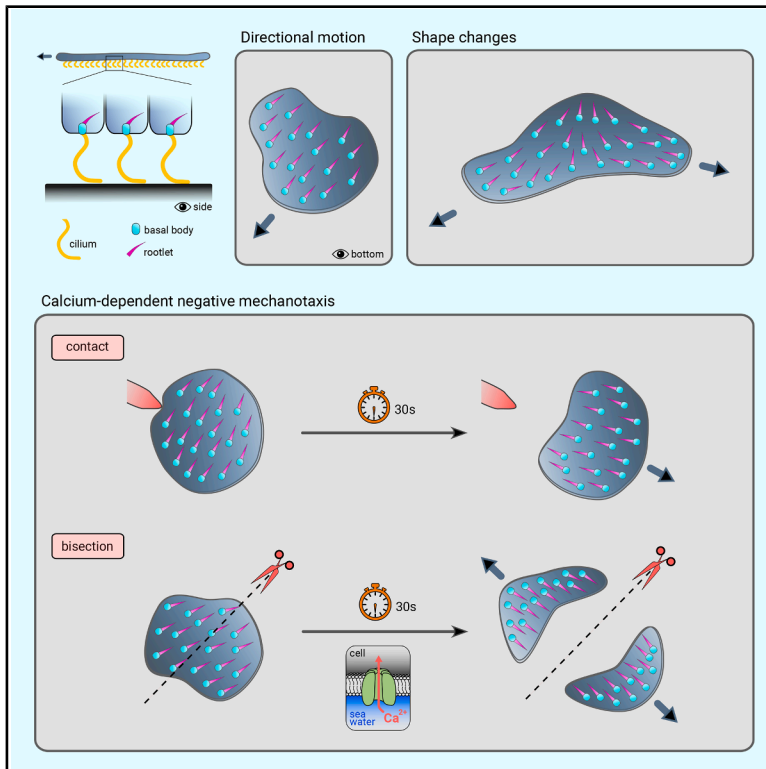


Current Biology

Fast mechanosensitive and Ca^{2+} -dependent reorientation of motile cilia basal bodies in the placozoan *Trichoplax*

Graphical abstract



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In brief

Leria et al. show that mechanical cues trigger Ca^{2+} -dependent, coordinated, and fast reorientation of the ciliary basal bodies in *Trichoplax*. This enables swift changes in ciliary beat direction and therefore in the direction of animal movement. Thus, simple cellular events underlie emergent complex mechanotactic behaviors in a nerveless animal.

Highlights

- Basal body orientation dynamically determines locomotion direction in *Trichoplax*
- Gradients of basal body orientation drive animal-scale shape changes
- Basal body reorientation enables a negative mechanotactic response within seconds
- Mechanical stimuli trigger coordinated Ca^{2+} -dependent reorientation of basal bodies

Report

Fast mechanosensitive and Ca²⁺-dependent reorientation of motile cilia basal bodies in the placozoan *Trichoplax*

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SUMMARY

Locomotion driven by ciliary beating is widespread among animals and represents one of the most ancient movement strategies.¹ The direction of ciliary beating is largely set by the orientation of the ciliary basal bodies (BBs),^{2–9} itself determined by the ciliary rootlet.⁹ In most animals, BB orientation is set along global body axes.¹⁰ The placozoan *Trichoplax* challenges this view. This small flat marine animal lacks a defined body plan^{11–14} yet efficiently crawls on solid substrates and continuously changes shape thanks to the thousands of cilia on its lower epithelium that can swiftly change beating direction.^{15–20} This raises a key question: how is ciliary beating orientation coordinated and reoriented across scales in the absence of a fixed body plan? Here, we immunostain rootlets to map BB orientation across the entire *Trichoplax* lower epithelium. Combining this approach with animal tracking reveals that BBs are globally aligned with the instantaneous direction of animal movement and that large-scale spatial variation of BB orientation underlies body-shape changes. Strikingly, BBs can also reorient collectively in a few seconds in response to external mechanical stimuli, revealing a coupling between mechanical cues and ciliary beating direction that enables rapid negative mechanotactic responses. Treatment with pharmacological inhibitors shows that this phenomenon depends on the activity of voltage-gated calcium channels. Together, our results uncover a rapid and coordinated mechanism of BB reorientation that links subcellular organization to whole-animal behavior, providing a framework to understand how ciliary locomotion emerges in the absence of fixed body axes.

RESULTS

Organization of lower epithelium BBs in *Trichoplax*

The lower epithelium of *Trichoplax* is covered by thousands of motile cilia (Figure 1A), which propel the animal over solid substrates (Video S1). *Trichoplax* typically move over a distance of one millimeter/minute, in a seemingly random fashion and with occasional stops (Figures S1A and S1B; Video S2). Given the absence of antero-posterior polarity and the morphological plasticity of *Trichoplax*, these movements are accompanied by various degrees of body-shape changes (Figure S1C; Video S3). Velocity slightly increases with animal size, but no effect of shape compactness can be detected (Figure S1D).

Transmission electron microscopy (TEM) showed that in the lower epithelium each basal body (BB) is associated with a striated rootlet (Figures 1B and 1C) and that the length (typically 1.5–2 μm) and striation pattern periodicity (6,895 ± 9,44 nm; *n* = 166) of *Trichoplax* ciliary rootlets are consistent with those of other organisms.^{9,22} Immunofluorescence (IF) with antibodies against acetylated tubulin, labeling the ciliary axoneme, and against

the BB component centrin confirmed the presence of a BB at the base of each cilium (Figure 1D). IF with antibodies against centrin and against the membrane-associated guanylate kinase (MAGUK) proteins, which label the cell contours, showed that, except for the round-looking lipophile cells,²¹ each lower epithelium cell carries a centrin-positive BB (Figure 1E). IF with antibodies against rootletin and centrin revealed rootletin-positive elongated structures associated with each BB and consistent with ciliary rootlets (Figure 1F).

Thus, the structure of the *Trichoplax* motile cilia BB does not substantially differ from that reported for other metazoans, and the rootlet can be used as a BB orientation marker.

