

1 **Title:** PREDICTION OF PRIMARY SOMATOSENSORY NEURON ACTIVITY

2 DURING ACTIVE TACTILE EXPLORATION

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12 **ABSTRACT**

13 Primary sensory neurons form the interface between world and brain. Their function is well-
14 understood during passive stimulation but, under natural behaving conditions, sense organs
15 are under active, motor control. In an attempt to predict primary neuron firing under natural
16 conditions of sensorimotor integration, we recorded from primary mechanosensory neurons
17 of awake, head-fixed mice as they explored a pole with their whiskers, and simultaneously
18 measured both whisker motion and forces with high-speed videography. Using Generalised
19 Linear Models, we found that primary neuron responses were poorly predicted by kinematics
20 but well-predicted by rotational forces acting on the whisker: both during touch and free-air
21 whisker motion. These results are discrepant with previous studies of passive stimulation, but
22 could be reconciled by differences in the kinematics-force relationship between active and
23 passive conditions. Thus, simple statistical models can predict rich neural activity elicited by
24 natural, exploratory behaviour involving active movement of the sense organs.

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26

27 **INTRODUCTION**

28 A major challenge of sensory neuroscience is to understand the encoding properties of
29 neurons to the point that their spiking activity can be predicted in the awake animal, during
30 natural behaviour. However, accurate prediction is difficult without experimental control of
31 stimulus parameters and, despite early studies of awake, behaving animals (Hubel, 1959),
32 subsequent work has most often effected experimental control by employing anaesthesia
33 and/or passive stimulation. However, the active character of sensation (Gibson, 1962; Yarbus
34 1967), based on motor control of the sense organs, is lost in reduced preparations. Recent
35 methodological advances permit a way forward: in the whisker system, it is now possible to
36 record neuronal activity from an awake mouse, actively exploring the environment with its
37 whiskers, whilst simultaneously measuring the fundamental sensory variables (whisker
38 kinematics and mechanics) likely to influence neuronal activity (O'Connor et al 2010).

39 Our aim here was to predict spikes fired by primary whisker neurons (PWNs) of awake mice
40 engaged in natural, object exploration behaviour. The manner in which primary neurons
41 encode sensory information fundamentally constrains all downstream neural processing
42 (Lettvin et al. 1959). PWNs innervate mechanoreceptors located in the whisker follicles
43 (Zucker and Welker 1969; Rice et al. 1986) and project to the cerebral cortex, analogously to
44 other modalities, via trisynaptic pathways through the brainstem and thalamus (Diamond et
45 al. 2008). Here, we show that primary neuron responses are well-predicted by a rotational
46 force ('moment') acting on the whisker, but not by whisker angle and its derivatives – a
47 finding at odds with passive stimulation studies (Gibson 1983, Lichtenstein et al 1990; Bale
48 et al 2013).

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50

51 **RESULTS:**

52 **Primary whisker neuron activity during object exploration is predicted by whisker
53 bending moment**

54 We recorded the activity of single PWNs from awake mice (Figure 1A, E` , Figure 1-figure
55 supplement 1) as they actively explored a metal pole with their whiskers (N = 20 units). At
56 the same time, we recorded whisker motion and whisker shape using high-speed videography
57 (1000 frames/s, Figure 1D, Figure 1-figure supplement 2). Since each PWN innervates a
58 single whisker follicle, we tracked the ‘principal whisker’ of each recorded unit from frame
59 to frame, and extracted both the angle and curvature of the principal whisker in each video
60 frame (total 1,496,033 frames; Figure 1B-E; Bale et al. 2015). Whiskers are intrinsically
61 curved, and the bending moment on a whisker is proportional to how much this curvature
62 changes due to object contact (Birdwell et al. 2007): we therefore used ‘curvature change’ as
63 a proxy for bending moment (O’Connor et al. 2010a). Whisker-pole contacts caused
64 substantial whisker bending (curvature change), partially correlated with the whisker angle
65 (Figures 1E, 4E) and, consistent with Szwed et al. (2003) and Leiser and Moxon (2007),
66 robust spiking (Figures 1E, 2E).

67 To test between candidate encoding variables, our strategy was to determine how accurately
68 it was possible to predict PWN activity from either the angular position (kinematics) or
69 curvature change (mechanics) of each recorded unit’s principal whisker. To predict spikes
70 from whisker state, we used Generalised Linear Models (GLMs) (Figure 2A). GLMs, driven
71 by whisker angle, have previously been shown to provide a simple but accurate description
72 of the response of PWNs to passive stimulation (Bale et al. 2013) and have mathematical
73 properties ideal for robust parameter-fitting (Truccolo et al. 2005; Paninski et al. 2007).

74 For each recorded unit (median 69,672 frames and 550 spikes per unit), we computed the
75 GLM parameters that maximised the regularised likelihood of the unit's spike train given the
76 whisker angle time series (8 fitted parameters) and assessed the quality of the best-fitting
77 GLM by feeding it with the whisker angle time series as input and generating a predicted
78 spike train in response (using cross-validation). We then compared the recorded spike train to
79 the GLM-predicted one (Figure 2B-C) and quantified the similarity between the smoothed
80 spike trains using the Pearson correlation coefficient (PCC). This is a stringent, single-trial
81 measure of model prediction performance (Figure 2-figure supplement 1B). We then repeated
82 this entire procedure for the whisker curvature time series. Although angle GLMs predicted
83 spike trains of a few units moderately well (2/20 units had PCC > 0.5), they performed poorly
84 for the majority (median PCC 0.06, IQR 0.019-0.3; Figure 2B-D, orange). This was not
85 simply because of non-linear tuning to whisker angle, since quadratic GLMs fared only
86 marginally better (median PCC 0.097, IQR 0.042-0.31; p=0.044, signed-rank test, Figure 2-
87 figure supplement 1A). In contrast, we found that the curvature GLMs were substantially
88 more accurate than the angle GLMs (median PCC 0.52, IQR 0.22-0.66; p=0.0044, signed-
89 rank test; Figure 2B-D, blue) with prediction accuracy up to PCC 0.88. This result was robust
90 to the number of fitted parameters: a GLM sensitive to instantaneous curvature exhibited very
91 similar prediction accuracy (Figure 2-figure supplement 1C).

92 Although the activity of most units was better predicted by whisker curvature change than by
93 whisker angle, there were a few units for which the angle prediction performance was
94 appreciable (Figure 2D). However, we found that this could largely be attributed to
95 redundancy. When a mouse whisks against an object, curvature change and angle fluctuate in
96 concert (Birdwell et al. 2007; Bagdasarian et al. 2013; Pammer et al. 2013; Figures 1E, 4E
97 and Figure 4-figure supplement 1). When we fitted GLMs using both curvature change and
98 angle as input, these GLMs predicted the spike trains no more accurately (median PCCs 0.53

99 IQR 0.40-0.62; p=0.067, signed-rank test; Figure 2D) than GLMs based on curvature change
100 alone.

101 In principle, neurons might also be sensitive to the axial force component (parallel to the
102 whisker follicle) and/or lateral force component (orthogonal to axial) associated with
103 whisker-object contact (Figure 1B-C; Solomon and Hartmann, 2011; Pammer et al. 2013).
104 We restricted our analysis to bending moment since, under our experimental conditions,
105 axial/lateral force components were near-perfectly correlated with bending moment (Figure
106 2-figure supplement 2) and bending moment is likely to have a major influence on stresses in
107 the follicle due to mechanical advantage (Pammer et al. 2013).

108 To further test the curvature-encoding concept, we asked whether curvature GLMs could
109 account for the response of PWNs to whisker-pole touch. To this end, we parsed the video
110 data into episodes of ‘touch’ and ‘non-touch’. Units fired at a higher rate during touch than
111 otherwise (Szwed et al. 2003; Leiser and Moxon, 2007). Without any further parameter-
112 adjustment, the curvature-based GLMs reproduced this effect (Figure 2E): the correlation
113 coefficient between recorded and GLM-predicted firing rate for touch episodes was 0.96.
114 Collectively, the above results indicate that, during active touch, the best predictor of whisker
115 primary afferent firing is not a kinematic parameter (whisker angle or its derivatives), as has
116 been consistently reported in studies using passive stimulation, but rather a mechanical
117 parameter – the bending moment.

118 Primary whisker neuronal activity during whisking is predicted by moment

119 During free whisking - in the absence of whisker-pole contact - whisker curvature, and
120 therefore bending moment, changed little (Figure 1E, Figure 4-supplement figure 1A);
121 consistent with previous studies (Knutsen et al. 2008; Quist et al. 2014). Yet, 50% of
122 recorded units ('whisking-sensitive units') were significantly modulated by whisking

123 amplitude (Figure 3A). This suggests that moment due to whisker bending is not the only
124 force that influences afferent activity. A likely candidate is the moment associated with the
125 rotational motion of a whisker: this ‘inertial’ moment is proportional to the whisker’s angular
126 acceleration (Quist et al. 2014). Consistent with this possibility, we found that whisking-
127 sensitive units were tuned to angular acceleration (Figure 3B) and that 60% of these were
128 phase-modulated (Figure 3C). Angular acceleration tuning was diverse: some units fired to
129 acceleration in a particular direction (rostral or caudal), whilst others responded to
130 acceleration in both directions (Figure 3B). Moreover, for whisking-sensitive units (but not
131 the whisking-insensitive ones), quadratic GLMs trained on data from non-touch episodes
132 were able to predict spikes using whisker angle acceleration as input (Figure 3D-E; whisking
133 sensitive units, median PCC 0.38, IQR 0.22-0.56; non-whisking sensitive median PCC -
134 0.006, IQR -0.03-0.03, p=0.001 rank-sum test). These findings indicate that, in the absence of
135 whisker-object contact, responses of PWNs to whisking itself can be accounted for by
136 sensitivity to the moment associated with angular whisker acceleration.

