushion, 60% glycerol and 400 μ M PMSF at 35°C, and ultracentrifuged at 200,000g for 20 min at 35°C. The MTs were washed, without resuspension, with 3 ml of PEM50 (35°C) (50 mM PIPES, pH 6.7, 1 mM EGTA, 1 mM MgCl₂, 1 μ M pepstatin and 400 μ M PMSF). Cold PEM50 (600 μ l) the pellet was depolymerized in ice for 15 min before resuspension. Finally, the resulting tubulin suspension was ultracentrifuged at 200,000g for 10 min at 4°C, and the supernatant was collected. The samples were aliquoted and stored at -20°C until use.

Isolation of primary cilia from LLC-PK1 renal epithelial cells. Wild-type LLC-PK1 renal epithelial cells were cultured as previously described [8] in DMEM supplemented with 3% FBS without antibiotics. Cells were seeded onto glass coverslips and grown at 37°C, in a humidified atmosphere with 5% CO₂ to reach full confluence. Confluent cells (2–3 weeks) were used for immunocytochemical studies. Wherever the use of isolated primary cilia was indicated, Confluent monolayers were scraped with Ca²⁺-free phosphate-buffered saline and centrifuged for 5 min at 52g.

The Primary Cilium is an Electrical Antenna

The cell pellet was suspended in a high Ca^{2+} “deciliation” solution containing 112 mM NaCl, 3.4 mM KCl, 10 mM CaCl_2 , 2.4 mM NaHCO_3 , 2 mM HEPES, pH 7.0. Resuspended cells were shaken in this solution for 10 min at 4°C. Ciliary membranes were separated by centrifugation at 7,700g for 5min. The supernatant was loaded on top of a 45% sucrose solution in a high Ca^{2+} saline solution and centrifuged for 1 h at 100,000g. The sucrose-supernatant interface band was collected and diluted (1:10) and centrifuged again for 1 h at 100,000g. The pellet was resuspended in a normal saline solution at pH 7.0 and supplemented with 2.0 mM EGTA and 0.5 mM sucrose. Samples were aliquoted and stored at -80°C until further use.

Immunochemical labeling of MT structures. Isolated primary cilia were labeled with an anti-acetylated- α -tubulin antibody (Santa Cruz Biotechnology) to visualize the axoneme. The antibody raised from rabbit against amino acids 149-448 of human α -tubulin was obtained from Santa Cruz Biotechnology Inc (H-300, sc-5546) and used at 1:500 dilutions as previously reported [22]. The secondary antibody used for tubulin staining was bovine anti-rabbit IgG-R (sc-2367, Santa Cruz Biotechnology Inc, CA) used at a 1/1000 dilution. Samples were viewed under an Olympus IX71 inverted microscope connected to a digital CCD camera C4742-80-12AG (Hamamatsu Photonics KK, Bridgewater, NJ). Images were collected with the IPLab Spectrum (Scanalytics, Viena, VA) acquisition and analysis software, running on a Dell-NEC personal computer.

Reagents. Unless otherwise stated, chemical reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA) and diluted to their final concentrations as indicated.

Electrophysiological data acquisition and analysis of ciliary structures. The electronic setup to obtain electrical recordings from permeabilized primary cilia consisted of an E-patch amplifier (Elements, Cesena, Italy) directly apposed to the sample via a saline-containing patch pipette, as previously reported [23, 30]. Experiments were conducted with one of two solutions, namely, either “intracellular” or “extracellular” solutions. “Intracellular” solution contained, in mM: KCl 140, NaCl 5, EGTA 1.0, and Hepes 10, adjusted to pH 7.18 with KOH. “Extracellular” solution contained, in mM: NaCl 135, KCl 5.0, EGTA 1.0, and HEPES 10, adjusted to pH 7.23 with NaOH. Patch pipettes were made from soda-lime capillary tubes (Biocap, Buenos Aires, Argentina) with a 1.25 mm internal diameter and a tip diameter of $\sim 4 \mu\text{m}$. Voltage clamp protocols included gap-free data acquisition at various holding potentials) from zero mV. Electrical signals were acquired, digitized, and stored in a personal computer. Data were analyzed with the software suite pCLAMP 10.0 (Molecular Devices. Sigmaplot Version 10.0 (Jandel Scientific, Corte Madera, CA) was used for statistical analysis and graphics.

