

Please cite the Published Version

Grant, RA, Breakell, V and Prescott, TJ (2018) Whisker touch sensing guides locomotion in small, quadrupedal mammals. *Proceedings of the Royal Society B: Biological Sciences*, 285 (1880). ISSN 0962-8452

DOI: <https://doi.org/10.1098/rspb.2018.0592>

Publisher: The Royal Society

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1 **Whisker touch sensing guides locomotion in small, quadrupedal mammals**

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10

11 **Abstract**

12 All small mammals have prominent facial whiskers that they employ as tactile sensors to
13 guide navigation and foraging in complex habitats. Nocturnal, arboreal mammals tend to
14 have the longest and most densely-packed whiskers, and semi-aquatic mammals have the
15 most sensitive. Here we present evidence to indicate that many small mammals use their
16 whiskers to tactually guide safe foot positioning. Specifically, in eleven, small, non-flying
17 mammal species we demonstrate that forepaw placement always falls within the ground
18 contact zone of the whisker field, and that forepaw width is always smaller than whisker
19 span. We also demonstrate commonalities of whisker scanning movements (whisking) and
20 elements of active control, associated with increasing contact with objects of interest, across
21 multiple small mammal species that have previously only been shown in common laboratory
22 animals. Overall, we propose that guiding locomotion, alongside environment exploration, is
23 a common function of whisker touch sensing in small, quadrupedal mammals.

24

25 **Keywords:** whisking, forepaw, rodents, arboreal, nocturnal, semi-aquatic

26 **Background**

27 All mammals have facial whiskers, with the exception of great apes and humans. Whiskers
28 are sensitive tactile hairs that guide behaviours, such as navigation, locomotion, exploration,
29 hunting and social touch (Prescott et al. 2011; Grant & Arkley 2016). The overall layout of
30 the whiskers and their specialist facial musculature is conserved from marsupials (Grant et al.
31 2013), to rodents (Haidarliu et al. 2010; 2017) to nocturnal primates (Muchlinski et al. 2008).
32 Small, social, arboreal and nocturnal mammals tend to have the longest and densest whiskers
33 (Muchlinski et al. 2010) and aquatic mammals the most sensitive whiskers (Dehnhardt et al.
34 1999). Therefore, mammals that forage and navigate in dark, complex habitats are likely to
35 use their whiskers more, and are also often able to actively position and move them
36 (Muchlinski et al. 2013; 2018). Indeed, Brown rats (*Rattus norvegicus*), Golden hamsters
37 (*Mesocricetus auratus*), House mice (*Mus musculus*) and many other small mammals actively
38 move their whiskers in a bilateral, cyclic motion, called whisking, which is one of the fastest
39 movements that mammals can make, occurring at speeds of up to 25 Hz in mice (Mitchinson
40 et al. 2011).

41

42 Whisker positioning and movement has strong associations with locomotion. While adult rats
43 will whisk bilaterally and symmetrically during forward locomotion, at higher speeds (> 150
44 cm/s) they will reduce whisker amplitudes and position their whiskers more forward, in order
45 to focus the whiskers in front of their snout, in a behaviour termed 'look ahead' (Arkley et al.
46 2014). The same strategy can be seen when Hazel dormice (*Muscardinus avellanarius*) and
47 House mice (*Mus musculus*) make large jumps and stretch across gaps (Jenkinson and
48 Glickstein 2000; Arkley et al. 2017), where the whiskers are focussed forward to act as
49 collision detectors and protect the delicate area in front of the snout (Arkley et al. 2014).

50 Stretching the whiskers out in front of the face also reduces the time to collision (Arkley et al.
51 2014), which increases the time in which to prepare for a safe landing following a jump
52 (Arkley et al. 2017). During climbing or walking on a flat floor, whiskers are often thought to
53 scan ahead and guide safe foot positioning (Arkley et al. 2014; 2017). This has been observed
54 in Brown rats (*Rattus norvegicus*, Arkley et al. 2014), Hazel dormice (*Muscardinus*
55 *avellanarius*, Arkley et al. 2017) Long eared jerboas (*Euchoreutes naso*) and Northern three-
56 toed jerboas (*Dipus sagitta*) (Sokolov and Kulikov 1987). Indeed, Sokolov and Kulikov
57 (1987), found that nocturnal, terrestrial Jerboas used their whisker tips to scan along the floor
58 directly where their paws fell, suggesting that the whiskers provided information about where
59 the animal would subsequently place its feet. However, these observations have yet to be
60 fully quantified.

61

62 The degree to which the whiskers are moved and controlled varies greatly from species to
63 species. Brown rats (*Rattus norvegicus*) and House mice (*Mus musculus*) whisk, and can
64 control their whiskers in robust and repeatable ways during locomotion and object
65 exploration, by altering the timing, spacing and positioning of their whiskers (Arkley et al.
66 2014; Berg & Kleinfeld 2003; Grant et al. 2009; 2013; Mitchinson et al. 2007; 2011). The
67 effect of these active whisker control strategies may be to increase the number of controlled
68 whisker contacts with surfaces of interest. For instance, by asymmetrically modulating the
69 amplitude of whisker movements on the two sides of the snout when a surface is encountered
70 unilaterally, termed contact-induced asymmetry (CIA), animals can increase the number of
71 contacts whilst avoiding excessive whisker bending (Mitchinson et al., 2007; 2011). Some
72 elements of whisker control are absent in the whisking, nocturnal, arboreal grey short-tailed
73 opossum, *Monodelphis domestica* (Grant et al. 2013; Mitchinson et al. 2011), which is
74 considered to be useful model of early mammals. Specifically, although *M. domestica* shows

75 whisking and CIA it is unable to alter whisker spread, another strategy thought to increase the
76 number of whisker contacts (Grant et al., 2009). Diurnal, terrestrial Domestic Guinea pigs
77 (*Cavia porcellus*) do not whisk and can only make few, asymmetric twitches of their
78 whiskers, rather than the bilateral, cyclic movements associated with whisking (Grant et al.
79 2017). However, the striking presence of whiskers in all small mammals, even in diurnal
80 terrestrial mammals, as well as the conservation of their arrangement and facial whisker
81 musculature, suggests that they might be still functional in all small mammals (Grant et al.
82 2017). We propose in this study that in addition to environment exploration, guiding
83 locomotion might be a common function of whiskers in small mammals.

84

85 This study will, for the first time, compare whisker movements and control during
86 locomotion in a range of diurnal, nocturnal, crepuscular and cathemeral small mammals, with
87 varying substrate preferences (arboreal, terrestrial and semi-aquatic) focusing on the role of
88 facial whiskers in guiding locomotion and foot positioning.

89

90 **Methods**

91 *Animals*

92 Eleven species of small mammals were considered in this study (59 individuals,
93 Supplementary Material, Supplement 1). This included the nocturnal, arboreal Hazel
94 dormouse (*Muscardinus avellanarius*), Etruscan shrew (*Suncus etruscus*), Woodmouse
95 (*Apodemus sylvaticus*) and Yellow-necked mouse (*Apodemus flavicollis*); the crepuscular,
96 arboreal Harvest mouse (*Mycromys minutus*); the cathemeral, arboreal Brown rat (*Rattus*
97 *norvegicus*), cathemeral, semi-aquatic Water shrew (*Neomys fodiens*) and cathemeral,
98 terrestrial Pygmy shrew (*Sorex minutus*); the diurnal semi-aquatic Water vole (*Arvicola*

99 *amphibious*), the diurnal, terrestrial Bank vole (*Myodes glareolus*) and the Domestic Guinea
100 pig (*Cavia porcellus*). The Etruscan shrews (*Suncus etruscus*) were wild-caught and
101 maintained at the Bernstein Center for Computational Neuroscience, Berlin, Germany.
102 Domestic Guinea pigs (*Cavia porcellus*) were domestic and maintained at Heeley City Farm,
103 Sheffield, UK. The rest of the animals were tested at the Wildwood Trust, Kent, UK, and
104 were either part of breeding programs, rehabilitation programs or for visitor displays. All
105 animals were adult, with males and females represented where possible. Whisker movements
106 were assumed to be sexually monomorphic.