137

138 **Relation between kinematics and mechanics is different in active vs passive touch and**
139 **has implications for neural encoding**

140 We found, during active object exploration, that bending moment, but not whisker angle,
141 predicts PWN firing. In apparent contrast, studies using the acute, passive stimulation
142 paradigm have reported that PWNs encode whisker angle (and its temporal derivatives,
143 Zucker and Welker, 1969; Gibson and Welker, 1983; Lichtenstein et al. 1990; Jones et al.
144 2004; Arabzadeh et al. 2005; Bale and Petersen, 2009; Lottem and Azouz, 2011; Bale et al.,
145 2013). We wondered whether the discrepancy might be due to differences in whisker
146 mechanics between passive and active stimulation conditions. To test this, we analysed the

147 relationship between angle and curvature change during active touch and compared it to that
148 during passive whisker stimulation. During active pole exploration, angle and curvature
149 change were, over all, only loosely related (median correlation coefficient 0.20, IQR 0.079-
150 0.39, Figures 4D-E). Important contributory factors were that the angle-curvature relationship
151 was both different for touch compared to non-touch (Figure 4-figure supplement 1A) and
152 dependent on object location (Figure 4- figure supplement 1B). In contrast, during passive
153 stimulation, whisker angle was near perfectly correlated with curvature change (for C2,
154 correlation coefficients 0.96 and 0.94 respectively; similar results for C5; Figures 4C-D and
155 Figure 4E, inset); consistent with properties of cantilevered beams (Birdwell et al., 2007).
156 Simulations confirmed that, due to the tight relationship between the variables, a unit tuned
157 only to curvature change can appear tightly tuned to angle (Figure 4- figure supplement 2).
158 The implication is that apparent sensitivity to whisker angle under passive stimulation
159 conditions can be accounted for by moment-tuning.

160

161 DISCUSSION

162 Prediction of spikes fired by sensory neurons under natural conditions

163 In the endeavour to understand how neurons encode and process sensory information, there is
164 a basic tension between the desire for tight experimental control and the desire to study
165 animals under natural, unconstrained conditions. Theories of sensory encoding suggest that
166 neural circuits have evolved to operate efficiently under natural conditions (Simoncelli and
167 Olshausen, 2001; Reinagel 2001). Previous studies have succeeded in predicting/decoding
168 spikes evoked by passive presentation of natural sensory stimuli to anaesthetised/immobilised
169 animals (Lewen et al. 2001; Arabzadeh et al. 2005; Pillow et al. 2008; Mante et al. 2008;
170 Lottem and Azouz, 2011; Bale et al., 2013), but it has been difficult to extend this approach

171 to encompass natural, active movement of the sense organs. Here we have addressed this
172 general issue, taking advantage of experimental possibilities recently created in the whisker
173 system (O'Connor et al., 2010a), and the ability of computational methods to uncover
174 stimulus-response relationships even from data with complex statistical structure (Paninski et
175 al., 2007; Fairhall and Sompolinski, 2014). Our main finding was that responses of PWNs,
176 recorded as an awake mouse actively explores an object with its whiskers, can be predicted
177 from the forces acting on the whiskers. Given that, for each unit, we were attempting to
178 predict the entire ~70 s time course of activity, the variability of the behaviour of untrained
179 mice (O'Connor et al., 2010a), and the lack of trial-averaging as a noise reduction strategy, it
180 is remarkable that we found model prediction correlation coefficients up to 0.88.

181 **Mechanical framework for tactile coding**

182 Our finding of force-encoding is at odds with previous passive stimulation studies but
183 consistent with previous studies using electrical whisking and biomechanical modelling.
184 Pushing a whisker against an object triggers spiking in many PWNs (Szwed et al. 2003,
185 2006; Leiser and Moxon, 2007). Modelling by Hartmann and co-workers accounts for this by
186 a biomechanical framework where the whisker is conceptualised as an elastic beam,
187 cantilever-mounted in the skin (Birdwell et al. 2007; Quist et al. 2014). When such a beam
188 pushes against an object, the beam bends, causing reaction forces at its base. Our data are in
189 striking agreement with the general suggestion that mechanoreceptor activity is closely
190 related to such reaction forces. Previous studies reported that PWNs encode both whisker-
191 object contact and whisker motion itself (Szwejda et al. 2003; Leiser and Moxon, 2007; Khatri
192 et al. 2009). Our results show that moment associated both with contact-induced whisker
193 bending and with whisker rotation predicts PWN spiking. Thus, a common framework
194 accounts for diverse PWN properties.

195 In apparent contrast, previous studies using passive stimulation in anaesthetised animals have
196 consistently reported a tight relationship between whisker kinematics and PWN response. In
197 the cantilever whisker model, passively induced changes in whisker angle correlate highly
198 with whisker bending. We confirmed that this applies to real whiskers *in vivo* and
199 demonstrate that moment-sensitive units can thereby appear angle-tuned. In this way,
200 moment-encoding can account for primary neuron responses not only during active touch but
201 also under passive stimulation. More generally, our results highlight the importance of
202 studying neurons under natural, active sensing conditions.

203 It is axiomatic that mechanoreceptors are sensors of internal forces acting in the tissue within
204 which they are embedded (Abraira and Ginty, 2013) and therefore valuable to be able to
205 measure mechanical forces in the awake, behaving animal. In general, including the
206 important case of primate hand-use, the complex biomechanics of skin makes force-
207 estimation difficult (Phillips and Johnson, 1981). In contrast, for whiskers, the quasi-static
208 relationship is relatively simple: the bending moment on a whisker is proportional to its
209 curvature and this has the important implication that reaction forces can be directly estimated
210 from videography *in vivo* (Birdwell et al. 2007; O'Connor et al. 2010a; Pammer et al. 2013).
211 Our results are the first direct demonstration that such reaction forces drive primary sensory
212 neuron responses – likely involving Piezo2 ion channels (Woo et al. 2014; Poole et al. 2015;
213 Whiteley et al. 2015). Our findings show that sensitivity to touch and self-motion in the
214 somatosensory pathway (Curtis and Kleinfeld, 2009; O'Connor et al. 2010b; Curtis and
215 Kleinfeld, 2009; Huber et al. 2012; Petreanu et al. 2012; Peron et al. 2015), arises directly at
216 receptor level, indicating a direct connection from receptor mechanisms to behaviour.

217 **Moment-based computations in tactile behaviour**

218 Extraction of bending moment is a useful first step for many tactile computations. Large
219 transients in bending moment signal object-touch events, and the magnitude of bending is
220 inversely proportional to the radial distance of contact along the whisker (Solomon and
221 Hartmann, 2006). As illustrated by our results on the statistics of active touch, if integrated
222 with cues for whisker self-motion, whisker bending can be a cue to the 3D location of an
223 object (Szwed et al. 2003, Szwed et al. 2006, Birdwell et al. 2007; Bagdasarian et al 2013;
224 Pammer et al., 2013). Bending moment can permit wall following (Sofroniew et al., 2014)
225 and, if integrated across whiskers, can in principle be used both to infer object shape
226 (Solomon and Hartmann, 2006) and to map the spatial structure of the environment (Fox et
227 al., 2012, Pearson et al., 2013).

228 **Summary and Conclusion**

229 We have shown that the responses of primary whisker neurons can be predicted, during
230 natural behaviour that includes active motor control of the sense organ, from forces acting on
231 the whiskers. These results provide a bridge linking receptor mechanisms to behaviour.

232

233 **EXPERIMENTAL PROCEDURES**

234 All experimental protocols were approved by both United Kingdom Home Office national
235 authorities and institutional ethical review.

236 **Surgical procedure**

237 Mice (C57; N=10; 6 weeks at time of implant) were anesthetized with isoflurane (2% by
238 volume in O₂), mounted in a stereotaxic apparatus (Narishige) and body temperature
239 maintained at 37°C using a homeothermic heating system. The skull was exposed and a
240 titanium head-bar (19.1mm x 3.2mm x 1.3mm; O'Connor et al., 2010a) was first attached to
241 the skull ~1 mm posterior to lambda (Vetbond), and then fixed in place with dental acrylic
242 (Lang dental). A craniotomy was made (+0.5mm to -1.5mm posterior to bregma, 0mm to
243 3mm lateral) and sealed with silicone elastomer. Buprenorphine (0.1 mg/kg) was injected
244 subcutaneously for postoperative analgesia and the mouse left to recover for at least 5 days

245

246 **Behavioural apparatus**

247 The behavioural apparatus was adapted from O'Connor et al. (2010a). A mouse was placed
248 inside a perspex tube (inner diameter 32 mm), from which its head emerged at one end, and
249 immobilised by fixing the head-bar to a custom mount holder. The whiskers were free of the
250 tube at all times. The stimulus object was a 1.59 mm diameter metal pole, located ~3.5mm
251 lateral to the mouse's snout. To allow control of its anterior/posterior location, the pole was
252 mounted on a frictionless linear slide (Schneeberger NDN 2-50.40) and coupled to a linear
253 stepper motor (Zaber NA08B30). To allow vertical movement of the pole into and out of
254 range of the whiskers, the pole/ actuator assembly was mounted on a pneumatic linear slide
255 (Festo SLS-10-30-P-A), powered by compressed air. The airflow was controlled by a relay

256 (Weidmüller). In this way, the pole moved rapidly (~0.15 s) into and out of range of the
257 whiskers. The apparatus was controlled from Matlab via a real-time processor (TDT, RX8).