Loose-patch clamp configuration. The loose-patch-clamp configuration was used to patch the axoneme of permeabilized cilia as previously reported for MT bundles and hippocampal neurites [23]. Loose patches have considerably small seal resistances ($\text{M}\Omega$ range) than tight patches obtained from intact cilia or MT sheets. Significant currents flow through the seal and affect the magnitude of the tip potential (Fig. 4e). In our preparations, the command voltage (V_{cmd}), which is the holding potential driven by the patch amplifier, is not the one “sensed” by the tip potential on the surface of the primary cilium. The difference will be inversely proportional to the magnitude of the seal resistance. A simple model circuit analysis was used to obtain the voltage at the pipette tip (V_p), which is given by

$$V_{tip} = V_{cmd} \left(\frac{R_{seal}}{R_{seal} + R_{pip}} \right) = V_{cmd} \times B$$

R_{pip} , R_{seal} , and V_{cmd} are the pipette resistance, seal resistance, and command voltages. Under such conditions, where R_{seal} is of the order of magnitude of R_{pip} , the voltage at the pipette tip will thus be reduced from the command voltage by a factor of B (note that when $R_{seal} \gg R_{pip}$, then $V_{pip} \approx V_{cmd}$, as in the tight-seal case).

Other current analyses. Unless otherwise stated, electrical tracings shown throughout the study were unfiltered data. Average currents at various holding potentials were obtained by integrating one-second tracings and expressed as mean $\pm$ SEM values, where (n) represented the number of experiments analyzed for a given condition. Power spectra of unfiltered data were obtained by Fourier transform with a subroutine from Clampfit 10.0. Limit cycles were constructed using the unfiltered tracings' time delay (τ) approach. The lag time τ was chosen arbitrarily at $2f$, where f was the sampling frequency of data acquisition. Three-dimensional phase space diagrams were constructed in Sigmaplot 10.0.

Spectral analysis. Fourier Power Spectrum and signal filtration was performed using Clampfit 10.0 (Molecular Devices). SigmaPlot 10.0 Software (Systat Software Inc., San Jose, CA) was used as a graphing tool.

Continuous Wavelet Transform (CWT) analysis of the data. To compare the electrical behavior of LLC-PK1 MT structures from different cell domains, namely the cytoplasm (MT sheets) and the primary cilium (axoneme), we used the CWT [75], which maps the original time series as a function of one variable, time, into two variables, time and frequency.

The mother function ψ behaves like a wave with a decaying property, which contrary to the Fourier transform, decomposes the signal in terms of sines and cosines. This property provides an effective localization in both time and frequency. Starting with a mother wavelet ψ , a family $\psi_{\tau,s}$ of “wavelet daughters” can be obtained by scaling and translating ψ , such that:

$$\psi_{\tau,s}(t) = \frac{1}{\sqrt{|s|}} \psi\left(\frac{t-\tau}{s}\right), \quad s, \tau \in \mathbb{R}, s \neq 0, \quad (1)$$

where s is a scaling factor that controls the width of the wavelet and τ is a translation parameter controlling the wavelet's location. Given a time series $x(t) \in L^2(\mathbb{R})$, its *continuous wavelet transform* (CWT) concerning the wavelet is a function of two variables, $W_{x,\psi}(\tau,s)$:

$$W_{x,\psi}(\tau,s) = \int_{-\infty}^{\infty} x(t) \frac{1}{\sqrt{|s|}} \psi^*\left(\frac{t-\tau}{s}\right) dt. \quad (2)$$

The (local) wavelet power spectrum or scalogram (periodogram) is defined as

$$(WPS)_x(\tau,s) = |W_x(\tau,s)|^2. \quad (3)$$

To describe the time-frequency localization properties of the CWT, we have to assume that both the wavelet $\psi(t)$ and its Fourier transform $\Psi(\omega)$ are well-localized functions.

Strictly speaking, the CWT provides us a time-scale representation of the function being analyzed and not a time-frequency model.

Matlab software (v 2019a) was used for these analyses and implemented its "cwt" function.