107

108 *Whisker movements on a flat floor*

109 All eleven species were used in this section of the study. Animals were placed in to a Perspex
110 arena (Figure 1a) using cardboard tubes to prevent excessive handling. They were filmed
111 directly from above or below using a high-speed, high-resolution video camera at 500 fps
112 (either Phantom Miro ex2 or Photron Fastcam) (Figure 1a, left; Supplementary Material,
113 Supplement 1). Animals that were filmed from below, were imaged through the
114 pedobarograph floor (Figure 1a). An infrared light-box illuminated the arena, allowing video
115 clips to be collected in semi-darkness. In some instances, a Perspex block was introduced to
116 the arena to promote object exploration. Multiple video clips were collected opportunistically
117 (by manual trigger) when each animal was locomoting around or exploring the block, and
118 range from 0.6-1.6 seconds in length. Recording stopped when the camera memory was full,
119 the animal stopped exploring, or became stressed. In total, 780 clips were collected from 59
120 individuals. The number of clips and the number of individuals filmed can be seen in
121 Supplementary Material, Supplement 1. The activity pattern (nocturnal, crepuscular,
122 cathemeral, diurnal and substrate preference (arboreal, terrestrial, semi-aquatic) were also

123 recorded for each species in Supplementary Material, Supplement 1. These groupings can
124 often be difficult to strictly define. Indeed, here we refer to cathemeral animals as those
125 species which are flexible enough to be active at many time of the day and not strictly just at
126 night time; including *Rattus norvegicus*, *Sorex minutus*, and *Neomys fodiens*.

127

128 From the 780 clips collected, those suitable for whisker tracking were selected resulting in
129 two to eight clips per individual and a total of 207 clips (Supplementary Material,
130 Supplement 1). These clips included episodes where the animal was locomoting and not
131 contacting a vertical surface with its whiskers, such as the block or arena wall. In addition,
132 the snout and both whisker arrays had to be clearly visible throughout the clip selection, with
133 minimal head pitch or roll. The whiskers and head were tracked semi-automatically using the
134 BIOTACT Whisker Tracking Tool (Perkon et al. 2011) (Figure 1b), the mean whisker
135 angular positions (relative to the head) was derived for each side of the head. To estimate
136 *amplitude* the mean value was removed from the mean whisker angular positions, and the
137 root mean square value was computed to give the root-mean-square (RMS) whisking
138 amplitude. As the mean whisker angular positions were approximately sinusoidal, the “peak-
139 to-peak whisking amplitude” was estimated by multiplying the RMS whisking amplitude by
140 $2\sqrt{2}$ (Chatfield 2003). This estimate of amplitude is reasonably robust to departures from a
141 purely sinusoidal pattern (Grant et al. 2014). The *whisking frequency* was estimated from a
142 Fourier transform of the mean whisker angular position data. The *whisker offset*, was
143 calculated as the mean whisker angular positions. Mean angular *retraction* and *protraction*
144 *speeds* were also calculated as the average velocity of all the backward (negative) and
145 forward (positive) whisker movements, respectively. Mean amplitude, frequency, speeds and
146 offset were calculated for left and right whiskers and then averaged to give a per clip
147 measure. Locomotion speed (m/sec) was also approximated from the position of the nose tip.

148 Refer to the methods section in Grant et al. (2014) for more information on the whisker
149 variables.

150

151 Each of the 780 clips were also reviewed to see if certain whisking behaviours were present
152 or absent for a particular species. These categorical whisking behaviours were *whisking*,
153 *spread reduction*, *contact-induced asymmetry (CIA)* and they were reviewed using scales
154 developed in Grant et al. (2012). Whisking was scored during clips where the animal was
155 locomoting forward, either as retractions and protractions present, or only retractions present.
156 Spread reduction and CIA were scored in clips where the animal's whiskers were contacting
157 the perspex block or arena walls. Spread reduction was scored as simply being present or
158 absent; CIA was scored as present, with both an increase in contralateral whisker angles and
159 decrease in ipsilateral whisker angles, or only the decrease in ipsilateral angles present. *Look*
160 *ahead* behaviour was also reviewed, which was the presence of a positive correlation
161 (Spearman's Rank) between locomotion speed and whisker offset.

162

163 *Whisker movements on an inclined plane*

164 Seven species were selected for inclusion in this section of the study, chosen for their larger
165 sample sizes. These included Guinea pig (*Cavia porcellus*), Water shrew (*Neomys fodiens*),
166 Water vole (*Arvicola amphibious*), Harvest mouse (*Mycromys minutus*), Brown rat (*Rattus*
167 *norvegicus*), Woodmouse (*Apodemus sylvaticus*) and Hazel dormouse (*Muscardinus*
168 *avellanarius*). Animals were filmed from below, through the pedobarograph (Figure 1a), in
169 the arena with a flat floor, and then two to four animals of each species were filmed again the
170 next day in the same arena inclined at an angle of 10 degrees (Figure 1a, right). Measures of
171 whisking amplitude, frequency, speed, offset and locomotion speed were extracted in the

172 same way as for the flat floor section of the study to enable a direct comparison. Whisker
173 span was measured from the footage, as the smallest whisker width in the video, when the
174 whiskers were at maximum protraction (Figure 1b). The forepaw width was also measured as
175 the width between the two forepaws (Figure 1c). These relative values are presented in the
176 text, where the size of the animal was controlled for by approximating the Geometric mean of
177 the head from head width and length ($GM = \text{square root}(\text{head width} \times \text{length})$) measurements
178 from the video (Supplementary Material, Supplement 2). It was not possible to identify
179 morphological features in the footage to guide these measurements, therefore the maximum
180 head width was identified, with the length then measured from this point to the nose tip.
181 Maximum shoulder and hip width was also measured for all species, from the videos and
182 presented as a ratio in Supplementary Material, Supplement 2, to get an idea of general body
183 shape; a value over one indicates that the hip width is larger than the shoulder width.

184

185 *Foot positions on a flat and inclined floor*

186 For the seven species filmed on both the flat and inclined plane, it was possible to identify
187 foot contacts using the pedobarograph, which is a glass floor, illuminated with a strip of red
188 LEDs to highlight foot contacts (Figure 1c). Foot positions and nose positions of each species
189 were tracked manually in three example clips when the animal locomoted forward across the
190 floor using the program Tracker (Tracker 4.80, Brown and Wolfgang 2013,
191 <http://www.cabrillo.edu>). The minimum distance of foot placements to the nose tip was
192 calculated, as well as the time it took from the nose point to move from the minimum
193 distance point and foot placement to arrive. The gait cycle was also calculated (in Hz) from
194 the time a front paw contacted the ground to when the same paw contacted the ground again.

