

The mechanical variables underlying object localization along the axis of the whisker

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Abstract

Rodents move their whiskers to locate objects in space. Here we used psychophysical methods to show that head-fixed mice can localize objects along the axis of a single whisker, the radial dimension, with one-millimeter precision. High-speed videography allowed us to estimate the forces and bending moments at the base of the whisker, which underlie radial distance measurement. Mice judged radial object location based on multiple touches. Both, the number of touches (1-17) and the forces exerted by the pole on the whisker (up to 573 μN ; typical peak amplitude, 100 μN) varied greatly across trials. We manipulated the bending moment and lateral force, pressing the whisker against the sides of the follicle, and the axial force, pushing the whisker into the follicle, by varying the compliance of the object during behavior. The behavioral responses suggest mice use multiple variables (bending moment, axial force, lateral force) to extract radial object localization. Characterization of whisker mechanics revealed that whisker bending stiffness decreases gradually with distance from the face over five orders of

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magnitude. As a result, the relative amplitudes of different stress variables change dramatically with radial object distance. Our data suggest that mice use distance-dependent whisker mechanics to estimate radial object location. This estimate does not require precise control of whisking, is robust to large variability in whisker forces, and is independent of object compliance and object movement. More generally, our data imply that mice can measure the amplitudes of forces in the sensory follicles for tactile sensation.

Introduction

Psychophysical methods have revolutionized our understanding of visual system function. For example, much of what we know about vision near threshold comes from simple detection tasks in humans (Hecht et al., 1942). These experiments have laid the groundwork for 70 years of cellular and systems neurophysiology in the retina (Field et al., 2005). Psychophysical experiments have also played an important role in linking neural activity and somatosensory perception (Mountcastle et al., 1990; Stuttgen et al., 2006). However, comparable experiments have not been done in the context of active somatosensation, where the animal controls its sensory input, mainly because stimulus control has remained an unmet challenge. Here we used quantitative head-fixed behavior (Schwarz et al., 2010) and high-speed whisker tracking (Clack et al., 2012; Knutsen et al., 2005; Mitchinson et al., 2007; O'Connor et al., 2010a; Ritt et al., 2008; Voigts et al., 2008) to study how forces acting on whiskers could underlie an estimation of object distance.

Diverse mammals use their whiskers for navigation (Brecht et al., 1997; Dehnhardt et al., 2001; Vincent, 1912), object localization (Celikel and Sakmann, 2007; Huber et al., 2012; Hutson and Masterton, 1986; Knutsen et al., 2006; Krupa et al., 2001; Mehta et al., 2007; O'Connor et al., 2010a; Petreanu et al., 2012), texture discrimination (Carvell and Simons, 1990; von Heimendahl et al., 2007), and object recognition (Anjum et al., 2006; Brecht et al., 1997). Object localization is critical for navigation and object recognition (Solomon and Hartmann, 2006). Rodents have thirty-five long mystacial vibrissae (whiskers), arranged in a raster pattern on each side of the face. These whiskers move through the space surrounding the head to locate objects (Hutson and Masterton, 1986; Knutsen et al., 2006; Mehta et al., 2007; O'Connor et al., 2010a). Whiskers are elastic, conical beams (Birdwell et al., 2007) and all mechanotransduction occurs within follicles in the mystacial pad (Dorfl, 1982). Contact between whiskers and objects produces time-varying stresses at the base of the whiskers (Birdwell et al., 2007) that are transduced by mechanoreceptors in the follicles into action potentials (Gibson and Welker, 1983; Stuttgen et al., 2008; Szwed et al., 2003; Szwed et al., 2006; Zucker and Welker, 1969).

Whiskers move mainly in the horizontal plane by rotating around a pivot point in the skin. The problem of three-dimensional whisker-based object localization is therefore naturally represented in cylindrical coordinates (Knutsen and Ahissar, 2009). Object elevation (z) may be determined by the vertical location of the particular whisker contacting the object. Rodents can accurately discriminate object locations in the anterior-posterior dimension, even with a single whisker (Celikel and Sakmann, 2007; Hutson and Masterton, 1986; Knutsen et al., 2006; Mehta et al., 2007; O'Connor et al., 2010a). It has been proposed that rodents solve this problem by extracting the azimuthal angle of the whisker base (θ_{base}) at which the whisker first touches the object (θ_{touch}). θ_{touch} could be computed from the timing of touch between object and whisker, referenced against a whisker position-related signal; for example an efference copy of the motor command or a reafference signal (Fee et al., 1997; von Holst and Mittelstaedt, 1950), could underlie object localization. This view is inspired by neural measurements throughout the somatosensory lemniscal pathway, which show temporally extremely sharp responses upon touch (Armstrong-James et al., 1992; Panzeri et al., 2001; Simons, 1978; Szwed et al., 2003), modulated by whisking phase (Curtis and Kleinfeld, 2009).

The strategies and neural codes underlying object localization in the radial dimension (r) are less well understood. For example, radial distance could be derived from the force amplitudes at a single follicle that result from the interactions between whisker and object (Figure 1A, B). Because the whisker is tapered (Ibrahim and Wright, 1975), the stresses in the follicle are expected to be strongly distance-dependent (Birdwell et al., 2007; Knutsen and Ahissar, 2009; Solomon and Hartmann, 2006). We use the term ‘force-dependent radial distance measurement’ to describe the situation where estimates of force amplitudes are the basis of radial object localization. Alternatively, mice could avoid precise force measurements and derive radial distance based on measurement of θ_{touch} and triangulation (Solomon and Hartmann, 2011). In that case radial object localization would require either contact between an object and multiple whiskers simultaneously (Figure 1C), or single whiskers over multiple contacts accompanied by movements of the follicle (e.g. the head) (Figure 1D) (Krupa et al., 2001). In these scenarios, θ_{touch} , integrated over multiple whiskers (Figure 1C) or multiple contacts with the same whisker

over time (Figure 1D), could provide information about radial object distance in combination with a binary representation of touch; measurement of whisker force magnitude is not necessary. Distinguishing these mechanisms requires experimental situations where θ_{touch} is nearly identical for different object locations. Force-dependent radial object localization has not been demonstrated experimentally. Understanding radial object localization demands precise control of the relative locations of object and whisker, and measurement of whisker movements and forces during object localization.

We trained head-fixed mice to locate a pole with a single whisker (O'Connor et al., 2010a). The pole was placed at one of several locations along the whisker; for all object locations the whisker contacted the pole at nearly identical azimuthal angles. Whisker tracking based on high-speed video recordings (Knutsen et al., 2005; O'Connor et al., 2010a; Voigts et al., 2008) revealed the whisking strategies and the mechanical forces supporting radial object localization. Our studies demonstrate that mice can perform force-dependent radial object localization, and suggest that simultaneous measurement of lateral and axial stresses in the skin jointly underlie radial distance perception.

Materials and Methods

Mice

The behavioral procedures were similar to a previous study probing object localization in the anterior-posterior dimension (O'Connor et al., 2010a). Adult (> P60) male mice (C57BL/6CrJ; Charles River, MA USA) (n = 8) were housed individually in cages containing tunnels and bedding material, in a reverse light cycle room. For 10 days prior to training and on days without behavioral testing, mice were maintained on 1 ml of water/day (O'Connor et al., 2010a). On days with behavioral sessions, mice obtained all water for the day during the session and performed until sated. The amount of water consumed was determined by weighing the mouse before and after the session. The volume consumed was approximately 1 ml. The weight and health of the mice were monitored daily. All procedures were in accordance with protocols approved by the Janelia Farm Institutional Animal Care and Use Committee.

A custom head post was mounted to the skull under isoflurane anesthesia (~1.5-2 % by volume in O₂). Bupivacaine or lidocaine HCl (10 µl, 0.5 % S.C.) was applied at the incision site. The scalp and periosteum were removed from the dorsal surface of the skull. A thin layer of cyanoacrylate adhesive was applied to the skull and covered with dental acrylic (Lang Dental Jet Repair Acrylic 1223, Henry Schein P/N: 1251546). Headposts were fixed to the skull with dental acrylic. Buprenorphine HCl (0.05-0.10 mg/kg I.P., Bedford Laboratories) was used for post-operative analgesia. Ketoprofen (5 mg/kg) was administered at the time of surgery and post-operatively to control inflammation. Mice were allowed 10 days to recover from surgery prior to water restriction.

Apparatus

Mice judged the distance to a thin steel pole (diameter 0.4 mm) (Figure 2A). In some experiments (Figures 9, 10) a cleaned, straightened rat whisker was substituted as a ‘compliant’ pole, which is deformed by contact with the mouse whisker. A pair of stepper motors (Zaber, P/N: NA08B30) and linear sliders (Schneeberger, Bedford, MA, P/N: NDN 2-50.40) moved the pole in the horizontal (x-y) plane. This assembly was mounted on a pneumatic linear slider (Festo SLS-10-30-P-A Mini slide, P/N: 170496), which rapidly (~ 250 ms) brought the pole into and out of reach of the whiskers along the z-direction, triggered by a computer controlled solenoid valve (Festo CPE 10-M1BH-5L-QS-6, P/N: 196883). The entire behavioral apparatus was enclosed in a light- and sound-isolation box.

The apparatus was controlled by software (<http://brodylab.princeton.edu/bcontrol>). A real-time Linux system interfaced with valves and recorded licking responses using a PCI-6025E data acquisition board (National Instruments, Austin, TX). The stepper motor was driven by its controller in response to commands sent through a serial port of a Windows computer running MATLAB.