258 **Electrophysiology**

259 To permit reliable whisker tracking (see below), before each recording session, A, B and E
260 whisker rows were trimmed to the level of the fur, under brief isoflurane anaesthesia. The
261 trigeminal ganglion was targeted as previously described (Bale et al., 2015). The silicone seal
262 was removed and a 3/4 shank tungsten microelectrode array (FHC, recording electrodes 8MΩ
263 at 1kHz, reference 1MΩ; tip spacing ~500 µm) was lowered through the brain (angle 4 ° to
264 vertical in the coronal plane) using a micromanipulator (Scientifica, PatchStar) under
265 isoflurane anaesthesia. Extracellular potentials were pre-amplified, digitised (24.4 kHz),
266 filtered (band pass 300-3000 Hz) and acquired continuously to hard disk (TDT, RZ5). The
267 trigeminal ganglion was encountered 6-7 mm vertically below the pial surface and whisker-
268 response units identified by manual deflection of the whiskers with a small probe. Once a
269 well-isolated unit was found, the whisker that it innervated (the ‘principal whisker’) was
270 identified by manual stimulation. At this point, anaesthesia was discontinued. Once the
271 mouse was awake, we recorded neuronal activity during repeated presentations of the pole
272 (‘trials’). Before the start of each trial, the pole was in the down position, out of reach of the
273 whiskers. The pole was first moved anterior-posteriorly to a position chosen randomly out of
274 a set of 11 possible positions, spanning a range ±6 mm with respect to the resting position of
275 the base of the principal whisker. A trial was initiated by activating the pneumatic slide relay,
276 thus moving the pole up into the whisker field, where it remained for 3s before being
277 lowered. At the end of a recording session, the microelectrode array was withdrawn, the
278 craniotomy sealed with silicone elastomer, and the mouse returned to its home cage.

279 **High-speed videography**

280 Using the method of O'Connor et al. (2010a) to image whisker movement/shape, whiskers
281 ipsilateral to the recorded ganglion were illuminated from below using a high-power infrared
282 LED array (940 nm; Roithner, LED 940-66-60) via a diffuser and condensing lens. The
283 whiskers were imaged through a telecentric lens (Edmunds Optics, 55-349) mounted on a
284 high speed camera (Mikrotron, LTR2; 1000 frames/s, 0.4 ms exposure time). The field of
285 view of the whiskers was 350x350 pixels, with pixel width 0.057mm.

286 **Response to touch and non-touch events**

287 Mouse whisking behaviour during the awake recording was segmented into 'touch', and 'non-
288 touch' episodes. Touches between the principal whisker of each unit and the pole were scored
289 manually in each frame of the high-speed video. Any frame not scored as a touch was scored
290 as non-touch. Touch and non-touch firing rates for a given unit were computed by averaging
291 activity over all corresponding episodes.

292 **Whisker tracking**

293 Since the trigeminal ganglion lacks topography, it is difficult to target units that innervate a
294 specific whisker, and therefore desirable for a whisker tracker to be robust to the presence of
295 multiple rows of whiskers. However, since neurons in the ganglion innervate individual
296 whiskers, it is sufficient to track only one whisker (the principal one) for each recorded
297 neuron. To extract kinematic/mechanical whisker information, we therefore developed a
298 whisker tracker ('WhiskerMan'; Bale et al., 2015) whose design criteria, different to those of
299 other trackers (Perkon et al., 2011; Clack et al., 2012), were to: (1) be robust to whisker
300 cross-over events; (2) track a single, target whisker; (3) track the proximal segment of the
301 whisker shaft. The shape of the target whisker segment was described by a quadratic Bezier
302 curve $\mathbf{r}(t,s)$ (a good approximation away from the zone of whisker-object contact; Quist et al.,
303 2012; Pammer et al., 2013): $\mathbf{r}(t,s) = [x(t,s), y(t,s)]$, where x, y are horizontal/vertical

304 coordinates of the image, $s = [0,..,1]$ parameterises (x,y) location along the curve and t is
305 time. We fitted such a Bezier curve to the target whisker in each image frame using a local,
306 gradient-based search. The initial conditions for the search were determined by extrapolating
307 the solution curves from the previous two frames, assuming locally constant, angular
308 velocity. The combination of the low-parameter whisker description and the targeted, local
309 search makes the algorithm robust to whisker cross-over events. The ‘base’ of the target
310 whisker was defined as the intersection between the extrapolated Bezier curve and the snout
311 contour (estimated as described in Bale et al., 2015). The solution curve in each frame was
312 visually checked and the curves manually adjusted to correct occasional errors.

313 **Estimation of kinematic/force parameters**

314 The whisker angle (θ) in each frame was measured as the angle between the tangent to the
315 whisker curve at the base and the anterior-posterior axis (Figure 1B). Whisker curvature (κ)
316 was measured at the base as $\kappa = \frac{x'y'' - x''y'}{(x'^2 + y'^2)^{3/2}}$, where x' , y' and x'' , y'' are the first and second
317 partial derivatives of the functions $x(s)$ and $y(s)$ with respect to s (Figure 1B). Since reaction
318 force at the whisker base reflects changes in whisker curvature, rather than the intrinsic
319 (unforced) curvature (Birdwell et al., 2007), we computed ‘curvature change’ $\Delta\kappa = \kappa - \kappa_{\text{int}}$,
320 where κ_{int} , the intrinsic curvature, was estimated as the average of κ in the first 100 ms of the
321 trial (before pole contact; O'Connor et al., 2010a). During free whisking, whisker angle
322 oscillated with the characteristic whisking rhythm, but curvature changed little. The small
323 changes in whisker curvature during free whisking were consistent with torsional effects
324 (Knutsen et al. 2008).

325 Under conditions of whisking against a smooth surface, such as in the present study, the
326 frictionless quasi-static framework of Birdwell et al. (2007) applies, and bending moment is
327 proportional to $\Delta\kappa$. We estimated bending moment using the method of Pammer et al. (2013)

328 from $\Delta\kappa$, using the radius of the relevant whisker at the base and published data on Young's
329 modulus of mouse whiskers (Quist and Hartmann, 2012).

330 Axial (\vec{F}_{ax}) and lateral forces (\vec{F}_{lat}) at the whisker base were calculated, during periods of
331 whisker-pole contact, using the method of Pammer et al. (2013), from bending moment and
332 whisker-pole contact location. Pole location, in the horizontal plane, in each frame, was
333 identified as the peak of a 2D convolution between the video image and a circular pole
334 template. To localise whisker-pole contact, the whisker tracker was used to fit the distal
335 segment of the whisker close to the pole, seeded by extrapolation from the whisker tracking
336 solution for the proximal whisker segment, described above. Whisker-pole contact location
337 was defined as the point where this distal curve segment was closest to the detected pole
338 centre. Pole and contact locations were verified by visual inspection.

339 The 'inertial' moment associated with the rotational motion of a whisker is proportional to
340 angular acceleration (Quist et al. 2014). To quantify inertial moment during free whisking,
341 we therefore used angular whisker acceleration as a proxy. Acceleration was calculated from
342 the whisker angle time series after smoothing with a Savitzky-Golay filter (polynomial order
343 5; frame size 31 ms).

344

345 **Passive whisker deflection**

346 To determine how whiskers move/bend in response to passive deflection under anaesthesia, a
347 mouse was anesthetized (isoflurane 2%) and placed in the head-fixation apparatus. Individual
348 whiskers (C2 and C5, trimmed to 5 mm) were mechanically deflected using a piezoelectric
349 actuator as previously described (Bale et al., 2013; Bale et al. 2015). All other whiskers were
350 trimmed to the level of the fur. Each whisker, in turn, was inserted into a snugly fitting plastic

351 tube attached to the actuator, such that the whisker entered the tube 2 mm from the face. Two
352 stimuli were generated via a real-time processor (TDT, RX8): (1) a 10 Hz trapezoidal wave
353 (duration 3 s, amplitude 8 °); (2) Gaussian white noise (duration 3 s, smoothed by
354 convolution with a decaying exponential: time constant 10 ms; amplitude SD 2.1 °). During
355 the stimulation, the whiskers were imaged as detailed above (1000 frames/s, 0.2 ms exposure
356 time).

357 **Electrophysiological data analysis**

358 *Spike sorting:* Single units were isolated from the extracellular recordings as previously
359 described, by thresholding and clustering in the space of 3-5 principal components using a
360 mixture model (Bale and Petersen, 2009). A putative unit was only accepted if (1) its inter-
361 spike interval histogram exhibited a clear absolute refractory period and (2) its waveform
362 shape was consistent between the anaesthetised and awake phases of the recording.

363 *Responses to whisking without touch:* To test whether a unit responded to whisking itself, we
364 extracted non-touch episodes as detailed above and computed time series of whisking
365 amplitude and phase by band-pass filtering the whisker angle time series (6-30Hz) and
366 computing the Hilbert transform (Kleinfeld and Deschênes 2011). Amplitudes were
367 discretised (30 equi-populated bins) and the spiking data used to compute amplitude tuning
368 functions. Phases for bins where the amplitude exceeded a given threshold were discretised (8
369 equi-populated bins) and used to construct phase tuning functions. To determine whether a
370 unit was significantly amplitude-tuned, we fitted a regression line to its amplitude tuning
371 curve and tested whether the slope was statistically significantly different to 0 ($p=0.0025$,
372 Bonferroni corrected). To determine whether a unit was significantly phase-tuned, we
373 computed the maximum value of its phase tuning curve and compared this to the distribution
374 of maxima of chance tuning functions. Chance tuning functions were obtained by randomly
375 shifting the recorded spike sequences by 3000-8000 ms and recomputing tuning functions

376 (500 times). A unit was considered phase-tuned if its tuning function maximum (computed
377 using amplitude threshold of 2°) exceeded the 95th percentile of the shuffled distribution.