BB orientation is aligned with the direction of movement in freely moving *Trichoplax*

To assess the relation between BB orientation and the direction of animal movement, individual *Trichoplax* crawling on glass coverslips were filmed for 20–30 s, rapidly fixed, then processed for IF against rootletin and centrin and imaged by confocal microscopy. To obtain a high-resolution view of the entire lower

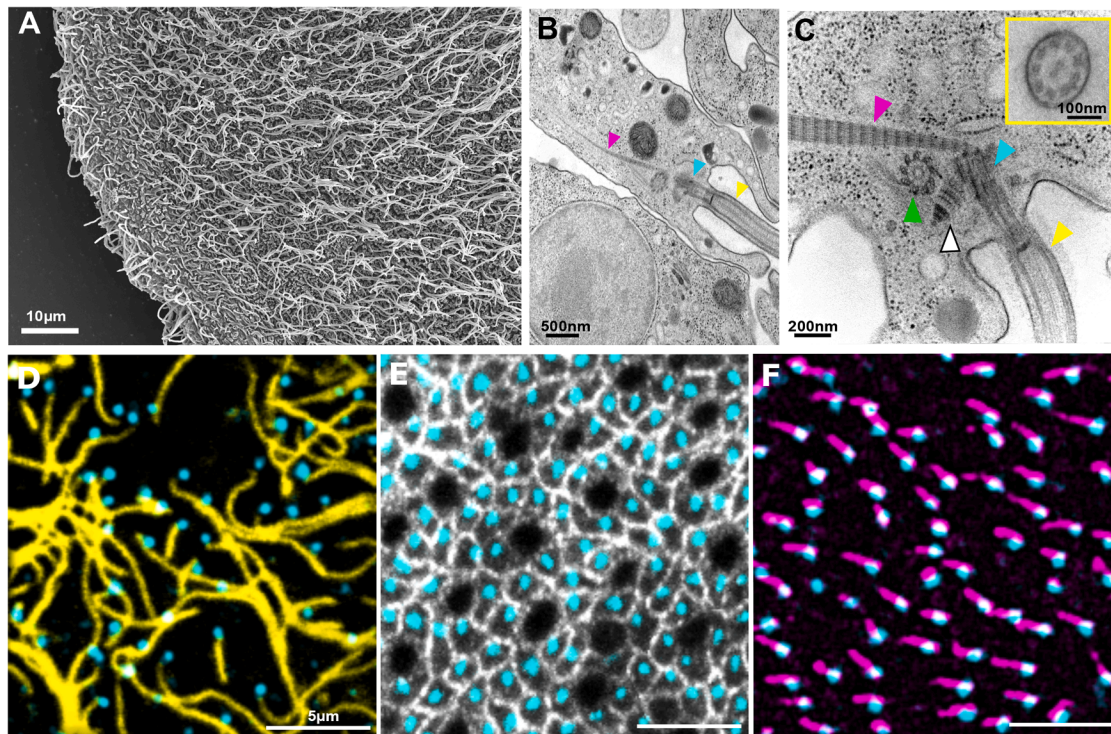


Figure 1. Cilia and associated structures in the *Trichoplax* lower epithelium

(A) Scanning electron microscopy image of the lower epithelium of *Trichoplax*.

(B) Transmission electron microscopy (TEM) image of a ciliated cell of the lower epithelium, showing the cilium (yellow arrowhead), the BB centriole (light blue arrowhead), and the ciliary rootlet (magenta arrowhead).

(C) Higher-magnification TEM image of a different ciliated lower epithelial cell. Color codes as in (B), plus a white arrowhead pointing at the basal foot and a green arrowhead pointing at the sister centriole. Inset: cross-section of the ciliary axoneme showing the typical 9 + 2 configuration.

(D) *En face* view of the lower epithelium, showing that BB centrioles (light blue, anti-centrin Ab) are each located at the basis of a motile cilium (yellow, anti-acetylated tubulin Ab).

(E) *En face* view of the lower epithelium, showing the apical outlines of lower epithelial cells (white, anti-MAGUK Ab) and the BB centrioles (light blue, anti-centrin Ab). The black cells not showing a centrin signal are the lipophile cells.²¹

(F) A view of the lower epithelium showing the BB centrioles (light blue, anti-centrin AB) and the corresponding rootlets (magenta, anti-rootletin Ab).

Scale bars in (E) and (F) are the same as in (D).

See also [Figure S1](#) and [Videos S1, S2, and S3](#).

epithelium of each animal, images taken at single-cell resolution were stitched together ([Figure 2A](#)). Using automated segmentation of the centrin and rootletin signals, we defined for each BB a polarity vector reporting the orientation of the rootlet and pointing toward the centrin signal ([Figures 2B–2G](#)). Since *Trichoplax* lower epithelia typically consist of tens of thousands of cells, polarity vectors were binned into $10 \times 10 \mu\text{m}$ square subregions, each containing approximately 20–25 ciliated cells, allowing better visualization. Thus, for each analyzed individual, we obtain color-coded maps and vector fields of BB orientation across the entire lower epithelium ([Figures 2H and S2A](#)). This approach revealed that the average orientation of BBs is aligned with the direction of animal movement ([Figures 2I–2K and S2B](#)). Remarkably, animals significantly changing their aspect ratio can display body-scale spatial variations of BB orientation ([Figures 2L–2N and S2A](#)), suggesting that body-shape changes result from tissue strain caused by variations in crawling orientation. Averaging animal-scale BB polarity data for $N = 4$ animals confirmed that the movement of the center of mass of the animal aligns with the average BB orientation ([Figure 2O](#)).

Fast reorientation of animal movements and BB orientation following mechanical perturbations

Chemo- and thermotactic cues are known to affect the directionality of *Trichoplax* movements,^{15,16,19,20,23–28} but the role of mechanical stimulation has never been explored. We applied two types of mechanical perturbations: poking and bisection. Gently poking the leading edge of freely moving animals ($N = 10$) with a round-tip microscalpel resulted in a reorientation of their movement in a direction on average opposite to that of the stimulus ([Figures 3A and 3B; Video S4](#)). This reorientation occurred a few seconds after the stimulus and persisted for at least 30 s, showing that the animals were not simply pushed but actively crawled away. To determine whether this active movement reorientation was correlated with a reorientation of the BBs, animals ($N = 3$) were fixed 30 s after the stimulation, then processed and analyzed as described in the previous paragraph. This revealed that 30 s after poking, BBs were on average aligned with the new direction of movement ([Figures 3C and S3A](#)). Manual bisection of animals ($N = 24$) with a microscalpel systematically resulted in the two halves