Cross-Spectral Density (CSD) analysis. The coherence of the electrical oscillations in cytoplasmic and axonemal MTs was also assessed for the presence of tandem oscillations or, more precisely, the phase relationship between the signals. The coherence between signals defines the degree of confidence by which one can predict the amplitude and phase of a wave at a point [37]. An analytical fluctuating signal $V(r, t)$, where r is a position vector at a t time, has a mutual coherence at two points (r_1 and r_2) as:

$$\Gamma(r_1, r_2, \tau) \equiv \langle V^*(r_1, t) V(r_2, t + \tau) \rangle \quad (4)$$

The mutual coherence function is the Fourier inversion and further integration with respect to t , which retrieves a Dirac δ function, such that:

$$\langle \psi^*(r_1, \nu) \psi(r_2, \nu') \rangle = \delta(\nu - \nu') W(r_1, r_2, \nu) \quad (5)$$

$$\text{where } W(r_1, r_2, \nu) = \int_{-\infty}^{\infty} \Gamma(r_1, r_2, \tau) e^{2\pi i \nu \tau} d\tau \quad (6)$$

$W(r_1, r_2, \tau)$ is the cross-spectral density function (CSDF), or cross-power spectrum (CPS), at the points $P_1 (r_1)$ and $P_2 (r_2)$ [76]. The mutual coherence function (4) describes the correlation in the space-time domain, while CSDF also characterizes a measure of the correlations in the space-frequency domain [76]. Briefly, CSDF is the Fourier Transform of the autocorrelation and cross-correlation function, the cross-correlation function that defines the relationship between two random signals [77].

The above analyses were carried out with the Matlab function "cpsd" without windowing, which uses Welch's averaged, modified periodogram method of spectral estimation.

References

1. Wheatley, D. N. Primary cilia in normal and pathological tissues. *Pathobiol* **63**(4), 222-238 (1995).
2. Afzelius, B.A. Cilia-related diseases. *J Pathol* **204**, 470-477 (2004).
3. Bisgrove, B. W. & Yost, H. J. The roles of cilia in developmental disorders and disease. *Development* **133**, 4131-4143 (2006).

4. Quinlan, R. J., Tobin, J. L. & Beales, P. L. Modeling ciliopathies: Primary cilia in development and disease. *Curr Top Develop Biol* **84**, 250-282 (2008).
5. Carrascosa Romero, C., Guerrero Solano, J. L. & De Cabo De La Vega, C. Ciliopathies: Primary cilia and signaling pathways in mammalian development in *Neuroimaging for Clinicians-Combining Research and Practice* (ed. Peres, J. F. P.) 125-144 (IntechOpen, 2011).
6. Hildebrandt, F., Benzing, T. & Katsanis, N. Ciliopathies. *N Engl J Med* **364**, 1533-1543 (2011).
7. Nauli, S. M., et al. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet* **33**(2), 129-137 (2003).
8. Raychowdhury, M. K., et al. Characterization of single channel currents from primary cilia of renal epithelial cells. *J Biol Chem* **280** 34718-34722 (2005).
9. Handel, M., et al. Selective targeting of somatostatin receptor 3 to neuronal cilia. *Neurosci* **89**, 909-926 (1999).
10. Brailov, I., et al. Localization of 5-HT(6) receptors at the plasma membrane of neuronal cilia in the rat brain. *Brain Res* **872**, 271-275 (2000).
11. Masyuk, A. I., et al. Cholangiocyte primary cilia are chemosensory organelles that detect biliary nucleotides via P2Y₁₂ purinergic receptors. *Am J Physiol Gastrointest Liver Physiol* **295**(4), G725-734 (2008).
12. Raychowdhury, M. K., et al. Vasopressin receptor-mediated functional signaling pathway in primary cilia of renal epithelial cells. *Am J Physiol* **296**, F87-97 (2009).
13. Milenkovic, L., Scott, M. P. & Rohatgi, R. Lateral transport of Smoothed from the plasma membrane to the membrane of the cilium. *J Cell Biol* **187**(3), 365-374 (2009).
14. Plotnikova, O. V., Pugacheva, E. N. & Golemis, E. A. Primary cilia and the cell cycle. *Methods Cell Biol* **94**, 137-60 (2009).
15. Mitchell, D. R. The evolution of eukaryotic cilia and flagella as motile and sensory organelles. *Adv Exp Med Biol*. **607**, 130–140 (2007).
16. Carvalho-Santos, Z., Azimzadeh, J., Pereira-Leal, J. B. & Bettencourt-Dias, M. Tracing the origins of centrioles, cilia, and flagella. *J. Cell Biol.* **194**(2), 165-175 (2011).
17. Dustin, P. *Microtubules*. (Springer-Verlag, 1984).
18. Desai, A. & Mitchison, T. J. Microtubule polymerization dynamics. *Annu Rev Cell Dev Biol* **13**, 83-117 (1997).
19. Amos, L.A. Microtubule structure and its stabilization. *Org Biomol Chem.* **2**, 2153-2160 (2004).