378 *Generalised Linear Model (GLM)*: To investigate how well primary whisker neurons encode
379 a given sensory variable (e.g., whisker angle, curvature), we fitted single unit activity to a
380 GLM (Nelder and Wedderburn, 1972; Truccolo et al., 2005; Paninski et al., 2007), similarly
381 to Bale et al. (2013). For each unit, a ‘stimulus’ time series (x) (whisker angle or whisker
382 curvature change) and a simultaneously recorded spike time series (n) were discretized into
383 1ms bins: x_t and n_t denote respectively the stimulus value and spike count (0 or 1) in bin t .

384 GLMs express how the expected spike count of a unit depends both on the recent stimulus
385 history and on the unit’s recent spiking history. The standard functional form of the model we
386 used was:

$$387 \quad y_t = f(\vec{k}^T \vec{x}_t + \vec{h}^T \vec{n}_t^* + b), \quad (1)$$

388 Here n_t^* , the output in bin t , was a Bernoulli (spike or no-spike) random variable. The
389 probability of a spike in bin t , y_t , depended on three terms: (1) the dot product between the
390 stimulus history vector $\vec{x}_t = (x_{t-L_k+1}, \dots, x_t)$ and a ‘stimulus filter’ \vec{k} (length $L_k = 5\text{ms}$); (2) the
391 dot product between the spike history vector $\vec{n}^* = (n_{t-L_h+1}^*, \dots, n_t^*)$ and a ‘spike history filter’
392 \vec{h}_t (length $L_h = 2\text{ms}$); (3) a constant b , which set the spontaneous firing rate. $f(\cdot)$ was the
393 logistic function $f(z) = (1 + e^{-z})^{-1}$.

394 To consider whether units might encode multiple sensory variables (e.g., both whisker angle
395 and whisker curvature change), we used a GLM with multiple stimulus history terms, one for
396 each sensory variable:

$$y_t = f(\vec{k}_1^T \vec{x}_{t;1} + \vec{k}_2^T \vec{x}_{t;2} + \vec{h}_t^T \vec{n}^* + b)$$

397 Here the indices 1,2 label the sensory variables.

398 Training and testing of the GLM was done using a cross-validation procedure. For each unit,
399 half of the trials were assigned randomly to a training set and half to a testing set. The
400 training set was used to fit the parameters (\vec{k} , \vec{h} and b), while the testing set was used to
401 quantify the similarity between the spike train of the recorded unit and that predicted by the
402 GLM. GLM fitting was achieved by finding the parameter values (\vec{k} , \vec{h} and b), which
403 minimized a cost function consisting of the sum of the negative log-likelihood and a
404 regularizing term $-\alpha \|\vec{k}\|^2$. For all units, model prediction performance on the test set was
405 robust to variation of α over several orders of magnitude: α was therefore set to a standard
406 value of 0.01. To quantify the performance of the model, the sensory time series of the testing
407 set was used as input to the best-fitting GLM to generate a ‘predicted’ spike train in response.
408 Both real and predicted spike trains were then smoothed by convolution with a 100ms box-
409 car filter and the similarity between them quantified by the Pearson correlation coefficient
410 (PCC). For each unit, the entire training/testing procedure was repeated for 10 random
411 choices of training/testing set and the final prediction accuracy defined as the median of the
412 10 resulting PCC values.

413 To test whether a given ‘actual’ PCC was statistically significant, we tested the null
414 hypothesis that it could be explained by random firing at the same time-averaged rate as that
415 of the recorded unit. To this end, the recorded spike sequences were randomly shifted by
416 3000-8000 ms and the training/testing procedure above applied to this surrogate data. This
417 was repeated 10 times and the resulting chance PCCs compared to the actual PCC using a
418 signed-rank test, $p=0.0025$ (Bonferroni corrected).

419 *Quadratic GLM:* To test whether the units might exhibit nonlinear dependence on the
420 stimulus parameters, we adapted the GLM defined above (equation 1) to include quadratic
421 stimulus variables (Rajan et al., 2013). This was important to assess whisker angular

422 acceleration during free whisking, since a subset of units exhibited U-shaped acceleration
423 tuning functions (Figure 3B). Given a stimulus time series x_t , the quadratic stimulus history
424 vector was $[x_{t-Lk+1}, \dots, x_t, x_{t-Lk+1}^2, \dots, x_t^2]$. Fitting methods were otherwise identical to those
425 detailed above.

426 *Effect of angle-curvature correlations on apparent neuronal stimulus encoding in the passive*
427 *stimulation protocol:* If, in a given recording, sensory variable X correlates with sensory
428 variable Y, a neuron responsive purely to X will tend to appear tuned to Y. To investigate
429 whether such an effect might produce apparent sensitivity to whisker angle in the passive
430 stimulation paradigm, we simulated the response of curvature-tuned neurons to the whisker
431 curvature change time series measured during passive white noise stimulation. To minimise
432 free parameters, constrained GLMs (4 free parameters) were used, sensitive either to
433 instantaneous curvature ($\vec{k} = [\gamma]$) or to its first order derivative ($\vec{k} = \gamma [-1 1]$), where γ was a
434 signed, gain parameter. Parameters (\vec{h} , b , γ) were adjusted to produce two spike trains (one
435 for training, the other for testing) with a realistic white noise induced firing rate (~50
436 spikes/s; Bale et al., 2013). We then attempted to predict the simulated, curvature-evoked
437 (training) spike train by fitting GLMs (length 5 stimulus filter, 8 free parameters) using as
438 input either angle or curvature change. Cross-validated model accuracy was computed as the
439 PCC between the predicted spike train and the testing spike train (both smoothed by
440 convolution with a 5 ms box-car).

441 *Effect of single-trial approach on GLM prediction performance:* The objective of encoding
442 models, such as GLMs, is to obtain an accurate description of the mapping between a
443 stimulus and the neuronal spike trains it evokes. Since the random component of a neuron's
444 response is inherently unpredictable, the best any model can do is to predict the probability of
445 the spike train. To enable this, encoding models have generally (with few exceptions; Park et

446 al, 2014) been applied to a ‘repeated-trials’ paradigm, where a stimulus sequence (e.g., frozen
447 white noise) is repeated on multiple ‘trials’ (Arabzadeh et al., 2005; Lottem and Azouz, 2011;
448 Bale et al., 2013; Petersen et al., 2008; Pillow et al., 2008). Model accuracy can then be
449 quantified, largely free of contamination from random response variability, by comparing
450 (using PCC or otherwise) the trial-averaged response of the model to the trial-averaged
451 response of the neuron.

452 In contrast, in the present study of awake, actively whisking mice, the precise stimulus (time
453 series of whisker angle/curvature) was inevitably different on every pole presentation: there
454 were no precisely repeated trials to average over. Our standard model performance metric
455 (PCC) was computed by comparing the response on a single long, concatenated ‘trial’ to the
456 corresponding GLM predicted response. Such a PCC is downwards biased by random
457 response variability.

458 To gauge the approximate magnitude of this downward bias, we used a simulation approach.
459 By simulating the response of model neurons, we could deliver identical, repeated trials and,
460 thereby compare model prediction performance by a metric based on trial-averaging with that
461 based on the single-trial approach. To this end, for each recorded unit, we used the best-
462 fitting curvature change GLM to generate 100 trials of spike trains evoked by the curvature
463 time series measured for that unit. Data from the first of these trials was used to fit the
464 parameters of a minimal ‘refitted GLM’ (stimulus filter length 1, spike history filter length 2;
465 total 4 free parameters), and the single-trial performance quantified, using the approach of the
466 main text (Figure 2-figure supplement 1B, left). Next, we used the refitted GLM to generate
467 100 repeated trials of spike trains evoked by the curvature time series. Repeated-trials
468 performance was then quantified as the PCC between PSTHs obtained by trial-averaging
469 (Figure 2, -figure supplement 1B right).

470

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477

478 **AUTHOR CONTRIBUTIONS:**

479 DC and RSP designed the study. DC and AE performed the experiments. DC, MHE and RSP
480 analyzed the data. MRB, AE, DC and RSP developed the experimental methods. DC, MHE
481 and RSP wrote the manuscript, with input from all authors.

482

483 **CONFLICT OF INTERESTS:**

484 The authors declare no competing financial interests.

485

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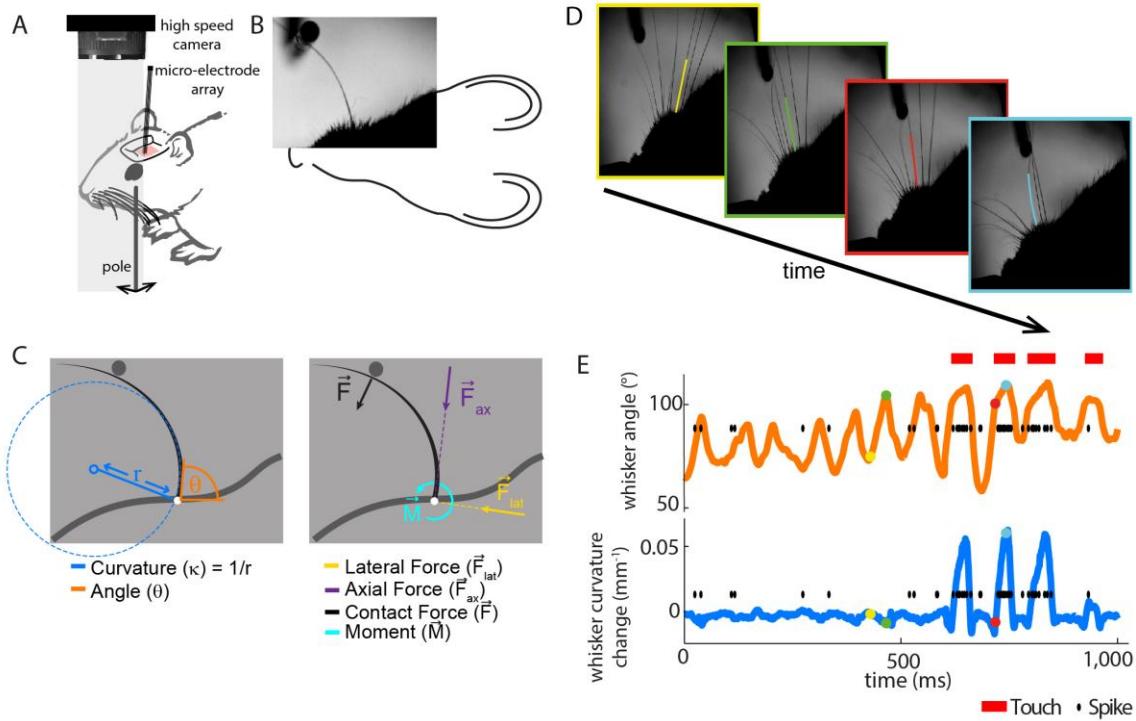
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630 **Figure 1. Electrophysiological recording from single primary whisker units in awake,**
631 **head-fixed mice and simultaneous measurement of whisker kinematics/mechanics.**