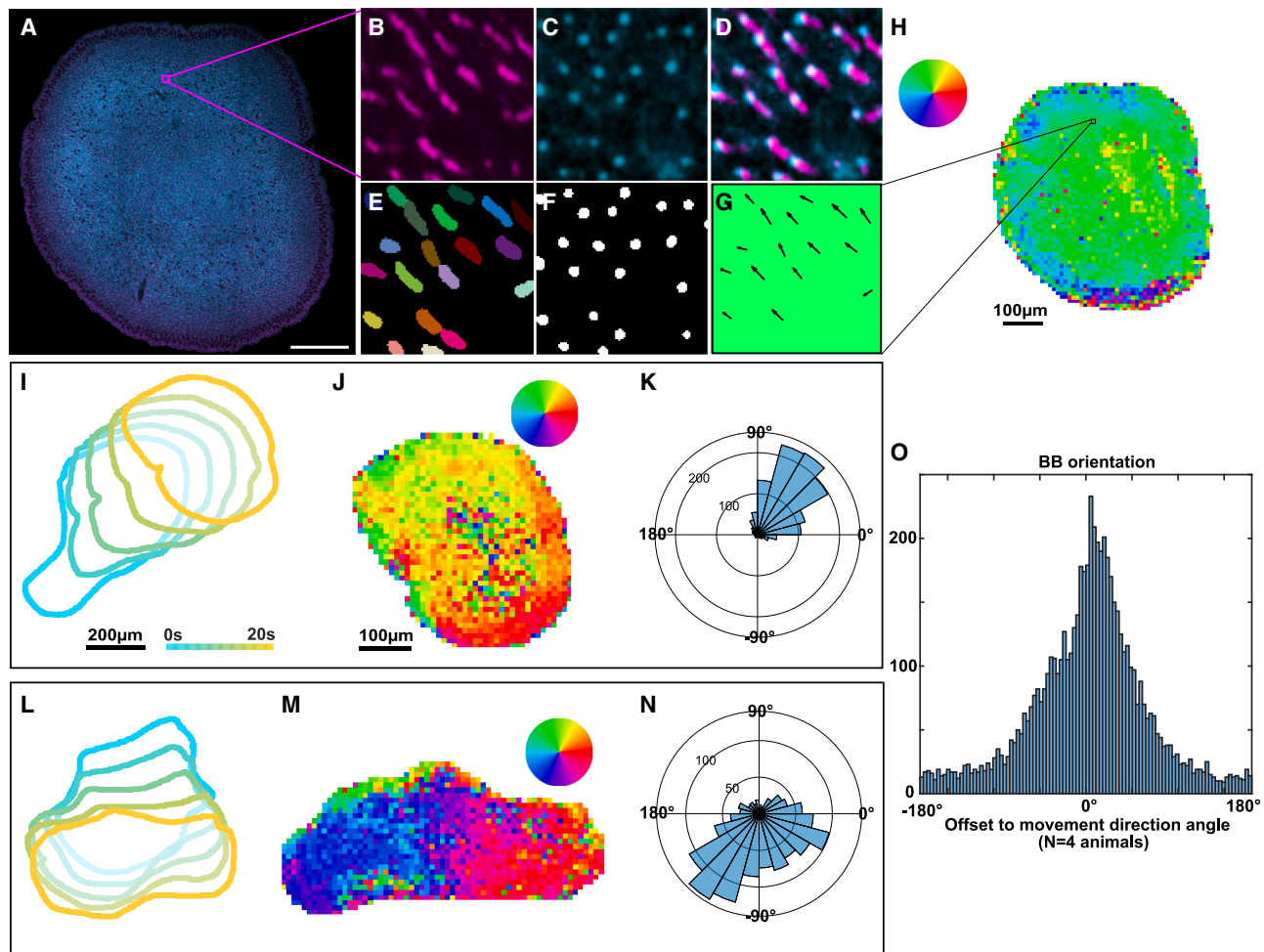


Figure 2. Orientation of BBs in *Trichoplax*

(A) En face view of the whole lower epithelium of a *Trichoplax* stained with anti-centrin (light blue) and anti-rootletin (magenta) Abs. Image reconstructed by stitching together nine high-resolution confocal images.
 (B) A close-up of the boxed area in (A), showing the rootletin signal.
 (C) A close-up of the same boxed area, showing the centrin signal.
 (D) Merge of the centrin and rootletin signals.
 (E) Segmentation of the rootletin signal shown in (B).
 (F) Segmentation of the centrin signal shown in (C).
 (G) BB orientation vectors computed from the segmentation shown in (E) and (F).
 (H) Color-coded map of BB orientation for the animal shown in (A).
 (I) Body outlines of a moving *Trichoplax*, drawn at 5-s intervals to show the animal displacement. The color code corresponds to the different times at which the outlines were drawn.
 (J) Color-coded map of BB orientation for the animal in (I).
 (K) Polar histogram of BB orientation for the same animal, showing a global alignment with the direction of movement.
 (L) Body outlines of an individual combining movement and lateral spreading.
 (M) Color-coded map of BB orientation for the animal in (L), showing a spatial variation of orientation corresponding to spreading.
 (N) Polar histogram of BB orientation for the same animal, showing a higher spread of the polarity distribution.
 (O) Histogram of BB orientation offset relative to the direction of movement (data pooled from $N = 4$ animals). BB orientation aligns on average with the direction of movement.
 See also Figure S2.

adopting a crescent-like shape and crawling away from each other, approximately perpendicular to the bisection axis (Figures 3D and 3E; Video S4). Averaging BB orientation in both halves of bisected animals ($N = 3$) revealed that 30 s after bisection, the BBs in each half had already reoriented away

from the cutting plane and pointed toward the new directions of movement (Figure 3F). Subsequent ciliary reorientation was confirmed with electron microscopy (EM) (Figure S3B). No systematic cell shape anisotropies were detected adjacent to the cut region (Figure S3C). Interestingly, comparing BB orientation

