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**The evolution of active vibrissal sensing in mammals: evidence from
vibrissal musculature and function in the marsupial opossum *Monodelphis
domestica***

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28 List of abbreviations:

29	A	Attachment place
30	CCO	Cytochrome Oxidase
31	DAB	Diaminobenzidine
32	FB	Fibrous bundles
33	FBP	Furry Buccal Pad
34	FE	Fibrous extensions
35	HF	Horizontal fascicles (of intrinsic muscles)
36	M.	musculus
37	ML	M. maxillolabialis
38	MP	Pars maxillaris profunda
39	MS	Pars maxillaris superficialis
40	N	Nostril
41	NL	M. nasolabialis
42	NS	Nostril
43	NV	Nasal Vibrissae
44	OF	Oblique fascicles (of oblique intrinsic muscles)
45	PB	Phosphate Buffer
46	PIP	Pars interna profunda
47	PM	premaxilla
48	PMI	Pars media inferior
49	PMS	Pars media superior
50	R	Rostral
51	RCS	Royal College of Surgeons
52	V	Ventral

53

54

55 **Summary**

56 Facial vibrissae, or whiskers, are found in nearly all extant mammal species and are likely to
57 have been present in early mammalian ancestors. A sub-set of modern mammals, including
58 many rodents, move their long mystacial whiskers back-and-forth at high speed whilst
59 exploring in a behaviour known as “whisking”. It is not known whether the vibrissae of early
60 mammals moved in this way. The gray short-tailed opossum, *Monodelphis domestica*, is
61 considered a useful species from the perspective of tracing the evolution of modern
62 mammals. Interestingly, these marsupials engage in whisking bouts similar to those seen in
63 rodents. To better assess the likelihood that active vibrissal sensing was present in ancestral
64 mammals we examined the vibrissal musculature of the opossum using digital microscopy to
65 see if this resembles that of rodents. Although opossums have fewer whiskers than rats, our
66 investigation found that the vibrissal musculature is similar in both species. In particular, in
67 both rats and opossums, the musculature includes both intrinsic and extrinsic muscles with
68 the intrinsic muscles positioned as slings linking pairs of large vibrissae within rows. We
69 identified some differences in the extrinsic musculature which, interestingly, matched with
70 behavioural data obtained through high-speed video recording, and indicated additional
71 degrees of freedom for positioning the vibrissae in rats. These data show that the whisker
72 movements of opossum and rat exploit similar underlying mechanisms. Paired with earlier
73 results suggesting similar patterns of vibrissal movement, this strongly implies that early
74 therian (marsupial and placental) mammals were whisking animals that actively controlled
75 their vibrissae.

