Deflection of a vibrissa leads to a gradient of strain across mechanoreceptors in a mystacial follicle

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Rodents have a set of long, flexible hairs, known as macrovibrissae or whiskers, that are arranged as Manhattan-style grids on both sides of their face. The vibrissae serve to detect and potentially recognize objects near the face of the animal. Each vibrissa is held in a follicle-sinus complex, and during contact, the actively applied forces cause the vibrissa shaft to bend (Hires et al. 2013; Quist and Hartmann 2012). The change in curvature and the obstruction of a vibrissa from its intended angular path are invariant with respect to the latitudinal location of objects (Bagdasarian et al. 2013; O’Connor et al. 2010). The vibrissa-follicle junction is rigid in vivo (Bagdasarian et al. 2013), so all mechanical signals are transduced into neuronal signals within the follicle and to a lesser extent, the surrounding tissue. Histological labeling of mechanoreceptors. We examined the distribution of mechanoreceptor types in transgenic mice that express fluorescent proteins in sensory nerve endings to compare the gross features of the mouse follicle with those of other species (Ebara et al. 2002; Rice et al. 1986). AdvillinCre+/− knock-in mice were crossed with red fluorescent protein (RFP) reporter mice (A114) (Madsen et al. 2010) to generate a mouse line that selectively labeled Merkel cells. These are located in the outer root sheath (ORS) of the follicle at the
Denote boundaries between the layers labeled above the image. Scale bar, 100 μm, based on DAPI labeling: the MS, GM, and ORS. The inner root sheath (IRS) was never labeled by DAPI. Directions are: R, rostral; M, medial. Yellow lines. 500 μm. Vibrissal nerve; IM, intrinsic muscle; CS, cavernous sinus; VS, vibrissa shaft; HB, hair bulb; HP, hair papilla. Directions are: C, caudal; L, lateral. Scale bar, 500 μm. DAPI-labeled layers as in Fig. 1. Mouse follicle-sinus complex anatomy and mechanoreceptor distribution. A: anatomical features of the mouse follicle. Merkel cells were selectively labeled with red fluorescent protein (RFP) in Advillin\textsuperscript{Cre\textsuperscript{+}} knock-in mice and 100-μm-thick serial sections imaged on a light microscope. RRC, rete ridge collar; OCB, outer conical body; ICB, inner conical body; MDR, Merkel cell-dense region; MS, mesenchymal sheath; RS, ring sinus; RW, ringwulst; DVN, deep vibrissal nerve; IM, intrinsic muscle; CS, cavernous sinus; VS, vibrissa shaft; HB, hair bulb; HP, hair papilla. Directions are: C, caudal; L, lateral. Scale bar, 500 μm. B: maximum projection of a confocal image stack through the MDR of an Advillin\textsuperscript{Cre\textsuperscript{+}} knock-in mouse crossed with an RFP reporter mouse. The Merkel cells are located at the level of the RS between the RW and the ICB. Scale bar, 100 μm. Inset (red boxes): magnified view of a single confocal layer close to the edge of the RS, demonstrating that Merkel cells are located in the outer root sheath (ORS). Glassy membrane (GM; unlabeled) is located between the ORS and the MS. Scale bar, 10 μm. C: maximum projection of a confocal image stack of the RS region in a Thy1-TN-XXL transgenic mouse with labeled Merkel and lanceolate-ending afferents. Inset: zoomed-in region of Merkel and lanceolate endings at the level of the MDR. Scale bar, 100 μm. D: schematic of a microdissected follicle row, pinned to a silicone base, immersed in artificial cerebrospinal fluid (aCSF) for 2-photon laser-scanning microscope (TPLSM) imaging. The imaged follicle was suspended above a gap in the silicone base to minimize friction during vibrissa deflection (arrow). E: maximum projection of a TPLSM-acquired image stack of a 4’6-diamidino-2-phenylindole (DAPI)-labeled vibrissa follicle. A window was opened in the vibrissa capsule above the dorsal aspect of the RS to expose the region between the RW and the ICB. Scale bar, 100 μm. Inset: zoomed-in region containing horizontally elongated cell nuclei that were classified as putative Merkel cells. Scale bar, 10 μm. F: longitudinal cross-section through the image stack in E. Layers of tissue were identified based on DAPI labeling: the MS, GM, and ORS. The inner root sheath (IRS) was never labeled by DAPI. Directions are: R, rostral; M, medial. Yellow lines denote boundaries between the layers labeled above image. Scale bar, 100 μm. G: radial cross-section through the image stack in E, demonstrating the same DAPI-labeled layers as in F. Scale bar, 20 μm. H and F: fixed and sectioned follicle tissue labeled with the membrane dye 5-hexadecanoylamino-fluorescein. The mouse was perfused and fixed, while the vibrissa was deflected in the caudal (H) or rostral (I) directions. Note how the VS buckles and bends in the region of the CS only during rostral deflections. Black arrows indicate the intrinsic sling muscle. Scale bars, 500 μm.

