SUPPLEMENTARY MATERIAL

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"Variation in Young's modulus along the length of a rat vibrissa"

Part I. Apparatus to consistently mount the vibrissa in the mechanical tester to ensure uniaxial loading

Vibrissae have a tendency to blow away during handling. They are also difficult to align. To ensure repeatable axial loading of the vibrissa into the micromechanical tester, we constructed a sample preparation device. The device consisted of a base "platform" and a top "cover."

Figure S1A illustrates the base platform, constructed of 1'' thick Delrin. The base platform has four "clamping" screws, two guidepost nails, and four "top-cover" screws used to mount the top cover to the base platform. A centerline was drawn along the base platform. The centerline was used to position a laser level (Straight-Line Inc., model 64001), which had a small midline groove. With the laser level turned on, we had a visual line to assist in aligning the vibrissa sample.

Figure S1B illustrates the top cover. It consists of two pieces of sheet metal with holes bored near the edges. A wooden stick, taken from a cotton swab, was placed in the exact center of both pieces and permanently epoxied in place. The device serves to hold the vibrissa sample in place when applying the epoxy.

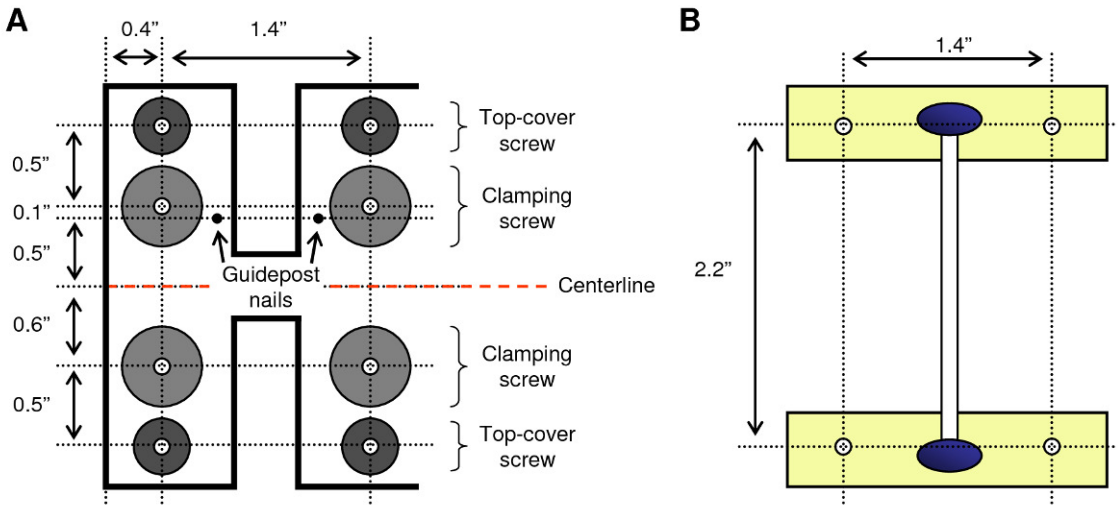

Figure S1: Device for vibrissa alignment**.** Drawings are not to scale. **(A)** Base platform. **(B)** Top cover.

The steps to mount each vibrissa are illustrated in Figure S2. We first cut thin sheets of aluminum into small flat plates. The edges of each plate were sanded to provide a rough surface for the epoxy to grip. Plates were thoroughly cleaned to remove all residues from the surface. The plates were placed on the base platform, with their top edge pressed firmly against the nails that served as guideposts and then clamped with the clamping screws to the base platform.

Next, a vibrissa segment was placed along the centerline defined by the laser-level. With the vibrissa in place, the top cover was secured to the base platform to gently hold the vibrissa in the correct orientation. Epoxy was then applied to secure the vibrissa segment to the two sanded metal plates. This step is simple because all components have been secured in their correct orientation. Otherwise, the extremely sticky epoxy can be quite difficult to work with.

Finally, after the epoxy has dried, two small binder-clips are used to hold the two sanded-metal plates together. The top cover is removed. The two metal plates, with the attached vibrissa sample, can now be removed from the base platform. The binder clips ensure that the plates do not shift relative to each other while they are transported to the micromechanical tester. Once the sanded metal plates are secured within the tester (Methods, Figure 1), the binder clips are removed and the sample is tested.