632 **A.** Schematic of the preparation, showing a tungsten microelectrode array implanted into the
633 trigeminal ganglion of a head-fixed mouse, whilst a metal pole is presented in one of a range
634 of locations (arrows). Before the start of each trial, the pole was moved to a randomly
635 selected, rostro-caudal location. During this time, the whiskers were out of range of the pole.
636 At the start of the trial, the pole was rapidly raised into the whisker field, leading to whisker-
637 pole touch. Whisker movement and whisker-pole interactions were filmed with a high-speed
638 camera.

639 **B and C.** Kinematic (whisker angle θ) and mechanical (whisker curvature κ , moment \vec{M} ,
640 axial force \vec{F}_{ax} and lateral force \vec{F}_{lat}) variables measured for the principal whisker in each
641 video frame.

642 **D.** Individual video frames during free whisking (yellow and green) and whisker-pole touch
643 (red and cyan) with tracker solutions for the target whisker (the principal whisker for the
644 recorded unit, panel **E**) superimposed (coloured curve segments).

645 **E.** Time series of whisker angle and curvature change, together with simultaneously recorded
646 spikes (black dots) and periods of whisker-pole contact (red bars). Coloured dots indicate
647 times of correspondingly coloured frames in **D**.

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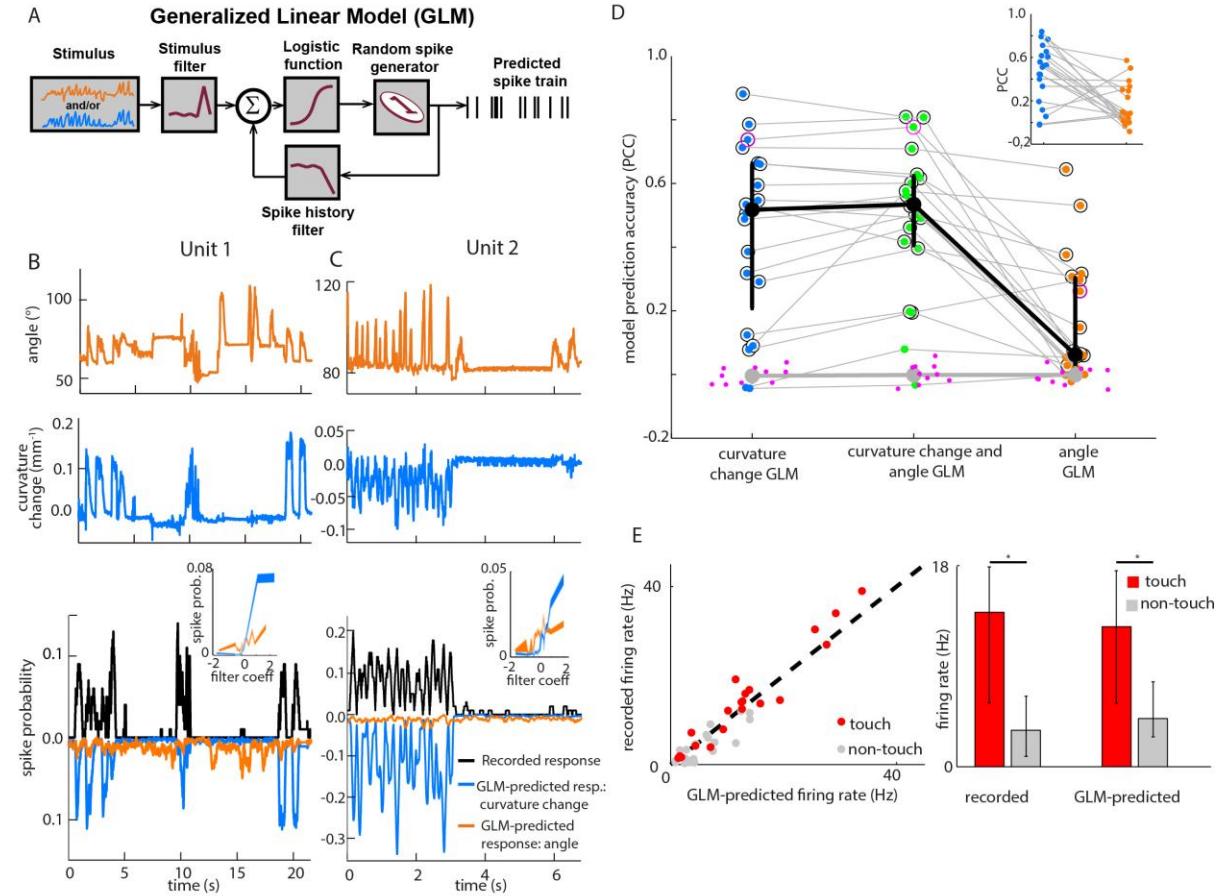
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657 **Figure 2. Primary whisker neurons encode whisker curvature, not whisker angle,**
658 **during active sensation.**

659 **A.** Schematic of the Generalized Linear Model (GLM).

660 **B.** For an example unit, whisker angle (top panel), whisker curvature change (middle panel)
661 and simultaneously recorded spike train (bottom panel, black), together with predicted spike
662 trains for the best-fitting angle GLM (bottom panel, orange) and curvature change GLM
663 (bottom panel, blue). Spike trains discretized using 1 ms bins and smoothed with a 100 ms
664 boxcar filter. Prediction performance (Pearson correlation coefficient, PCC) for this unit was
665 0.59. Inset shows tuning curves for both GLMs, computed by convolving the relevant sensory
666 time series (angle or curvature change) with the corresponding GLM stimulus filter to
667 produce a time series of filter coefficients, and estimating the spiking probability as a
668 function of filter coefficient (25 bins).

669 **C.** Analogous to panel **B** for a second example unit. Prediction performance PCC for this unit
670 was 0.74.

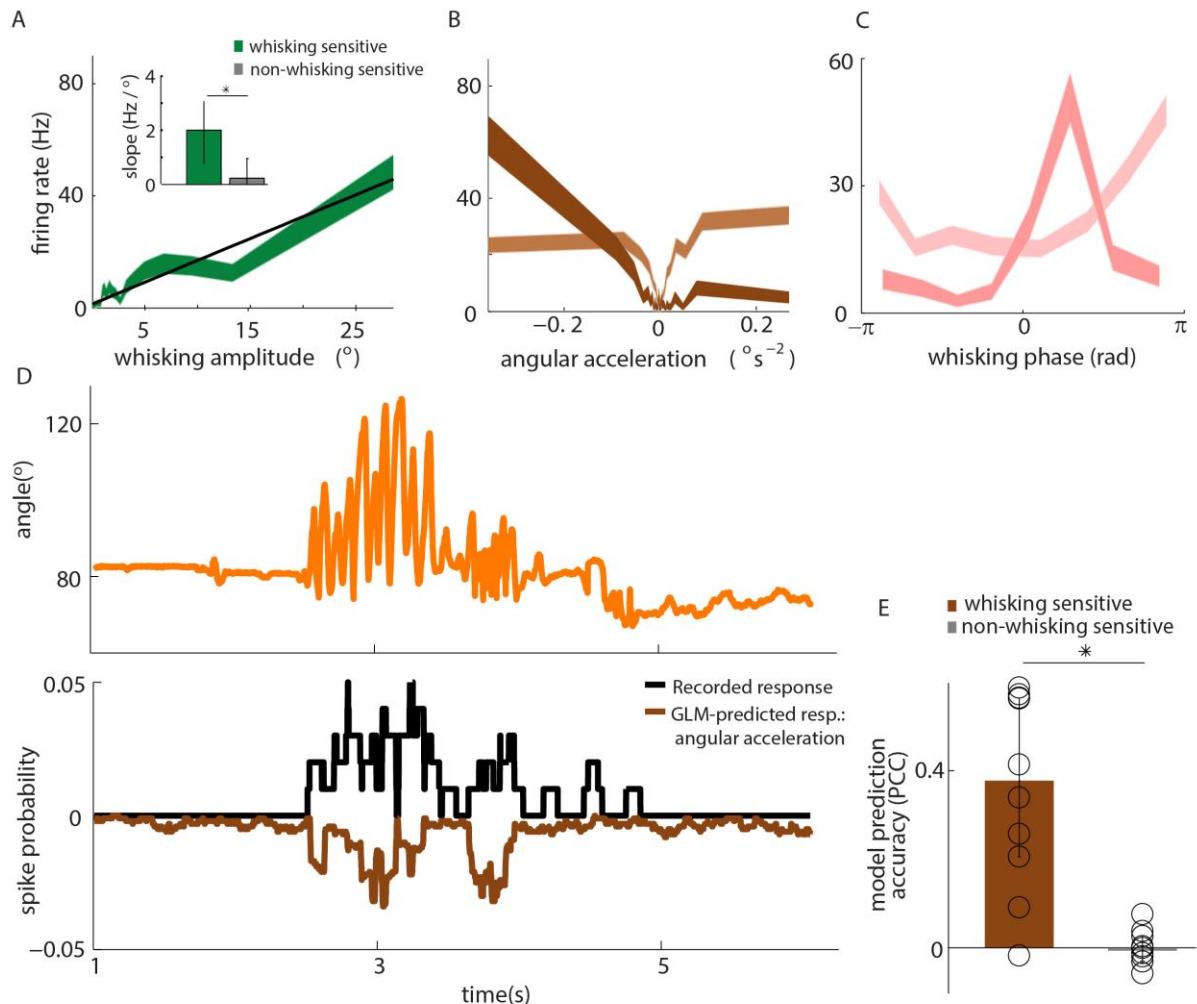
671 **D.** Prediction performance between predicted and recorded spike trains) compared for GLMs
672 fitted with three different types of input: curvature change alone; angle alone; both curvature
673 change and angle. Each blue/orange/green dot is the corresponding PCC for one unit: large
674 black dots indicate median; error bars denote inter-quartile range (IQR). To test statistical
675 significance of each unit's PCC, the GLM fitting procedure was repeated 10 times on spike
676 trains subjected each time to a random time shift: magenta dots show these chance PCCs for
677 the unit indicated by the magenta circle; the mean chance PCC was computed for each unit,
678 and the large gray dot shows the median across units. Black circles indicate units whose PCC
679 was significantly different to chance (signed-rank test, Bonferroni corrected, $p < 0.0025$). To
680 facilitate direct comparison between results for curvature change GLM and angle GLM, these
681 are re-plotted in the inset.

