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Supplementary Information for:

Feedback Control in Active Sensing: Rat Exploratory Whisking is Modulated by Environmental Contact

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Summary

Sensing in animals is often an active process in which the position and orientation of the sensory apparatus is controlled so as to enhance the organism's capacity to obtain behaviourally-relevant information. The rat whisker system is considered to be a paradigmatic example of such an "active sense system", although our understanding of the strategies that guide whisker movements during natural behaviour is limited, in part due to the difficulty of accurately observing whisker positions in freely moving animals. We used digital video tracking and wireless recording of mystacial electromyogram signals to assess the effects of whisker-object contact on whisking in untrained animals exploring simple environments. Our results show that contact leads to reduced protraction (forward whisker motion) on the side of the animal ipsilateral to an obstruction and increased protraction on the contralateral side. Reduced ipsilateral protraction occurs rapidly and in the same whisk cycle as the initial contact. We conclude that whisker movements are actively controlled so as to increase the likelihood of environmental contacts whilst constraining such interactions to involve a gentle touch. That whisking pattern generation is under strong feedback control has important implications for understanding the nature of the signals reaching upstream neural processes.

In this supplement we present additional details concerning the methods used and results obtained. Four short video clips are also provided to illustrate our data and analysis techniques and are described here. The order of presentation in this supplement is i) detailed methods, (ii) additional results, (iii) commentary on the video material.

I. DETAILED METHODS

Experiment 1: High speed videography of contact-induced whisking modulation

Animals. Twenty-two male Royal College of Surgeons (RCS) dystrophic rats aged 4-14 months and weighing between 250 and 350g were used in this study. These animals display normal whisker function but also a genetically-induced retinal degeneration (dystrophy) such that they had minimal visual capacity at the time of testing (Hetherington et al., 2000). Animals were kept in a 12-h dark/light cycle environment at a temperature of 22°C with food and water *ad libitum*, and were tested during the active (dark) period of their daily cycle. Animals were habituated to handling by the experimenters prior to testing.

Apparatus and recording procedure. Multiple recordings of behaving animals were obtained using a Photron Fastcam PCI high speed digital video camera at 250 frames per second (fps), 1ms shutter, backlit by high-frequency fluorescents. The animal was between the lights and the camera in a glass and plastic box, approximately 450×400×100mm internally, and otherwise unrestrained. To prompt unilateral object contacts, for some recordings a rectangular wooden box or Perspex block was placed in the arena with the camera aligned with two of its vertical surfaces. On other occasions, a front-silvered mirror, placed behind a glass wall, and angled obliquely relative to the camera, was used to obtain a view of the animal in the vertical plane in addition to the overhead view. 4 second clips were recorded opportunistically at 1024x1024 resolution whenever the rat entered the camera's field of view which covered an area of the arena approximately 15cm square.

Review and tracking. Each of the clips obtained with the rectangular box or block was reviewed to determine whether it met the following criteria: (i) During protraction, one of the rat's forward whiskers (column 3 or higher) on one side appeared to make an unambiguous contact with a vertical surface that was aligned to the camera's field of view. (ii) The whiskers on both side of the snout were visible throughout the whisking cycle during which this contact occurred. In each of the matching clips three judges independently identified the frame in which the contacting whisker was first visibly deflected. Additionally, for an interval safely bracketing the deflection, they each tracked, using a custom-made tracking tool, the position of a central point on one rearward (column 1 or 2) whisker on each side of the snout, along with that of the tip of the snout and of the mid-point of the head. Tracked whisker angles, calculated using the estimated midline of the head as a reference, were boxcar-smoothed over a three sample window before further processing.