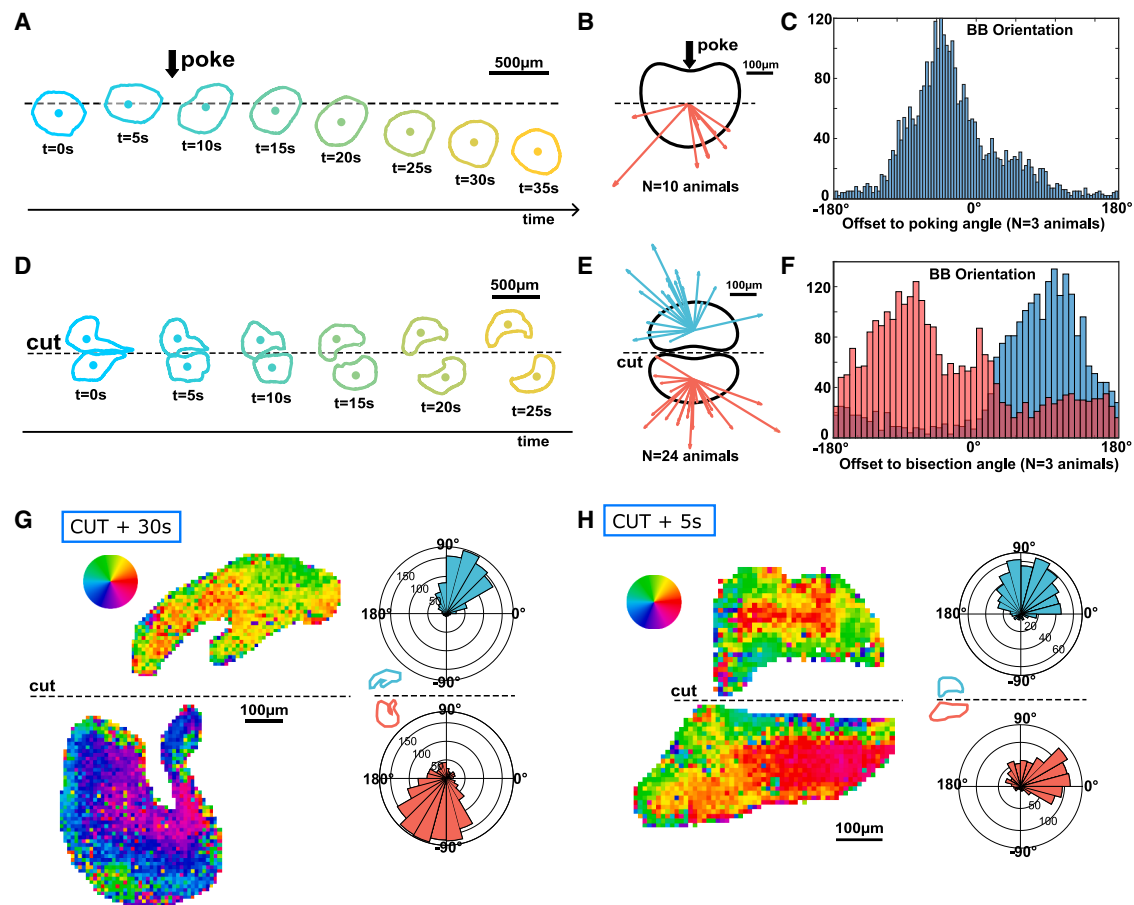


Figure 3. Mechanosensitive reorientation of BBs in *Trichoplax*

(A) Body outlines of a moving *Trichoplax* reorienting following poking (black arrow), drawn at 5-s intervals along the x axis (time). The color code corresponds to the different times at which the outlines were drawn, and the centroid of each outline is indicated.

(B) Centroid displacement following poking for $N = 10$ individuals. The black contour schematizes an idealized poked individual. All experiments are aligned so that the poking direction is always vertical. The black arrow represents the poking direction. Arrows represent individual centroid displacements, from the initial position upon poking to the final position after 30 s. Post-poking movement aligns on average with the direction of poking.

(C) Histogram of post-poking (30 s) BB orientation offset relative to the direction of poking (data pooled from $N = 3$ animals). BB orientation aligns on average with the direction of poking.

(D) Body outlines of a bisected *Trichoplax*.

(E) Centroid displacements following bisection for $N = 24$ individuals. The black contour schematizes an idealized bisected individual. All experiments are aligned so that the bisection axis is always horizontal. The dotted line represents the bisection axis. Arrows represent individual centroid displacements of the two halves, from the initial position upon bisection to the final position after 30 s.

(F) Histogram of post-bisection (30 s) BB orientation offset relative to the bisection axis (data pooled from $N = 3$ animals). BB orientation in bisected halves is on average perpendicular to the bisection axis.

(G) Color-coded map and polar histogram of BB orientation 30 s after bisection.

(H) Color-coded map and polar histogram of BB orientation 5 s after bisection, showing incomplete reorientation.

See also [Figure S3](#) and [Video S4](#).

patterns 30 and 5 s after bisection suggests that a few seconds are required to propagate BB reorientation across the animal halves ([Figures 3G, 3H, S3D, and S3E](#)). Asymmetric or partial cuts show that the severed regions consistently move away from the cutting plane, regardless of the cut geometry ([Video S4](#)). Together, these results show that *Trichoplax* can actively change its crawling direction in response to mechanical stimuli. This negative mechanotactic behavior is enabled by the reorientation of BBs, which takes place across the entire lower epithelium on the timescale of seconds.

Calcium is required for BB reorientation and negative mechanotaxis

Animal-scale BB reorientation induced by local mechanical stimuli implies long-range propagation of a reorientation signal starting from the stimulation point. Given the rapidity of reorientation, we reasoned that planar cell polarity signaling is unlikely to play a role and instead chose to investigate the possible role of Ca^{2+} . Indeed, Ca^{2+} waves play a role in several instances of coordinated multicellular responses, and across evolution Ca^{2+} signaling mediates a number of cellular responses to external