Fig. 1. Mouse follicle-sinus complex anatomy and mechanoreceptor distribution. A: anatomical features of the mouse follicle. Merkel cells were selectively labeled with red fluorescent protein (RFP) in Advillin\textsuperscript{Cre\textsuperscript{+}} knock-in mice and 100-μm-thick serial sections imaged on a light microscope. RRC, rete ridge collar; OCB, outer conical body; ICB, inner conical body; MDR, Merkel cell-dense region; MS, mesenchymal sheath; RS, ring sinus; RW, ringwulst; DVN, deep vibrissal nerve; IM, intrinsic muscle; CS, cavernous sinus; VS, vibrissa shaft; HB, hair bulb; HP, hair papilla. Directions are: C, caudal; L, lateral. Scale bar, 500 μm. B: maximum projection of a confocal image stack through the MDR of an Advillin\textsuperscript{Cre\textsuperscript{+}} knock-in mouse crossed with an RFP reporter mouse. The Merkel cells are located at the level of the RS between the RW and the ICB. Scale bar, 100 μm. Inset (red boxes): magnified view of a single confocal layer close to the edge of the RS, demonstrating that Merkel cells are located in the outer root sheath (ORS). Glassy membrane (GM; unlabeled) is located between the ORS and the MS. Scale bar, 10 μm. C: maximum projection of a confocal image stack of the RS region in a Thy1-TN-XXL transgenic mouse with labeled Merkel and lanceolate-ending afferents. Inset: zoomed-in region of Merkel and lanceolate endings at the level of the MDR. Scale bar, 100 μm. D: schematic of a microdissected follicle row, pinned to a silicone base, immersed in artificial cerebrospinal fluid (aCSF) for 2-photon laser-scanning microscope (TPLSM) imaging. The imaged follicle was suspended above a gap in the silicone base to minimize friction during vibrissa deflection (arrow). E: maximum projection of a TPLSM-acquired image stack of a 4’6-diamidino-2-phenylindole (DAPI)-labeled vibrissa follicle. A window was opened in the vibrissa capsule above the dorsal aspect of the RS to expose the region between the RW and the ICB. Scale bar, 100 μm. Inset: zoomed-in region containing horizontally elongated cell nuclei that were classified as putative Merkel cells. Scale bar, 10 μm. F: longitudinal cross-section through the image stack in E. Layers of tissue were identified based on DAPI labeling: the MS, GM, and ORS. The inner root sheath (IRS) was never labeled by DAPI. Directions are: R, rostral; M, medial. Yellow lines denote boundaries between the layers labeled above image. Scale bar, 100 μm. G: radial cross-section through the image stack in E, demonstrating the same DAPI-labeled layers as in F. Scale bar, 20 μm. H and F: fixed and sectioned follicle tissue labeled with the membrane dye 5-hexadecanoylamino-fluorescein. The mouse was perfused and fixed, while the vibrissa was deflected in the caudal (H) or rostral (I) directions. Note how the VS buckles and bends in the region of the CS only during rostral deflections. Black arrows indicate the intrinsic sling muscle. Scale bars, 500 μm.
Two-photon imaging. Cell nuclei throughout the dissected tissue were labeled with the blue fluorescent dye DAPI during microdissection and transferred to a two-photon laser-scanning microscope (TPLSM) for fluorescence imaging at an excitation wavelength of 800 nm. The microscope objective was positioned over the micro-dissected window, which included a region of the RS that extended from the level of the ringwulst (RW) out to the medial aspect of the inner conical body (ICB; Fig. 1E). For each experimental vibrissa deflection, we scanned a 512 × 512 × 180 pixel Z-stack at a resolution of 1 μm/pixel in X and Y and 1.875 μm/pixel in Z, for a 512 × 512 × 338 μm3 ~ 90-nl vol. Each image stack required ~10 min of acquisition time. A single experiment included six to 24 image stacks.