76 **Introduction**

77 The early evolution of mammals is associated with a number of significant milestones such as
78 the onset of thermoregulation, restructuring of the inner ear, and the emergence of a six-
79 layered cortex. Hair also appears in mammals for the first time, probably having a role in
80 tactile sensing prior to the appearance of pelage hair as a means of maintaining body
81 temperature (Maderson, 1972, 2003). Indeed, some of the first hairs may well have been
82 facial vibrissae, or whiskers, similar to those found in nearly all extant mammal species
83 (Pocock, 1914; Ahl, 1986). Many terrestrial mammals that have well-developed mystacial
84 whiskers, termed macrovibrissae, move them back-and-forth at high-speed during
85 exploration, a behaviour known as “whisking” (Wineski, 1985; Mitchinson et al., 2011).
86 Whisking is a form of active tactile sensing (Prescott et al., 2011) and, in rodents such as rats
87 and mice, is regulated by sensory feedback in a manner that appears to boost the sensory
88 information obtained by the animal (Mitchinson et al., 2007). We recently showed
89 (Mitchinson et al., 2011) that similar patterns of rhythmic macrovibrissal movement, also
90 under sensory guidance, are present in *Monodelphis domestica* (Wagner, 1842), a small
91 ground-dwelling marsupial native to South America, whose last common ancestor with
92 modern rodents was an early mammal of the Triassic period living more than 160 million
93 years ago (Luo et al., 2011). The presence of whisking in both rodents and marsupials
94 therefore presents the intriguing possibility that a common ancestor to all modern therians
95 (marsupial and placental mammals) may have employed active vibrissal sensing. However,
96 by observing behaviour alone it is not possible to rule out the possibility of convergent
97 evolution of whisking in rodents and the opossum. That is, both lines may have evolved the
98 capacity to actuate their whiskers at a later time, due to similar evolutionary pressures. Fossil
99 evidence is not able to resolve this issue as the few fossilized skulls of early mammals that
100 have so far been found have not preserved evidence of facial musculature (Ji et al., 2002;
101 Luo, 2007; Luo et al., 2011). The alternative strategy, pursued here, is to explore this
102 question via comparative physiology. Specifically, the hypothesis of convergent evolution of
103 active vibrissal sensing in both rodents and marsupials would appear much less likely if we
104 can obtain evidence that the underlying mechanisms are similar in these two groups of
105 whisking animals. To that end, in the current study we studied the anatomy of the opossum
106 mystacial pad making direct comparisons between the vibrissal musculature of this animal
107 and that of the common rat.

108 Whereas the vibrissal musculature of the rat has recently been described in detail (Haidarliu,
109 2010; Hairadliu, 2012), that of the opossum had not hitherto been mapped, we therefore
110 replicated the methodology of our earlier study of the rat facial musculature using
111 cytochrome oxidase (CCO) staining (Haidarliu, 2010) to obtain digital microscope images of
112 the opossum mystacial pad that could be directly compared with those of rodents. This
113 method identifies interesting similarities and differences in the vibrissal muscles of the two
114 species. Most importantly, we investigate here whether the large facial whiskers are moved
115 by an intrinsic sling-like musculature that connects each whisker follicle to the adjacent one
116 along each whisker row. There are many ways in which muscles might be configured in order
117 to provide whisker protraction, if this specific mechanism is present in both rodents and
118 marsupials it strongly indicates that whisking in rats and opossum is not convergent but
119 represents a shared inherited trait.

120

121 As well as controlling back and forth whisking movements, recently described extrinsic
122 muscles also control more complex whisker behaviours that can occur in both the protraction
123 and retraction phases of the whisk (Haidarliu et al. 2010). We have previously shown
124 (Mitchinson et al., 2011) that the periodic whisker movements of rats, mice, and opossums,
125 become bilaterally asymmetric (i.e. left and right whisker fields protract by different
126 amounts) under conditions where the animal is making a head-turn (head-turning asymmetry)
127 or making contact with an object at an oblique angle (contact-induced asymmetry). This
128 modulation is consistent with active sensing strategies that promote exploration in the
129 direction of movement, in the case of head-turning asymmetry, or increase the likelihood of
130 further whisker-object contacts in the case of contact-induced asymmetry. In an earlier study
131 (Grant et al., 2009) we also showed that when rats are exploring a vertical surface they also
132 reduce the angular separation, or *spread*, of their whiskers by differentially controlling the
133 velocity of movement of the more rostral and more dorsal whiskers (and possibly also by
134 reshaping the mystacial pad, see Haidarliu et al. 2010). Spread reduction also results in an
135 increased number of contacts with a surface that is being investigated and thus can be
136 understood as a further element of active sensing control. In this investigation, we explore
137 whether the corresponding extrinsic musculature is present in the opossum and similar to the
138 rat. In addition, we obtain new videographic data for the opossum to determine whether it is
139 able to differentially control the velocities of rostral and caudal vibrissae so as to modify
140 angular spread and retraction velocities.

141

142 Comparative analysis of the vibrissal musculature in these two distantly related mammalian
143 groups therefore casts new light both on the sensory capabilities of early mammals and, at the
144 same time, helps us to understand how modification of the facial musculature in vibrissal
145 specialists such as the rat has perhaps enabled modern rodents to better exploit this uniquely
146 mammalian sense.