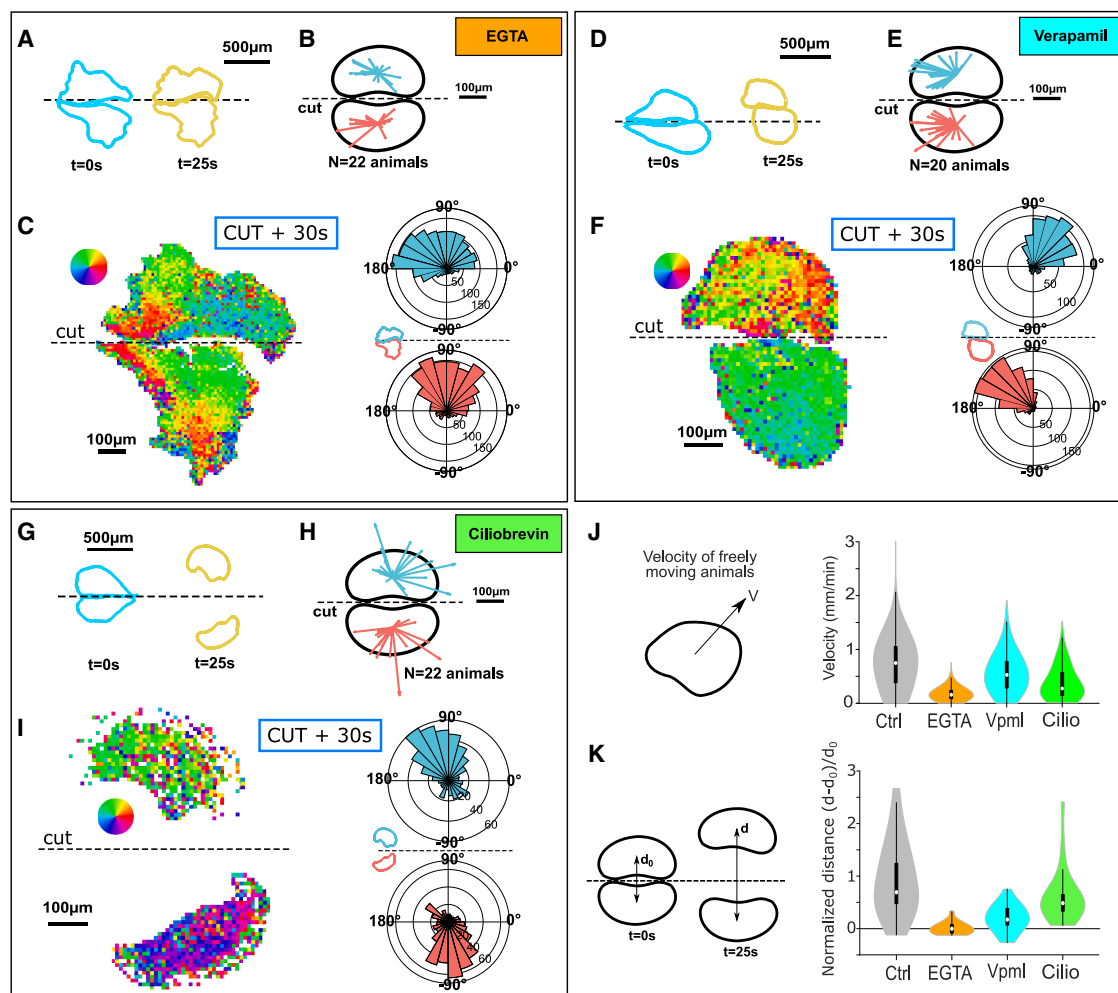


Figure 4. Calcium-dependent reorientation of BBs in *Trichoplax*

(A) Body outlines at t = 0 s and t = 25 s after bisection in an EGTA-treated individual.
 (B) Centroid displacements following bisection for N = 22 EGTA-treated individuals.
 (C) Color-coded map and polar histograms of BB orientation for the animal in (A).
 (D) Body outlines at t = 0 s and t = 25 s after bisection in a verapamil-treated individual.
 (E) Centroid displacements following bisection for N = 20 verapamil-treated individuals.
 (F) Color-coded map and polar histograms of BB orientation for the animal in (D).
 (G) Body outlines at t = 0 s and t = 25 s after bisection in a ciliobrevin-treated individual.
 (H) Centroid displacements following bisection for N = 22 ciliobrevin-treated individuals.
 (I) Color-coded map and polar histograms of BB orientation for the animal in (G).
 (J) Velocity of freely moving, non-bisected individuals in all conditions.
 (K) Normalized separation distance post-bisection (25 s) in all conditions. Negative values indicate that bisected halves get closer to each other.
 See also [Figure S4](#) and [Video S1](#).

cues,²⁹ notably the fast reorientation of flagellar BBs in unicellular green algae.³⁰ We investigated the possible requirement of Ca^{2+} for BB reorientation using two distinct perturbations: treatment with EGTA, a Ca^{2+} chelator, or with verapamil,³¹ a pharmacological inhibitor of voltage-gated Ca^{2+} channels (VGCCs), crucial actors in fast cellular responses to external stimuli that have been described in *Trichoplax*.³² Following bisection of animals treated with 50 mM EGTA, the two halves failed to move away from each other (N = 22) (Figures 4A, 4B, and 4K), and the rapid reorientation of BBs did not occur (N = 3) (Figures 4C

and S4A). Instead, BBs showed random orientations in the bisected halves. To understand whether the failure of halves to separate following EGTA treatment was due to BB reorientation defects or also to decreased motility, we tracked movements and analyzed BB orientation in uncut EGTA-treated animals. This revealed that coordination of BB orientation across the animal was preserved (Figure S4B), but overall motility and ciliary beating were strongly reduced (Figures 4J and S4C; Video S1). Thus, although the EGTA concentration and incubation time used here did not affect *Trichoplax* viability and tissular

cohesion, it is possible that the impairment of negative mechanotaxis is mostly due to a general decrease of motility following the rather crude and nonspecific Ca^{2+} depletion treatment.

75 μM verapamil-treated *Trichoplax* ($N = 20$) also displayed defective negative mechanotaxis and impaired BB reorientation following bisection (Figures 4D–4F and S4D). However, ciliary beating and overall motility were much less affected in verapamil-treated uncut animals than in EGTA-treated ones, with an average velocity close to that of untreated controls (Figures 4J, S4E, and S4F; Video S1). Moreover, following bisection of verapamil-treated animals, the two halves are still able to move, albeit with random orientation (Figure 4K), suggesting that in this case the impaired negative mechanotaxis does not result from a decrease in motility but from a specific defect of BB reorientation. To directly test the impact of motility defects on negative mechanotaxis and to understand whether ciliary beating itself is required to propagate BB reorientation, we treated *Trichoplax* with 25 μM ciliobrevin D, a dynein inhibitor known to impair ciliary beating.³³ This resulted in an average decrease of motility in both uncut animals and in animal halves following bisection, confirming that reduction of ciliary beating indeed affects the locomotion velocity (Figure 4J; Video S1). However, the reorientation of movement of the bisected animal halves was not affected, in sharp contrast with verapamil treatments (Figure 4K). Analysis of BB orientation in these conditions was complicated by the fact that animals tend to detach from the substrate, making bisection difficult or impossible. However, a milder phenotype of partially reduced ciliary beating and partially decreased motility allows bisection and results in animal halves showing normal BB reorientation (Figure 4I). Altogether, these results show that VGCC-mediated Ca^{2+} influx is required for bisection-induced negative mechanotaxis, and that BB reorientation can occur even in animals with reduced ciliary beating. Whether animal-scale BB reorientation is possible in the complete absence of ciliary beating remains to be determined.

DISCUSSION

Ciliary locomotion is an evolutionarily ancient strategy in which the beating of motile cilia allows organisms to swim within liquids or glide over solid surfaces. In most metazoans, the orientation of the BBs, a major factor in defining the orientation of ciliary beating,⁷ is established during embryonic development, is aligned along the main body axes, and is maintained throughout life. The most notable exceptions are metamorphosis and some regenerative processes.¹⁰ Here, we examine the curious case of *Trichoplax*, which lacks major body axes and moves by ciliary walking while constantly changing body shape. The cilia on its lower epithelium can coordinately change their beating direction in a few seconds, thus allowing changes in the direction of locomotion,^{15,16,18,20,23–27,34} but the molecular underpinnings of this beating reorientation had never been addressed.