Vibrissa deflections. The vibrissa emerging from the micro-dissected follicle was cut to 30% of its original length and inserted 100 μm into a glass pipette that was coupled to a micrometer-resolution manipulator (MPC-200; Sutter Instrument, Novato, CA; Fig. 1D). The average distance of the glass-pipette mouth to the vibrissa-follicle junction was 7 ± 2 mm, a distance at which the vibrissa is rigid and thus the axial force during deflections minimal (Quist and Hartmann 2012). The vibrissa was deflected at a distance corresponding to 10° or 20° of angle at the base in the rostral (forward) or caudal (backward) direction. The vibrissa remained in the deflected position while a two-photon image stack was acquired. Each deflection was followed by a return to the rest angle, which was also imaged for comparison and calculation of relative displacements. Each deflection condition was repeated three to 12 times on a single follicle.

Data analysis. Relative displacements of DAPI-labeled cell nuclei were estimated by computing rigid follicle movements from TPLSM image stacks with the vibrissa in reference and deflected positions and then performing particle image velocimetry between the pairs of aligned and transformed image stacks. All data and statistical analysis were performed with MATLAB (MathWorks, Natick, MA) and used computational resources at the San Diego Supercomputer Center. Unless otherwise stated, averages refer to arithmetic means, and tests for significance were performed using two-sample t-tests. Comparisons between displacement fields as a function of follicle location and deflection direction and interactions thereof were evaluated by one- or two-way repeated-measures ANOVA.

RESULTS

Tissue labeling and mechanoreceptor distribution. Individual follicles are innervated by two sets of nerve (Fig. 1). With reference to visualization of the follicle from an AdvillinCre/+ mouse line crossed with a RFP reporter (Fig. 1, A and B) and in agreement with prior studies (Ebara et al. 2002; Rice et al. 1986; Sakurai et al. 2013), a single, large deep vibrissa nerve (DVN) innervates Merkel cells that are located in the ORS. The nerve attaches to these cells at the level of the RS between the RW and the ICBs. The MDR and the RW below it are the focus of this study. The afferent attachments to the Merkel cells, as well as longitudinal lanceolate endings, are preferentially labeled in Thy1-TN-XXL mice (Fig. 1C). The DVN further innervates the MS, to which the RW is attached with club-like endings, and innervates the CS as free-nerve endings (Ebara et al. 2002; Sakurai et al. 2013). In contrast to the deep nerve, small, superficial vibrissa nerves innervate Merkel cells at the rete ridge collar (RRC), ICB, and outer conical body and attach as both lanceolate and free-nerve endings.

The structure of the MDR was investigated in a whole mount with part of the outer connective tissue sheath of the follicle microdissected (Fig. 1D) and all nuclei stained with DAPI (Fig. 1E). Longitudinal and radial cross-sections revealed four distinct layers of labeled tissue (Fig. 1, F and G). An outer ring, 10–20 μm thick and contiguous with the RW, was identified as the MS. An unlabeled, 10 μm-thick ring, identified as the glassy membrane, separated the MS from the ORS. We identified two DAPI-labeled cylindrical shells within the ORS with different thicknesses and nuclear densities. The external cylinder was 10–15 μm and contained the elongated cell nuclei of putative Merkel cells. The internal cylinder was ~10 μm thick and sparsely populated by cell nuclei of an unknown type. The IRS, which surrounds the vibrissa shaft, was unlabeled. Thus we conclude that DAPI labeling was restricted to cylindrical layers that are known to be innervated by DVN afferents (Ebara et al. 2002; Rice et al. 1986). The restricted and bright labeling of nuclei within the MDR by DAPI, as opposed to genetic labeling of the cytoplasm throughout the Merkel cells (cf. Fig. 1, B and E), suggests that the former labeling is a better choice for our analysis of displacement fields.