682 **E. Left.** Firing rate during touch episodes compared to that during non-touch episodes for
683 each unit, compared to corresponding predicted firing rates from each unit's curvature change
684 GLM. **Right.** Medians across units: error bars denote IQR; * denotes differences significant
685 at $p < 0.05$ (signed-rank test).

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690 **Figure 3. Primary whisker neurons encode whisker angular acceleration during free**
691 **whisking**

692 **A.** Mean response of an example whisking-sensitive unit to whisking amplitude, computed
693 during non-contact episodes (dark green, shaded area shows SEM) with regression line
694 (black). Inset shows regression line slopes (median and IQR) for whisking sensitive (green)
695 and non-whisking sensitive (grey) units. * indicates statistical significance rank-sum test
696 ($p=0.05$).

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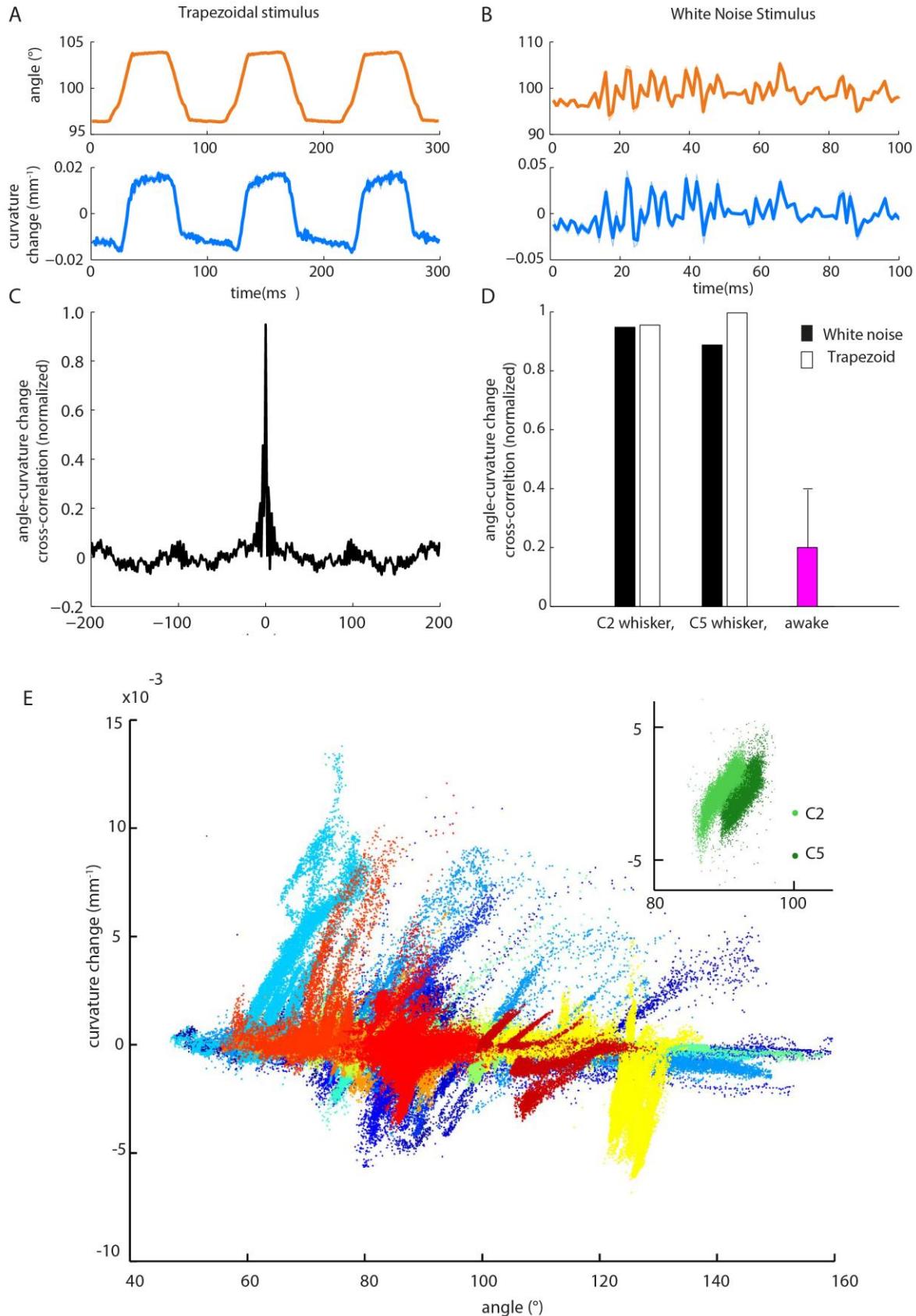
698 **B.** Mean response of two example units as a function of angular acceleration. The dark brown
699 unit is the same as that shown in A.

700 **C.** Mean response of two example units as a function of whisking phase. The dark pink unit
701 is the same as that reported in A; the light pink unit is the same as that shown as light brown
702 in B.

703 **D.** An excerpt of free whisking (**orange**) along with activity of an example, whisking-
704 sensitive unit (black) and activity predicted by a GLM driven by angular acceleration
705 (brown). The unit is the same as that shown in A.

706 **E.** GLM prediction accuracy (PCC) for all whisking sensitive (brown) and whisking
707 insensitive units (grey). Bars and vertical lines denote median and IQR respectively.

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719 **Figure 4. Whisker angle and whisker curvature change are highly correlated during**
720 **passive whisker deflection, but decoupled during active touch.**

721 **A.** Whisker angle (**top**) and whisker curvature change (**bottom**) time series, due to passive,
722 trapezoidal stimulation of C2 whisker in an anaesthetised mouse, estimated as mean over 10
723 repetitions. Note that errorbars (showing SEM) are present but very small.

724 **B.** Corresponding data for low-pass filtered white noise (hereafter abbreviated to ‘white
725 noise’) stimulation of the same whisker.

726 **C.** Cross-correlation between curvature change and angle during white noise stimulation, for
727 C2 whisker.

728 **D.** Cross-correlation between angle and curvature change at zero lag, for both passive
729 stimulation under anaesthesia and awake, active sensing (median of absolute cross-
730 correlation for each unit; error bar denotes IQR).

731 **E.** Joint distribution of whisker angle and whisker curvature change in awake, behaving mice
732 (1 ms sampling). Different colours denote data corresponding to different recorded units.
733 **Inset:** Analogous plot for passive, white noise whisker deflection in an anaesthetised mouse.
734 Different colours indicate data from different whiskers.

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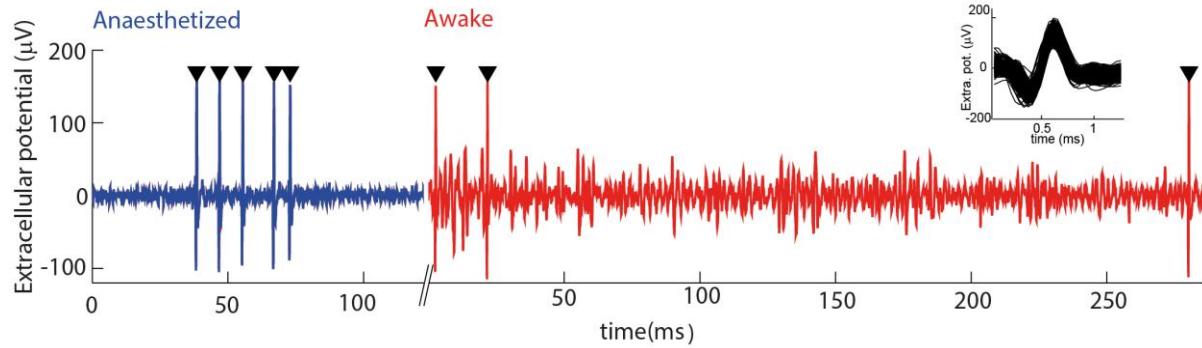
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740 **SUPPLEMENT**

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743 **Figure 1-figure supplement 1. Electrophysiological recording from trigeminal primary
744 neurons of awake, head-fixed mice.**

745 Extracellular potential recorded from the same single unit during both anaesthetized and
746 awake epochs. Spikes belonging to the cluster of the target unit are shown by black triangles.
747 Inset shows overlay of all waveforms belonging to this cluster.