Data analysis. For each clip, estimates of bilateral protraction onset and cessation times, relative to the time of the initial deflection, were calculated by a semi-automatic process. Specifically, the time of the deflection event (D) was calculated first as the average of the estimates obtained (to the nearest frame)

from the three judges. The *ipsilateral protraction of interest* (IPI) was then defined as the protraction bracketing D on the side of the snout where the deflection occurred, and the *contralateral protraction of interest* (CPI) as the protraction on the undeflected side nearest in time to the IPI (note that bilateral protractions are not always perfectly in phase). Within each identified IPI and CPI the time of protraction *cessation* was calculated next as the frame in which a maximum occurred in the tracked protraction angle. Note that, in each case, the tracked whiskers did not touch the contacted surface at any time so cessation of protraction was not due to physical obstruction of their movement. Finally, in the majority of cases the time of protraction *onset* was calculated as the previous minimum of protraction angle. In a small number of cases, however, a brief reversal in protraction occurred during the protraction of interest on one side (this phenomenon has previously been described by Carvell and Simons, 1990). In each of these latter cases, the next earlier minimum protraction angle was used to estimate protraction onset, as the observed behavior was considered to be best described as a single, momentarily interrupted, *tri-phasic* whisk than as two individual whisks.

Statistical analysis. Protraction onset and cessation times were compared bilaterally using the Wilcoxon signed ranks with an alpha value of 0.05. Reported p-values are two-tailed.

Experiment 2: Wireless EMG monitoring of whisking behavior

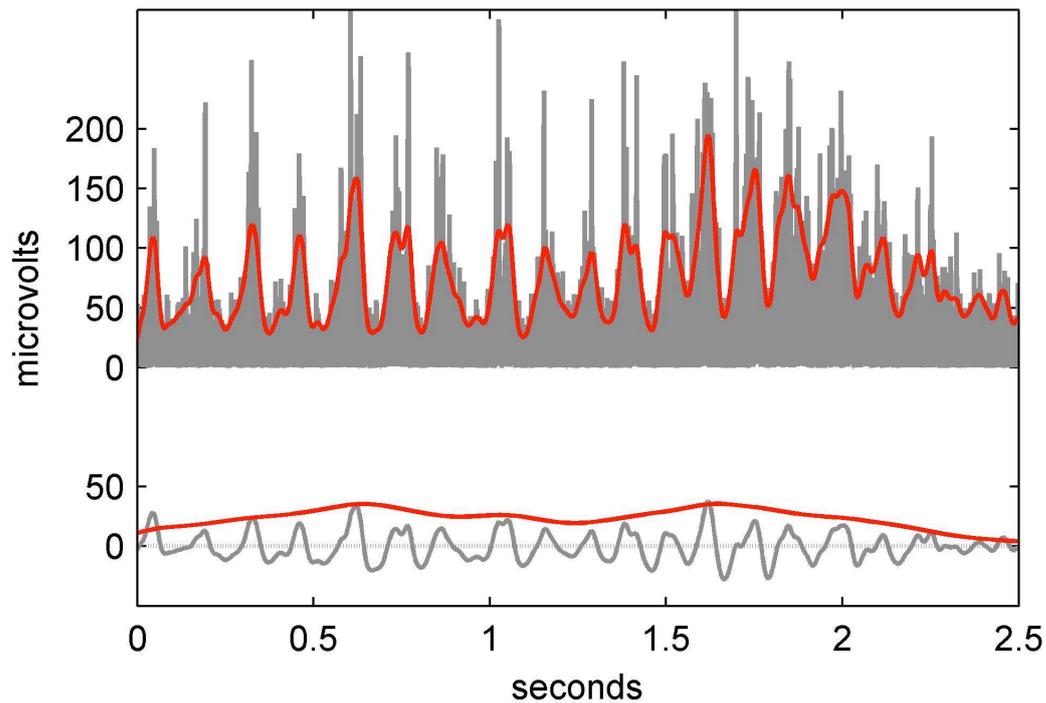
Animals and electrode implantation. Three of the dystrophic RCS rats used in Experiment 1 were implanted bilaterally for recording of whisking EMG as follows. Animals were anaesthetized with an intraperitoneal injection of ketamine and xylazine (1.1ml/kg). Under anesthesia, a pair of EMG recording electrodes (Teflon coated tungsten wire, diameter 0.05mm, Advent Research Materials Ltd., Oxford, UK) was then chronically implanted into the intrinsic muscles of each mystacial pad. The preparation and implantation of the recording electrodes was very similar to the procedure described by (Berg and Kleinfeld, 2003a). The wires were fed subcutaneously to a connector unit that was attached to the skull as follows. Animals were placed in a stereotaxic frame (Kopf Instruments) and the skull exposed by a midline incision of the scalp. Stainless steel screws were inserted into burr holes made in the skull using a dental drill. The burr holes and screws (two ~4mm rostral to bregma, one ~3mm caudal to lambda) were carefully placed so as to avoid damaging the underlying brain tissue. The connector unit was positioned on the top of the skull and set into a layer of dental acrylic, which was applied so as to engulf the skull screws. The midline incision was closed around the implanted connector. Animals were treated with an analgesic (Rimadyl, 0.05ml s.c.) and allowed to recover for a minimum of 3 days prior to any experimentation.