147

148 **Materials and Methods**

149 **Muscle Staining**

150 *Animals*

151 Four 1-year-old opossums (*Monodelphis domestica*) were used in this study, 1 female and 3
152 males. The animals were obtained from a colony maintained by the University of Trieste, and
153 all procedures required for keeping and sacrificing the animals were approved by the local
154 Ethics Committee and communicated to the Ministerial Office, Trieste. For the purpose of the
155 current study the animals were anaesthetised with urethane (Urethane, 25%, 0.65 mL/100g
156 body weight) and perfused transcardially (100ml of phosphate buffer (PB) pH=7.4 followed
157 by a mixture of 2.5% glutaraldehyde, 0.5% paraformaldehyde and 5% sucrose in 0.1 M PB).
158 They were then decapitated, placed in the perfusion solution to which an additional 25%
159 sucrose was added and refrigerated overnight. The mystacial pads were then removed
160 bilaterally, by cutting down the sagittal plane and cutting around each pad (about 2 mm each
161 side of the pad). Any pieces of bone were removed from the pads and they were placed flat
162 between stainless steel grids in perforated plastic histology cases (Medex Supply) to prevent
163 curling. The histology cases were then put into a solution of 20% sucrose in 0.1M Phosphate
164 buffer pH 7.4 for 2 days and refrigerated. In addition to facial vibrissae the opossum also has
165 prominent genal (cheek) vibrissae. In one male and one female we therefore also removed
166 the genal whisker area and stored them in the same way.

167

168 *Staining of the mystacial pads*

169 After fixing, each of the pads was sectioned with a *Microm cryostar* cryostat into 50 μ m
170 slices. As there were eight pads (from four animals), six pads were sliced tangentially and
171 two pads were sliced coronally. The four genal areas were all sliced tangentially. All slices
172 were stained for CCO activity (based on Haidarliu et al. 2010) as follows. The slices were

173 floated in a solution of 10 ml of 0.1 M PB containing 0.75 mg Cytochrome C, 40 µl catalase
174 solution and 5 mg Diaminobenzidine (DAB) and 0.5 ml distilled water. They were then
175 placed in an incubator at 37°C on a shaking platform for 1-2 hours until the stain developed
176 (i.e. until there was a strong difference between reactive and unreactive tissues). The slices
177 were then rinsed in 0.05 M PB, mounted in distilled water and left to air dry briefly before
178 coverslipping with Entellan.

179 Figures of the stained musculature were prepared from digital images. A Nikon light
180 microscope with objectives 1x, 2x, 20x, 40x was used to obtain the images which were
181 collected in SPOT and exported as .tif images. Some panels in Figure 4 were collected on
182 Leica fluorescent microscope with a blue filter. Only small adjustments in contrast and
183 brightness were made to the figures. The intrinsic muscle figure (in Figure 2a) was made by
184 combining two consecutive slices.

185

186 **Xylene clearing of skin**

187 One male 2-year-old opossum was used to get a clear picture of the pad layout, by clearing
188 with xylene as follows. The mystacial pads and genal areas were removed from each side, as
189 above. The samples were then shaved, the fatty layers removed from the bottom with a
190 scalpel, pressed flat and dehydrated with ethyl alcohol (50%, 70%, 95% and 100%), then
191 immersed in xylene until they became transparent. The prepared samples were then lit from
192 below with a spotlight and photographed using a Canon digital camera.