A custom lickport, which provided the water reward and recorded licking, was placed within reach of the mouse’s tongue. Licks were recorded as interruptions in the light path between a LED (Panasonic, LN77L; 860 nm) and a phototransistor (Fairchild Semiconductor, L14G1). The phototransistor and LED signaled interruptions to the control computer.

Water was delivered by gravity into the lickport through a 1.3 mm diameter steel tube, under solenoid valve control (The Lee Company, Westbrook, CT). Excess water was sucked out of the lickport through a tube (0.8 mm ID) using a peristaltic pump (Rainin Instrument Co Inc., Rabbit Plus peristaltic pump). Punishment was provided by puffs of compressed air (typically 10 psi) delivered through a small metal tube (~ 2.3 mm inner diameter) pointed at the face from a distance of several centimeters. The air was controlled by a solenoid valve (The Lee Company, Westbrook, CT) connected to a compressed air source.

During behavior mice were crouched in a natural position in acrylic (2.9 cm ID; McMaster P/N: 8486K433) tubes such that their heads protruded out at the front and they could use their front paws to grip the tube's edge. The headpost was secured to the bench using a kinematic mount, guaranteeing precise positioning of the head across sessions. Whiskers were free to move around the space surrounding the heads.

Whiskers were gradually (over 12-27 days) trimmed down to the C2 whisker under light isoflurane anesthesia. Whiskers were retrimmed every 2-4 days.

Imaging whiskers

A high-speed videography system using CMOS cameras (Basler AG, Germany, P/N: A504 K, 200x310 pixels, 500-1000 fps, 0.2 ms exposure, 8-bit depth; or AOS Technologies, Switzerland, AOS Imaging Studio software & AOS X-PRI camera, 180x250 pixels, 500 fps, 0.5 ms exposure, 8-bit depth) measured the position and shape of whiskers during behavior. Pixel size was either 0.07 mm or 0.06 mm. For every trial a movie of 1.5 s length was recorded, starting 83 ms before the triggering of the pole descent (Figure 2C). Illumination was with an infrared LED (Roithner Lasertechnik, Vienna, Austria, P/N ELJ-940-211; 940 nm). The light was delivered through a diffuser and a condenser lens and pointed directly into the camera.

Behavior

Head-fixed mice judged the distance to a metal pole that was presented at one of two positions (Figure 2A, B). The sound of the pneumatic slider controlling the vertical pole position indicated trial start. The pole locations were arranged to lie along the whisker, in

the radial dimension. A proximal position was defined as the Go position; licking and breaking the beam of the lickport triggered a water reward (scored as a hit). One or several distal positions were defined as No Go positions; licking and breaking the beam triggered a time-out (2-10 s) and an airpuff as mild punishment (false alarm). Trials in which mice did not lick were neither rewarded nor punished in both Go (miss) and No Go (correct rejection) trials.

Licking triggered reward or punishment only during the answer period, which began some time after the pole became available for sampling (sampling period) (Figure 2C). When there was no lick response (correct rejections and misses), the pole ascended and the trial was over. The sampling period was adjusted across and during sessions (typically between 0.5 and 1.25 s) to improve the mouse's performance. The answer period ended 2 seconds after the start of the pole descent. During a hit trial the water valve opened, dispensing a drop of water (~ 8 μ L) and terminating the answer period. A drinking period was granted to allow the mouse to drink before the end of the trial. The probability of each of the two trial types was typically 50 %, but was sometimes adjusted during training to suppress false alarms. To prevent discouraging mice with a succession of No Go trials, the number of successive trials of one type was limited to three.

Probing the mouse's ability to perform radial distance discrimination requires that the azimuthal angle is as similar as possible for all object locations. The Go position (target) and No Go position (distracter) were selected based on whisker video. The target was always 5 mm from the follicle. For the standard task the distracter was 8 mm from the follicle (Figures 2D). For the experiments of Figure 2E & F the distracter positions were in the range of 6-9 mm from the follicle; only one distracter position was used per session. For the experiments of Figure 7E-G, azimuthal jitter was introduced for both Go and No Go trials. In the absence of jitter we defined a vector from the follicle to the point of contact between whisker and pole (length $r = 5$ mm, Go; $r = 8$ mm, No Go). We then defined an arc by rotating the vector about the follicle by $+3^\circ$ and -3° (Figure 7E). Pole positions across trials were then chosen randomly from points on that arc. For the experiments of Figure 9 & 10C distracter positions were randomly chosen across trials from the range 7-13 mm, which generated a large range of bending moments and axial

forces in the No Go positions. This large range of forces relaxed the demands on the properties of the flexible object for creating appropriate illusion trials.

To define the reaction time for a session (Figures 2C, 6, 7, 8, 9, 10C) we constructed a histogram of the times of first lick across trials. The reaction time was the time between the pole coming into reach (earliest frame of contact in the session, typically 250 ms) and the mode of the histogram. For individual hit and false alarm trials the reaction time was defined as the time from earliest frame of contact in the session to the animal's first lick in that trial. Since mice did not lick in correct rejection and miss trials, we used the mean reaction time of the hit trials as a proxy. For correct rejection and miss trials we defined the time point of the animal's decision as the time of first contact with the pole plus the mean reaction time on hit trials (time between first contact and first lick).

Measurement of whisker stiffness

Mice performed all experiments with a single C2 whisker. After conclusion of the behavioral experiments we analyzed the structure of three whiskers with light and electron microscopy (Figure 5). The whisker radius was measured as a function of distance from the follicle. The taper was remarkably linear ($n = 3$, $r = -0.988$) and the whisker can thus be modeled as a cone. The second moment of inertia is thus (Birdwell et al., 2007):

$$I_p = \frac{\pi}{4} (a_p)^4 = \frac{\pi}{4} \left(a_{base} \left(1 - \frac{s_p}{length_{whisker}} \right) \right)^4 \quad (M1)$$

where a_{base} is the radius at the base of the whisker, s_p is the length along the contour of the whisker from the base to point p , and $length_{whisker}$ is the total length of the whisker.

The whisker bending stiffness was probed by pushing the whisker, which was mounted on a micromanipulator by its root, against a metal pole on a high resolution balance (Mettler Toledo, Mx5, readability $1 \mu\text{g}$, repeatability at full load, $0.9 \mu\text{g}$) in 1 mm increments along the whisker. The measured weights ranged from $< 1 \mu\text{g}$ (0.1 mm displacement 1 mm from the tip) to 630 mg (0.5 mm displacement at 10 mm from the follicle). 87 % of measurements were greater than $20 \mu\text{g}$, and therefore have expected errors $\leq 2.5 \%$. Force on the whisker was calculated as:

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$$F_{scale} = mass(kg) \cdot 9.8m / s^2 \quad (M2)$$

The whisker contour was extracted from photographs. For small displacements (< 1 mm), force scaled linearly with deflection.

The bending stiffness EI_p was estimated by comparing measured deflections to a numerical model (Birdwell et al., 2007). Model whisker deflection was iteratively calculated from contact point back to whisker base in 1 micrometer segments, using the relationship (Solomon and Hartmann, 2006)

$$d\kappa_p = \frac{|\vec{r}_p \times \vec{F}|}{EI_p}. \quad (M3)$$

For each set of deflections at a horizontal distance x from the base, numerical estimates of whisker shape were generated for a range of E . The model error was calculated as the ratio (A2/A1) of the area (A2), between the model deflection and actual whisker deflection from base to pole, and the area (A1) between the actual whisker deflection and the undeflected whisker from base to pole (Birdwell et al., 2007). The E that minimized the error at each x was tightly clustered around 5 GPa for whisker JF25395, and ranged from 4-8.5 GPa for whisker JF25403. We used $E = 5$ GPa to calculate whisker forces.

Calculation of forces based on measurements from high-speed video

In the main text we described the equations that allow forces to be calculated based on the idealized geometry of whisker and contacted object. However, in practice critical details need to be considered in extracting these geometrical quantities from image data. In this section we describe at the “algorithmic” level how we calculated forces, starting with the representation of the whisker as a sequence of points representing its medial axis (i.e. starting after “whisker tracking”).

Tracking whiskers in raw video images was performed as described (Clack et al., 2012; O'Connor et al., 2010a). Equations given below for angles and follicle coordinates reflect the specific videographic conditions of our experiments, in which the mouse face appeared at the top of the image and protraction involved rightward motion of the whisker within the image. After tracking, the whisker’s medial axis is represented as a

sequence of n points (x_i, y_i) . To overcome discretization noise for computing derivatives, the shape was approximated as a parametric curve:

$$c(l) = (x(l), y(l)) \quad (\text{M4})$$

where $l \in [0, 1]$, and $x(l)$ and $y(l)$ are fifth-degree polynomials. The polynomials were computed by fitting x_i and y_i , respectively, as a function of l_i , where, $l_1 = 0$ and

$$l_i = \frac{\sum_{k=2}^i \sqrt{(x_k - x_{k-1})^2 + (y_k - y_{k-1})^2}}{\sum_{k=2}^n \sqrt{(x_k - x_{k-1})^2 + (y_k - y_{k-1})^2}} \quad (\text{M5})$$

For subsequent evaluation of $x(l)$ and $y(l)$, l was evaluated at 100 evenly spaced points in the interval $[0, 1]$.

Arc-length distance along the whisker (s ; in mm) is derived from $c(l)$ at each time point (i.e. for each video frame) as:

$$s(l) = \int_0^l \sqrt{x'^2 + y'^2} dl. \quad (\text{M6})$$

Tracking of the face-most edge of the whisker can be unreliable due to movement of the fur on the whisker pad. In some early experiments a small segment of whisker was obscured by the lickport. For these reasons we added a “mask” (Figure 3, Figure 4). For all analyses presented here the mask consisted of a horizontal line, chosen so that neither fur nor lickport would obscure the beginning of the whisker. This allows the whisker to be effectively “truncated” at the intersection of a particular location on the whisker and the mask.