Apparatus. A domestic Sony DV camera for normal speed video recording was ceiling-mounted and positioned such that its field of view encompassed a 400×380×80mm rectangular, experimental arena. The lid and floor of this arena were made from *Datastop*TM radio frequency shielded glass (Tempest Security Systems) which has a transparent conductive coating allowing the surfaces to function as a radio

antenna. A light-weight (~5 grams), battery-powered, purpose-built, stereo telemetry transmitter module was attached to the head connector unit on the animal's head at the start of each recording session. The two channels were transmitted via two CFM-8 FM telemetry transmitters from *Biotelemetry* (biotelemetry.com) that were electrically independent of each other (other than through the animal tissue). Receivers were two ICOM PCR-1000 (icomamerica.com). Overall, the telemetry system was found to have a signal-to-noise ratio of at least 40dB and a flat response from 200Hz to 3kHz.

Procedure. Each animal was allowed to roam freely in the experimental arena on four or five separate days, until it lost interest in exploring. During each session bilateral mystacial EMG was continuously recorded along with normal speed digital video. Thirteen recording sessions were performed in total (5/4/4 using each of the three animals), each session lasting between 5 and 30 minutes, being terminated when the animal settled into a prolonged period of inactivity. In addition, to verify correct EMG implantation, several short recordings were made with each animal of simultaneous high speed digital video and bilateral EMG using a modified (*Datastop* glass lid and floor) version of the arena used in Experiment 1.

EMG Processing. Raw EMG signals were processed using standard techniques to recover both fast-varying and slow-varying proxy measures of rat whisking behavior. The received signals were first digitized at 10kHz then high-pass filtered at 200Hz to remove mains hum and major harmonics, to give the "motor unit signal". The medium-term (several whisks) average of this signal is the medium-term (several whisks) average muscle force, which we refer to as the 'set-point'. The instantaneous "whisker protraction drive signal" was then extracted by rectifying and low-pass filtering this signal at 20Hz, to leave only features that represent whisking (5-15Hz) and variations in muscle tone, or set-point (down to 0Hz). This fast-varying signal was used for validation of the EMG signal as a proxy for whisker angle with parameters (lag, gain and offset) selected to minimize the mean square error on the estimate (lag was 20ms for both channels). To obtain a slow-varying measure of the instantaneous amplitude of whisking (i.e. discarding phase and set-point information), we performed the following steps: First, we rectified and band-pass filtered the motor unit signal at 2-20Hz, to leave only features representing whisking and not set-point changes (note that, without this step, small amplitude rostral whisking would generate a similar result to large amplitude caudal whisking). Second, we rectified this new signal and low-pass filtered at 2Hz to leave only features representing variations in amplitude of whisking over time (the 2Hz threshold was chosen as this is approximately half the lowest reported rate of natural exploratory whisking). Third, to correct for variability in the implants across animals, we took the log value and normalized this independently on each channel of each animal (but across sessions) such that the extreme deciles of each set lay on zero and unity. The resulting slow-varying signal is referred to below as the *whisking amplitude*. All filters were 2nd order Butterworth; those with non-negligible delay (i.e. all except the mains hum filter) were applied once in each direction to maintain temporal correspondence. Supplementary Figure 1 illustrates the signal extraction process for a 2.5 second sample of unilateral EMG.