20. Freedman, H., Huzil, J. T., Luchko, T., Ludueña, R. F. & Tuszynski, J. A. Identification and characterization of an intermediate Taxol binding site within microtubule nanopores and a mechanism for tubulin isotype binding selectivity. *J Chem Inf Model* **49**, 424-436 (2009).
21. Freedman, H., et al. Model of ionic currents through microtubule nanopores and the lumen. *Phys Rev E Stat. Nonlin Soft Matter Phys* **81(5 Pt 1)**, 051912 (2010).
22. Cantero, M. R., Perez, P. L., Smoler, M., Villa Etchegoyen, C. & Cantiello, H. F. Electrical oscillations in two-dimensional microtubular structures. *Sci Rep* **6**, 27143 (2016).
23. Cantero, M. R., Villa Etchegoyen, C., Perez, P. L., Scarinci, N., & Cantiello, H. F. Bundles of brain microtubules generate electrical oscillations. *Sci Rep* **8(1)**, 11899 (2018).
24. Gutierrez, B. C., Cantiello, H. F. & Cantero, M. R. Electrical oscillations of brain microtubules. Preprint at <https://www.biorxiv.org/content/10.1101/2020.04.21.054155v2> (2020).
25. Cantero, M. R. & Cantiello, H. F. Microtubule electrical oscillations and hippocampal function. *J Neurol Neuromed* **5(3)**, 1-5 (2020).
26. Cantero, M. R., Gutierrez, B. C. & Cantiello, H. F. Actin filaments modulate electrical activity of brain microtubule protein two-dimensional sheets. *Cytoskeleton (Hoboken)* **77(3-4)**, 167-177 (2020).
27. Cantero, M. R., Perez, P. L., Scarinci, N. & Cantiello, H. F. Two-dimensional brain microtubule structures behave as memristive devices. *Sci Rep* **9**, 12398 (2019).
28. Vissol-Gaudin, E., et al. Electrical behavior and evolutionary computation in thin films of bovine brain microtubules. *Sci Rep* **11(1)**, 10776 (2021).
29. Fourest-Lieuvin, A. Purification of tubulin from limited volumes of cultured cells. *Protein Expression and Purification* **45(1)**, 183-90 (2006).
30. Gutierrez, B. C., et al. Honeybee brain oscillations are generated by microtubules. The concept of a brain central oscillator. *Front Mol Neurosci* **14**, 727025 (2021).
31. Dai, X. Q., et al. External Ca^{2+} regulates polycystin-2 (TRPP2) cation currents in LLC-PK1 renal epithelial cells. *Exp Cell Res* **350(1)**, 50-61 (2017).
32. Miyoshi, K., Kasahara, K., Miyazaki, I. & Asanuma, M. Lithium treatment elongates primary cilia in the mouse brain and in cultured cells. *Biochem Biophys Res Commun* **388(4)**, 757-762 (2009).
33. Perez, P. L., Scarinci, N., Cantiello, H. F. & Cantero, M. R. Polycystin-2 (TRPP2) regulates primary cilium length in LLC-PK1 renal epithelial cells. Preprint in <https://www.biorxiv.org/content/10.1101/2020.02.24.962860v1> (2020).
34. Cantero, M. R. & Cantiello, H. F. Effect of lithium on the electrical properties of polycystin-2 (TRPP2). *Eur Biophys J* **40(9)**, 1029-1041 (2011).