Our results show that the predominant orientation of the BBs of the lower epithelium motile cilia corresponds to the overall direction of animal locomotion. We also observed that spatial variations of BB orientation result in in-plane tissue strain and body-shape changes as well as underpin more elaborate phenomena, such as fold flattening³⁵ or epithelial fractures.³⁴ How such spatial variations of BB orientation appear is unclear, but

they might result from biochemical signals²⁷ and/or from a trade-off between body size and spatial coordination.³⁶ These observations suggest that the rapid changes in body shape and direction of movement observed in *Trichoplax* require coordinated BB reorientation, which results in ciliary beating redirection. We also found that fast reorientations of BBs and of *Trichoplax* movement occur upon mechanical perturbations. Such rapid BB reorientation events have never been observed in metazoans and could be exclusive to the morphologically plastic placozoans. An appealing hypothesis, to be further investigated, is that this might represent an original mechanosensitive stress-response strategy allowing *Trichoplax*, which lacks muscles and a nervous system, to escape noxious mechanical stimuli. Indeed, the mechanical stimuli we provided, although admittedly rather harsh, are in the range of what *Trichoplax* might encounter in the ecologically complex biotopes where they live.³⁷

Although environmental Ca^{2+} and VGCC-mediated cellular Ca^{2+} influx are necessary for fast BB reorientation following mechanical stimulation, it remains unclear whether they are required for the mechanosensitive events that trigger BB reorientation, for the reorientation itself, or for both. A possible scenario is that polarized Ca^{2+} influx via VGCCs affects the rotation of BBs and of the ciliary complex by creating a torque powered by motor proteins. Which proteins are involved and how they can create the required torque will have to be further determined, but actin and tubulin, known to determine and stabilize the position of BBs in mono- and multiciliated cells,^{38–40} will be tested as possible candidates. The BB-associated protein centrin also undergoes conformational changes upon Ca^{2+} binding, and centrin or centrin-like proteins have been involved in extremely fast, Ca^{2+} -induced structural changes in unicellular organisms.^{30,41–43} Finally, Ca^{2+} -dependent contractility of ciliary rootlets themselves, resulting in BB displacement, has been described in unicellular flagellate algae.⁴⁴ A possible alternative interpretation, that BB reorientation reflects the active rotation of individual cells, with BBs passively following, is unlikely for several reasons. First, cell rotation would require rapid and tissue-wide disengagement of both cell-substrate and cell-cell adhesions, implying intercellular junction remodeling processes that occur on much longer timescales, even in the rapidly rearranging fly epithelium.⁴⁵ Second, the rotational drag associated with an epithelial cell is orders of magnitude larger than that of a slender structure such as the BB rootlet, resulting in substantially longer rotation timescales. Finally, internal forces cannot generate net angular momentum without an external torque. Cell rotation would therefore require interfacial force-generating structures that transmit torque to the environment. By contrast, BB-rootlet complexes are slender, torque-transmitting structures coupled to the cytoskeleton, far more susceptible to rapid reorientation driven by intracellular tension anisotropies.

Trichoplax lower epithelium being composed of tens of thousands of monociliated cells, the coordinated response to a local mechanical stimulus requires the propagation of a BB reorientation signal from the stimulation point across the entire epithelium. It is tempting to speculate that Ca^{2+} waves could be involved in such a long-range process. Indeed, tissue-wide Ca^{2+} waves in response to mechanical stimulation are an evolutionarily conserved feature,^{46–51} and their propagation speed matches the timescale of BB reorientation described here. However,

Trichoplax lacks gap junctions, a widespread strategy of Ca^{2+} wave intercellular propagation, and thus alternative hypothetical propagation mechanisms must be envisaged. These might imply the release of secreted molecules such as nitric oxide, monoamine neurotransmitters, and soluble neuropeptides, as well as the direct transmission of mechanical or electrical signals, as observed in glass sponges and choanoflagellates.^{50,52} Strikingly, our bright-field movies show an opacification wave that propagates in reorienting animals, starting from the stimulation point and concomitant with BB reorientation. This is visible not only upon bisection (Video S4) but also when two animals make physical contact and change directions (Video S3). Opacification reflects a local density increase, suggesting a mechanical wave propagating across the tissue upon redirection. Of note, asymmetric bisection experiments suggest that although negative mechanotaxis is initiated at the same time in the two animal fragments, it takes longer for the larger fragment to display consistent, directed movement (Video S4), indicating a delay due to propagation over longer distances. A simple interpretation is that the first rows of cells deformed by the stimulus initiate the reorientation of their BBs upon Ca^{2+} entry, thus changing their crawling direction and pushing on the next rows of cells, causing deformation and ciliary torque, Ca^{2+} entry and BB reorientation, and so on, creating a BB reorienting mechanochemical wave sweeping across the epithelium.

Altogether, this work sheds new light on the cellular and molecular bases of shape changes and locomotion in the early-diverging metazoan phylum Placozoa. It also questions the mechanisms used by *Trichoplax* to sense its environment and modify its behavior accordingly. Further exploring the cellular basis of rapid BB rotation and the mechanisms underpinning the reorientation wave propagation will be key to informing integrative theories for the emergence of locomotion behaviors in this neuronless, muscleless animal.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Andrea Pasini (andrea.pasini@univ-amu.fr).

Materials availability

Animals used in this study are available from the lead contact. There are restrictions on the availability of the guinea pig polyclonal anti-rootletin antibody because of limited stocks.

Data and code availability

- Full-size acquisitions, processed datasets, and orientation maps have been deposited at Mendeley Data and are publicly available as of the date of publication at <https://doi.org/10.17632/wwh9fdpwmh.1>.
- All original code has been deposited at Mendeley Data and is publicly available at <https://doi.org/10.17632/wwh9fdpwmh.1> as of the date of publication.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, M.L., R.C., and A.P.; methodology, M.L., M.D., M.R., F.R., N.B., R.H., R.C., and A.P.; data curation, M.L. and R.C.; visualization, M.L., R.C., and A.P.; software, M.L. and R.C.; resources, A.L.B. and A.P.; supervision, R.C. and A.P.; project administration, A.L.B., R.C., and A.P.; funding acquisition, A.L.B., M.L., R.C., and A.P.; writing, M.L., R.C., and A.P.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- EXPERIMENTAL MODELS AND SUBJECT DETAILS
 - *Trichoplax* collection and maintenance
- METHOD DETAILS
 - *Trichoplax* movement recording for trajectory tracking
 - Scanning and Transmission Electron Microscopy
 - Mechanical stimulation and pharmacological treatments
 - High-speed live imaging of ciliary beating
 - Antibodies
 - Immunofluorescence staining
 - Confocal microscopy
- QUANTIFICATION AND STATISTICAL ANALYSIS
 - Tracking
 - Polarity analysis