Supplementary Figure 1: Illustration of EMG processing. Upper plot shows rectified motor unit signal (grey) with the fast-varying whisker protraction drive signal overlaid (black). Lower plot shows whisker protraction drive signal with set-point removed (grey, i.e. rectified motor unit signal filtered between 2-20Hz), with the slow-varying whisking amplitude signal overlaid (black). Both black overlays are magnitude-scaled for visual clarity.

Video tracking. To facilitate video tracking the radio transmitter carried two brightly colored tags that were easily discriminated against the otherwise low color-saturated environment. This allowed the location and bearing of the head to be tracked automatically using custom software. The location of the tip of the snout was found by searching forward from the head tags. Manual frame-by-frame review of substantial sections of the tracked video confirmed the accuracy of the automatic tracking. Additionally, head pitch could be coarsely obtained from the separation of the tags, though without polarity, allowing periods of extreme head pitch to be recognized. Walls were manually located in the footage from each recording session allowing their distance from and orientation to the rat to be calculated for each frame.

Pairing EMG and video recordings. Video and EMG recordings were synchronized electronically. Data from each session were then reviewed so as to eliminate periods of experimental noise, ambiguous tracking, irrelevant behavior (e.g. grooming), and extreme head pitch. The remaining video frames were culled by two thirds to give a frame rate of 8.33fps which is towards the middle of the observed frequency range for rat exploratory whisking (5-15Hz, Berg and Kleinfeld, 2003b). The instantaneous whisking amplitude values coincident with each frame were then calculated as the proxy whisking protraction measures for that sample. Finally, to eliminate frames where the animal was whisking either weakly or