35. Kleene, S. J. & Van Houten, J. L. Electrical signaling in motile and primary cilia. *Biosci* **64**(12), 1092-1102 (2014).
36. Graps, A. An introduction to wavelets. *IEEE Computational Science & Engineering* **2**(2), 50-61 (1995).
37. Sharma, A., Ghatak, A. K. & Kandpal, H. C. Coherence- Overview. *Encyclopedia of Modern Optics* (ed. Guenther, B. & Steel, D.) 84-99 (Elsevier Ltd, 2005).
38. Lee, J. H. & Gleeson, J. G. The role of primary cilia in neuronal function. *Neurobiol Dis* **38**(2), 167-172 (2010).
39. Hou, X., et al. Cystin, a novel cilia-associated protein, is disrupted in the *cpk* mouse model of polycystic kidney disease. *J Clin Invest* **109**, 533-540 (2002).
40. Yoder, B. K., Hou, X. & Guay-Woodford, L. M. The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. *J Am Soc Nephrol* **13**, 2508-2516 (2002).
41. Murcia, N. S., et al. The Oak Ridge Polycystic Kidney (*orpk*) disease gene is required for left-right axis determination. *Development* **127**(11), 2347-2355 (2000).
42. Pazour, G. J., San Agustin, J. T., Follit, J. A., Rosenbaum, J. L. & Witman, G. B. Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in *orpk* mice with polycystic kidney disease. *Curr Biol* **12**, R378-R380 (2002).
43. Ma, M., Gallagher, A-R. & Somlo, S. Ciliary mechanisms of cyst formation in polycystic kidney disease. *Cold Spring Harb Perspect Biol.* **9**(11), a028209 (2017).
44. Woo, D. D., Tabancay, A.P. Jr & Wang, C. J. Microtubule active taxanes inhibit polycystic kidney disease progression in *cpk* mice. *Kidney Int* **51**(5), 1613-1618 (1997).
45. Scarinci, N., Perez, P. L., Cantero, M. R. & Cantiello, H. F. Ca^{2+} -dependent regulation by the cyclic AMP pathway of primary cilium length in LLC-PK1 renal epithelial cells. Preprint in <https://www.biorxiv.org/content/10.1101/2020.05.11.088336v1> (2020).
46. Roach, M. C., et al. Preparation of a monoclonal antibody specific for the Class I isotype of β -Tubulin: The β isotypes of tubulin differ in their cellular distributions within human tissues. *Cell Motility and the Cytoskeleton* **39**, 273-285 (1998).
47. Renauld, J., Johnen, N., Thelen, N., Cloes, M. & Thiry, M. Spatio-temporal dynamics of β -tubulin isotypes during the development of the sensory auditory organ in rat. *Histochem Cell Biol* **144**, 403-416 (2015).
48. Chakraborti, S., Natarajan, K., Curiel, J., Janke, C. & Liu, J. The emerging role of the Tubulin Code: From the tubulin molecule to neuronal function and disease. *Cytoskeleton* **73**, 521-550 (2016).

49. Bonacina, J., Carabajal, M. P. A., Cantero, M. R. & Cantiello, H. F. The bacterial tubulin homolog FtsZ forms 2D-sheets that sustain electrical oscillations. *Biophys J* **118**: 126A (2020).
50. Priel, A., Ramos, A. J., Tuszynski, J. A. & Cantiello, H. F. A biopolymer transistor: electrical amplification by microtubules. *Biophys J* **90**, 4639-4643 (2006).
51. Priel, A., Ramos, A. J., Tuszynski, J. A. & Cantiello, H. F. Effect of calcium on electrical energy transfer by microtubules. *J Biol Physics* **34(5)**, 475-485 (2008).
52. Poole, C. A., et al. Confocal analysis of primary cilia structure and colocalization with the Golgi apparatus in chondrocytes and aortic smooth muscle cells. *Cell Biol Int* **21(8)**, 483-94 (1997).
53. Kim, J., Kato, M. & Beachy, P. A. Gli2 trafficking links Hedgehog-dependent activation of Smoothened in the primary cilium to transcriptional activation in the nucleus. *Proc Natl Acad Sci USA* **106**, 21666-21671 (2009).
54. Praetorius, H. A. & Spring, K. R. Removal of the MDCK cell primary cilium abolishes flow sensing. *J Membr Biol* **191**, 69-76 (2003).
55. Cantiello, H. F. Regulation of calcium signaling by polycystin-2. *Am J Physiol* **286**, F1012-F1029 (2004).
56. Li, Q., et al. Polycystin-2 cation channel function is under the control of microtubular structures in primary cilia of renal epithelial cells. *J Biol Chem* **281(49)**, 37566-37575 (2006).
57. Praetorius, H. A. & Spring, K. R. Bending the MDCK cell primary cilium increases intracellular calcium. *J Membr Biol* **184**, 71-79 (2001).
58. Köttgen, M., et al. TRPP2 and TRPV4 form a polymodal sensory channel complex. *J Cell Biol* **182(3)**, 437-447 (2008).
59. Delling, M., et al. Primary cilia are not calcium-responsive mechanosensors. *Nature* **531**, 656-660 (2016).
60. Jin, X., et al. Cilioplasm is a cellular compartment for calcium signaling in response to mechanical and chemical stimuli. *Cell Mol Life Sci* **71(11)**, 2165-2178 (2013).
61. Su, S., et al. Genetically encoded calcium indicator illuminates calcium dynamics in primary cilia. *Nature Methods* **10**, 1105-1107 (2013).
62. Karr, T. L., Kristofferson, D. & Purich, D. L. Calcium ion induces endwise depolymerization of bovine brain microtubules. *J Biol Chem* **255**, 11853-11856 (1980).
63. Brown, E. M., et al. Cloning and characterization of an extracellular Ca²⁺-sensing receptor from bovine parathyroid. *Nature* **366**, 575-580 (1993).
64. Brown, E. M. & MacLeod, R. J. Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev* **81**, 239-297 (2001).