195

196 *Statistical Considerations*

197 Whisking results for all eleven species are presented in Table 1 and Supplementary Material,
198 Supplement 4 as mean values \pm standard deviations. Whisking variables and locomotion
199 speed were compared on flat and inclined floors using a MANOVA, individual multivariate
200 ANOVAs were conducted for each of the seven species that were tested on the flat and
201 inclined planes. Locomotion speed was correlated against amplitude, offset and frequency for
202 the nocturnal, crepuscular, cathemeral, and diurnal species groupings, using a Spearman's
203 Rank Correlation. Whisker span, foot span and offset were also correlated for the nocturnal,
204 crepuscular, cathemeral, diurnal species groupings, using a Spearman's Rank Correlation.

205

206 **Results**

207 *Whisker movements in small mammals*

208 All eleven of the small mammals can control movements of their whiskers to some extent.
209 Rhythmic whisking was observed in all of the species tested, apart from *Cavia porcellus*
210 (Table 1), which made isolated unilateral whisker twitches instead. This can be clearly seen
211 in the example whisker traces in Figure 2a, vii, where the left whiskers of *Cavia porcellus* (in
212 blue) made low amplitude, rhythmic movements, but the right whiskers (in red) were just
213 slowly moving forward, with no rhythmic movements. On review of the video footage,
214 whisking in *Neomys fodiens* looked to only consist of retraction movements, but all other
215 species engaged in rhythmic, bilateral, forward and backward whisker sweeps (Table 1).
216 Examples of these whisker sweeps can be seen in Figure 2a, and varied between the species
217 in terms of amplitude, frequency, offset asymmetry and speeds (Table 1). For example,
218 *Rattus norvegicus* and *Apodemus flavicollis* had large amplitude whisks (Figure 2a, iv and ii ,

219 respectively), while *Muscardinus avellanarius* had the most forward facing whiskers, with
 220 the largest offset values (Table 1, Figure 2a, i).

221 **Table 1 Whisker measurement results for each species on a flat floor**, shown as mean \pm s.d. Grey
 222 boxes in the species column show the animals that were also tested on the inclined floor; grey boxes
 223 in the whisker variable columns, correspond to variables that significantly altered when the same
 224 animals were filmed on an inclined floor.

Species	Loco Speed m/sec	Freq Hz	Off deg	Amp deg	Asym deg	Pro Speed deg/ms	Re Speed deg/ms	Whisk	Spread	CIA	Look ahead
European Dormouse (<i>Muscardinus avellanarius</i>)	0.53 \pm 0.48	9.60 \pm 3.88	126.19 \pm 8.21	37.83 \pm 16.30	18.25 \pm 7.41	1.57 \pm 0.28	0.24 \pm 0.16	Present	Present	Present	Present
Etruscan Shrew (<i>Suncus etruscus</i>)	0.26 \pm 0.10	10.13 \pm 3.03	87.56 \pm 11.49	29.56 \pm 4.41	16.45 \pm 8.49	1.41 \pm 0.09	0.20 \pm 0.08	Present	Absent	Ips. Only	Present
Woodmouse (<i>Apodemus sylvaticus</i>)	0.34 \pm 0.26	16.08 \pm 7.05	117.28 \pm 10.09	36.57 \pm 7.26	11.95 \pm 3.78	1.69 \pm 0.31	0.43 \pm 0.23	Present	Present	Present	Present
Yellow-necked mouse (<i>Apodemus flavicollis</i>)	0.40 \pm 0.20	13.67 \pm 2.14	101.68 \pm 12.72	46.06 \pm 11.27	19.00 \pm 10.26	1.57 \pm 0.10	0.53 \pm 0.19	Present	Present	Present	Present
Harvest Mouse (<i>Micromys minutus</i>)	0.28 \pm 0.07	12.03 \pm 4.49	99.76 \pm 6.77	45.67 \pm 11.86	17.82 \pm 3.65	1.87 \pm 0.41	0.51 \pm 0.21	Present	Present	Present	Absent
Brown Rat (<i>Rattus norvegicus</i>)	0.27 \pm 0.15	8.80 \pm 0.76	107.23 \pm 8.90	44.22 \pm 7.96	17.95 \pm 3.00	1.34 \pm 0.10 ^l	0.15 \pm 0.05	Present	Present	Present	Absent
Water Shrew (<i>Neomys fodiens</i>)	0.39 \pm 0.19	8.08 \pm 4.81	104.45 \pm 6.80	39.22 \pm 13.39	16.04 \pm 4.71	1.74 \pm 0.41	0.42 \pm 0.37	Ret. only	Absent	Present	Absent
Pygmy Shrew (<i>Sorex minutus</i>)	0.64 \pm 0.22	14.80 \pm 4.46	91.78 \pm 10.30	39.25 \pm 8.51	15.42 \pm 6.14	1.84 \pm 0.24	0.54 \pm 0.20	Present	Present	Ips. Only	Absent
Water Vole (<i>Arvicola amphibious</i>)	0.15 \pm 0.12	8.27 \pm 3.47	117.51 \pm 7.49	40.97 \pm 23.97	26.56 \pm 9.47	2.03 \pm 2.44	0.20 \pm 0.22	Present	Present	Ips. Only	Absent
Bank Vole (<i>Myodes glareolus</i>)	0.83 \pm 0.61	13.75 \pm 3.18	126.08 \pm 14.14	27.84 \pm 10.52	9.65 \pm 3.12	1.37 \pm 0.18	0.27 \pm 0.23	Present	Present	Present	Absent
Domestic Guinea pig (<i>Cavia porcellus</i>)	0.26 \pm -0.07	n.a.	104.22 \pm 7.11	36.68 \pm 23.51	30.75 \pm 13.69	n.a.	n.a.	Absent	Absent	Absent	Absent

225

226 As well as whisking, other elements of whisker control also varied between the species.
227 Spread reduction was absent in *Suncus etruscus* and *Neomys fodiens*, and contact-induced
228 asymmetry was limited to only a decrease in ipsilateral whisker angles, without any increases
229 in contralateral angles, in *Suncus etruscus*, *Sorex minutus* and *Arvicola amphibious*. *Cavia*
230 *porcellus* did not engage in spread reduction or contact-induced asymmetry. When the
231 animals were placed on an inclined floor, aspects of whisker position and movement were
232 significantly altered in all of the tested species, apart from *Cavia porcellus* (Table 1,
233 Supplementary Material, Supplement 4). While locomotion speed was not significantly
234 affected in any of the species ($F(1,106)=0.748$, $p=0.389$), generally, whisker speeds were
235 reduced as well as whisker amplitudes. Supplementary Material, Supplement 4 shows three
236 example whisker traces from *Micromys minutus*, *Arvicola amphibious* and *Neomy fodiens*,
237 who all showed significant reductions in amplitude on the inclined floor compared to the flat
238 floor.

239

240 The lengths of the whiskers varied between species, even when controlling for body size.
241 Figure 2c shows a diagram taken from tracing around the head and whiskers, and exact
242 whisker lengths (controlled for by body size) can be seen in Supplementary Material,
243 Supplement 2. *Rattus norvegicus* had the longest whiskers (relative length: 2.82 ± 0.26 , Figure
244 2c), followed by *Apodemus sylvaticus* (relative length: 2.24 ± 0.36 , Figure 2c) and
245 *Muscardinus avellanarius* (relative length: 2.15 ± 0.26 , Figure 2c). *Micromys minutus* and
246 *Neomy fodiens* had very similar whisker lengths (relative length: 1.69 ± 0.23 and 1.68 ± 0.21 ,
247 respectively, Figure 2c), followed by *Arvicola amphibious* (relative length: 1.61 ± 0.11), and
248 *Cavia porcellus* having the smallest whiskers (relative length: 0.88 ± 0.15).