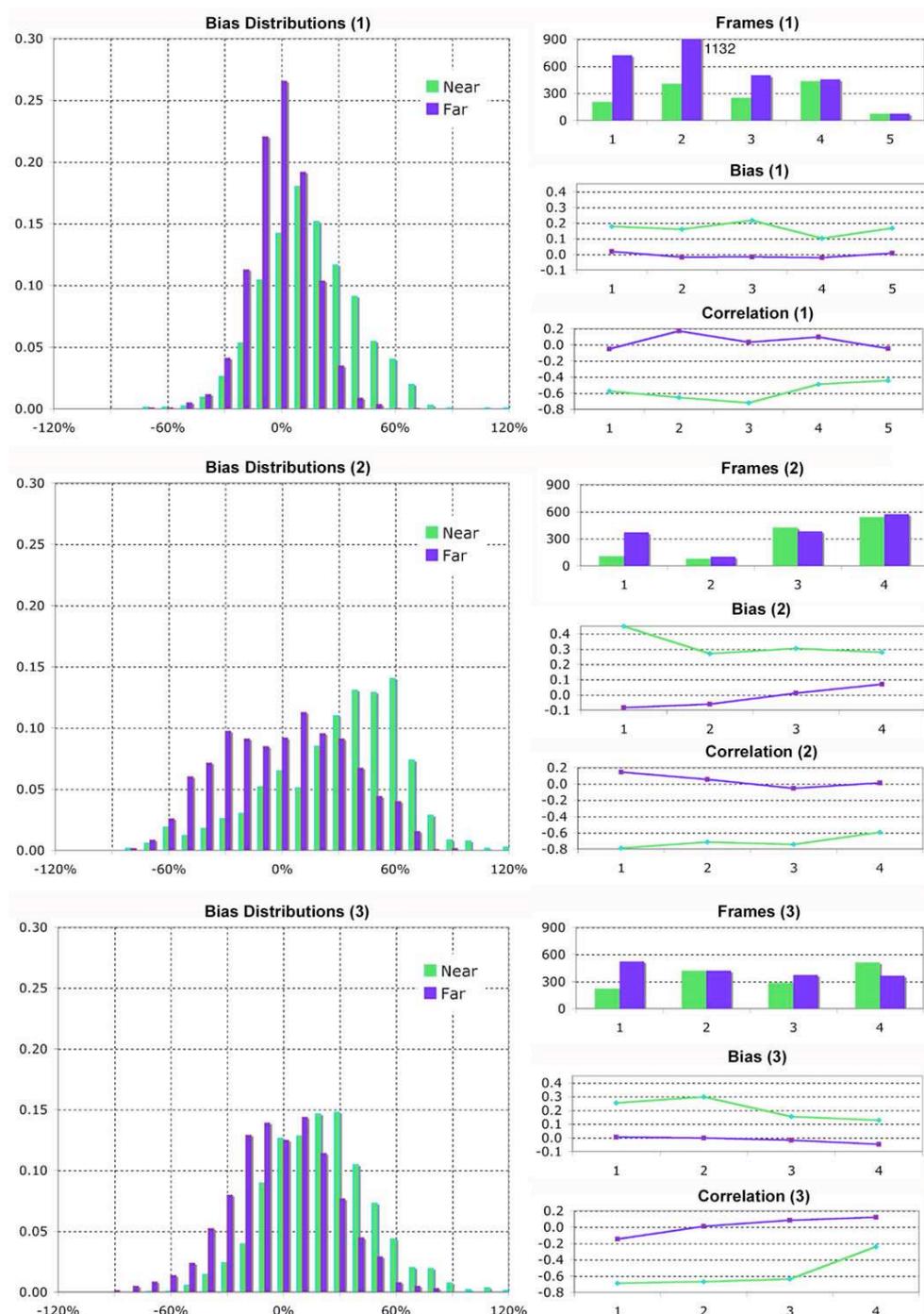
not at all (which would effectively be EMG noise frames), we removed all frames in which whisking was not robustly detected (mean whisking amplitude across channels greater than 0.7).

Statistical analyses. Since whisking amplitude data is strongly auto-correlated within sessions we could not directly test the significance of correlations, or of differences between means, for dependent measures based on the whisking EMG recordings. Instead we performed repeated measures ANOVA using values of these measures computed on a per-session basis using the first four session values for each rat (see Results and Supplementary Data for details). It is worth noting that this represents a very conservative estimate of the within-subjects degrees of freedom in the whisker amplitude data (one per session). When multiple ANOVA analyses are performed (here two), a corrected alpha is appropriate to reduce the risk of Type I error, however, the standard Bonferroni correction is known to be too conservative for studies with correlated outcomes. Therefore a partial Bonferroni-corrected alpha of 0.048 (corrected from $\alpha=0.05$ for a correlation between two variables of 0.92) was used in the reported statistical tests.