The arc-length distance at the intersection of the whisker and the mask was subtracted from $s(l)$. I.e., the arc-length origin becomes the intersection of the whisker and the mask. Thus, considering the mask, $s(l)$ was redefined as:

$$s(l) = \int_0^l \sqrt{x'^2 + y'^2} dl - \int_0^{l_{\text{intersection}}} \sqrt{x'^2 + y'^2} dl \quad (\text{M7})$$

where $l_{intersection}$ is the value of l at which $c(l)$ intersects the mask.

Angle (θ ; in radians) at each point l along the whisker, for each time point, is:

$$\theta(l) = \tan^{-1}\left(\frac{x'(l)}{y'(l)}\right) \quad (M8)$$

where x' , y' denote derivatives with respect to l .

θ gives the azimuthal angle in the horizontal plane. Protraction corresponds to increasing θ . $\theta = 0$ is perpendicular to the midline of the mouse.

Angle at the whisker base (θ_{base} ; in radians) at each time point is:

$$\theta_{base} = \theta(l_{base}) \quad (M9)$$

where $l_{base} = \arg \min(|s(l)|)$.

Angle at the point of contact between whisker and pole ($\theta_{contact}$; in radians) at each time point is:

$$\theta_{contact} = \theta(l_c) \quad (M10)$$

where l_c is the value of l at which the whisker is closest to the center of the contacted pole, calculated for each time point as:

$$l_c = \arg \min\left(\sqrt{(x(l) - x_0)^2 + (y(l) - y_0)^2}\right). \quad (M11)$$

(x_0, y_0) is the center of the contacted pole object. The point of finding the location along the whisker nearest the center of the contacted pole was to (a) define operationally the location of contact during periods of contact, and to (b) help identify such periods.

In order to help identify periods of whisker-pole contact, we calculated for each time point a quantity (d_{pole} ; in mm) to estimate the distance between whisker and pole:

$$d_{pole} = \sqrt{(x(l_c) - x_0)^2 + (y(l_c) - y_0)^2} - barRadius \quad (M12)$$

where $barRadius$ is the radius in pixels of the pole presented to the mouse.

The coordinates of the contact point were defined as:

$$(x_c, y_c) = (x(l_c), y(l_c)). \quad (\text{M13})$$

Note that for the data presented here, (x_c, y_c) was not extrapolated to accommodate any failure of whisker tracking near the pole (the shadow of the pole could cause termination of whisker tracing at points very near the contact point). However, we have found this extrapolation useful in analysis of similar videography datasets, and to illustrate our general methods for extracting forces and moments from videography, we illustrate this step in Figure 3E. When extrapolated, Eqns. M11 and M13 were generalized to define the whisker-object contact point as the point along the whisker or its extrapolation that was closest to the center of the bar.

To define curvature, we could in principle use the “primary” fitted whisker ($c(l)$; Eqn. M4). However, there can be variations in the extent of the whisker actually tracked from frame to frame, mainly because (a) the field of view does not necessarily capture the entire whisker, and the whisker can therefore be more or less fully in the field of view; and (b) the shadow of the pole could terminate tracing of the whisker near the whisker-pole intersection, such that the whisker was not traced distal to the pole. Thus, $c(l)$ could be fitted to different segments of the whisker in different frames. This could generate subtle changes in the shape of $c(l)$ even at a fixed point on the whisker, and thus spurious changes in apparent curvature (Figure 4). To mitigate this problem in measuring curvature, we fitted a “secondary” parametric curve

$$\hat{c}(l) = (\hat{x}(l), \hat{y}(l)) \quad (\text{M14})$$

to a segment of the whisker falling within a constant region of arc-length (Figure 4D); that is, where $\hat{x}(l)$ and $\hat{y}(l)$ are polynomials fitted to x_i and y_i respectively, as a function of l_i , for i such that $a \leq s(l_i) \leq b$. Here we used second-degree polynomials, fitted over the interval $[a, b]$. Thus, $\hat{c}(l)$ was fitted over a constant length of whisker, although subject to imperfections due for instance to pixilation and to variability in tracking the whisker near the face. The interval $[a, b]$ was chosen after inspection of the residuals

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between $\hat{c}(l)$ and the raw (x_i, y_i) . Over small intervals second-degree polynomial fits were simple and accurate.

At each time point, we derived curvature (κ_p ; in mm^{-1}) from $\hat{c}(l)$ at a user-defined arc-length distance (in mm) along the whisker, s_p , as:

$$\kappa_p = \frac{\hat{x}'(l_p)\hat{y}''(l_p) - \hat{y}'(l_p)\hat{x}''(l_p)}{\left(\hat{x}'(l_p)^2 + \hat{y}'(l_p)^2\right)^{\frac{3}{2}}}, \quad (\text{M15})$$

for l_p such that $s(l_p) = s_p$.

Because whiskers can have intrinsic curvature, a more directly useful quantity ($\Delta\kappa_p$; in mm^{-1}) is the change in curvature (Birdwell et al., 2007):

$$\Delta\kappa_p = \kappa_p - \frac{\sum_i \kappa_p(t_i)}{N}, \quad (\text{M16})$$

where t_i is a set of N time points during which the whisker is not bent.

Whisker follicle position coordinates (x_f, y_f) were estimated by linearly extrapolating past the end of the tracked whisker, typically from the intersection with the mask; i.e. proceeding from $c(l_{intersection})$. The extrapolation was based on θ_{base} . The distance to extrapolate (typically 1 mm) was estimated based on inspection of the video data.

$$(x_f, y_f) = \left(-d_{extrap} \sin(\theta_{extrap}) + x(l_{base}), -d_{extrap} \cos(\theta_{extrap}) + y(l_{base})\right) \quad (\text{M17})$$

where d_{extrap} is the distance to extrapolate, and $\theta_{extrap} = \theta_{base}$.

Follicle position along the face (d_f) was estimated by taking the x-coordinate (anterior-posterior axis) value of follicle position, $d_f = x_f$.

The lever arm from location of curvature measurement to location of whisker-object contact, for each time point, is:

$$\vec{r}_p = \left(x_c - x(l_p), y_c - y(l_p)\right). \quad (\text{M18})$$

The angle (in radians) of this vector is:

$$\theta_p = \tan^{-1} \frac{x_c - x(l_p)}{y_c - y(l_p)}. \quad (\text{M19})$$

Similarly, the lever arm from follicle to whisker-object contact location:

$$\vec{r}_0 = (x_c - x_f, y_c - y_f). \quad (\text{M20})$$

And the angle (in radians) of this vector is:

$$\theta_0 = \tan^{-1} \frac{x_c - x_f}{y_c - y_f}. \quad (\text{M21})$$

At each time point, we calculated the magnitude of the force applied to the whisker (F ; in 1×10^{-6} Newtons) using Eqn. 4. Magnitude of moment at the follicle (M_0 ; in 1×10^{-6} Newtons * 1×10^{-3} m) was calculated using Eqn. 5. Magnitude of the axial force (F_{ax} ; in 1×10^{-6} Newtons) pushing into the follicle along the axis of the whisker was calculated using Eqn. 6. Magnitude of the lateral force (F_{lat} ; in 1×10^{-6} Newtons) was calculated using Eqn. 7.

During whisking in air, the forces acting on the follicle are dominated by the whisker's moment of inertia and its angular acceleration. These forces are much smaller than the forces produced by contact between object and whisker and not relevant to object localization. The forces were therefore set to zero between contacts (Figure 6B, D).

We excluded trials in which the whisker contacted the pole during retraction from behavior and whisker analyses (Figures 6-10). We also excluded contacts after the decision.

Calculating the protraction parameter

To describe protraction with a single parameter, we combined azimuthal motion and follicle translation in a single parameter, θ_{total} (Figure 8A). For the frame of first touch of each contact we defined a line with origin in the follicle and along θ_{touch} . The intersection point between this line and a line along the anterior-posterior axis passing through the approximate contact point for the Go position (5 mm from the follicle) was determined. For each video frame of a contact the angle between the base of the whisker (i.e. a line along θ_{base}) and a line connecting the follicle to the intersection point was calculated. This

angle is θ_{total} . θ_{total} at first touch is thereby by definition 0.

Statistics

Statistics were computed as indicated in the text. Comparisons of forces and bending moments for target and distracter locations (Figures 6, 8, 9, 10) were based on bootstrap methods. For every session we analyzed frames of contact in which θ_{total} was ≥ 2 degrees. We restricted the analysis to these protraction amplitudes because whisker tracking could be unreliable near the moment of whisker-pole contact (corresponding to $\theta_{total} = 0$ degrees).

Analysis of whether the slope of $M_0(\theta_{total})$, $F_{ax}(\theta_{total})$, and $F_{lat}(\theta_{total})$ was larger for Go than for No Go trials (Figure 8B-D) proceeded as follows, separately for each of three mice.

For $M_0(\theta_{total})$, $F_{ax}(\theta_{total})$, and $F_{lat}(\theta_{total})$ time series, N_{nogo} trials were drawn with replacement 10,000 times from both the Go and No Go trials (after pooling trials across two behavioral sessions for one of the three mice), where N_{nogo} is the number of No Go trials. Thus, each bootstrap sample comprised N_{nogo} randomly matched Go and No Go trials. For each bootstrap sample, we calculated the maximum likelihood estimate of the fraction of trial pairs where the Go trial had a slope greater than that of the matched No Go trial (i.e., $slope_{go} > slope_{nogo}$) and the binomial 95% confidence interval for this fraction (MATLAB ‘binofit’). We then averaged the means and confidence intervals across all 10,000 bootstrap repetitions. For each of $M_0(\theta_{total})$, $F_{ax}(\theta_{total})$, and $F_{lat}(\theta_{total})$, and for all mice, the 95% confidence intervals did not contain 0.5. Slopes were thus larger for Go trials.