249

250 *Whiskers and locomotion in small mammals*

251 Despite variations in the length of their whiskers and the animals' abilities to move and
252 control them, the forepaw placements of all the species tested always fell within an area that
253 the whiskers had previously scanned. Indeed, in all the species tested, the forepaw placements
254 fell 4-25 mm of where the nose tip had previously been. Analysis of the timings indicate that
255 in small mammals the nose tip, and whisker field, scan an area 47-367 ms ahead of forepaw
256 placements. There was more variation in rear paw placement, with the majority of rear paw
257 placements falling 7-62 mm from a previous nose tip position, with a delay of 203-674 ms
258 behind the nose tip scan. Some rear paw placements occurred outside the whisker field in
259 *Muscardinus avellanarius*, *Rattus norvegicus*, *Arvicola amphibious*, *Cavia porcellus* and
260 *Neomys fodiens* (Figure 2c). Figure 2b shows the distance of the fore (in blue) and hind (in
261 red) paw placements from a previous nose placement. Forepaw placements fell closer to a
262 previous nose tip location than hindpaw placements in all species tested. Figure 2c
263 diagrammatically shows this, with mean paw positions (in bold colour) and standard
264 deviations (in lighter shading) approximated on the traced whisker field for each species.

265 The animals travelled at varying speeds with their gait cycles varying from 1.76 Hz in rat, to
266 5Hz in guinea pig (Supplementary Material, Supplement 2); the gait cycle was not associated
267 with species' whisking frequency (Spearman's Rank Correlation: $r=0.143$, $df=6$, $p=0.787$),
268 such that species that moved quicker did not necessarily move their whiskers quicker.

269 There was no significant difference between footfall placement positions (Wilcoxon Signed
270 Rank: $W(12)=29$, $p=0.4328$) or timings ($W(12)=22$, $p=0.1823$) with respect to previous nose
271 tip positions, when comparing locomotion on a flat or inclined plane for any of the species
272 tested. On an inclined floor, the same pattern was observed that forepaw placements fell
273 closer to previous nose tip positions (6-34 mm) than hind paws (10-51 mm), with the nose

274 position being 38-213 ms ahead of forepaw placements, compared to 119-382 ms ahead of
275 hind paw placements. As there was no difference between paw placements on a flat and
276 inclined plane, the data was combined in Figure 3a and b to explore the relationship between
277 whisker span and forepaw width. In all species, forepaw width was always smaller than
278 whisker span, indicating that forepaw placements fell within the whisker field (Figure 3a).
279 Forepaw width and whisker span was also significantly correlated, with larger whisker spans
280 being associated with larger foot widths in all the species tested, including nocturnal,
281 crepuscular, cathemeral and diurnal individuals (Figure 3a, Supplementary Material,
282 Supplement 3, all p-values <0.05). As whisker position impacts whisker span, with higher
283 offset values being associated with smaller, more focussed whisker spans (Figure 3d) (Arkley
284 et al. 2014), whisker offset was plotted against whisker span (Figure 2b). Whisker span was
285 not correlated to mean whisker offset values (Figure 3a, Supplementary Material, Supplement
286 3, all p-values >0.05), although the nocturnal species showed the general trend that higher
287 offset values were associated with smaller whisker spans (solid trendline in Figure 2b),
288 especially in *Apodemus sylvaticus* and *Muscardinus avellanarius* (Figure 3b).

289

290 *Whisker control varies in small mammals*

291 While whisker offset was not correlated to forepaw width in any species (Supplementary
292 Material, Supplement 3, all p-values >0.05), it was correlated to locomotion speed in all of
293 the nocturnal species *Muscardinus avellanarius*, *Suncus etruscus*, *Apodemus sylvaticus* and
294 *Apodemus flavicollis* (Figure 3c, Supplementary Material, Supplement 3, all p-values >0.05).
295 Specifically, at higher locomotion speeds, the nocturnal species protracted their whiskers
296 further forward, with higher offset values, which can be seen by comparing the example

297 screenshots in the nocturnal *Muscardinus avellanarius* (Figure 3d) to the crepuscular
298 *Micromys minutus* (Figure 3e) and the diurnal *Arvicola amphibious* (Figure 3f).

299

300 **Discussion**

301 Our results revealed that all the small mammals in this study could move their whiskers
302 somewhat, although the degree of movement and control varied between species. All the
303 species placed their forepaws on the floor, where their whiskers had previously scanned. This
304 suggests that whiskers are likely to be functional and important in many small mammals,
305 especially for guiding quadrupedal locomotion.

306 Whisker studies are often associated with nocturnal, or cathemeral, arboreal mammals, and
307 this study is the first to consider whisker movement and control in a range of species. Indeed,
308 this is the only study to have described whisker movements in *Apodemus sylvaticus*,
309 *Apodemus flavicollis*, *Micromys minutus*, *Neomys fodiens*, *Sorex minutus*, *Arvicola*
310 *amphibious*, *Myodes glareolus* and to have quantitatively confirmed the presence of whisking
311 in a large number of small mammals.

312

313 *Whisker position is associated with locomotion*

314 We found that all the species tested placed their forepaws in to an area that the whiskers had
315 previously passed through. This has been suggested to occur in *Rattus norvegicus* (Arkley et
316 al. 2014), *Muscardinus avellanarius* (Arkley et al. 2017), *Euchoreutes naso* and *Dipus sagitta*
317 (Sokolov and Kulikov 1987), however, it was not fully quantified until now. In our species,
318 all forepaw placements occurred within 4-25 mm of where the nose tip had previously been
319 47-367 ms before. We also found that forepaw widths were always smaller than the whisker

320 span in the species we have tested, and that they were also correlated. This correlation
321 suggests that if an animal increases the span of their whiskers, by spreading them out and
322 reducing offset values, then the forepaw placements were also more spread out.

323

324 In our data, whisker scanning sometimes occurred one entire gait cycle ahead of the foot
325 placement, but was much more likely to take place while the foot is off the floor, just prior to
326 its placement on the ground. The most extreme example can be seen in *Micromys minutus*,
327 where the gait cycle takes around 235 ms to complete (4.26 Hz), but the nose scanned only
328 47 ms ahead of the foot placement (Supplementary Material, Supplement 2). In rat (*Rattus*
329 *norvegicus*), it can take 88-224 ms to make an action from a whisker contact, including
330 discriminating textures or jumping on to a platform (von Heimendahl 2007; Diamond et al.
331 2008). These studies looked at discretely triggered actions, however, modulation of ongoing
332 action may take place at multiple levels of the neuraxis from the brainstem through to cortex,
333 at even shorter latencies. For instance, the latencies for whisker responses in rat
334 somatosensory and in the midbrain superior colliculus can be as little as 5ms (Zhu and
335 Connors 1999; Cohen et al. 2008), allowing whisker sensory processing to influence motor
336 outputs well within the duration of typical gait cycle. Since whisking frequency can be more
337 than twice as fast as stride frequency, whisker contacts over multiple cycles can be useful in
338 guiding foot placements. In the laboratory house mouse (*Mus musculus*), aspects of whisking
339 frequency have been found to be correlated to the gait cycle (Sofroniew et al. 2014). We did
340 not observe any association between gait cycle and whisker frequency here, so species that
341 moved faster did not necessarily whisk quicker.