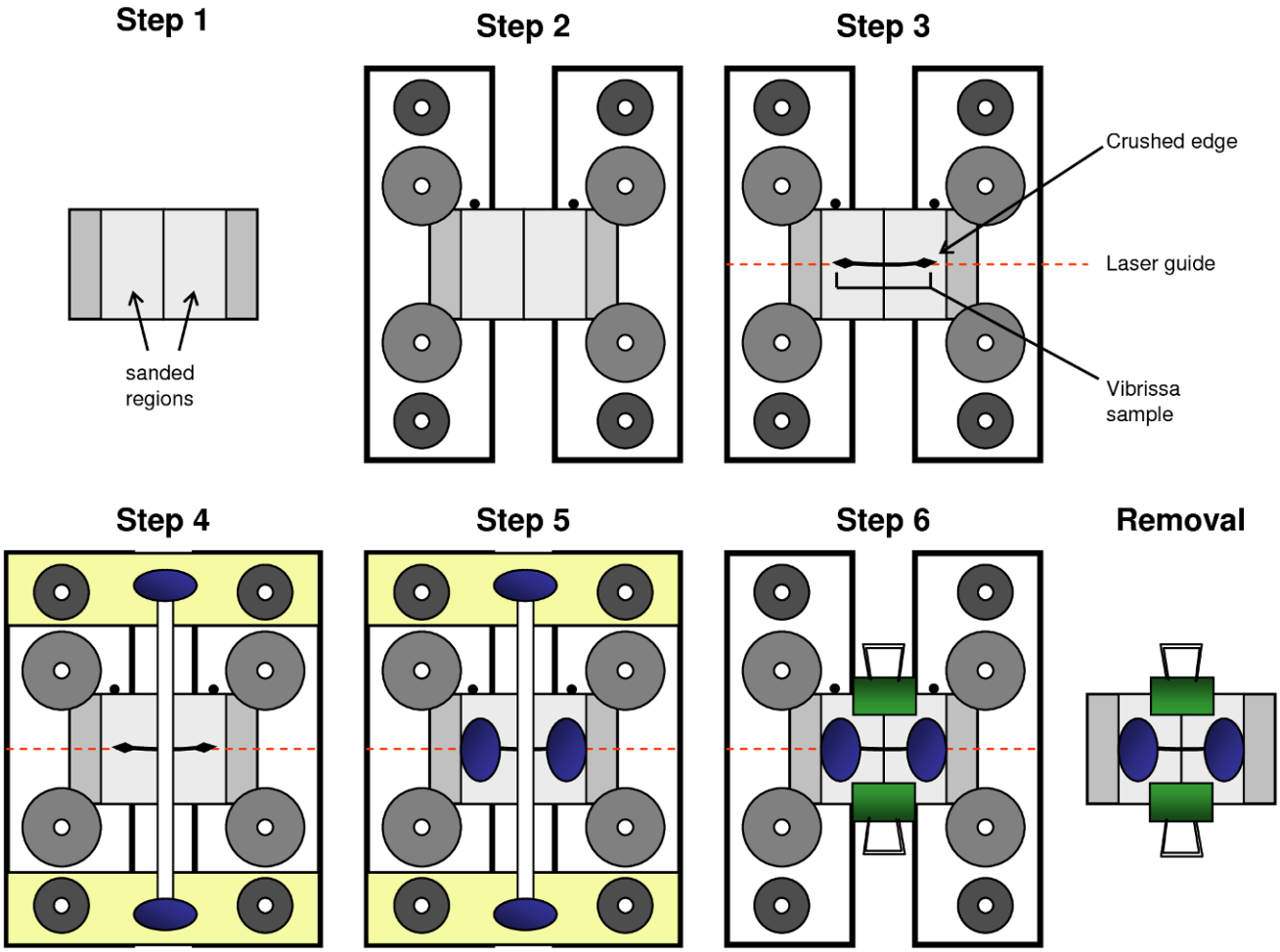

Figure S2: Steps in sample preparation. (1) Metal plates are sanded **(2)** Plates are secured to the base platform. **(3)** The vibrissa segment is aligned along the centerline with a laser level. **(4)** The top cover is secured to the base platform, gently holding the vibrissa in place while **(5)** epoxy is applied. **(6)** After epoxy has set, the top cover is removed and binder clips hold the edges of the metal plates together. At this point, the metal plates (with vibrissa sample attached) can be removed and positioned in the micromechanical tester. Binder clips are removed and the sample is tested.