Analysis of which force/moment cues could underlie performance on the illusion experiments (Figures 9, 10) proceeded similarly, separately for each of two mice. For $M_0(\theta_{total})$, $F_{ax}(\theta_{total})$, and $F_{lat}(\theta_{total})$ time series, $N_{illusion}$ trials were drawn with replacement 10,000 times from both the illusion and No Go trials (after pooling trials across three behavioral sessions for each mouse), where $N_{illusion}$ is the number of illusion trials. Thus, each bootstrap sample comprised $N_{illusion}$ randomly matched illusion and No Go trials. By bootstrapping in this way we avoided the problem of having to decide which particular trials to compare between illusion and No Go trial types (since the number of trials of

each type was not matched and there was no logical way to pair them). We restricted analysis to No Go trials in which the pole was in one of the two locations closest to the Go location. The rationale for choosing locations close to the Go location is that to achieve its level of illusion-trial task performance, the mouse must have been able to discriminate illusion trials (in which a flexible object was in the Go location) from even the most similar No Go trials. On the other hand, we chose the two closest No Go positions, rather than only the closest, in order to have more No Go trials for analysis (there was no obvious dependence of performance on the particular No Go location). For each bootstrap sample, we calculated the maximum likelihood estimate of the fraction of trial pairs where the illusion trial had a slope greater than that of the matched No Go trial (i.e., $\text{slope}_{\text{illusion}} > \text{slope}_{\text{nogo}}$) and the binomial 95% confidence interval for this fraction. We then averaged the means and confidence intervals across all 10,000 bootstrap repetitions. For analysis of $F_{ax}(M_\theta)$, the procedure was identical except that we estimated the fraction of trial pairs where the illusion trial had a slope less than that of the matched No Go trial (i.e., $\text{slope}_{\text{illusion}} < \text{slope}_{\text{nogo}}$).

For the data shown in Figure 6G-I, we asked whether performance depended on absolute force amplitudes. Calculations were done separately for each trial type of each session. We fitted a generalized linear model for binomial data with a logit link function (MATLAB ‘glmfit’) to force vs. response. The reported p-values are for the null hypothesis that response does not depend on force.

Glossary

a_{base}	Radius of whisker cross section at the base
a_p	Radius of whisker cross section at p
$c(l)$	Parametric curve fitted to sequence of (x_i, y_i) pairs returned by tracking
$\hat{c}(l)$	Parametric curve fitted to sequence of (x_i, y_i) pairs from tracker, within an arc-length region of interest
d_f	Position of the follicle along the face
d_{pole}	Distance between whisker and pole
E	Young's Modulus
\vec{F}	Force exerted on the whisker by the object
\vec{F}_{ax}	Component of F pushing the whisker into the follicle
\vec{F}_{lat}	Component of F pushing the whisker against the caudal wall of the follicle
F_{scale}	Force acting on the scale when pushing the whisker against it
\vec{F}_t	Friction-caused force pulling the whisker out of the follicle
I_p	Second moment of inertia at p
\vec{M}_0	Bending moment acting on the follicle
\vec{M}_p	Bending moment acting on p
p	Point along the whisker
\vec{r}_0	Vector (lever arm) from the follicle to the point of contact
\vec{r}_p	Vector (lever arm) from p to the point of contact
s	Arc-length distance along whisker
s_p	Length of the whisker along its contour from the base to point p

x_c	Image x-coordinate of whisker-object contact point
x_f	Image x-coordinate of whisker follicle
y_c	Image y-coordinate of whisker-object contact point
y_f	Image y-coordinate of whisker follicle
θ	Angle of whisker, defined at all points along whisker
θ_0	Angle of \vec{r}_0
θ_{base}	Angle at the base of the whisker
$\theta_{contact}$	Whisker angle at the point of contact between object and whisker
θ_p	Angle of \vec{r}_p
θ_{total}	Parameter quantifying protraction; combines angular rotation and translation
θ_{touch}	θ_{base} at the time of first touch during a contact period
κ_p	Curvature of the whisker at p
$\Delta\kappa_p$	Change in curvature of the whisker at p
μ	Friction coefficient

Results

Head-fixed mice perform radial object localization

We trained head-fixed mice in one of several variations of a Go/No Go vibrissa-based object localization task (Figure 2A-C). Mice reported the presence of a vertical pole within a target position (the “Go stimulus”, proximal) or in a distracter position (the “No Go stimulus”, distal) by either licking (Go response) or withholding licking (No Go response). In each trial, the pole was presented at a single location, and the mouse had to judge object location based on its memory of the positions. Our task probes memory-guided object localization in a head-centered reference frame (O'Connor et al., 2010a). Whiskers were trimmed so that mice performed the task with a single whisker (C2). Target and distracter locations were carefully arranged for each mouse along the ‘radial’ axis. Therefore whiskers contacted the pole in both locations at nearly identical azimuthal angles (angle difference: mean 1.6°; range 0.2°-2.9°; 3 mice, 5 sessions) (Figure 2B). The

remaining azimuthal differences are likely too small to be discriminated (Knutsen et al., 2006; O'Connor et al., 2010a).

All mice (8/8) reached a 90 % correct response criterion on the standard task (single target and distracter locations; distance from the follicle: target, 5 mm; distracter, 8 mm) over hundreds of trials per session (Figure 2D). To estimate reaction times we measured the time elapsed between the pole coming within reach and the first (answer) lick on Hit trials (Figure 2C). These reaction times were $\sim 640 \pm 79$ ms (mean \pm std, 3 mice), longer than for object localization in the anterior-posterior axis with a single whisker ($\sim 470 \pm 210$ ms) (AH, DHO, KS unpublished data). This difference suggests that radial object localization requires more extensive interactions between whisker and object compared to anterior-posterior localization.

We next explored the accuracy of vibrissa-based radial object localization. Psychometric curves relate performance to the size of the offset between target and distracter locations. All mice generalized from the standard task to smaller distances between distracter and target stimuli (target, 5 mm; distracter, 6-9 mm) (Figure 2E), and performed above chance even with target and distracter locations separated by one millimeter (mean fraction correct = 57 %, all mice $p < 5 \times 10^{-3}$, one-sided binomial test) (Figure 2F).

Computing the forces acting on the follicle

We used high-speed videography (O'Connor et al., 2010a) to determine the mouse's motor strategies, and also to measure the mechanical forces acting on the whiskers that must support radial object localization. Automated whisker tracking provided the contour of the whisker. To account for imperfections in videography and whisker tracking (pixilation, noisy tracking close to the face due to fur, limited field of view, failure of tracking near the pole due to shading) we developed a number of practical procedures to enable force estimates from video data (Figure 3 A-E; see Materials and Methods). Changes in the whisker curvature in the plane of whisking were used to estimate forces acting on the follicle (Figure 3F-I).

Whiskers are cantilevered beams, with one end embedded in the follicle in the whisker pad (Figure 1A). As the whisker protracts against the pole, the pole exerts a force on the whisker (\vec{F}), bending it and causing stresses in the follicle (Figure 1B). With negligible

friction, \vec{F} will act in a direction normal to the whisker at the point of contact. We can calculate the relationship between whisker shape and the force on the whisker in the quasi-static regime. This means that we ignore small force transients on millisecond time scales (Den Hartog, 1947; Timoshenko et al., 1974). We also assume that the C2 whisker is contained in the plane of whisker movement (Knutsen et al., 2008; Quist and Hartmann, 2012). Since the whisker is a long lever arm, lateral stresses in the face (i.e. perpendicular to the whisker) are dominated by the bending moment (\vec{M}_0) acting on the follicle (Birdwell et al., 2007),

$$\vec{M}_0 = \vec{r}_0 \times \vec{F}. \quad (1)$$

\vec{r}_0 is the lever arm connecting the whisker base to the point of contact. \vec{M}_0 rotates the follicle in the horizontal plane around a fulcrum in the whisker pad. For thin elastic beams, such as whiskers (Figure 5), the bending moment acting on a point (p) along the contour of the beam (\vec{M}_p) is proportional to the change in curvature ($\Delta\kappa_p$) at p (Birdwell et al., 2007) (Figure 3F, G). The constants of proportionality are governed by the shape and material properties of the beam (Landau and Lifshitz, 1986). Coefficients of friction for hair are generally low (0.1 - 0.3) (Bhushan et al., 2005). We therefore neglect friction in the subsequent calculations. Numerical simulations show that our conclusions are expected to be qualitatively similar with sliding friction taken into account (data not shown). With the help of standard trigonometric identities one can arrive at the expression:

$$M_p = r_p F \cos(\theta_p - \theta_{contact}) = (\Delta\kappa_p)(EI_p). \quad (2)$$

r_p is the length of the lever connecting p and the point of contact with the pole, θ_p is the angle of the lever, $\theta_{contact}$ is the angle of the whisker at the contact point and determines the direction of the applied force. (EI_p) is the bending stiffness of the whisker at p . E is the Young's Modulus, which characterizes the elasticity of the whisker. I_p , the second moment of inertia at p , is a purely geometric quantity:

$$I_p = \frac{\pi a_p^4}{4}, \quad (3)$$

where a_p is the whisker radius at p . We measured E and a_p for whiskers used by mice during the experiment (Figure 5; Material and Methods). The time-dependent $\Delta\kappa_p$ was calculated for each whisker video frame. Equations 2 and 3 then allow us to extract the magnitude of \vec{F} from measured quantities:

$$F = \frac{(\Delta\kappa_p)(EI_p)}{r_p \cos(\theta_p - \theta_{contact})}. \quad (4)$$

The bending moment at the follicle (\vec{M}_0) has magnitude:

$$M_0 = r_0 F \cos(\theta_0 - \theta_{contact}), \quad (5)$$

where θ_0 is the angle of the lever arm (Figure 3H). \vec{M}_0 primarily causes lateral stresses in the skin. In addition, the axial component of the force, \vec{F}_{ax} , causes axial stresses by pushing the whisker into the follicle (Figure 3I), with magnitude:

$$F_{ax} = F \sin(\theta_{base} - \theta_{contact}), \quad (6)$$

where θ_{base} is the angle of the whisker at the base, and reflects the orientation of the follicle.