342

343 However, other aspects of whisker positioning were controlled during locomotion. Whilst
344 whisking and locomotion was generally similar on flat and inclined surfaces, all of the
345 species, apart from *Cavia porcellus*, altered some aspects of whisker positioning or speed
346 during locomotion on an inclined slope, compared to a flat floor. Moreover, at higher
347 locomotion speeds *Rattus norvegicus* and *Mus musculus* have been found to reduce whisking
348 and protract their whisker forward, in a process called ‘look ahead’, which is thought to focus
349 the whiskers in front of the snout and prevent collisions with this sensitive area (Arkley et al.
350 2014; Sofroniew et al. 2014). This behaviour was observed in our data only in the truly
351 nocturnal species, irrespective of substrate preference, including *Muscardinus avellanarius*,
352 *Suncus etruscus*, *Apodemus flavicollis* and *Apodemus sylvaticus*, and might serve to prevent
353 collisions during high-speed locomotion in these nocturnal animals. This ‘look ahead’
354 behaviour would increase offset angles (Arkley et al. 2014) and decrease whisker span, which
355 can be seen in Figure 3b, however, this relationship was not significant. Whisker span,
356 therefore, is probably associated with a number of parameters, including both offset and
357 whisker length.

358

359 While the foot placements always fell within the whisker field, it is worth bearing in mind
360 that whiskers are a discrete set of point sensors, and that the positioning of a whisker tip
361 might not necessarily coincide at exactly the same place as a footfall. Data collection was
362 carried out within the first five minutes of the animals being introduced to the experimental
363 arena, this is an exploration phase where the animals locomoted forwards with their heads
364 down to explore the floor, and only raised their heads to better investigate objects or vertical
365 surfaces (Grant et al. 2009). Locomoting with their head down enables a large number of
366 whisker contacts (see Figure 6, left, in Grant et al. 2009, and Figure 1a in Arkley et al. 2014)
367 and increases the likelihood of a whisker contact coinciding in space with a foot placement.

368 The head was positioned downwards towards the floor in the majority of our data collection.
369 Raising the head, as occurs during running and habituation to an environment (Arkley et al.
370 2014), lifts the smaller whiskers off the floor and enables floor contact only at the tips of the
371 longer whiskers, with no contact beneath the snout (see Figure 6, right, in Grant et al. 2009,
372 and Figure 1b and c in Arkley et al. 2014) . This head raising is associated with the look-
373 ahead strategy, focussing the whiskers to detect impacts in front of the snout, rather than
374 beneath it. Understanding how whisker layout, length and positioning affects whisker
375 contacts with the ground, especially on small structures such as branches, would be an
376 interesting direction for future work.

377

378 Longer whiskers are associated with small, nocturnal, arboreal mammals (Muchlinski et al.
379 2013). We can see in our data that the arboreal mammals tended to have relatively longer
380 whiskers (especially *Rattus norvegicus*, *Muscardinus avellanarius* and *Apodemus sylvaticus*),
381 with the terrestrial, diurnal *Cavia porcellus* having the smallest whiskers, and the semi-
382 aquatic species (*Arvicola amphibius* and *Neomys fodiens*) being somewhat intermediary
383 (Supplementary Material, Supplement 2). Similarly, climbing rodents have longer digits and
384 higher joint mobility than semi-aquatic rodents (Samuels and Van Valkenburgh 2008), to
385 enable good grasping during climbing. Having longer whiskers might ensure that the
386 placement of these long, flexible digits can still be guided by whisker touch in arboreal
387 mammals. We can also see in our data that the diurnal semi-aquatic *Arvicola amphibius* and
388 *Neomys fodiens*, have shorter whiskers, and hence smaller whisker spans. Semi-aquatic
389 rodents tend to have smaller forepaws than arboreal rodents, but larger hind limbs for
390 paddling (Samuels and Van Valkenburgh 2008). Therefore, their smaller whisker span should
391 be sufficient for guiding their smaller forepaws, although the semi-aquatic nature of their
392 lifestyle may also be impacting on whisker length, for instance, longer whiskers may be

393 harder to control in water. The terrestrial *Cavia porcellus* appears to have the smallest
394 whiskers, relative to the other species examined here. It would be interesting to further
395 explore how whisker length is associated with quadrupedal locomotion strategies and skeletal
396 structures in a larger number of small mammal species. For example, the hip width of all the
397 species here, are wider than the shoulder widths (Supplementary Material, Supplement 2).
398 Therefore, the hind limbs may well naturally have a wider stance than the forelimbs, and be
399 positioned outside of the whisker field, especially in animals with shorter whiskers, such as
400 *Arvicola amphibius*, *Neomys fodiens* and *Cavia porcellus*.

401

402 *Whisker movement and control*

403 All of the species in this study whisked bar one. The exception was the diurnal, terrestrial
404 *Cavia porcellus* which could only make unilateral whisker twitches, agreeing with previous
405 observations of *Cavia porcellus* whisker movements (Jin et al. 2004; Grant et al. 2017). In the
406 whisking species, whisker movements had clear protraction (forward) and retraction
407 (backward) phases in all the animals apart from *Neomys fodiens*, where only retractions were
408 present. Whisking is often more associated with nocturnal and arboreal species, although
409 terrestrial and diurnal species can also whisk (Arkley et al. 2014; Grant et al. 2017;
410 Muchlinski et al. 2018). In this study, the largest whisker movements, with the highest
411 amplitudes, were observed in *Rattus norvegicus* and *Apodemus flavicollis*, which are both
412 arboreal species (although they also burrow and run on the ground). Whisking is thought to
413 enable rapid sampling during spatial exploration (Knutsen, 2015) and is associated with
414 larger infraorbital nerves and higher tactile sensory acuity in small mammals (Muchlinski et
415 al. 2018), which may well be important for tactually guiding climbing in complex
416 environments, such as trees and hedgerows. Many of the arboreal species in this study

417 engaged in all of the tested control strategies, including *Muscardinus avellanarius*, *Apodemus*
418 *sylvaticus*, *Apodemus flavicollis*, *Rattus norvegicus* and *Micromys minutus*. Semi-aquatic
419 mammals have highly sensitive whiskers (Dehnhardt et al. 1999), and we do see that *Arvicola*
420 *amphibius* and *Neomys fodiens* engaged in many control behaviours, such as spread reduction
421 and whisking. The terrestrial, diurnal *Cavia porcellus* engaged in the fewest control
422 behaviours. Therefore, our data supports the idea that whisker-use is associated with complex
423 habitats, including arboreal and aquatic environments.

424

425 As well as variations in whisking movements, aspects of whisker control also differed
426 between species. Extensive studies in House mice (*Mus musculus*) and Brown rats (*Rattus*
427 *norvegicus*) have revealed that whisker movements can be actively controlled during
428 locomotion and object exploration. During object exploration, rats reduce the spacing, or
429 spread, of their whisker, so that they bunch up on a surface and enable more whisker contacts
430 (Grant et al. 2009; 2013). This behaviour is absent in the Grey short-tailed opossum,
431 *Monodelphis domestica*, which lacks the muscular control to enable spread reduction (Grant
432 et al. 2013). Our data found no evidence of this behaviour in *Suncus etruscus*, *Neomy fodiens*
433 and *Cavia porcellus*. The absence of spread reduction in *M. domestica* and other small
434 mammals suggests that it may have evolved after whisking accompanied by some changes in
435 the whisking musculature (Grant et al. 2013; Muchlinski et al. 2018). Asymmetry, or more
436 specifically contact-induced asymmetry (CIA), also often occurs following a unilateral
437 contact and can be seen in *Mus musculus*, *Rattus norvegicus* and *Monodelphis domestica*
438 (Mitchinson et al. 2011). It is characterised by the whiskers contralateral to the contact
439 increasing in amplitude and the whiskers ipsilateral to the contact decreasing in amplitude,
440 enabling asymmetry between the two whisker fields. In our data, we saw no evidence of this
441 behaviour in *Suncus etruscus*, *Sorex minutus* and *Arvicola amphibius*. CIA appears to allow

442 animals to increase the number of contacts with vertical surfaces of interest (Mitchinson et
443 al., 2007). Since Mitchinson et al. (2011) found evidence of bilateral CIA in the marsupial
444 opossum, *M. domestica*, it may have been present in early mammals, in which case it may
445 have been lost in some modern-day species. The relationship between lifestyle and the
446 ability to express different forms of CIA may be worth investigating further in different
447 mammalian species, for example, the semi-aquatic lifestyle of *Sorex minutus* and *Arvicola*
448 *amphibious* may explain some changes in aspects of whisker control.