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cub.2026.04.054>.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal anti-Centrin	Sigma-Aldrich	Cat# 04-1624;
Mouse monoclonal anti-Acetylated α -Tubulin	Sigma-Aldrich	Cat# T6793; RRID: AB_477585
Mouse monoclonal anti-MAGUK	Sigma-Aldrich	Cat# MABN72
Guinea pig polyclonal anti-Rootletin	This paper	N/A
Goat anti-mouse IgG2a Alexa Fluor 647	Invitrogen	Cat# A21241
Goat anti-mouse IgG2b Alexa Fluor 488	Invitrogen	Cat# A21141
Donkey anti-guinea pig IgG Alexa Fluor 488	Jackson ImmunoResearch	Cat# 706 545 148; RRID: AB_2340472
Donkey anti-mouse IgG Alexa Fluor 647	Jackson ImmunoResearch	Cat# 715 605 151; RRID: AB_2340863
Chemicals, peptides, and recombinant proteins		
Verapamil	TargetMol	Cat# T20656; CAS: 52-53-9
Ciliobrevin-D	MedChem Express	Cat# HY-122632; CAS: 1370554-01-0
OsO ₄ 4% Aqueous Solution	https://www.emsdiasum.com	#19190
Glutaraldehyde 25% Aqueous Solution, specially purified for Electron Microscopy	https://www.sigmaaldrich.com/	#G5882-50ML
Deposited data		
Whole-animal Centrin/Rootletin stainings	This paper	https://data.mendeley.com/datasets/wwh9fdpwmh
Experimental models: Organisms/strains		
<i>Trichoplax</i> spH2	This paper	N/A
Software and algorithms		
Codes computing orientation vectors fields and averaged orientation maps	This paper	https://data.mendeley.com/datasets/wwh9fdpwmh
Other		
Instant Ocean Sea Salts	https://www.aquariumsystems.eu	#218035

EXPERIMENTAL MODELS AND SUBJECT DETAILS

Trichoplax collection and maintenance

Trichoplax were collected from racks of glass microscope slides that were placed on the bottom of the aquariums of a local pet store in Marseille, France. After three weeks, the slides were recovered, transferred to the laboratory and observed with a binocular dissection microscope. Placozoans were identified, retrieved and transferred by pipetting to large glass Petri dishes containing 100ml filtered artificial sea water (FASW) made by dissolving 33g/L Instant Ocean Sea Salt (#218035, Aquarium Systems, Sarrebourg, France) in milliQ H₂O, where they were maintained at 23°C under a 12hrs/12hrs dark-light cycle. Animals were fed weekly a mix

of *Rhodomonas salina* (gifts from Adriano Senatore, University of Toronto Mississauga, Canada and Harald Gruber-Vodicka, University of Kiel, Germany), *Nannochloropsis oculata* (#T0055, Teramer, Montpellier, France) and *Dunaliella salina* (#T0057, Teramer, Montpellier, France). Water was changed weekly.

For genotyping, 1 and 10 animals were lysed and amplification of mitochondrial 16S *rDNA* gene was performed by PCR using the primers and conditions described by Signorovitch and her colleagues.⁵³ DNA sequences obtained from PCR fragments were BLASTed against the NCBI database resulting in a match with the *Trichoplax* H2 haplotype.

METHOD DETAILS

Trichoplax movement recording for trajectory tracking

To track the movement of freely moving individuals, they were placed in a drop of FASW on round glass coverslips, left to settle and adhere for a few minutes, then filmed for variable amounts of time under a Leica S4E stereomicroscope equipped with a DinoEye camera or under a Leica S9i stereomicroscope with an integrated camera.

Scanning and Transmission Electron Microscopy

For Scanning Electron Microscopy, *Trichoplax* were placed in a 100 μl drop of FASW on glass coverslips, left to adhere, then fixed with Osmium Fixative (NaCl 460mM, Na Cacodylate 100mM, OsO₄ 78mM, Glutaraldehyde 0,3% in milliQ H₂O) for 20min on ice. After rinsing in FASW, samples were postfixed in 0,3% Glutaraldehyde in FASW overnight at 4°C, rinsed again in milliQ H₂O, dehydrated by incubation in progressively increasing EtOH concentrations (from 25% v/v in milliQ H₂O to absolute EtOH), washed three times with Hexamethyldisilazane, then left to dry overnight. Samples were then sputter-coated with gold using a Edwards S150B Sputter Coater and observed with a FEI Teneo™ VS Scanning Electron Microscope (5,00 kV, spotsize 6-7, dwell time 1-3 μs , High Vacuum).

For Transmission Electron Microscopy, individual *Trichoplax* were transferred to 1,4mm diameter, 0,05mm thick, sapphire coverslips (#16706849, Leica Microsystems), left to adhere, submitted to High-Pressure Freezing fixation on a LEICA EMPACT-2 HPF machine, then cryosubstituted with (2% (v/v) OsO₄ in Acetone) in a Leica AFS2 Freeze Substitution and Low Temperature Embedding System using the following program: -90 °C for 24 hours; -90 °C => -60 °C over 12 hours; -60 °C for 8 hours; -60 °C => -30 °C over 12 hours; -30 °C for 8 hours; -30 °C => -5 °C over 1 hour and 15min. At the end of the cryosubstitution process, the samples were washed with Acetone, embedded in Hard Plus Resin-812 following a multi-step protocol from 25% to 100% resin in Acetone, cooked at 60°C for 48hours then cut in 90nm-thick sections with a Leica EM UC7 Ultramicrotome. Sections were contrasted by incubation for 5min in Lead Citrate Trihydrate 3% in milliQ H₂O, washed in milliQ H₂O and observed on a Tecnai G2 Transmission Electron Microscope running at 200kV.

Mechanical stimulation and pharmacological treatments

Trichoplax were deposited on round glass coverslips as described above, except that the coverslips were placed within small custom-made baskets to facilitate their subsequent handling. For bisection experiments, animals were cut in two halves with a hand-held microscalpel mounted on a pin holder (Phymep, France) then freeze-substituted (see below for details) immediately after bisection (within 4s) or after 30s. ‘Poking’ experiments were performed by gently touching the rim of an animal with a hand-held round-tip microscalpel mounted on a pin holder (Phymep, France), followed by freeze-substitution 30s after.

For calcium signaling inhibition experiments, EGTA 0.5M pH8 was added to a final concentration of 50mM in seawater for 1hour. To block Voltage-Gated Calcium Channels, Verapamil (T20656, TargetMol) was added to a final concentration of 75 μM in seawater for 1hour.

For ciliary beating inhibition experiments, Ciliobrevin D (HY-122632, MedChemExpress) was added to a final concentration of 25 μM for 15 min.