Part II. Mechanical Testing and Analysis

As described in Supplementary Material Part I, each vibrissa sample was mounted across two thin aluminum plates (thickness 0.02", 9536K18, McMaster-Carr). These plates provided a gripping surface for the clamps of the micromechanical tester, and ensured that the sample was precisely aligned with the direction of the applied load.

It was difficult to precisely align each vibrissa sample across the two plates. We therefore constructed a sample preparation device to ensure repeatable placement of the vibrissa. This device is described in Part I of this Supplementary Material.

After the vibrissa sample was placed across the plates, an epoxy (8265-S, JB-Weld) was used to adhere the flattened ends of the sample to each plate and allowed to cure overnight. Flattening the vibrissa ends helped prevent the sample from slipping out of the epoxy during mechanical testing. The epoxy is extremely rigid compared to the vibrissa, and therefore will not deform when the sample is being pulled.

Before mechanical testing, each mounted sample was scanned on a flatbed scanner and digitally photographed with a 10x microscope. The photos were imported into Matlab for image processing. In Matlab, we measured the length of the sample between the epoxy (from the scan) as well as three diameters at different points along the sample (from the microscope images): at the top juncture with the epoxy, at the bottom juncture with the epoxy, and at the center between the epoxies.

To obtain an error estimate on the measurement of diameter, we repeated the measurements of diameter for the base and tip segments of one vibrissa ten times. The standard deviation was approximately $\pm$1 μm for a 124 μm diameter base segment. Similar results were found for measurements of the tip-segment. We therefore assume ~1% (1/124) measurement error for all vibrissa diameters.

Testing the sample was straightforward. Setscrews in the clamps (Figure 1A in main text) were tightened to grip the top and bottom aluminum plates. The test was then run using Mach-1 Motion software from Biosyntech, which performed both the data acquisition and actuator control.

Young's modulus of a sample was then determined from the stress-strain curve generated from these uniaxial loading tests.

Stress is the force divided by the cross-sectional area of the sample. To calculate stress, we used the cross-sectional area of the segment calculated from the measurement of diameter in the middle of the segment. This was a reasonable approximation because the exposed region of each segment was short (average exposed region = 2.5 $\pm$ 0.4 mm). Across all samples, the diameter change for the exposed region was 4.3 $\pm$ 2.4 μm (4.7 $\pm$ 2.7 % of the larger diameter).

To calculate strain, we divided the sample's change in length (as recorded by the micromechanical tester) by the original sample length. Note that strain is a ratio and therefore dimensionless.

Part III. Experimental Considerations

There are four main considerations in the interpretation of the present study.

First, we could divide the vibrissa into only two segments: a tip-segment and a base-segment. Ideally, we would have further divided each segment to get an even more precise correlation between Young's modulus and sample diameter, but it was not possible to properly grip a segment of shorter length during the uniaxial tensile test.

Second, because each sample had a significant exposed length (2.5 mm on average), there was some variation in segment diameter. Ideally, each sample would have had constant diameter along the entire region of loading. However, the percent change in diameter along any segment was quite small, around 4.7%.

Third, we did not directly control the ambient humidity of the experimental environment or of the sample itself. It is well known that Young's modulus of a keratinized structure will be greater if its moisture content is lower (Fraser and Macrae 1980). The moisture content of a sample is influenced by the relative humidity of the surrounding environment. We were therefore careful to measure ambient humidity during experiments. We found that although ambient humidity varied by ~11%, (1.9% / 17.6%) measurements of elasticity did not correlate with ambient humidity readings.

Finally, because the tensile tests were performed on plucked vibrissae, the computed values for Young's modulus may be slightly higher than those found *in vivo.* It seems likely that *in vivo* the follicle-sinus-complex will provide an internal source of moisture for the vibrissa, which would tend to decrease the effective Young's modulus. However, the effect of humidity may be counterbalanced by our relatively low strain rate. As described in Methods, increased strain rates are typically associated with higher values for Young's modulus.

References:

Fraser, R. D. and T. P. Macrae, 1980. "Molecular structure and mechanical properties of keratins." Symposia of the Society for Experimental Biology 34: 211-246.