Internal vibrissa shaft deformations. The vibrissa-follicle junction is reported to be rigid during whisking against an object (Bagdasarian et al. 2013), and the vibrissa shaft is reported to flex within the follicle during passive vibrissa deflection (Ebara et al. 2002; Wrobel 1965). We confirmed both of these observations in follicles fixed with preservative in vivo, while all vibrissae were deflected in either the caudal or rostral directions (Fig. 1, H and I). We observe that the superficial internal segment of the vibrissa shaft, which extends from the RRC down to the RW, is indeed rigid and that the deep segment, which extends below the level of the RS, bends during rostral but not caudal deflections. These observations are consistent with the report by Ebara et al. (2002) that “the follicle is soft at the lower level of the cavernous sinus and gradually becomes more rigid toward and through the level of the ring sinus.” In the present study, it is of relevance that the shaft of the vibrissa remains straight across the MDR (Fig. 1I).

Relative displacements during static vibrissa deflection. Freshly dissected follicles, with a window cut through the outer capsule wall, were stained with DAPI and pinned so that TPLSM image stacks of DAPI fluorescence could be acquired during rest and with 10° and 20° deflections of the vibrissa in both the caudal and rostral directions (Fig. 1D). We alternated the acquisition of data between the rest position and a given deflection (Fig. 2A). We assumed that the total transformation describing the motion of the follicle in response to a deflection of the vibrissa is the sum of a rigid body transformation and localized deformations.

Automated cell tracking was used to locate the centroids of labeled nuclei (Fig. 2B). Approximately 150 corresponding nuclei/image stack, evenly distributed throughout the field of view, were manually matched across reference and deflection image stacks for each cell. The nuclei in the deflected stack, with position vectors ẋ, were optimally aligned to the corresponding nuclei in the reference stack, with position vectors x, by adjusting three translational and
The difference between the reference stack and the optimally realigned deflection stack defines the local displacement of the tissue caused by deflection of the vibrissa. We determined the displacement vectors with particle image velocimetry calculated with the use of 25 × 25 × 25 μm³ voxels that typically contained three or more reference cell nuclei. The spatial lags of the cross-correlation between the reference and aligned deflected data sets were computed continuously for each image pixel at location (x,y,z). Each cross-correlation typically contained a single, local peak, whose offset from the origin corresponded to the displacement vector field \( u(x,y,z) \) (Fig. 2).
The displacement vectors were the essential result of the analysis. For the data of Fig. 2, B and C, the root-mean-square length of the displacement vectors was $4.4 \pm 2.5 \mu m$ (mean $\pm$ SD; Fig. 2D).

The displacement vectors were conditioned before further analysis. First, vectors with magnitudes $>3$ SD above the mean, i.e., ~12 $\mu m$ for the data of Fig. 2, B–D, were considered outliers and removed. Second, the field formed by the displacement vectors was slightly smoothed with a Gaussian filter, with $\sigma = 15 \mu m$; voxels without a cell nucleus or otherwise incomplete data were not interpolated.

Lastly, we fitted a cylindrical annulus that was aligned to the principal axis of the vibrissa shaft (Fig. 2E) to extract only the relevant tissue that further may be mapped onto a plane for improved visualization. A $95 \pm 5-\mu m$-thick region, which exclusively encompassed the ORS, MS, and glassy-membrane parts of the RW, was extracted for further analysis (Fig. 1F). The displacement fields were transformed from their Cartesian coordinates into cylindrical coordinates as radial projections (Fig. 2E). The radial distance, $r$, is the perpendicular distance from the principal axis, and the radial displacement, $\Delta r$, is the change in $r$ after deflection of the vibrissa (Fig. 2, F and G). The polar angle, $\alpha$, is the offset from the vertical axis, such that $-\pi/2$ and $\pi/2$ indicate the caudal and rostral aspects of the follicle, respectively, and the polar displacement, $\Delta\alpha$, is the change in angular offset after deflection. The longitudinal coordinate, $l$, is the location along the axis, and the longitudinal displacement, $\Delta l$, is the change in this coordinate after deflection.