High-speed live imaging of ciliary beating

Individual *Trichoplax* were deposited on a glass coverslip in a drop of FASW, EGTA-supplemented FASW, Verapamil-supplemented FASW or Ciliobrevin D-supplemented FASW, left to adhere, covered with a second coverslip using adhesive-tape spacers to prevent squeezing, then observed and filmed in brightfield on a Zeiss Axio M2 microscope, using a Freefly Ember S5K camera (acquisition speed 500 images/s). Movies were treated with Blackmagic’s DaVinci Resolve (<https://www.blackmagicdesign.com/products/davinciresolve>) to adjust the color temperatures, increase contrast, selecting high-detail frequencies to highlight the cilia and low-detail frequencies to smoothen the contrast of the background epithelial cells.

Antibodies

The following primary commercial antibodies were used: mouse monoclonal against Centrin (#04-1624, Sigma-Aldrich); mouse monoclonal against Acetylated α -Tubulin (#T6793, Sigma-Aldrich); mouse monoclonal against pan-MAGUKs (#MABN72, Sigma-Aldrich).

Polyclonal antibodies against *Trichoplax* Rootletin were generated by Eurogentec (Eurogentec SA, Seraing, Belgium) by immunising guinea pigs with the two peptides -CTNYSSPLKSKYGGRT and -CKSPSKVSPRRQTKYK identified in the predicted protein sequence of *Trichoplax* spH2 Rootletin (Uniprot accession number: A0A369RWE8; NCBI accession number: RDD38860.1). Antibodies were affinity purified and diluted to a final concentration of 50% glycerol.

Immunofluorescence staining

For cilia staining, the glass coverslips with adherent *Trichoplax* were transferred to 3.7% formaldehyde in FASW for 1 hour at room temperature (RT), then washed 3 times in PBS 1X and permeabilized for 20 min in PBS containing 0.1% Triton-X100. Samples were transferred to blocking buffer (goat serum 3%, donkey serum 3%, bovine serum albumin 1% in PBS) for 30 min to 1 hour then incubated overnight at 4°C with anti-Acetylated α -Tubulin (dilution 1:200) and anti-Centrin (dilution 1:1000) antibodies. Samples were washed 3 times in PBS 1X for 10 min and were then incubated in blocking buffer with secondary antibody goat anti-mouse IgG2a Alexa Fluor 647 (A-21241, Invitrogen) and goat anti-mouse IgG2b 488 (A-21141, Invitrogen). They were washed 3 times in PBS for 10 min and mounted with Prolong Gold antifade reagent with DAPI (P36935, Life Technologies).

For BBs and rootlets staining, we adapted a previously described freeze-substitution protocol.^{21,54} *Trichoplax* on glass coverslips were plunged into dry ice-cold acetone overnight, transferred to 3.7% formaldehyde in MetOH at -20°C for 3 hours, then incubated 5–10 min at -20°C in 2% trichloroacetic acid in MetOH to improve Rootletin staining. Samples were rehydrated through downgrading baths of EtOH (from absolute EtOH to 25% EtOH in PBS 1X) every 10 min, washed 3 times in PBS 0.1% Triton X-100 for 10 min. After transfer into blocking buffer for 1 hour, the samples were incubated with a mix of the two anti-Rootletin antibodies (each diluted 1:200) and the anti-Centrin antibody (1:1000) in blocking buffer overnight at 4°C. They were rinsed 3 times every 10 min with PBS 1X and then incubated 3–4 hours at room temperature or overnight at 4°C, in blocking buffer with anti-guinea pig AlexaFluor 488-conjugated antibodies (#706 545 148, JIR) and AlexaFluor 647-conjugated anti-mouse antibodies (#715 605 151, JIR). The same protocol, but replacing acetone with tetrahydrofuran, was used to stain cell outlines with anti-pan MAGUK antibodies diluted at 1:500.

Confocal microscopy

For visualisation of Rootletin and Centrion staining across the whole lower epithelium, *Trichoplax* were observed with a Zeiss LSM 880 Airyscan confocal microscope equipped with a 40x water objective, using the Tile Scan function with 10% overlap, and with short z-stack, bidirectional line scanning, average 4, laser scanning speed 7, sampling 2140x2140, and simultaneous acquisition of two channels (with lasers 488 nm and 633 nm). All other images were acquired with the same confocal microscope or with a Zeiss LSM 780, with objectives 63x oil or 40x water. All images were subsequently processed and analyzed with FIJI.

QUANTIFICATION AND STATISTICAL ANALYSIS

Tracking

Tracking was performed by manually contouring animal outlines every 20 seconds in FIJI and following their center of mass. The compactness deficit (Figure S1D) is defined as $\frac{p^2}{4\pi A} - 1$, so that it is dimensionless, equal to 0 for a circular shape, and increases when the perimeter increases.

To track the movement of poked/bisected animals following stimuli, we contoured animal outlines every 5 s for 30 s following poking/bisection and followed their center of mass. The normalized post-bisection separation distance (Figures 4K and 4L) is defined as $\frac{d(t) - d_0}{d_0}$, so that negative values indicate that bisected halves move closer to each other. Measurements of velocity distributions in different conditions are made by measuring the velocity along the tracks of tracked animals.

Polarity analysis

A dedicated image analysis pipeline was developed to perform BB polarity analysis. For each tiled image, the z-stacks were individually extracted and saved as TIFF files (<https://gist.github.com/lacan/16e12482b52f539795e49cb2122060cc>), then stitched together using the Grid/Collection Stitching function in FIJI.⁵⁵ Maximum intensity projection was performed to optimize signal intensity.

A model was trained in ilastik⁵⁶ to recognize the Centrion signal corresponding to the BB. The Centrion signals from all the images were segmented using the ilastik plugin (<https://github.com/ilastik/ilastik4ij#ilastik-imagej-modules>) in FIJI, the obtained probability map was thresholded and centroid xy-coordinates extracted using the Analyse Particle function. For the Rootletin signal detection, a model was trained in Cellpose⁵⁷ based on the pre-trained CP model. The Rootletin signals from all the images were segmented using Cellpose through the FIJI BIOP plugin (<https://github.com/BIOP/ij-utilities-wrappers>). ROIs were obtained from the segmentation, fitted to an ellipse and the centroid xy-coordinates, the major axis length and the major axis angle were extracted with the Measure function.

For each BB, the polarity was determined by computing a vector going from the centroid of the Rootletin signal to its extremity closest to the Centrion signal (see provided code). The lower epithelial surface of the whole animal was then subdivided into 10 μm x 10 μm square subregions, each containing around 20–25 ciliated cells and in which the BB polarity vector was averaged, allowing to extract an angle for each square subregion. The polarity orientation of each box was mapped by attributing a colour-code determined by a hsv wheel around the trigonometric circle. Violin plots were made with BoxPlotR⁵⁸ and all the other plots were made with MATLAB. The relevant data, codes and full-size BB orientation vector fields are provided as separate data files (see [data and code availability](#)).