193

194 **Behaviour Filming**

195 *Animals*

196 Behavioural clips were collected for a previous study (Mitchinson et al. 2011), but re-
197 analysed for the current study. 26 opossums (*Monodelphis domestica*), each around one year
198 old, were filmed using high-speed videography at the University of Trieste animal facility.
199 The experimental setup was as described in Mitchinson et al. (2011). Specifically, a high-
200 speed, high-resolution (1024x1024) digital video camera (*Photron Fastcam*) was suspended
201 above a transparent viewing arena illuminated from below with a custom-built light box.
202 Animals were placed into the arena, one at a time, and allowed to freely explore. In half the
203 trials, a perspex block was placed in the arena beneath the camera field-of-view. Video data
204 was collected in near darkness using an infrared light box for illumination. Multiple 1.6
205 second video clips, at 500 frames per second, were collected opportunistically (by manual
206 trigger) when the animal moved beneath the field of view of the camera. Comparison data

207 from nineteen rats (12 Royal College of Surgeons (RCS) dystrophic rats, 7 Hooded Listers)
208 was collected under similar circumstances, at University of Sheffield animal facilities, with a
209 light-box operating in the visible light spectrum. Previous investigations have found that the
210 whisker movements of genetically-blind (dystrophic) and sighted animals are similar in these
211 conditions with both strains displaying modulation of whisker movement indicating the use
212 of active touch sensing strategies (Mitchinson et al., 2007, 2011; Grant et al., 2009).

213

214 *Clip selection and whisker tracking*

215 Clip selection was based on the criteria given in Mitchinson et al. (2011). Specific clips were
216 chosen to include i) episodes of *regular whisking*, when the animal was moving its whiskers
217 periodically without contacting anything but the smooth floor; and/or ii) episodes of *contact*
218 *whisking*, where the animal was contacting one corner of a perspex block with its whiskers,
219 with no nearby obstructions, other than the floor. The animal's snout and whiskers were
220 tracked in each clip episode, using the BIOTACT Whisker Tracking Tool (Mitchinson et al.,
221 2011, Perkon et al., 2011). The output of the tracker, for each video frame, is the orientation
222 and position of the snout, and a set of "whisker curves", from which whisker angular position
223 (relative to the midline of the head), for each identified whisker, can be derived. For the
224 opossum, tracking parameters were set such that only the mystacial vibrissae were tracked
225 and not the genal vibrissae (but see below for measurements of genal whisker motion).
226 Tracking was validated by manually inspecting the tracking overlaid on to the video frames.

227 Our analyses focus on the movement of the entire whisker field on each side of the snout
228 using a measure of *mean angular position* calculated as the unsmoothed mean of all the
229 tracked whisker angular positions on each side and in each frame. We also computed a
230 measure of horizontal dispersion of the whiskers in each field termed the mean *angular*
231 *spread* and calculated as the standard deviation of all the tracked whisker angular positions in
232 each frame. Finally, we computed mean angular *retraction* and *protraction velocities*,
233 calculated from the angular position, as the average velocity of all the backward (negative)
234 whisker movements, and forward (positive) whisker movements, respectively. These
235 measures were all averaged to give a mean value per clip. Note that there was no tracking of
236 whiskers across frames, therefore the number of identified whiskers varied across frames and
237 clips. Our previous investigation (Mitchinson et al., 2011) demonstrated that these automated
238 estimates of whisker motion provide a good proxy for estimates calculated by tracking by
239 eye. In the current study, both the mean angular position and angular spread measures were
240 further validated by examining video tracking and plots of each clip. To reduce noise, we also

241 included clips only if there were four or more whiskers tracked on each side of the face, for
242 the entire episode of interest. This led to the inclusion of 30 and 51 regular whisking clips,
243 and 23 and 43 contact whisking clips, respectively, for rat and opossum. Examples of raw
244 data can be found in Figure 7. Identical tracking and analysis routines were used with both
245 opossum and rat clips which should ensure similar levels of noise in the tracking data.

246

247 To examine the capacity for movement in the genal whiskers we also tracked, by visual
248 inspection using a manual whisker tracking tool as described in Mitchinson et al. (2007), one
249 genal whisker and two macrovibrissae (one in each of the left and right whiskers fields) in
250 twelve clips of regular whisking, between 0.55 and 1.3 seconds in length.