Example displacement fields computed from images of the follicle at rest and during a 10° caudal deflection are shown in Fig. 2G. The upper and lower halves of the displacement fields correspond approximately to the MDR and RW regions, respectively (Fig. 2F). Three main characteristics were observed. First, tissue was radially displaced outward and inward along the caudal and dorsal aspects of the follicle, respectively, suggesting a relative flattening of the follicle (Fig. 2G). Second, the tissue underwent a relative counter-rotation in the MDR and RW regions of the MS and ORS (Fig. 2G). Lastly, the RW and MDR differed in the direction of longitudinal displacement, such that the deeper RW tissue was displaced laterally outward toward the skin, whereas the more superficial MDR was shifted medially inward away from the skin (Fig. 2G). Individual trials within a single experiment were highly repeatable, as shown by the SE compared with displacement magnitudes (Fig. 2G).

Displacement maps for each condition of deflection and amplitude were aligned and averaged across experiments. Image stacks acquired with right-side follicles were mirrored before averaging. As there are no sharp boundaries to delineate regions along the principal axis of the follicle, seven naive observers manually aligned the data sets by matching pairs of DAPI fluorescence images. Alignments were in agreement across observers, and the optimal offset $d_j$ for each image to a reference image was found by minimizing the sum of squares across $U$ users and $N$ images, i.e., $\min \sum \frac{(O_{ijU}-(d_j-d_j))^2}{d}$, where $O_{ijU}$ is the alignment for one pair of images from one observer.

Radial tissue displacements. The tissue displaced outward along the caudal edge of the follicle during caudal deflections and outward toward the rostral edge during rostral deflections. Thus radial displacements in the RS region follow the direction of vibrissa deflection at a ratio of ~0.3 $\mu m$/degree (Fig. 3); these effects are significant at the location of the RW, i.e., $F(1,36) = 8.3 (P = 0.007)$, and the MDR, i.e., $F(1,36) = 34.5 (P < 0.001)$. Additionally, we observed inward radial displacements on the order of 0.1 $\mu m$/degree along the dorsal edge of the follicle that were invariant of deflection direction.

Polar tissue displacements. Polar displacements in the MDR had an opposite sign in the dorsocaudal and dorsorostral quadrants, with $F(1,36) = 15.0 (P < 0.001)$, independent of the direction of vibrissa deflection. Displacements throughout the RW region, however, were statistically different during caudal and rostral deflections, with $F(1,36) = 17.0 (P < 0.001)$ but not as a function of location. Thus MS and ORS tissue rotate about the axis of the vibrissa in the direction of vibrissa deflection at a ratio of ~0.17° of rotation/degree of vibrissa deflection, where the direction of rotation in the RW is dependent on deflection direction (Fig. 3).

Longitudinal tissue displacements. Longitudinal displacements in the MDR were invariably in the medial direction, i.e., inward, regardless of vibrissa deflection direction and amplitude. In the RW region, the direction of longitudinal displacement differed between directions of the deflection, with $F(1,36) = 5.74 (P = 0.022)$, but the displacement was not significantly different between the dorsocaudal and dorsorostral quadrants. Thus the MDR undergoes inward longitudinal displacement during vibrissa deflection that is invariant of direction, whereas the RW region undergoes directional-selective longitudinal displacements. These displacements are on the order of ~0.3 $\mu m$/degree (Fig. 3).

Strains during vibrissa deflection. The displacements in the follicle that we observed were coherent over length scales much larger than that of single cells (Fig. 3). Thus sites of mechanotransduction during vibrissa deflection cannot be inferred from displacement measurements alone. As a means of estimating local volumetric deformations, we calculated the volumetric strain field, which is a scalar quantity measuring uniform dilation or compression at a point in space, from the measured displacements. The volumetric strain field is found by computing the spatial derivatives of the displacement vector field that contribute to the fractional change in volume (Landau and Lifshitz 1959), i.e., $\Delta V/V = \sum_{x,y,z} \frac{\partial u_x(x,y,z)}{\partial x} + \frac{\partial u_y(x,y,z)}{\partial y} + \frac{\partial u_z(x,y,z)}{\partial z}$, where the index labels the direction of the vector at each point $(x,y,z)$.