449

450 **Conclusions**

451 Our data demonstrate that many small mammals use their whiskers to tactually guide safe
452 foot positioning. Specifically, we have demonstrated that forepaw placement always falls
453 within the whisker field of all the small mammals tested here, and that forepaw width is
454 always smaller than whisker span. We have also demonstrated that nocturnal, arboreal and
455 semi-aquatic mammals all show elements of active whisker control during object exploration
456 and locomotion with arboreal mammals having the longest whiskers and full ability to control
457 whisker spread and contact asymmetry. Overall, we propose that guiding locomotion, along
458 with environment exploration, might be common functions of whisker touch sensing in small
459 non-flying mammals.

460

461 **Ethics Statement**

462 All procedures were purely observational and therefore approved by the UK Home Office,
463 under the terms of the UK Animals (Scientific Procedures) Act, 1986, and consistent with
464 ethical guidelines at Manchester Metropolitan University (MMU), University of Sheffield,
465 and approved by the local ethics committees at the Wildwood Trust and Bernstein Center for

466 Computational Neuroscience. All procedures complied with general handling and husbandry
467 guidelines at the Wildwood Trust; animals were handled by Wildwood Trust Staff who had
468 appropriate licenses, especially for dormice handling.

469 **Data Accessibility**

470 Data is shown as mean and standard deviation values in the Table 1 in the main text and in
471 Supplement 2 and 4. All raw data is available on Dryad (doi:10.5061/dryad.92t4802).

472 **Competing Interests**

473 We have no competing interests.

474 **Authors' Contributions**

475 RG conceived the study, carried out the data collection, analysis and wrote the paper. VB
476 supported data collection and manuscript drafting. TP Supported the conception of the study.
477 All authors gave final approval for publication.

478 **Acknowledgements**

479 Many thanks go to the Wildwood Trust for access to animals and their continued support for
480 the project, especially to Angus Carpenter for his comments on the manuscript and Hazel
481 Ryan for help with experimental design and animal handling. We are also grateful to Eddie
482 Gill and Heeley City Farm for supporting us with filming the guinea pigs. We are thankful to
483 Dr Heather Driscoll for designing and building the pedobarograph and to Dr Ben Mitchinson
484 for designing and building the initial portable set-up. We are also grateful to Kendra Arkley,
485 Mariane Delaunay, Fraser Combe and Adele Tomasz for their support during data collection
486 and animal handling. We would also like to acknowledge Professor Michael Brecht at
487 Bernstein Center for Computational Neuroscience for supporting us in filming the Etruscan
488 shrews.

489 **Funding**

490 Video analysis was performed using the BIOTACT Whisker Tracking Tool which was jointly
491 created by the International School of Advanced Studies in Trieste, the University of
492 Sheffield and the Weizmann Institute in Rehovot under the auspices of the FP7 BIOTACT
493 project (ICT 215910), which also partly funded this study, alongside a small project grant
494 from the British Ecological Society (BES) and an internal Research Incentivisation Grant
495 from Manchester Metropolitan University.

496

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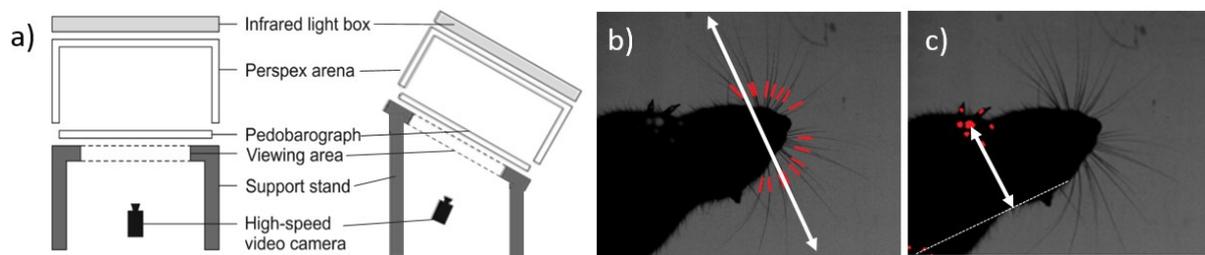
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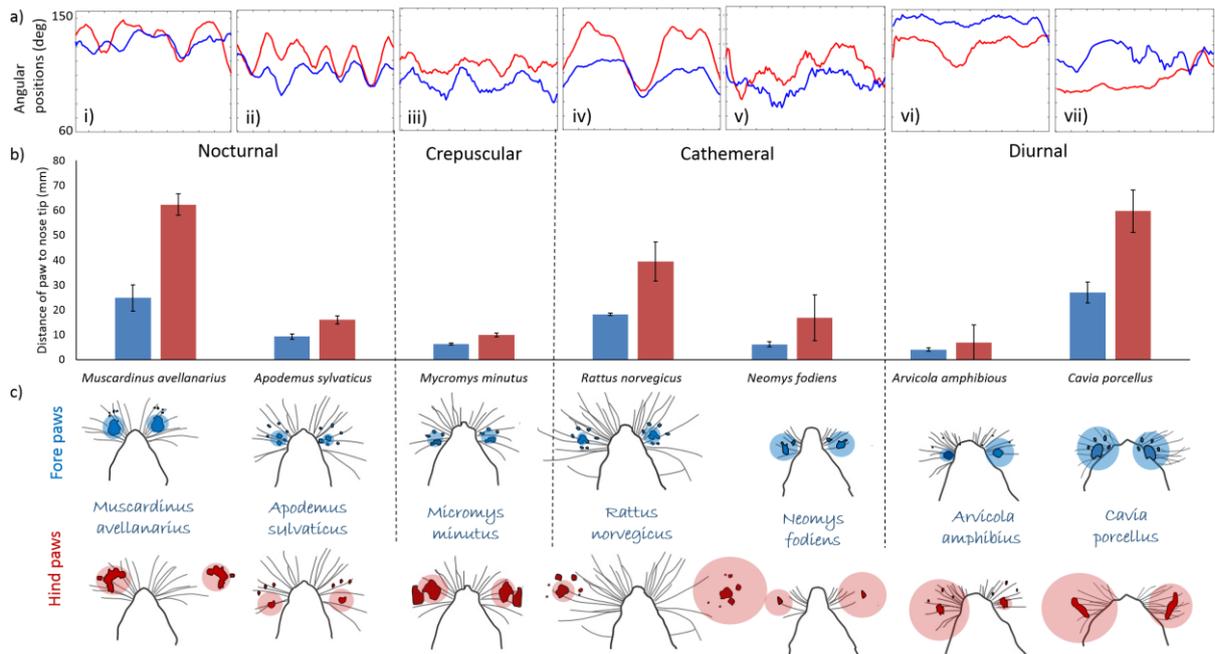
572 **FIGURES**



574 **Figure 1: Filming set-up and example video stills.** a) left: a diagram of the experimental arena.
 575 Animals could be filmed from below (when the pedobarograph was on) and from above (if the
 576 pedobarograph was not used). The experimental arena could also be tilted by 10 degrees to examine

577 locomotion and whisking on an inclined plane.(a, right) b) An example video still showing the
 578 whiskers and tracking; c) the same video-still with the foot placements indicated in red. The white
 579 lines show the whisker span (b) and forepaw width (c).