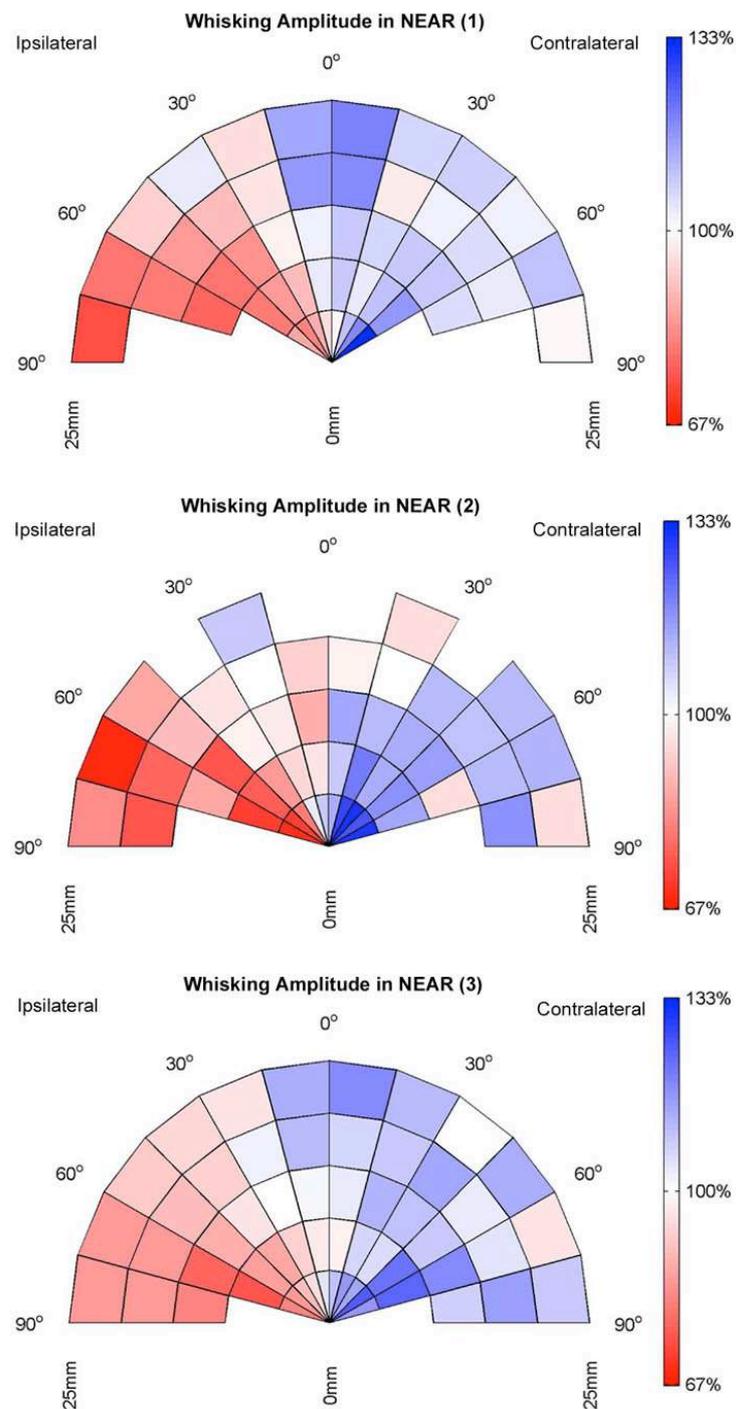
II. ADDITIONAL RESULTS

Results for each of three implanted rats were comparable. Supplementary Figure 2 shows results for each animal separately, with a further breakdown of mean values by session. In each animal, the distribution of whisking bias values (contralateral – ipsilateral amplitude) (left column) showed a marked tendency, in the NEAR sub-set only, towards increased whisking on the contralateral side that was observed in all sessions. Mean bias per animal was 15.5% (no. frames= 1371), 30.3% (1154), and 20.1% (1440) of average whisk strength; per-session values are given in the right column of the figure (centre panels). The negative correlation, in NEAR only, between the bearing to the nearby wall and the difference between left and right whisking amplitudes, was also a robust finding across individual rats ($r=-0.61$, -0.64 , and -0.65) and across sessions (see Figure right lower panels).

Finally, decomposition of the whisking amplitude data, in NEAR, in relation to both bearing and distance to the nearby wall is shown in Supplementary Figure 3. These plots confirm that the pattern of reduced ipsilateral whisking and increased contralateral whisking, when close to a wall, was characteristic of all three animals. Increased bilateral whisking when the wall is directly in front (bearing $\leq 15^\circ$) and at a distance of 15–25mm, was seen in two animals; in the third animal there was insufficient data in the relevant cells to confirm this pattern.