251

252 **Results**

253

254 **Whisker layout and general appearance**

255 Opossum whiskers are arranged in a grid-like pattern of rows and arcs (Figure 1a and c),
256 visually somewhat similar to that seen in rats (compare Figure 1b and d), but with twenty-
257 three whiskers in total on each side compared to thirty (typically) in the rodent species. In the
258 opossum, the whiskers form four rows, labelled A-D (Figure 1c), with three to seven large
259 whiskers in each, whereas the rat has five rows of up to seven whiskers each. The dorsal-most
260 row A of the opossum contains just three whiskers (A0, A1 and A2), row B, four whiskers
261 (B1 –B4), and each of the two ventral rows (C and D) has seven whiskers (C0 – C6, and D0 –
262 D6). The overall grid-like pattern is disturbed by the two “straddler” whiskers, α and β , at the
263 caudal edge of the pad, whose horizontal alignment is offset relative to the main whisker
264 rows such that α is positioned between the rows A to B, and β between B to C (Figure 1c).
265 Vertically, these whiskers are reasonably well aligned with the most caudal whiskers in rows
266 A, C, and D, such that they form part of the first whisker arc (A0, α , β , C0, D0) (Figure 1g).
267 For comparison, the rat whisker array has four straddlers offset vertically relative to the five
268 whisker rows (Figure 1d) and appearing to form their own arc. Overall, the mystacial pad is
269 generally less prominent in the opossum compared to the rat (compare Figures 1a and b), the
270 opossum also lacks the prominent microvibrissal array—the short non-actuated whiskers on
271 the chin and upper lip—that is seen in rodents such as rats and mice. The opossum does,
272 however, have a few nasal whiskers arranged in a horizontal row (Figure 1c, labelled NV),

273 which have rudimentary sling-like muscles (Figure 1e, on the most dorsal most follicles,
274 compare positions to NV in Figure 1c). In addition there is a furry buccal pad (FBP, the area
275 of the pad containing follicles ventral and caudal to the whisker pad), with hair follicles that
276 are not arranged into rows (Figure 1c). The whiskers of the furry buccal pad differ from the
277 whiskers of the mystacial pad in that they are smaller and have no intrinsic muscles. Part of
278 the buccal pad is positioned more caudally than the mystacial whiskers, and the ventral
279 fascicles of the musculus (*m.*) *nasolabialis* and *m. maxillolabialis* separate the furry buccal
280 pad from row D (Figure 1e, note the FBP is below the dark muscle staining in the ventral area
281 of the pad). Opossum whisker follicles have a well-defined capsule, and can contain both
282 cavernous and ring sinuses, sometimes the ringwulst (a doughnut shaped structure) can be
283 observed in large whiskers (see, e.g. Figure 2b), thus structurally they have many similarities
284 to the vibrissal follicles of the rat.

285
286

287 **Intrinsic Muscles**

288 In tangential slices cut through the entire opossum snout and stained for CCO activity,
289 intrinsic muscles are easily defined as short, dark-brown strips connecting the large
290 neighbouring whiskers in each row (Figure 1e and 2a, b and c). A key finding is that within
291 each of the rows, the whiskers are connected to each other by sling-like intrinsic muscles as
292 previously described for a variety of whisking rodents (see Discussion). Although there is
293 substantial similarity in this overall arrangement there are some interesting differences
294 between the opossum and rat musculature that we detail next.

295

296 In the rat, intrinsic muscles connect the straddler whisker β to both rows B and C, and γ with
297 both rows C and D (Haidarliu and Ahissar 1997, and Figure 1f). However, in the opossum,
298 the situation is inverted, as whisker B1 connects to, or “straddles” the two large caudal
299 whiskers (α and β), such that the dorsal-most part of the B1 intrinsic muscle attaches to the
300 ventral part of α , and the ventral-most part of the B1 intrinsic muscle attaches to the dorsal
301 part of β (Figure 2b and c). Due to these differences, henceforth we will refer to the α and β
302 whiskers as *inverted-straddlers*.