The lateral component of the force, $\vec{F}_{lat} = \vec{F} - \vec{F}_{ax}$, pushes the whisker against the posterior side of the follicle when protracting against an object, with magnitude:

$$F_{lat} = F \cos(\theta_{base} - \theta_{contact}). \quad (7)$$

The order of calculations is illustrated in Figure 3 (panels F–I).

Whisking and forces during radial object localization

Touch forces were quantified in a subset of sessions (3 mice, 5 sessions, 445 trials), for which contact between whisker and pole was scored manually in individual image frames (Figure 6A–D). On correct Go trials, whiskers contacted the pole on average 7.0 ± 2.0 (mean \pm std) times (range 1–17) before the mouse indicated its decision with licking (Figure 6B, E). On No Go trials the rate of contacts was similar, but the number of contacts was smaller (average, 5.4 ± 1.3 ; range 1–10; unpaired t-test $p < 10^{-3}$) (Figure 6D,

E). Behavioral performance was almost equally reliable for trials with small numbers of contacts as for trials with large numbers of contacts (Figure 6F). These observations suggest that mice palpate the object to accumulate evidence, until reaching a decision with a certain level of confidence.

Peak moments and forces were on the order of 1 μNm and 100 μN respectively (Figure 6G-I). The bending moments and forces also differed across trial types. On average, peak bending moments and forces were larger for the proximal Go location compared to the distal No Go location (F_{ax} : Go = $36 \pm 28 \mu\text{N}$, No Go = $19 \pm 19 \mu\text{N}$; F_{lat} : Go = $210 \pm 69 \mu\text{N}$, No Go = $39 \pm 14 \mu\text{N}$; M_θ : Go = $0.9 \pm 0.3 \mu\text{Nm}$, No Go = $0.3 \pm 0.1 \mu\text{Nm}$). This is largely due to the linear taper of rodent whiskers (Birdwell et al., 2007) (Figure 5; $r = -0.988$), which implies that the bending stiffness decreases steeply with distance from the follicle (Equation 3). M_θ , F_{ax} and F_{lat} therefore depend on object location: during a protraction against an object, bending moments and forces build up more rapidly for more proximal objects.

Although mice move their whiskers mainly by pivoting the follicles within the skin, causing changes in the azimuthal angle θ_{base} of the whisker, the position of a follicle (d_f) also translates horizontally along the face (Figure 2B, Figure 7A). θ_{base} and d_f are typically correlated, but they can also move independently (Harvey et al., 2001; O'Connor et al., 2010a). Indeed, follicles moved gradually forward over multiple whisker-object contacts within a trial; as a result, θ_{base} at the time of the first touch within each contact period (θ_{touch} ; measured from the first video frame scored as occurring during a touch event) decreased across successive contacts (Figure 7A). As a simple consequence of geometry (Figure 7B), these changes in θ_{touch} are always larger for more proximal object locations than for more distal locations, providing possible azimuthal cues for radial object location (Figure 1D; Figure 7C).

In our experiments, mice thus might avoid force-dependent radial object localization by relying on these azimuthal cues. Specifically, mice could theoretically track θ_{touch} (or a related parameter, such as θ_{base} averaged over one contact period) and d_f to extract object location (as illustrated in Figure 1D). Two observations argue against this possibility. First, we analyzed whether task performance depended on the relationship between θ_{touch}

and d_f over multiple contacts in individual trials. Mice generally performed at high levels in selected trials with relatively constant θ_{touch} (range of $\theta_{touch} < 1^\circ$, performance = 85 % correct, n = 39). Even on trials with single contacts mice performed radial object localization (performance = 84 % correct, n = 19) (Figure 7D). Second, we randomly varied the pole position in the azimuthal dimension (± 2 -3 degrees), while keeping radial distance constant (Figure 7E). This azimuthal jitter interfered with the object-location dependent relationship between θ_{touch} and d_f (cf. Figure 7C & F) but did not perturb performance of the radial distance task (Figure 7G). Performance was not reduced even in the first session in which jitter was introduced, excluding the possibility that mice relearned the radial task after introduction of jitter. Azimuthal jitter in the pole position also introduced uncertainty in the timing of contact events within a protraction (Go trials, ~ 4.9 ms; No Go trials, ~ 6.2 ms; calculated based on the mean velocity at contact and $\pm 3^\circ$ jitter).

The pneumatic sliders moving the pole vertically into reach of the whisker could cause distance-dependent vibrational cues. We performed control experiments as follows: During the first session behavioral training was under standard conditions (Go, 5 mm; No Go, 8 mm). For the next two sessions the pole was moved smoothly from an anterior out-of-reach position into the target (5 mm) or distracter (8 mm) locations in the horizontal plane. In these sessions, without sliders, performance was not reduced (fraction correct: control = 83.4 % (n = 543); without sliders = 85.0 % (n = 853); two sided binomial test, $p = 0.23$), arguing against vibrational cues. In addition, radial object localization is whisker dependent. In three highly performing mice we cut the C2 whisker. After trimming performance dropped to chance levels (fraction correct = 50.4 %; one sided binomial test, $p = 0.28$). Together these data argue that mice perform force-dependent radial object localization, based on measurement of the time-varying forces in the follicle.

The force cues underlying object localization

We next investigated the variables which could underlie force-dependent radial object localization. M_0 , F_{ax} and F_{lat} all increased with protraction against the object, and the rates of change depended on object location (Figure 8). Since protraction against the object includes changes in whisker angle (θ_{base}) as well as translation of the follicle along

the face (pad movement; Figure 2B, Figure 7A), we parameterized protraction as θ_{total} (Figure 8A, Materials and Methods). $M_0(\theta_{total})$, $F_{ax}(\theta_{total})$, and $F_{lat}(\theta_{total})$ all had larger slopes for the more proximal target location compared to the distracter location ($M_0, p < 0.05$; $F_{ax}, p < 0.05$; $F_{lat}, p < 0.05$; see Materials and Methods; Figure 8B-D) (we note that in the presence of sliding friction F_{ax} would pull on the whisker for small protractions, and then push the whisker into the face at larger protractions).

Since the durations of contacts were independent of object location (Go, 23.2 ± 13.8 ms; No Go, 20.3 ± 12 ms; mean \pm std; 1690 contacts; $p = 0.054$, unpaired two-sample t-test) the time-derivatives of the moment and force variables, which are likely more directly related to driving sensory responses, are also object location-dependent. Therefore mice could use the object location-dependent variables M_0 , F_{ax} , and F_{lat} (Figure 8B-D), together with knowledge about whisker movement, to compute radial object location.

Do changes in individual mechanical variables as a function of protraction code for object location? We tested this possibility by confounding the relationships between $M_0(\theta_{total})$, $F_{ax}(\theta_{total})$, and $F_{lat}(\theta_{total})$ and object location (Figure 9). Mice were trained to lick when the object was present in a proximal target location, and had to withhold licking when the object was in a distal distracter location. For every No Go trial the distracter location was chosen randomly from the range 7-13 mm, to produce a range of forces across different No Go trials. Within bins of thirty-two consecutive trials, two randomly chosen Go trials were not rewarded. In half of these trials a compliant object (a rat's whisker) was presented in the target location, with the stiff pole out of reach ('illusion' trials) (Figure 9A). In all other trials the compliant object was out of reach. Prior to every session the position of the compliant object (for illusion trials) was matched to the Go position of the stiff object based on video still frames. Differences in θ_{touch} were similar between contacts with the stiff and compliant objects in the target position (2 mice, 6 sessions, mean difference = 0.9° ; range = $0.1^\circ - 2^\circ$) and stiff object in the target and distracter positions (2 mice, 6 sessions, mean difference = 1.5° ; range = $0.3^\circ - 2.9^\circ$). In this type of experiment each session thus contained three trial types (Go, stiff; Go, compliant; No Go, stiff). In additional experiments the compliant

pole was presented in the middle of the distracter position range (No Go, compliant ; 4 mice, 8 sessions) (Figure 9B).

As the whisker protracts against the compliant object, bending moments and forces acting on the follicle build up more slowly compared to the stiff pole (cf. slopes of "Go" and "Illusion" data in Figure 9C-E). Object locations and the stiffness of the compliant object were chosen so that the bending moment and forces experienced in these illusion trials were in the range of those experienced in No Go trials. $M_0(\theta_{total})$, $F_{ax}(\theta_{total})$ and $F_{lat}(\theta_{total})$ could not be distinguished between cases where the compliant object was in the target location, or the stiff object was in the distracter location (Figure 9C-E; Materials and Methods).