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756 **Figure 1- figure supplement 2: Video of an awake mouse, exploring a pole with its
757 whiskers with simultaneous electrophysiological recording of a primary whisker neuron**

758 High-speed video of a head-fixed mouse (1000 frames/s) with sound of spikes fired by a
759 primary whisker unit. The pole is initially out of range of the whiskers. Whisker tracker
760 solution for the principal whisker of the recorded unit is overlaid in red.

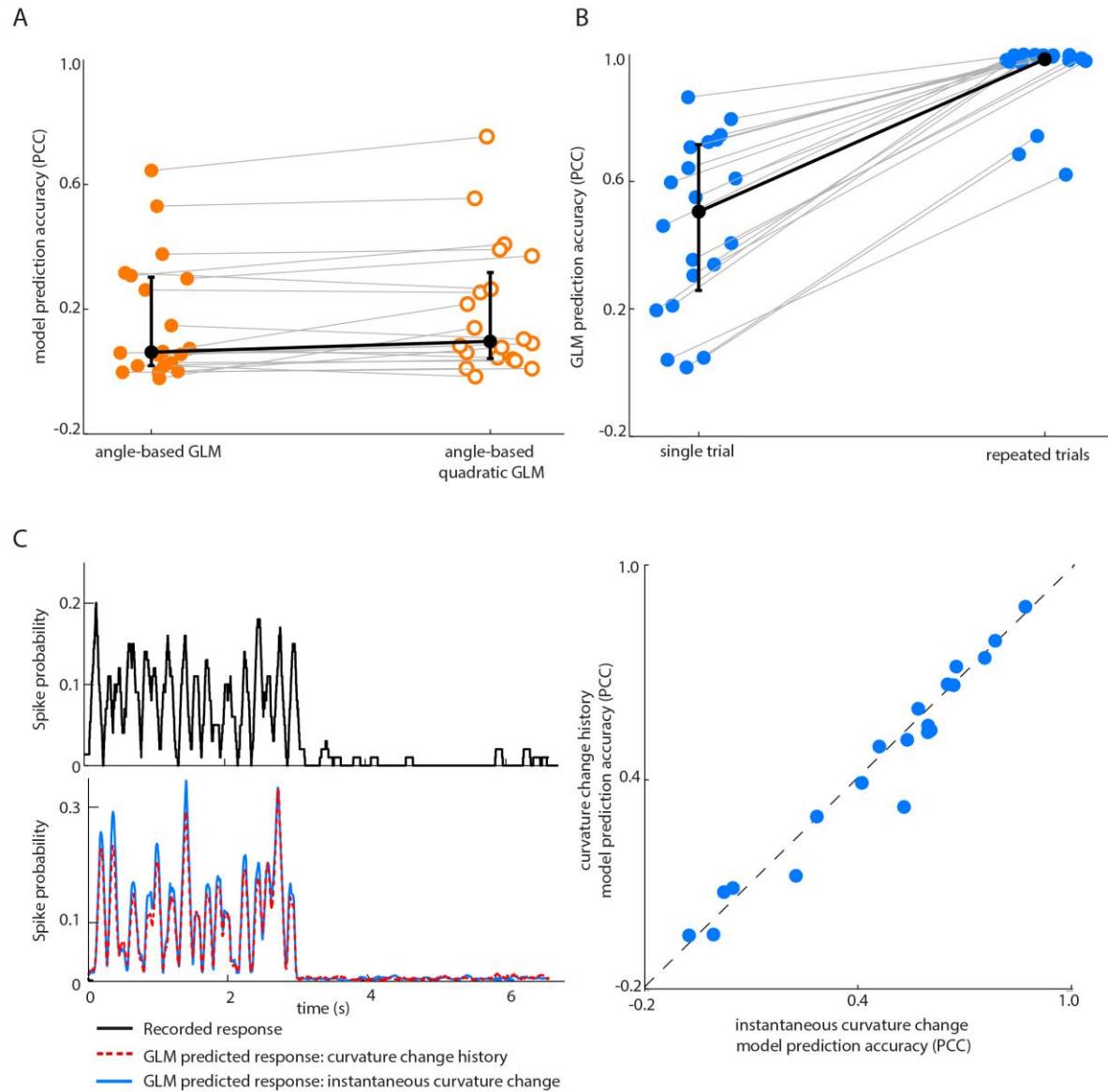
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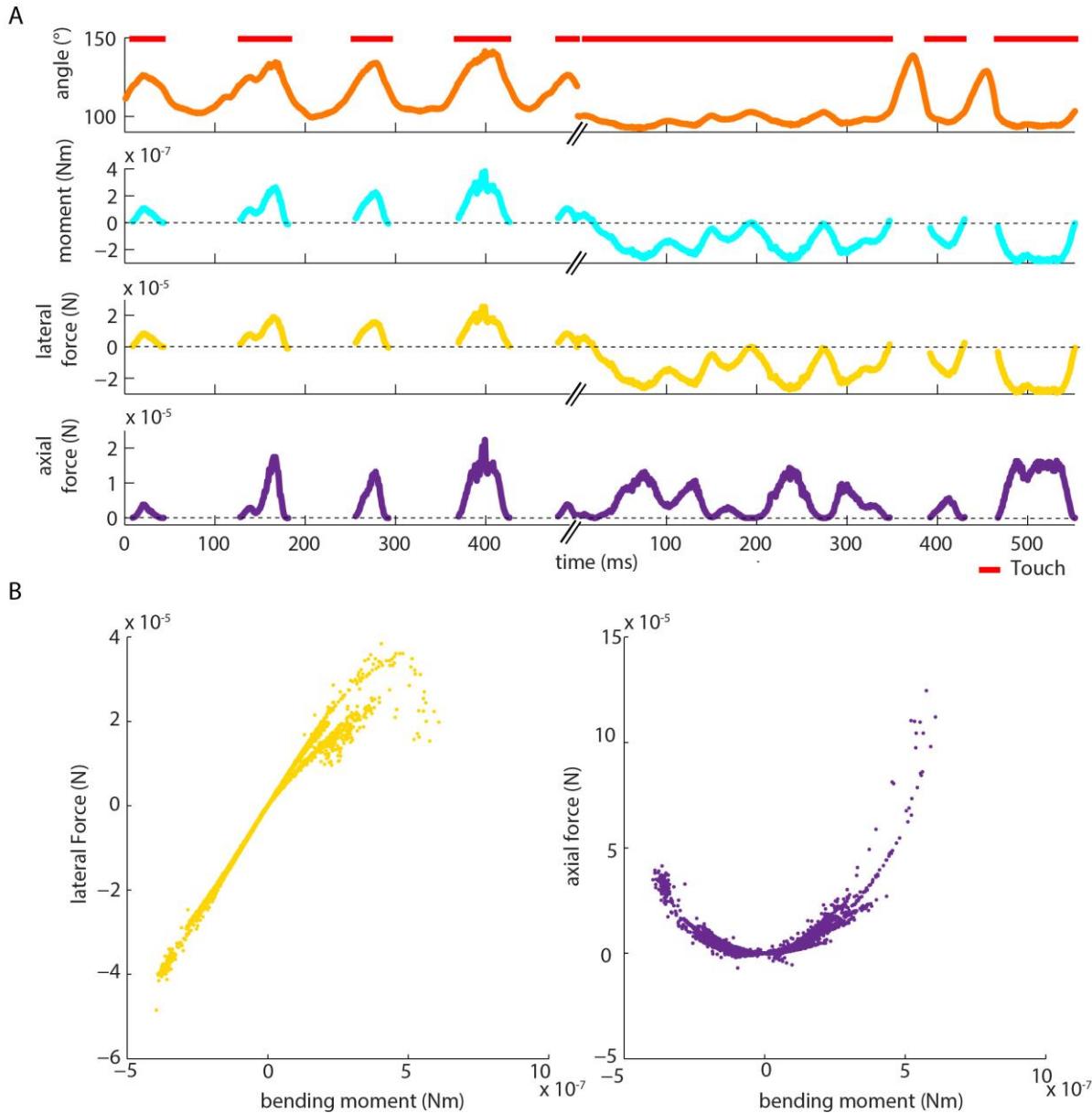
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768 **Figure 2- figure supplement 1. Effect on GLM performance of quadratic input terms,
769 simulated repeated trials and minimal stimulus filters**

770 **A.** Angle GLM prediction performance is robust to addition of quadratic stimulus-
771 dependence. Prediction accuracy (PCC) for standard angle GLM (same data as Figure 2C of
772 main text) in comparison to quadratic GLM (see Methods). Black dots denote medians, error
773 bars IQR.

774 **B.** Single-trial GLM prediction accuracy is limited by neuronal response variability.
775 Prediction accuracy (PCC) for simulated neurons. Each simulated neuron is the best-fitting
776 GLM, based on instantaneous curvature change, for its corresponding recorded unit (see
777 Methods). Prediction accuracy is quantified both using the single-trial approach of the main
778 text and using a repeated-trial method only possible by virtue of using a simulation. Black
779 dots denote medians, error bars IQR.