65. Hofer, A. M. & Brown, E. M. Extracellular calcium sensing and signaling. *Nat Rev Mol Cell Biol* **4**, 530-538 (2003).
66. Kleene, N. K. & Kleene, S. J. A method for measuring electrical signals in a primary cilium. *Cilia*; **1**, 17-27 (2012).
67. DeCaen, P. G., Dellling, M., Vien, T. N. & Clapham, D. E. Direct recording and molecular identification of the calcium channel of primary cilia. *Nature* **504**, 315-318 (2013).
68. Dellling, M., DeCaen, P. G., Doerner, J. F., Febvay, S. & Clapham, D. E. Primary cilia are specialized calcium signalling organelles. *Nature* **504**, 311-314 (2013).
69. Ichinomiya T. Frequency synchronization in random oscillator network. *Phys Rev E Stat Nonlin Soft Matter Phys* **70(2 Pt 2)**, 026116 (2004).
70. Hutu, F. D, Cauet, S. & Coirault, P. Robust synchronization of different coupled oscillators: Application to antenna arrays. *J The Franklin Institute* **346(5)**, 413-430 (2009).
71. BniLam, N., Steckel, J. & Weyn, M. Synchronization of multiple independent sub-array antennas: An application for angle of arrival estimation. *IEEE Transactions on Antennas and Propagation* **67(2)**, 1223 – 1232 (2019).
72. Lin, E. & Cantiello, H. F. A novel method to study the electrodynamic behavior of actin filaments. Evidence for cable-like properties of actin. *Biophys J* **65**, 1371-1378 (1993).
73. Tuszyński, J. A., Portet, S., Dixon, J. M., Luxford, C. & Cantiello, H. F. Ionic wave propagation along actin filaments. *Biophys J* **86**, 1890-1903 (2004).
74. Broer, H. W. Resonance and fractal geometry. *Acta Appl Math* **120**, 61-86 (2012).
75. Aguiar-Conraria, L. & Soares, M.J. The continuous wavelet transform: a primer. *NIPE WP* **16**, https://econpapers.repec.org/paper/nipnipewp/16_2f2011.htm (2011).
76. Mandel, L. & Wolf, E. Spectral coherence and the concept of cross-spectral purity. *J Opt Soc Am* **66(6)**, 529-535 (1976).
77. Oppenheim, A. V., Schaffer, R. W. & Buck, J. R. *Discrete-Time Signal Processing*. 2nd Ed. (Pearson, 1999).

Acknowledgements

This study was funded by grants Agencia, MinCyT (Argentina) PICT 2016-3739 and PICT 2018-3337 (HC).

Author Contributions

HFC and MdRC designed all the experiments, and wrote the main manuscript text. NS and BCG carried out all experimental procedures. NS, BCG and MdRC conducted the analysis of the experimental data, and prepared the Figures. All authors reviewed the manuscript.

The Primary Cilium is an Electrical Antenna

Competing interests.

The authors declare no competing interests.

Data Availability

The datasets used and analyzed during the current study available from the corresponding author on reasonable request.