Supplementary Figure 2: Whisking asymmetry in individual rats. Left column shows NEAR and FAR distributions of whisking bias for each animal (see Figure 4b for a combined plot). Right column shows statistics for each rat on a per-session and per sub-set basis. From the top down, for each animal, these are: the number of frames, the whisking bias (contralateral – ipsilateral amplitude), and the correlation between wall bearing and whisking amplitude difference (left – right). See text for cross-session values.



Supplementary Figure 3: Ipsilateral and contralateral whisking amplitude near to walls.

Here we present, for each animal, polar plots of ipsilateral (shown left of the midline) and contralateral (shown right of midline) mean whisking amplitude as a percentage of average whisk strength, binned for NEAR frames only, according to distance and bearing to the nearby wall (see Figure 4c for a combined plot). Cell counts as (total, median per cell, range over cells) are, from the top to the bottom, (1349, 48, 10-276), (1134, 29, 6-145), (1433, 41, 11-169).

III. SUPPLEMENTARY VIDEO MATERIAL

All videos are presented with all originally recorded frames present (250 per recorded second for the high-speed video, 25 per recorded second for the normal speed), regardless of playback speed.

Video 1: High-speed tracking. Here we present an example of bilateral tracking of whisker movement during a sequence in which a rat makes contact with a vertical surface, on a forward whisker, whilst proceeding across a featureless floor. Replay is at 1/50th normal speed. Frames from this sequence were also shown in Figure 1. Tracked points are indicated by filled squares. The approximate point of contact is indicated by an open circle, which turns solid in the first frame where deflection was judged to have occurred. Note that the video quality presented here is substantially poorer (due to compression and reduced resolution) than the 1024x1024 uncompressed digital video used in tracking.

Video 2: EMG validation. This video is one of the high-speed clips (250fps) used to verify the relationship between recorded EMG and whisker angle and is presented here with additional information overlaid. Replay is at 1/10th normal speed. The clip includes the frame sequence presented in Figure 3. The tracked points are marked in the video by filled squares whilst the instantaneous bilateral values of the EMG whisking envelope are represented by the height of the bars in the bottom left. Each channel of the soundtrack consists of a sine wave with its frequency modulated by the instantaneous value of the whisking protraction drive signal (high frequency corresponding to high EMG activity). The leftmost EMG indicator bar and the left audio channel represent the whisking signal on the left side of the snout. Neither visual nor audio indicators are lagged (in contrast to the presentation of this data in Figure 3), so the EMG indicators noticeably lead the response of the whiskers.

Video 3: Normal-speed video tracking. Here we present a short clip of the normal-speed video (25fps) to illustrate the automated tracking of the two tags on the animal head along with tracking of the nose. The stereo soundtrack contains the synchronised raw EMG signals.

Video 4: Pronounced asymmetry near a wall. Here we present a short clip of high speed video (250 fps) to illustrate the strong asymmetry we observe when the animal approaches a wall at an acute angle. Replay is at 1/10th normal speed. The upper half of each video frame shows the image of the animal in the vertical plane obtained using a front-silvered mirror, positioned behind a glass wall, and slanted at an oblique angle with respect to the camera. The lower half of each frame shows the view in horizontal plane, with the viewpoint of the camera aligned with the vertical surface of the wall. (Note that the dark strip across the centre of the frame therefore corresponds to the floor of the arena for the upper half of the frame, and the wall for the lower half.) Protraction is strongly asymmetric in a manner that appears to reduce bending of the whiskers on the side of the snout closest to the wall, while increasing the likelihood of surface contact by whiskers on the opposite side. A snap-shot from the video clip was presented as Figure 5 in the main article.

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