Therefore, if $M_0(\theta_{total})$, $F_{ax}(\theta_{total})$ and $F_{lat}(\theta_{total})$ by themselves were the mechanical variables underlying object localization, then mice would group the compliant object in the target location with the stiff object in the distracter location, causing a dramatic drop in behavioral performance; mice would incorrectly make No Go responses even though the compliant object was in the Go position (and therefore be fooled into misinterpreting the location of the compliant object; a somatosensory illusion).

Remarkably, mice interpreted the compliant object in the target location as a Go stimulus (% trials with licking; illusion, 77 ± 17 %; control, 94 ± 3 %; mean \pm std; unpaired t-test, $p = 0.06$) (Figure 9B), despite the fact that the mechanical variables at a given protraction were comparable to those produced by the stiff pole in the distracter (No Go) location (Figure 9C-E). Similarly, when the compliant object was presented in the distracter location it was correctly interpreted as a No Go stimulus (illusion, 2 ± 2 %; control, 9 ± 4 %), excluding the possibility that mice interpreted the compliant object as a novel stimulus triggering licking (Figure 9B).

These data exclude the possibility that $M_0(\theta_{total})$ and $F_{ax}(\theta_{total})$ are the solitary variables underlying object location. The situation is less clear for F_{lat} . The slope of $F_{lat}(\theta_{total})$ was slightly larger for illusion trials compared with No Go trials on average, although this difference was not significant at our level of statistical power ($p > 0.05$; Materials and Methods). However, we cannot rule out that mice perform a more precise measurements of F_{lat} than our apparatus, based on which they could group the compliant object in the

target location with the stiff object in the target location based on $F_{lat}(\theta_{total})$ alone (Figure 9E; Figure 10A). We note however that M_0 and F_{lat} both produce lateral stresses in the follicle (Figure 10A, B), where M_0 is expected to dominate because of mechanical advantage (Equation 1, 7). It therefore seems unlikely that F_{lat} could be sensed independently of M_0 to provide an estimate of object location.

Our findings are thus consistent with two mechanical models for radial distance perception (Figure 10A). The mouse could monitor lateral force (F_{lat}) alone (Figure 10A, left), or compare at least two variables in the three-dimensional force/moment space (Figure 10A, right). Because F_{ax} produces stresses primarily in a separate part of the follicle (towards the medial end of the follicle) from M_0 and F_{lat} (Figure 10B), and because the lateral stresses are dominated by M_0 (Equation 1, 7), the most likely possibility is a combination of M_0 and F_{ax} coding for radial object location (Figure 10C, D). During contact between whisker and pole, M_0 and F_{ax} increase at different rates with increasing protraction. M_0 increases roughly linearly with protraction (Figure 8B). In contrast, F_{ax} increases nonlinearly; F_{ax} becomes appreciable only after the whiskers are bent by interactions with the pole (Figure 8C, Equation 6). Since intrinsic whisker curvature in the plane of protraction was small ($\kappa_p = -5.6 \times 10^{-4}$; cf. peak values during contact, $\kappa_p = 4.2 \times 10^{-3}$), F_{ax} builds up with a delay compared to M_0 . For a conical whisker (Figure 5) the bending stiffness (EI_p) is a steep function of distance from the face (Equation 3). Forces applied by more distal objects will therefore produce more pronounced whisker bending and thus larger F_{ax} relative to M_0 for a given degree of protraction. Mice could therefore measure F_{ax} as a function of M_0 (Figure 10C, D) to extract object location.

Discussion

Object localization is a fundamental haptic behavior. Localization of object features (for example, corners and edges) underlies object recognition and whisker-based navigation. Rodents use their whiskers to judge object location in three dimensions in the vicinity of their heads (Knutsen and Ahissar, 2009). Behavioral studies have focused on object localization in the anterior-posterior direction. In these tasks rodents are asked to either compare object distance on two sides of the head within a trial (Knutsen et al., 2006) or compare object distance on one side of the head across trials (Mehta et al., 2007; O'Connor et al., 2010a; O'Connor et al., 2010b). Two distinct strategies might underlie object localization in the anterior-posterior direction. In one strategy rodents scan their whiskers over possible object locations. Neural contact signals are then interpreted with reference to a neural signal related to whisker location or phase (Fee et al., 1997; Knutsen et al., 2006; Mehta et al., 2007). In these schemes object location is encoded in the timing of touch-evoked action potentials. Electrophysiological data suggest how whisking and contact signals might be compared and combined to compute object location (Curtis and Kleinfeld, 2009). In another strategy mice use the fact that objects at different locations are engaged differently with the whiskers; for example, during a protraction an anterior object will produce weaker forces compared to a posterior object. Object-location dependent spike rates could code for object location (O'Connor et al., 2010b). However, it is unclear if mice use forces measured in the follicle for tactile perception, beyond detecting touch.

In addition to localization in the anterior-posterior direction, rodents could use the amplitudes of time-varying stresses in the follicle to extract radial object location along the shaft of the whisker (Birdwell et al., 2007; Solomon and Hartmann, 2006; Szwed et al., 2006). However, force-dependent radial object localization has not been demonstrated experimentally. In an aperture discrimination task, progressive whisker trimming dramatically reduced performance, suggesting that contact with multiple whiskers may be required for radial object localization (Krupa et al., 2001) (Figure 1C). But whiskers were not tracked and the motor strategy underlying aperture discrimination remains unknown.

The same whiskers may have contacted the aperture edge multiple times during head-movements or follicle movement, which would provide azimuthal cues about the location of the aperture edge (Figure 1D). Those experiments therefore did not reveal if rodents can judge radial object distance based on forces measured in one follicle (Figure 1B), or rather triangulate the distance to the aperture edge using multiple whiskers or by integrating over multiple contacts (Figure 1C, D). Our experiments show that mice perform force-dependent measurement of radial distance and, more generally, can perform force measurement for tactile perception.

Head-fixation limits whisking largely to a plane (Knutsen et al., 2008) and facilitates whisker tracking for stimulus control. These conditions make it possible to test the hypothesis that rodents can judge radial object distance with individual whiskers. We find that mice can perform force-dependent radial object localization to at least 1 millimeter (Figure 2E, F). Object localization typically involved multiple contacts between whisker and object. Individual contacts lasted 5-100 ms with peak forces up to 500 μ N. The number and duration of contacts, the azimuths at contact and the amplitudes of the forces as a function of protraction varied greatly across trials; however, these parameters did not correlate with behavioral performance (Figure 6F-I, 7B, 9). Even single contacts were sufficient for discrimination in some trials. Precise timing of contact events within the whisk cycle does not seem to be critical for radial distance discrimination, as ~ 5 ms uncertainty in the time of contact, introduced by azimuthal jitter, had no impact on the performance (Figure 7G). Since the whisker contacted the pole at the same azimuthal angle, our experimental conditions exclude possible roles for protraction-dependent torsional cues (Knutsen et al., 2008).

We note that our calculations of the absolute magnitudes of the contact forces between whisker and object have to be viewed as estimates. During their normal life cycle mouse whiskers change over days (Ibrahim and Wright, 1975), together with changes in the second moment of inertia and thus the bending stiffness (Equation 3). Yet we used the mechanical properties of representative C2 whiskers to extract forces from curvature measurements for C2 whiskers of all mice, ignoring time-dependent changes and differences across whiskers. The mechanical model of the whisker was imperfect

(Materials and Methods) (Birdwell et al., 2007; Quist et al., 2011). Estimates of the location of force measurement (p in Equations 2 & 3) were also approximate, for two reasons. First, the C2 whisker has intrinsic curvature which is not localized to the plane of whisking (Quist and Hartmann, 2012) (unpublished observations). Second, the fur on the face obscured the exact location of the end of the whisker. The absolute precision of the force measurements is expected to be no better than a factor of 2-3. More accurate force estimates will require three-dimensional whisker tracking and mechanical models (Quist and Hartmann, 2012).

In addition, we computed forces in the quasi-static regime, ignoring transient forces caused by vibrations in the whisker. Although these force transients are object location-dependent, they are relatively small and extremely rapid (~ 1 ms, in preparation) and it is unclear if sensory neurons in the trigeminal nucleus could detect them.

We varied the compliance of the object to confound the relationship between stresses in the follicle and object location (Figure 9). Remarkably, increasing object compliance did not confuse the mice, even though stresses on the whisker follicle were greatly reduced. This suggests that the value of individual stresses as a function of protraction, or their time derivatives, are not solitary cues underlying coding of radial object distance. Instead our data argue that mice effectively compare multiple variables at the follicle.

While our behavioral data (Figure 6, 8, 9) allow for the possibility that mice rely solely on lateral force, we consider this possibility unlikely (Figure 9, 10). The total lateral stresses are likely dominated by the bending moment, due to mechanical advantage (Equation 1, 7). Assuming distances between the fulcrum within the follicle and any given mechanoreceptor, in the range 20 μm and 400 μm , bending moments on the order of 1 μNm (Figure 8B) will produce forces between 2.5 mN and 50 mN, 10- to 150-fold higher than peak lateral forces (Figure 8D). Thus, for mice to use lateral force as a sole cue, they would have to tease out lateral force from the much larger lateral stresses induced by bending moment (Figure 10B). A better understanding of how forces are measured by the diverse sensory afferents in the follicle would help disambiguate these mechanisms.