303

304 In rows A and B, in the opossum, in addition to intrinsic muscles composed of a sling and
305 two caudally-directed horizontal muscle fascicles (HF in Figure 2c), each pair of whiskers is

306 also joined by a supplementary intrinsic muscle fascicle that connects the ventral capsular
307 surface of the more rostral whisker follicle with dorsal capsular surface of a more caudal
308 whisker follicle in the same row (OF in Figure 2c). From the capsule of the caudal-most
309 whisker in opossum row A (A0), a third muscle strip also originates. It is directed
310 dorsocaudal and inserted into the *corium*, the base layer of the mystacial pad. The location of
311 the origin and insertion sites of these oblique muscle strips suggests that they might serve for
312 torsional rotation of the whiskers in rows A and B during protraction, and simultaneously for
313 ventral whisker shaft deflection that may result in whisker palpation of the objects from
314 above. Interestingly, the presence of such oblique muscle strips, termed here *oblique intrinsic*
315 *muscles*, has not been reported in any other whisking species.

316

317 That the dorsal two whisker rows in the opossum are notably different to the ventral two rows
318 in their intrinsic musculature suggests that there might be a compartmentalisation of the
319 whisker pad. If so, the layout in the opossum could be considered as having a *nasal*
320 *compartment* containing two rows of whiskers (A and B) and the two inverted-straddlers, and
321 a *maxillary compartment* containing two rows of seven whiskers without straddlers (Figure
322 1c). In the nasal compartment, the two inverted-straddlers fortify the dorso-caudal section of
323 the mystacial pad, which is well-aligned with the position of the eye (Figure 1a, g); indeed,
324 Figure 1g shows that the inverted-straddler whiskers are arranged directly in front of the eyes
325 and might help to protect this sensitive area.

326

327 **Extrinsic Muscles**

328 We next compare the extrinsic muscles of the *Monodelphis domestica* mystacial pad with
329 those of the rat. The extrinsic muscles in the rodent mystacial pad have been called a variety
330 of names; the terminology used here agrees with the nomenclature used by Hairdarliu et al.
331 (2010), and also conforms to *Terminologia Anatomica* (1998).

332

333 *Superficial retracting muscles.* In the opossum, the most superficial muscles of the mystacial
334 pad, are the *M. nasolabialis* and *M. maxillolabialis* (Figure 3) which both attach caudal to the
335 mystacial pad. *M. nasolabialis* is a striated muscle (Figure 3a,b) that originates from the
336 maxilla and os nasale, rostral to the orbit. It runs between the caudal part of the whisker rows.
337 The *M. maxillolabialis* is also a striated muscle (Figure 3c,d) that is slightly deeper than the
338 *M. nasolabialis*. It originates from the ventrocaudal part of the maxilla, and its fibres pass
339 under the *M. nasolabialis* (Figure 3c), and then run rostrally, between the vibrissae rows,

340 from B to D. Contraction of these muscles pulls the corium of the mystacial pad caudally,
341 causing a retraction of the vibrissae. This is similar to what has been observed in the rat
342 (Haidarliu et al. 2010).