As a means to minimize inelastic deformations of the follicle from repeated vibrissa deflections, we computed strain fields from control trials, in which displacements were calculated across two image stacks with the vibrissa in the rest position taken before and after a vibrissa deflection. This control strain field was then subtracted from each strain field computed from displacement maps that compared a follicle in its rest and deflected positions. We then averaged computed strain fields across all follicles, grouped by vibrissa deflection direction and amplitude, as in the case of the underlying displacement fields (Fig. 3). SEs were typically on the order of the variations across the strain field for a single follicle and were generally larger along the caudal and rostral edges of the follicle, since fewer features were available for the strain computation. We
focus on the data sets with a $10^\circ$ deflection of the vibrissa, as these consistently showed less variability (Fig. 3).

Strain in the RW region was predominantly negative, indicating compression, and ranged between $-0.005$ and $-0.05$, on average, in magnitude for a $10^\circ$ deflection. In contrast, strain in the MDR varied from negative to positive (Fig. 4, A and B). We averaged and compared strain across the four quadrants of the dorsal half of the follicle, which approximately corresponded to the rostral and caudal RW ($R_{RW}$ and $C_{RW}$, respectively) and the rostral and caudal MDR ($R_{MDR}$ and $C_{MDR}$, respectively; Fig. 4A). We found no statistically significant difference in strain among quadrants, within or across deflection conditions. However, we found a statistically significant interaction among mean strains from diagonal quadrants, with $F(1,20) = 6.2$ ($P = 0.022$), which implied correlations in the variability across quadrants. This interaction is interpreted as a preferential gradient of strain, with a magnitude of $0.02\,\text{V/V}$ across the RS region that shifts in orientation between deflection direction (Fig. 4C). During caudal deflections, tissue is compressed in the RRW region and dilated in the CMDR (Fig. 4B). During rostral deflections, tissue is compressed in the CRW region and dilated in the RMDR (Fig. 4B). Similar results were observed for a $20^\circ$ deflection. This leads to the crux result; i.e., the direction of vibrissa deflection is encoded mechanically in the follicle by a longitudinally diagonal rostrocaudal gradient of strain. Thus, for a caudal deflection, the rostral portion of the RW is more compressed than the caudal portion of the Merkel dense region and vice versa.

DISCUSSION

We analyzed tissue displacements in cylindrical coordinates and found that the tissue is displaced differentially in the radial, polar, and longitudinal directions during vibrissa deflections (Fig. 2E). Specifically, we find that cells rotate about the axis of the vibrissa shaft and are displaced radially in the direction of deflection (Fig. 3). Furthermore, longitudinal displacements within the RW region reverse between caudal and rostral vibrissa deflections. Additionally, we observed significant direction invariant displacements (Fig. 3). As mechanoreceptors may not respond to tissue displacements, we computed volumetric strain as a measure of tissue deformation and thus an indirect predictor of mechanoreceptor activation. We find that vibrissa deflection leads to a gradient of strain across the MDR and RW regions and that the orientation of this gradient rotates when deflection direction changes (Fig. 3). Rice and Munger (1986) hypothesized that as a deflected vibrissa pivots about a fulcrum close to the skin and moves in the opposite direction in the RS, the MS and attached lanceolate endings are compressed on the leading edge and stretched elsewhere. Our observations are consistent with this prediction, as we find that
During a rostral deflection, this diagonal segment of the RW and dilates in the caudal region close to the ICB. During a rostral deflection, this diagonal gradient is mirrored.

The differential strain that we observe should exert different displacement patterns within the domain of the Merkel endings that originate from the axonal terminal field of a single neuron. It is of interest that a given Aβ fiber terminates on multiple neighboring Merkel cells that span only a fraction of the follicle, with different fibers labeling different parts of the follicle. In contrast, Aβ fiber innervation exhibits a much broader pattern in the vibrissae of the cat, which does not whisk (Ebara et al. 2002). In general, the amalgam of past anatomical data and the present results suggests that each of myriad directions and amplitudes of motion of the vibrissae that occur when a rodent sweeps its vibrissae across objects is encoded as a particular pattern of afferent input.