Our data further rule out that protraction-dependent bending moment by itself is used to determine radial distance (Birdwell et al., 2007). In contrast, a strategy in which axial stresses were compared with lateral stresses for a given protraction would be especially favorable. The whisker taper plays a critical role in this and related schemes. Bending stiffness is a steep function of whisker diameter. Since the taper of the whisker is linear (diameter from 70 μm at the base to 3-4 μm at the tip for a mature C2 whisker) the bending stiffness varies by a factor of almost 100,000 along the whisker (length 16 mm) (Equation 3). Close to the whisker tip small forces are sufficient to bend the whisker, whereas large forces are required close to the follicle. As the whisker bends, axial forces, pushing the whisker into the follicle, build up. For more distal object locations axial forces are relatively larger for a given bending moment. Mice could therefore extract object location by comparing lateral and axial stresses, independent of their absolute values. Consistent with this view, stiffening the whisker and eliminating its taper appeared to confuse mice and make them interpret distal objects as proximal objects (data not shown). Mice therefore appear to use the gradual whisker taper (Ibrahim and Wright, 1975) (Figure 5) as a ruler to estimate radial object location.

Coding radial distance by relying on the relative amplitude of multiple force variables would provide a robust algorithm for object localization. Mice are faced with measuring object location for objects with different properties, including different stiffness (i.e. another mouse compared to a rock). In addition, they may have to determine object distance under conditions where contact forces between whisker and object cannot be controlled accurately (i.e. while running through a tunnel). Algorithms relying on relative amplitudes (e.g. Figure 10C, D) can deduce object location independent of the compliance of the contacted object and whether or not it is moving.

We note one additional advantage of algorithms based on relative force measurements. Absolute forces can only be interpreted in terms of object location if the mouse has detailed knowledge of whisker protraction or has precise control of whisking. In contrast, coding of object distance by comparing two or more forces requires neither knowledge of whisker position nor precise control of whisking.

It has long been known that lateral stresses applied to whiskers excite trigeminal ganglion (TG) neurons in a direction- and velocity-dependent manner (Gibson and Welker, 1983; Stuttgen et al., 2008; Szwed et al., 2003; Szwed et al., 2006; Zucker and Welker, 1969). However, TG neurons also readily encode axial forces (Stuttgen et al., 2008). It is possible that different TG neurons are tuned to selectively detect lateral or axial stresses acting on the follicle and could thus underlie radial object localization.

We note that radial object localization with a single whisker presents a challenge for mice. Compared to object localization in the anterior-posterior direction, reaction times were longer (640 ms vs. 470 ms on average) and the average number of contacts per trial was larger (7.0 vs. 4.8; correct Go trials; AH, DHO, KS unpublished data). Learning was also relatively slow: at least 14 daily behavioral sessions were needed to train mice to perform the 3 mm radial distance discrimination task with a single whisker (C2) and an accuracy of >90% for at least 100 consecutive trials, much longer than training for anterior-posterior object localization (cf. Figure 3 in (O'Connor et al., 2010a)). Further, in an aperture discrimination task progressive whisker trimming dramatically reduced performance (Krupa et al., 2001). Together these observations indicate that under natural conditions rodents likely also rely on multi-whisker cues, such as triangulation, to judge object distance in three dimensions. Similarly, classic human psychophysics has shown that accuracy and speed in haptic object recognition increases with the number of fingers involved (Davidson, 1972).

The whisker system has become an important model system to study sensorimotor integration and active sensation (Diamond et al., 2008). An understanding of the computations performed by this system requires precise characterization of the forces acting on the whisker that are transduced in the follicle (Birdwell et al., 2007; Szwed et al., 2006). We combined measurements of these forces with mouse behavior to constrain the mechanisms of sensory transduction and the neural codes that might underlie radial object localization.

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Figure legends

Figure 1. Radial object localization.

Different strategies that could underlie radial object localization.

(A) Schematic of the experimental situation treated in this paper. A mouse protracts a single whisker (whisker C2) against a pole (highlighted in red) to determine its position. The pole exerts a force F on the whisker.

(B) Mice could estimate radial distance from time-dependent or protraction-dependent stresses in the follicles (force-dependent radial distance measurement). These are produced by the bending moment (M_0), the axial force (F_{ax}) and the lateral force (F_{lat}).

(C) Triangulation with multiple whiskers. In this example, mice touch the object (red) with two whiskers (w_1, w_2), separated by d . The radial distance D can be extracted as $d / \tan(\theta_{touch}(w_1) - \theta_{touch}(w_2))$.

(D) Triangulation with one whisker over multiple touches. In this example, mice touch the object two or more times (at times t_1 & t_2). The whisker follicle moves a distance d from time t_1 to t_2 . The radial distance D can be extracted as $d / \tan(\theta_{touch}(t_1) - \theta_{touch}(t_2))$.

Figure 2. Behavioral analysis of radial object localization.

(A) Schematic of the task. Left, the pole is out of reach. Right, pole is within reach of the spared (C2) whisker. Mice judge the radial distance to the pole and respond according to the pole's location (Go stimulus, lick, blue; No Go stimulus, no-lick, red).

(B) Top view of the pole positions. Trial types are defined by the location of the pole along the radial dimension (i.e. along the whisker). θ_{base} , azimuthal angle at the base of the whisker; d_f , position of the follicle along the face.

(C) Temporal structure of behavioral trials (top, correct No Go, 'correct rejection'; bottom, correct Go, 'hit'). During the sampling period (typically 0.7-1 s) mice explored the object with their whiskers and licking is not scored. During the answer period licking is scored as a response (circles, answer licks from three typical trials; mean reaction time of three mice, 5 sessions, 635 ms). After the response animals were allowed to drink

(2 s). If the animal failed to respond, as in the No Go trial, the pole ascended 2 s after the beginning of the trial.

(D) Performance during three typical behavioral sessions for three mice (fraction of trials correct, running average over 100 trials).

(E) Radial distance discrimination as a function of offset between Go and No Go stimuli for one mouse (16 sessions). Circles, mean performance averaged over one session (colors indicate the sequence of behavioral sessions across time). Black line, average.

(F) Average performance (mean \pm std; $n = 5$ mice; 16-18 sessions per mouse).

Figure 3. Derivation of bending moments and forces from single video frames.

(A) Raw video frame (same as in Figure 1A) showing a C2 whisker bent during contact with a pole (bottom view). The shadow of the mouse face is in the top left. All subsequent panels use the same video frame.

(B) The tracker extracts a subpixel-resolution series of points, (x_i, y_i) , representing the medial axis of the whisker.

(C) The (x_i, y_i) points were fitted by a parametric curve (magenta) comprising fifth-order polynomials fitted to both x - and y -coordinates as a function of arc length along the whisker. Derivatives used in subsequent steps for computing angle at different points along the whisker were based on this fitted curve.

(D) To avoid noise in whisker angle and length near the face, induced by fur on the whisker pad, we effectively truncated the whisker at an intersection with a ‘mask’ (cyan). Angle at the base of the whisker (θ_{base}) was estimated from the angle at the intersection between mask and whisker; this is warranted because the whisker is very stiff and straight close to the face. Similarly, the arc length origin was set to occur at this intersection, rather than at the first tracked point, which could vary depending on how far “into the fur” a whisker was tracked (see Figure 4 for more details). A second-order polynomial was fitted to x - and y -coordinates in a small region of interest defined by arc length (s) along the whisker. This region of interest was chosen to maximize signal-to-noise ratio in

curvature estimates (green region in panel F). Derivatives used in subsequent steps to measure whisker curvature were based on this 'secondary' parametric curve.

(E) The location of the whisker follicle (indicated by the red "x") must be known to compute moment at the follicle. The follicle location was estimated by linearly extrapolating for a fixed distance past the mask, based on the angle at the base of the whisker (left zoomed box). The location of whisker-object contact was computed by linearly extrapolating beyond the last tracked point along the whisker, and defining the contact point as the closest point along this line to the center of the pole (right zoomed box).

(F-I) Illustration of calculations. In these panels the raw image from (A-E) is omitted, but all fitted quantities are shown: raw whisker (x_i, y_i) points (blue, but obscured because of successful fitting by the magenta curve), primary fitted curve (magenta), secondary fitted curve (green), mask (cyan) and follicle location (red "x"). The pole is illustrated schematically by the red circle at bottom. Images are slightly enlarged from those in (A-E) for clarity.

(F) We defined azimuthal angles (θ) for the whisker and for vectors with respect to the axis perpendicular to the midline of the mouse (i.e. the medial-lateral axis; shown by grey dashed line). An angle of 0 implies parallelism with the medial-lateral axis. Protraction corresponded to increasing angle. The first step was to calculate the magnitude of moment at a point, p , along the whisker (M_p). This requires a measurement of change in curvature (from the undeflected case) ($\Delta\kappa_p$) and the bending stiffness (EI_p) at p . In principle we could use any p , but we chose p for a high signal-to-noise ratio for curvature measurement. Because the whisker is linearly tapered, the larger curvatures occur farther from the whisker base, leading to more reliable measures of curvature. However, p should not be too close to the pole because the lever arm inducing curvature eventually vanishes and tracking errors near the intersection of whisker and pole can interfere with curvature measurement. Bending stiffness of individual whiskers was estimated using measurements on isolated whiskers (see Materials and Methods; Figure 5).

(G) The magnitude of the contact force (F) can be calculated from M_p , together with the length of the vector (\bar{r}_p) connecting p to the site of whisker-object contact, and the angle

(Θ_1) between \vec{r}_p and the contact force vector (\vec{F}). Since contact force is perpendicular to the whisker at contact (with negligible friction), Θ_1 can be obtained using only the angle of \vec{r}_p (denoted θ_p) and the angle of the whisker at contact ($\theta_{contact}$).

(H) Having measured the contact force (F), the moment at the follicle (M_0) can be obtained after measuring the length of the vector (\vec{r}_0) connecting the point of contact to the follicle, and the angle (Θ_2) between \vec{r}_0 and contact force vector \vec{F} .