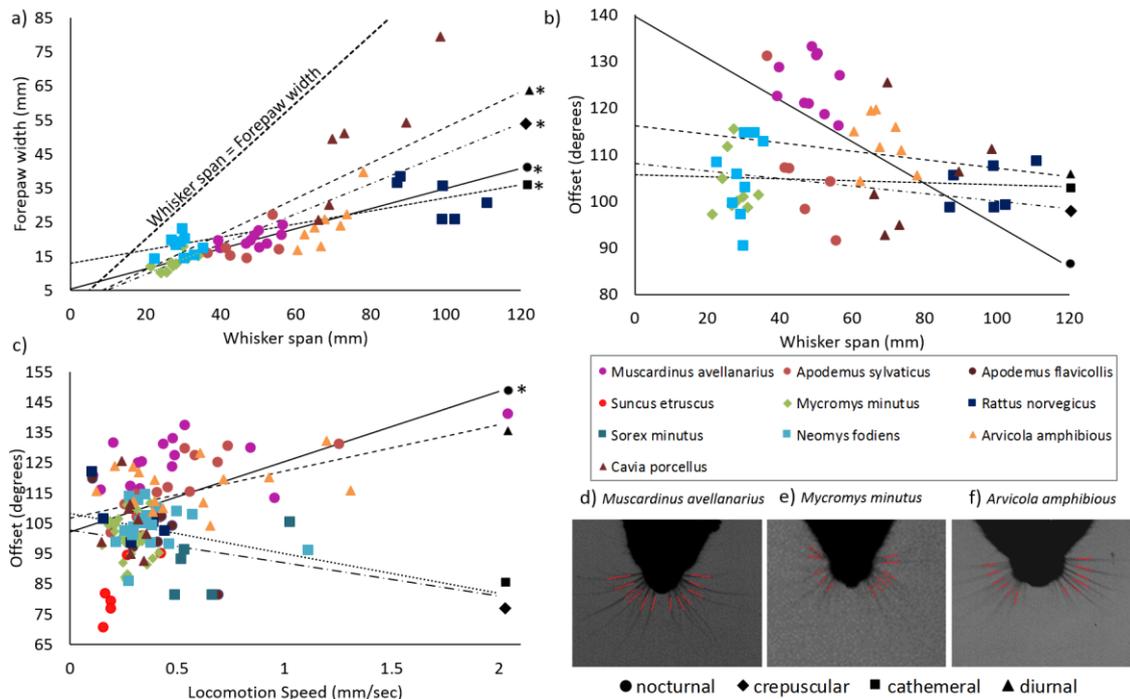
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582 **Figure 2. Whisker movements and paw placements in small mammals.** a) example mean whisker
 583 angular position traces (60-150 degrees) over 200 ms for the left (in blue) and right (in red) whiskers
 584 for each of the species tested with a pedobarograph. b) bar charts showing the mean distance (with
 585 standard error bars) of paw placement from previous nose tip positions, for the forepaws (in blue) and
 586 hind paws (in red). c) diagram of traced footprints and a projection of their positioning on the whisker
 587 field, with approximated mean forepaw positions (in bold) and standard deviations (in lighter
 588 shading), for each species. The whiskers scan ahead of paw placements and this diagram shows the
 589 locations that the head has been in and the feet have moved in to. Forepaw placements always fall
 590 within the whisker field. Hind paw placements are more variable, but often fall within the whisker
 591 field.

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593

594 **Figure 3 Whisker span and position are associated with elements of locomotion.** a) Forepaw
 595 width was correlated to whisker span in diurnal, cathemeral, crepuscular and nocturnal species, and
 596 was always smaller than the whisker span in all of the species tested. b) Forepaw width was not
 597 correlated to offset values. c) Offset was correlated to locomotion speed in nocturnal species, but not
 598 in diurnal, crepuscular or cathemeral species. d-f) Screen shots of *Muscardinus avellanarius*,
 599 *Micromys minutus* and *Arvicola amphibious* at maximally protracting their whiskers during a period
 600 of fast locomotion. *Muscardinus avellanarius* has more forward protracting whiskers, with higher
 601 offset values. Graphs show individual species (in colour), with diurnal (triangle), cathemeral (square),
 602 crepuscular (diamond) and nocturnal (circle) indicated by different shapes. Linear line of best fit were
 603 plotted though the scatter plots for diurnal (triangle, dashed line), cathemeral and crepuscular (square,
 604 dotted line, both grouped here), and nocturnal (circle, full line) species groupings.

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Supplementary Material

609 **Supplement 1:** The number of individuals filmed during the study, and the number of
 610 collected clips.

Common Name	Scientific Name	Activity pattern	Substrate preference	Filmed above	Filmed below ¹	No. individuals	No. clips collected	No. Clips Used
Hazel dormouse	<i>Muscardinus avellanarius</i>	Nocturnal	Arboreal	X	X	9	103	22
Etruscan shrew	<i>Suncus etruscus</i>	Nocturnal	Arboreal	X		6	106	7
Wood mouse	<i>Apodemus sylvaticus</i>	Nocturnal	Arboreal	X	X	5	71	18
Yellow-necked mouse	<i>Apodemus flavicollis</i>	Nocturnal	Arboreal	X		2	36	6
Harvest mouse	<i>Mycromys minutus</i>	Crepuscular	Arboreal	X	X	8	86	46
Brown rat	<i>Rattus norvegicus</i>	Cathemeral	Arboreal	X	X	6	54	10
Water shrew	<i>Neomys fodiens</i>	Cathemeral	Semi-aquatic	X	X	9	105	31
Pygmy shrew	<i>Sorex minutus</i>	Cathemeral	Terrestrial	X		2	60	5
Water vole	<i>Arvicola amphibious</i>	Diurnal	Semi-aquatic	X	X	7	77	26
Bank vole	<i>Myodes glareolus</i>	Diurnal	Terrestrial	X		1	12	2
Guinea pig	<i>Cavia porcellus</i>	Diurnal	Terrestrial	X	X	4	70	13
TOTAL:						59	780	

611 1. If filmed from below, also used the pedobarograph, and included being tested on the flat and
 612 inclined floor. Both whiskers and feet were measured in these species.

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625 **Supplement 2:** Measurements extracted from the manually tracked footage.

626 Relative whisker lengths and forepaw widths in all the species tested, with geometric mean
 627 (GM = $\sqrt{\text{head width} \times \text{head length}}$). Hip width divided by shoulder width. Gait cycle and the
 628 minimum time of nose position to forepaw placement. Values are mean \pm s.d.