780 **C.** Prediction accuracy of curvature-based GLMs is accounted for by tuning to instantaneous
781 curvature change. A GLM performs a temporal filtering operation on its sensory stimulus
782 input and the sensory feature(s) which it encodes is determined by this ‘stimulus filter’. The
783 stimulus filters can, in principle, be complex, but we found that the ability of a GLM to
784 predict spikes (**lower left**) from curvature change was fully explained by the simple case
785 where the action of the stimulus filter is simply to multiply the sensory input by a gain factor
786 (median 0.55, IQR 0.26–0.66; $p=0.35$ signed-rank test). Recorded spike train (**upper left**) and
787 curvature-predicted spike trains (**lower left**) both for a ‘curvature history’ GLM with a length
788 5 stimulus filter identical to Figure 2D of main text and for an ‘instantaneous curvature’
789 GLM with a length 1 stimulus filter. Data for unit 2 of main text Figure 2C. Prediction
790 accuracy of the curvature history GLM compared to that of the instantaneous curvature GLM
791 for every recorded unit (**right**).

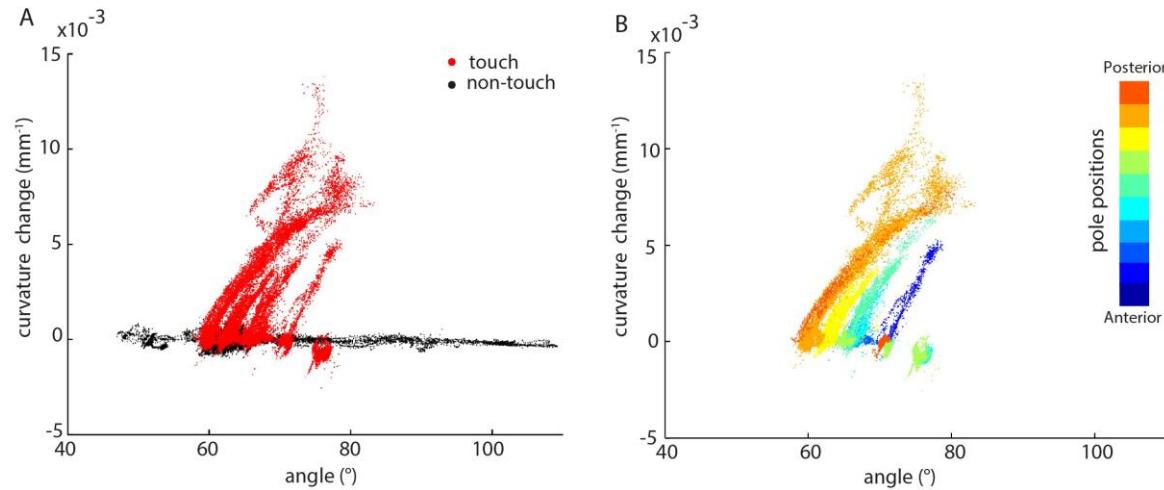


795 **A.** Two example time series for simultaneously measured whisker angle, bending moment,
796 lateral force and axial force (see Methods). Red bars indicate episodes of whisker-pole
797 contact.

798 **B.** Joint distribution of bending moment and lateral force (**left**), compared to that of bending
799 moment and axial force (**right**), for the same recording shown in **A**. Moment was highly

800 linearly correlated with lateral force (median absolute correlation coefficient across units
801 0.995, IQR 0.987-0.999, median R^2 of linear fit 0.99, IQR 0.97-1.0), and highly quadratically
802 correlated with axial force (median R^2 of quadratic fit 0.94, IQR 0.85-0.98). This indicates
803 that, during our conditions of pole exploration, axial force and lateral force are both
804 redundant with moment.

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807 **Figure 4-figure supplement 1. Natural statistics of active touch are rich: relationship**
808 **between whisker angle and whisker curvature change.**

809 **A.** Joint distribution of angle and curvature change for an example recording from an awake
810 behaving mouse, with samples registered during touch and non-touch distinguished by colour
811 (1 ms sampling).

812 **B.** Touch data of **A** classified according to pole position (dot colour).

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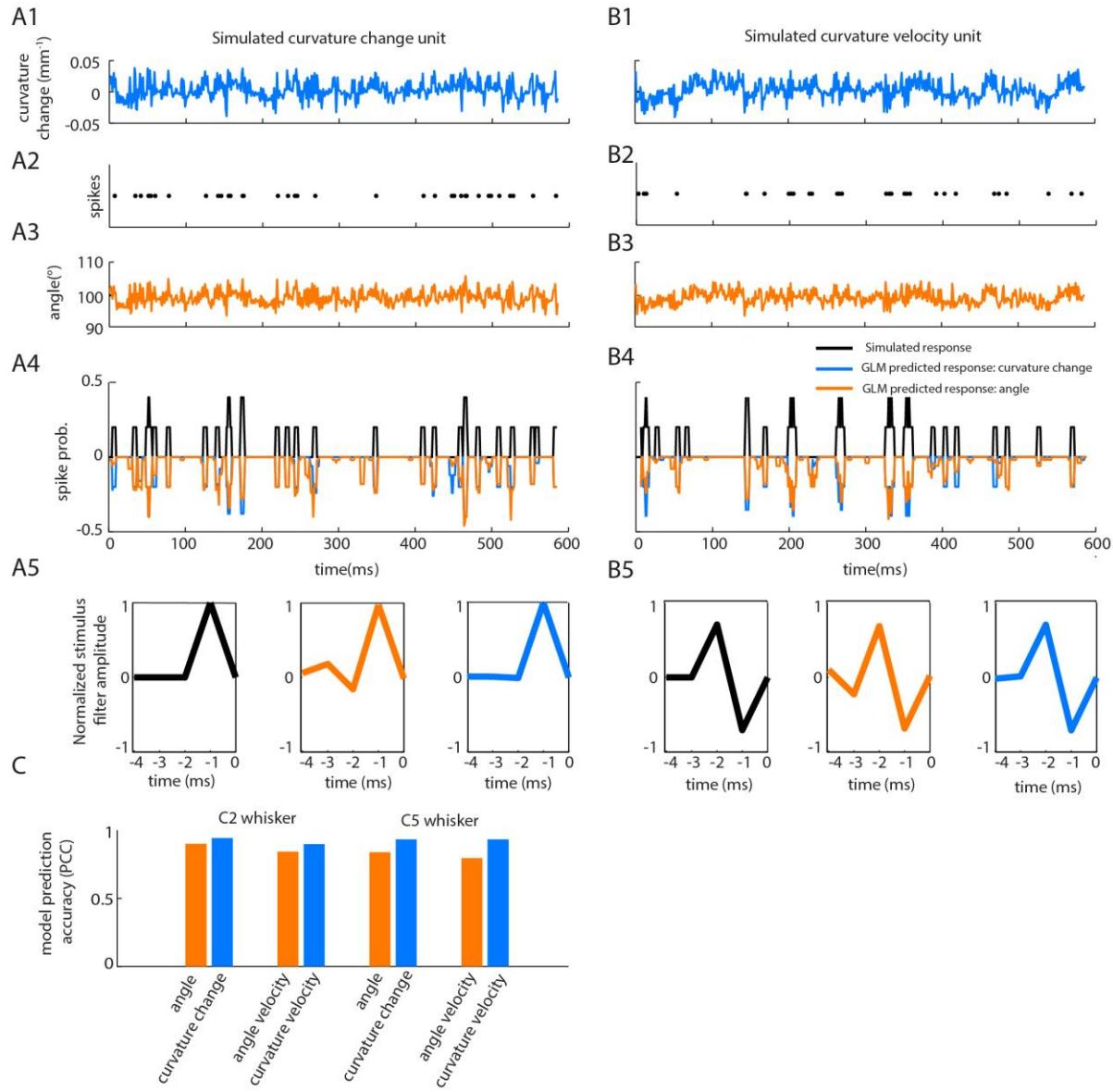
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826 **Figure 4-figure supplement 2: Correlations between angle and curvature change during**
827 **passive whisker stimulation can make curvature-tuned units appear angle-tuned.**

828 The data of Figure 4 show a strong correlation between whisker angle and whisker curvature
829 during passive stimulation of the whisker. To test whether this correlation might make
830 curvature-tuned units appear angle-tuned, we used a simulation approach. This allowed us to
831 generate responses from idealised neurons whose true tuning was known, by construction, to
832 be only to curvature. We simulated responses of such neurons to the curvature change time
833 series obtained from passive white noise stimulation (**A1-2**). We then trained a GLM to

834 predict these curvature-evoked spikes using only whisker angle as input (**A3-A4**). Despite
835 being fed the ‘wrong’ input, this GLM was able to predict the spikes accurately (for C2
836 whisker, angle PCC was 0.90, curvature change PCC 0.94; results similar for C5; **C**). This
837 result was robust to different choices of feature tuning (**B-C**).

838 **A1.** Whisker curvature change caused by the white noise stimulus applied to C2 whisker of
839 an anaesthetized mouse (same data as main text Figure 3, repeated for clarity).

840 **A2.** Spike train evoked by a simulated curvature-tuned neuron in response to the stimulus in
841 A1 (a GLM with the position filter shown in left panel of A5).

842 **A3.** Whisker angle time series corresponding to panel A1.

843 **A4.** Target response (black) compared to predicted response from best-fitting GLMs using
844 either angle (orange) or curvature change (blue) as input.

845 **A5. Left.** Stimulus filter used to generate the spike train of panel A2. **Middle-Right.** Best-
846 fitting stimulus filters (normalised to unit length) for GLMs trained on the spikes of panel A2
847 and the angle time series of panel A3 or the curvature change time series of panel A1
848 respectively.

849 **B1-5.** Results analogous to A1-5 for a simulated neuron tuned to curvature velocity.

850 **C.** Quantification of the GLM predictions shown in panels A4-B4.

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