(I) Magnitudes of the axial (F_{ax}) and lateral (F_{lat}) components of force can be calculated as the length of the projection of \vec{F} onto the long and short axes of the whisker at the follicle, respectively. Inset shows how the angles between vectors are computed using the angle of the whisker at base (θ_{base}) and the angle at contact ($\theta_{contact}$).

Figure 4. Schematics of video analysis methods.

(A) Illustration of the steps involved in measuring whisker angle and curvature from raw tracked video. Each image shows a projection through time across one trial. Raw x-y pairs representing the medial axis of the whisker (black) are first fitted with a parametric curve (magenta, shown overlaid on black). A secondary parametric curve (green) is then fitted over an arc-length region of interest (ROI). A “mask” (blue) truncates the whisker at its noisy edge near the fur on the face and defines the arc-length origin. Zoomed panel at right shows the pixilation of the raw x-y pairs (in black) and the smooth fitted curves (magenta and green).

(B-E) Illustration of contamination of whisker tracing by fur on the face. Small hairs on the whisker pad can cause spurious measurements of shape and angle at the base of the whisker.

(B) Example time-projection through a trial showing how contamination by fur and lickport occlusion can interfere with measurement of shape and angle at base, as well as interfere with defining a constant region of arc-length (note how the green arc-length ROI is staggered from frame to frame).

(C) Schematic of problem shown in (B), for a single pair of frames.

(D) Example from (B) now improved by the addition of a mask (blue), which effectively truncates the whisker at its intersection with the mask. The mask allows only the faithfully traced portion to be used for further analysis, and prevents the arc-length ROI (green) from being staggered from frame to frame.

(E) Schematic of the improvements shown in (D), for a single pair of frames.

(F-H) Illustration of the purpose of the secondary fitted curve (green), fitted over an arc-length ROI. A parametric curve will in general have a slightly different shape when fitted over different regions of even the same whisker in the same frame.

(F) Example of how the extent of the traced whisker in our videos could vary from frame to frame, for instance due to the shadow of the pole entering the image.

(G) Because of frame-to-frame variability in the extent of the traced whisker, the curvature of the primary fitted curve (magenta) at a point reflected not only the true whisker curvature but also fitting artifacts. Magenta shading at top indicates region of whisker fitted. Curvature is measured at the point (p) indicated by the arrow, and the local region around this point is magnified at bottom. Magenta curves at bottom show the fitted curve over the local, magnified region. Left and right columns indicate a frame in which the full whisker is traced (left) and a frame in which the whisker is traced only as far as the pole (right; pole indicated by gray circle).

(H) To minimize artifacts due to fitting variable lengths of whisker, we fitted a secondary parametric curve over a constant arc-length region of interest. Curvature measured from this secondary fitted curve was much more reliable. Green box at top indicates the arc-length region of interest over which the secondary curve was fitted. Green curves at bottom show the fitted secondary curve over the local, magnified region.

Figure 5. Structure of C2 whiskers.

Top and middle, scanning electron microscopy images of one C2 whisker towards the middle of the whisker (top; scale bar 20 μm) and the tip of the whisker (middle; scale bar, 10 μm). Bottom, radii of three C2 whiskers (x and *, light microscopy; o, electron

microscopy).

Figure 6. Whisking and contact forces underlying radial object location.

(A) Time-lapse sequence showing one contact (ms, milliseconds). The black line is the medial axis of the tracked whisker. The small circle indicates the cross section of the object (a thin pole). Distance from the pole to the follicle, 5 mm.

(B) Azimuthal angle at the whisker base (θ_{base}), bending moment (M_0), axial force (F_{ax}) and lateral force (F_{lat}) for one correct Go trial. Green, manually scored contact periods. The initial time point in a contact period is defined as the first touch (first video frame showing contact between whisker and object). Blue circles, licks. The first answer lick defines the reaction time. Grey patch, after reaction time.

(C-D) Same as (A-B) but for a correct No Go trial. Distance from the pole to the follicle, 8 mm.

(E) The distribution of the number of contacts for Go (blue) and No Go (red) trials before the reaction time (3 mice, 5 sessions).

(F) The fraction of trials correct as a function of the number of contacts for Go (blue) and No Go (red) trials (3 mice, 5 sessions). Bars denote performance (left y-axis), lines the number of trials with a given number of contacts (right y-axis).

(G-I) Moments and forces across trial types. Performance as a function of the maximum value of a mechanical parameter across trials for Go (blue) and No Go (red) trials (1 mouse, 1 session). Bars denote performance (left y-axis), lines show the number of trials with a particular maximum moment or force (right y-axis). Note that this mouse performed perfectly for the 100 consecutive trials shown.

(G) Maximum bending moment.

(H) Maximum axial force.

(I) Maximum lateral force.

Figure 7. Mice rely on stresses in the follicle for object localization.

(A) Spread of the azimuthal angle at first touch (θ_{touch}) across contacts of one session. Later (earlier) contacts within trials are coded by warmer (colder) colors. Distance from the pole to the follicle: Left, 5 mm, Go; right, 8 mm, No Go. Note that the spread of θ_{touch} is larger for proximal objects (left) compared to distal objects (right), producing a possible azimuthal clue for object localization.

(B) The larger spread of θ_{touch} with changing d_f for more proximal object locations is a consequence of geometry.

(C) Azimuthal angle at first touch (θ_{touch}) as a function of whisker pad movement, d_f for one session. Difference in the slope of Go (blue) vs. No Go (red) data points represents greater impact of d_f on θ_{touch} for more proximal objects (corresponding to Go). Target, 5 mm; distracter, 8 mm. Same session as in B. Striped pattern reflects finite resolution of whisker tracking due to fixed pattern noise in video images.

(D) Correct and incorrect trials as a function of the spread of θ_{touch} across contacts (3 mice, 5 sessions). Each point corresponds to a trial. Points are randomly displaced in the y-axis for clarity. Values of zero correspond to trials with single contacts.

(E) Schematic showing the introduction of 3° of azimuthal jitter. See Material and Methods for details.

(F) Azimuthal angle at first touch (θ_{touch}) as a function of d_f with azimuthal jitter ($\pm 3^\circ$). Target, 5 mm; distracter, 8 mm (1 mouse, 1 session). The slopes of Go (blue) vs. No Go (red) points are indistinguishable.

(G) Performance on sessions without (first 50 trials) and with (rest of the session, more than 100 trials) random azimuthal jitter (2 mice shown by black and grey data points, 5 sessions). Azimuthal jitter consisted of azimuthal angle offsets (range $\pm 3^\circ$) added to pole positions across trials.

Figure 8. The stresses on the follicle during object localization.

(A) Schematic illustrating computation of the protraction parameter θ_{total} . θ_{total} accounts for protraction due to changes in both θ_{base} and d_f . The same whisker is shown early (grey) and late (black) during a protraction. Grey “x” marks the intersection point of a line defined by θ_{touch} with a line running along the anterior-posterior axis at the approximate lateral distance of the contact point for the Go position (5 mm from the follicle).

(B-D) Bending moment (B), axial force (C), or lateral force (D) acting on the follicle as a function of the protraction parameter for Go (blue) and No Go (red) trials. All time-points corresponding to contact periods in one session are overlaid with partial transparency.

Figure 9. The amplitudes of bending moment and axial force are not the primary cues underlying object localization.

(A) Schematic of the ‘Illusion’ experiment. Mice discriminated a proximal Go position (5 mm) and a range of distal No Go positions (7-13 mm). In a subset of unrewarded ‘Illusion’ trials a compliant pole replaced the stiff pole in the Go position.

(B) Behavior for trials with stiff and compliant poles (5 mice, 36 sessions). The fraction of trials with licks during the answer period is plotted for different conditions.

(C-E) Parameters producing stresses in the follicle for Go trials with the stiff pole (blue), No Go trials with the stiff pole (red), and unrewarded Go trials with the compliant pole (grey) (one session).

(C) Bending moment (M_θ) as a function of protraction (θ_{total}).

(D) Axial force (F_{ax}) as a function of protraction.

(E) Lateral force (F_{lat}) as a function of protraction.

Figure 10. Force-dependent schemes for object localization.

(A) Forces and moments at the follicle that could be read out for force-dependent radial object localization. Left, lateral force (F_{lat}). Right, mice could use a combination of at

least two of the three force/moment variables. Bending moment (M_θ) and axial force (F_{ax}) are most likely.

(B) Schematic illustrating how forces and the bending moment might couple to different directional stresses in the follicle. The whisker (thick black line) is embedded in the follicle (pink). It is coupled to the whisker pad through springs and rotates around the fulcrum (F) (anterior, A; posterior, P; medial, M; lateral, L). The bending moment and lateral force mainly cause compression or rarefaction in springs along the A-P axis, whereas the axial force affects the spring along the M-L axis. Note that little is known about stresses in the follicle that are actually sensed by mechano-sensitive cells.

(C) Forces and bending moment in Go trials with the stiff pole (blue), No Go trials with the stiff pole (red), and Go trials with the compliant pole (grey) (same session as Figure 9D-F). Data from Go trials with the compliant pole (grey) is masked by the data from Go trials with the stiff pole (blue). Inset: The axial force as a function of bending moment for Go trials with the stiff pole, No Go trials with the stiff pole, and unrewarded Go trials with the compliant pole (same session as Figure 9D-F).

(D) Simulated forces and bending moment in Go trials with the stiff pole (blue) and No Go trials with the stiff pole (red). The dashed black line midway between Go and No Go indicates a putative decision boundary. Inset: Projection in the $F_{ax} - M_\theta$ plane. The decision boundary separates distal objects and proximal objects in our binary choice task.

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