Species	Rel. Whisker lengths	Rel. Forepaw Widths	Geometric Mean (mm)	Hip/Shoulder width	Gait Cycle (Hz)	Min time of forepaw to nose (ms)
<i>Muscardinus avellanarius</i>	2.15 \pm 0.26	0.90 \pm 0.10	22.65 \pm 2.09	1.01 \pm 0.04	2.09 \pm 1.96	223.01 \pm 201.56
<i>Apodemus sylvaticus</i>	2.24 \pm 0.36	0.88 \pm 0.23	20.57 \pm 1.31	1.23 \pm 0.09	3.12 \pm 0.55	88.10 \pm 12.24
<i>Micromys minutus</i>	1.69 \pm 0.23	0.81 \pm 0.15	16.36 \pm 1.77	1.07 \pm 0.10	4.26 \pm 0.38	47.00 \pm 5.22
<i>Rattus norvegicus</i>	2.83 \pm 0.26	0.94 \pm 0.16	34.53 \pm 0.76	1.22 \pm 0.06	1.76 \pm 0.01	225.58 \pm 20.78
<i>Neomys fodiens</i>	1.68 \pm 0.21	1.04 \pm 0.17	17.48 \pm 0.33	1.01 \pm 0.04	4.21 \pm 1.65	146.83 \pm 15.73
<i>Arvicola amphibious</i>	1.61 \pm 0.11	0.58 \pm 0.09	42.51 \pm 2.95	1.23 \pm 0.09	2.26 \pm 0.43	248.50 \pm 37.58
<i>Cavia porcellus</i>	0.88 \pm 0.15	0.54 \pm 0.22	88.44 \pm 4.13	1.24 \pm 0.11	5.00 \pm 0.00	366.25 \pm 143.25

629

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632 **Supplement 3:** Spearman's Rank correlation for offset, whisker span and forepaw width in
 633 nocturnal, crepuscular, cathemeral and diurnal mammals. Asterisks (*) refer to significant
 634 correlations, $p < 0.05$.

Species	Whisker Span vs. Forepaw Width	Whisker Span vs. Offset	Locomotion Speed vs. Offset
<i>Nocuturnal:</i>			
<i>Muscardinus avellanarius</i>	R=0.552, $p=0.027^*$	R=0.290, $p=0.276$	R=0.483, $p=0.001^{*a}$
<i>Apodemus sylvaticus</i>			
<i>Crepuscular:</i>			
<i>Micromys minutus</i>	R=0.850, $p=0.004^*$	R=0.000, $p=1.000$	R=-0.131, $p=0.500$
<i>Cathemeral:</i>			
<i>Rattus norvegicus</i>	R=0.693, $p=0.004^*$	R=0.038, $p=0.894$	R=-0.263, $p=0.160^b$
<i>Neomys fodiens</i>			
<i>Diurnal:</i>			
<i>Arvicola amphibious</i>	R=0.864, $p < 0.001^*$	R=-0.165, $p=0.573$	R=0.292, $p=0.148$
<i>Cavia porcellus</i>			

635 a. As well as the species mentioned, also includes data from *Apodemus flavicollis* and
 636 *Suncus etruscus* in the locomotion speed and offset correlation.

637 b. As well as the species mentioned, also includes data from *Sorex minutus* in the
 638 locomotion speed and offset correlation.

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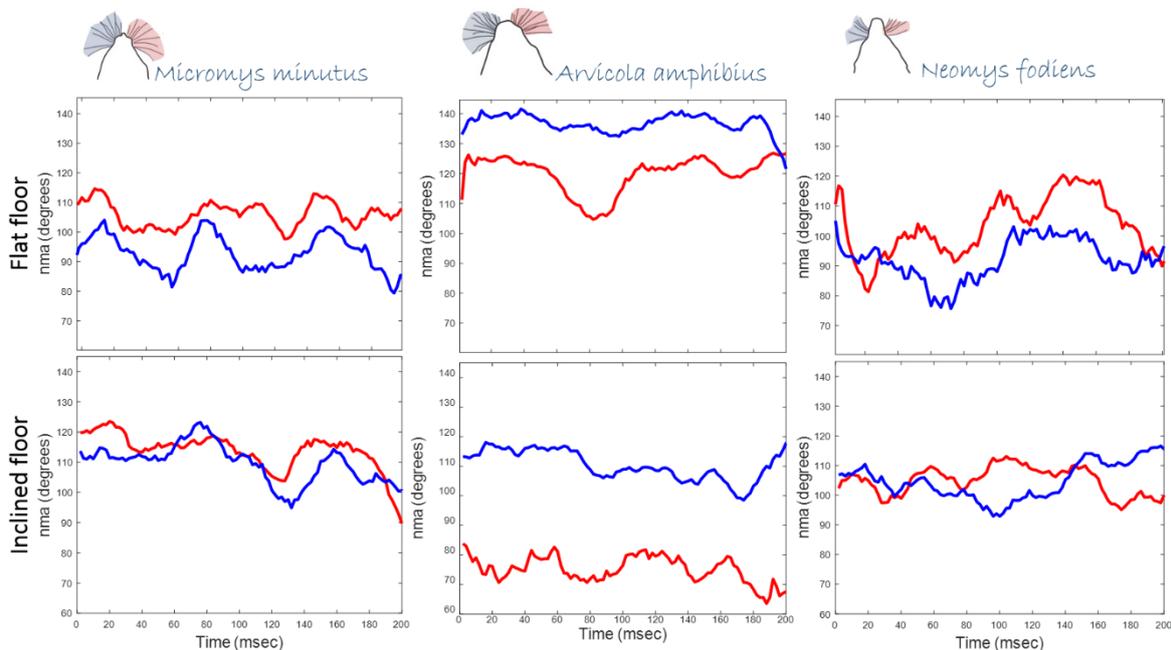
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641 **Supplement 4:** Whisker measurement results for each species locomoting on an inclined
 642 floor. Asterisks indicate a significant difference to the flat floor data in that variable. Values
 643 are mean \pm s.d.

Species	Loco Speed m/sec	Freq Hz	Off deg	Amp deg	Asym deg	Pro Speed deg/m s	Re Speed deg/m s
<i>Muscardinus avellanarius</i>	0.26 \pm 0.11	11.5 \pm 4.06	121.99 \pm 4.45	25.22 \pm 7.93	12.66 \pm 4.45	1.31 \pm 0.09*	0.10 \pm 0.08
<i>Apodemus sylvaticus</i>	0.34 \pm 0.26	16.08 \pm 7.05	117.28 \pm 10.09*	36.57 \pm 7.26*	11.95 \pm 3.78	1.48 \pm 0.14*	0.37 \pm 0.23
<i>Mycromys minutus</i>	0.33 \pm 0.14	12.88 \pm 6.44	102.10 \pm 5.41	34.49 \pm 8.75*	14.34 \pm 8.75*	1.60 \pm 0.22**	0.29 \pm 0.09**
<i>Rattus norvegicus</i>	0.32 \pm 0.14	10.13 \pm 2.29	99.39 \pm 12.48	29.44 \pm 8.98	14.34 \pm 3.43	1.25 \pm 0.04*	0.08 \pm 0.02*
<i>Neomys fodiens</i>	0.39 \pm 0.08	12.55 \pm 6.76	102.35 \pm 6.32	29.37 \pm 4.33*	12.18 \pm 2.07*	1.53 \pm 0.10	0.22 \pm 0.05*
<i>Arvicola amphibious</i>	0.61 \pm 0.24	9.55 \pm 1.56	114.73 \pm 7.27	26.54 \pm 12.51*	27.17 \pm 8.66	1.56 \pm 0.70	0.09 \pm 0.10*
<i>Cavia porcellus</i>	0.32 \pm 0.06	n.a.	106.60 \pm 16.99	49.30 \pm 24.45	30.72 \pm 23.52	n.a.	n.a.

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647 **Supplement 4, Figure 1.** Example mean whisker angle traces from the left (in blue) and right (in
 648 red) whisker field from three species locomoting on an inclined floor. Significant reductions could
 649 be observed in whisker amplitude when the animals were locomoting on the inclined floor, compared
 650 to the flat floor.

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