Methodological considerations. We labeled cells in the follicle-sinus complex with the fluorescent nuclear stain DAPI. Therefore, nerves were not labeled, and labeling did not distinguish between different mechanoreceptor types. Whereas this precludes direct measurements of mechanoreceptor deformation, we find that displacement and strain fields were coherent on spatial scales much larger than individual cells (Figs. 3 and 4). Applied mechanical pressure can be sensed by Merkel cells through layers of intervening cells (Ikeda and Gu 2014). Thus we assume that tissue deformations observed on the spatial scale of tens of microns reflect the stresses experienced by individual mechanoreceptors.

Head and body movements may substitute for vibrissa movements when scanning surfaces (Krupa et al. 2001). Furthermore, rats can make amplitude and velocity discriminations during passive vibrissa stimulation (Fassih et al. 2014; Stüttgen et al. 2006). Thus vibrissa deflection, without an active muscular contribution, is a feature of normal sensory experience. During whisking, the vibrissae are actively moved and pushed against surfaces by the contractile actions of facial muscles (Hill et al. 2008). Tissue mechanics and internal deformations of the follicle may therefore be very different during active touch compared with passive vibrissa deflection, as used here.

Relationship between strain measurements and mechanosensitivity. Mechanosensitivity of the Merkel-neurite complex and lanceolate endings in hairy skin is mediated by the Piezo2 mechanosensitive cation channel (Coste et al. 2010; Lou et al. 2013). Merkel cells in the rat follicle-sinus complex have been shown recently to transduce actively movements of the vibrissae via Piezo2, assumed to be located on Merkel cell processes, and to drive Aβ afferents via Ca2+-based action potentials and the presumptive release of an, as of yet, unidentified neurotransmitter (Ikeda et al. 2014; Ikeda and Gu 2014; Maksimovic et al. 2014). Mechanically activated currents have been measured in Piezo2, expressing cultured dorsal-root ganglion neurons (Coste et al. 2010) during cell-membrane displacements, down to 10 nm (Poole et al. 2014). As an order-of magnitude estimate of the associated volumetric strain, we take the radial cross-section (L) of ganglion neuron processes to be 2 μm, for which a 10-nm membrane deflection (ΔL) yields ΔV/V ~ ΔL/L ~ 10⁻⁴. We observe strain with magnitudes in the range of 0.02–0.05 during 10° angular vibrissa deflections (Fig. 4, B and C). The minimum deflection for an electro-physiological response in trigeminal fibers is stated to be 0.1° (Gibson and Welker 1983), which by linear extrapolation, is a strain with magnitude in the range of 2 × 10⁻⁴–5 × 10⁻⁴. Thus the sensitivity for vibrissa touch in the mouse is consistent with the threshold to activate Piezo2-mediated membrane currents in Merkel cell afferents.

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Fig. 4. Population analysis of strain fields. A: raw DAPI fluorescence image aligned and averaged across follicles and then transformed into cylindrical coordinates (see METHODS). The boundary between the RW and MDR is indicated by the curved, solid white line. Vertical, dashed line indicates the center axis of the VS. Scale bar, 100 μm. CMDR, caudal MDR; RMDR, rostral MDR; CWR, caudal RW; RWR, rostral RW. B: strain fields were averaged across follicles by vibrissa deflection direction (caudal or rostral). The number of follicles included in each panel is as in Fig. 3. Strain field averages were smoothed by a square median filter across 100 μm. The cartoons indicate the direction of deflection and the area (green) for which volume strains were computed (ΔV/V; see text). C: gradients of mean strain across diagonal quadrants in the follicle. Dashed lines are individual follicles, and solid lines are averages.

during a caudal deflection, the tissue compresses in the rostral (leading) segment of the RW and dilates in the caudal region close to the ICB. During a rostral deflection, this diagonal gradient is mirrored.
GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
P.M.K., D.W.M., and D.K. conception and design of research; S.J.W. and D.W.M. performed experiments; S.J.W., P.M.K., and D.K. interpreted results of experiments; S.J.W. and P.M.K. prepared figures; S.J.W. and P.M.K. drafted manuscript; S.J.W., D.K. edited and revised manuscript; P.M.K., D.W.M., and D.K. approved final version of manuscript.

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