343

344 *Deep retracting muscles.* In the rat, the deep retracting muscles originate deep and rostral to
345 the pad and then run most of the way down the entire pad, whereupon they insert deeply into
346 the mystacial fibrous plate (Haidarliu et al. 2010). The three muscles have been named, in
347 dorso-ventral order, as different parts of the *M. Nasolabialis profundus*—the *Pars interna*
348 *profunda*, *Pars maxillaris superficialis* and *Pars maxillaris profunda*. The opossum similarly
349 has three muscles also arranged dorso-ventrally that have very similar origins (Figure 4b).
350 Since these appear to correspond to the same muscles as those of the rat it is appropriate to
351 use the same nomenclature. All three *M. Nasolabialis profundus* muscles in opossum lie in a
352 rostral-to-caudal direction and are connected to fibrous collagenous bundles situated between
353 the vibrissal rows (Figure 4a, c, d). These bundles, which have been identified as collagen, as
354 they possess a blue autofluorescence at a typical wavelength (Figure 4e, f), have rosette-like
355 ends where they attach to the muscle fibres rostrally (Figure 4a, c, d, e) and belong to the
356 three parts of the *M. nasolabialis profundus* (see also Figure 10b). *Pars interna profunda*
357 attaches to a collagen bundle that runs between rows A and B, *Pars maxillaris superficialis*
358 attaches to a collagen bundle that runs between rows B and C, and *Pars maxillaris profunda*
359 attaches to a collagen bundle that runs between rows C and D (Figure 4f). Deep in the pad,
360 the bundles contact the fibrous mat beneath the mystacial pad (Figure 4g). If these muscles
361 contract, the collagenous bundles pull the mystacial fibrous plate rostrally, which should
362 retract the vibrissae in much the same way as in the rat. In terms of their general appearance,
363 these muscles in the opossum appear to have a similar function to those of the rat; however,
364 they are much shorter and actuate the pad via collagen bundles, unlike in the rodent species
365 where the muscles run the full length of the pad. This is therefore an important difference
366 between the two species.

367

368 *Extrinsic protraction muscles.* A further set of mystacial muscles comprises the extrinsic
369 protracting muscles which are also different parts of the *M. Nasolabialis profundus*. In
370 opossum, the *Pars media superior* (PMS) and the *Pars media inferior* (PMI) originate rostral
371 to the *Pars maxillaris profunda* origin from the septum intermusculare and rostral tip of the
372 premaxilla (os incisivum) (Figure 5a and b). These attachment points are indicated by the
373 black arrow in Figures 5a and 5b. These muscles are relatively short dorsoventrally (Wible,

374 2003) compared to the rat. Specifically, from their rostral attachment points, both muscle
375 parts run caudally, between whisker rows A to D, until vibrissal arc 2 (Figure 5c). Figure 5c
376 shows fragments of transected PMI and PMS which continue caudolaterally, and are inserted
377 into the corium of the mystacial pad between all vibrissal rows and ventral to row D. In
378 summary then, whereas in the rat the extrinsic protraction muscles are strongly inserted into
379 the corium throughout every whisker row, in the opossum these muscles have a weaker
380 presence throughout the pad and are absent from the more caudal sections.

381

382 **Genal Whiskers**

383 As well as the mystacial whiskers, the opossum also has genal whiskers. These are six
384 whiskers that are arranged in a horseshoe-shape in the cheek area (Figure 6a). The genal
385 whiskers have striated intrinsic muscles, but rather than being attached to each other, they are
386 attached mostly to the skin superficial collagenous layer. Genal whisker follicles have a
387 strong capsule, and can contain both cavernous (with mesh and trabeculae), and ring sinuses;
388 sometimes the ringwulst can be observed.

389

390 **Behavioural consequences of differences in mystacial musculature**

391 We have described above several interesting differences in mystacial muscle groups in the
392 opossum compared to those of the rat—in the intrinsic muscles, the deep retracting muscles
393 and the extrinsic protracting muscles. We next compare rat and opossum behavioural data
394 between regular (non-contact) whisking and contact episodes in order to establish whether
395 there is any identifiable consequence of the differences in mystacial musculature on
396 observable whisking and active sensing behaviour. In particular, we hypothesized that the
397 different extrinsic muscle organisation in the two species might impact on their capacity to
398 modify relative whisker movement velocities following contact, and so cause measurable
399 differences in the angular spread of the whiskers. As described in the methods, we calculated
400 the *mean angular whisker positions*, *mean angular spread*, *mean retraction velocity* and
401 *mean protraction velocity* for 30 and 51 regular (non-contacting) whisking clips, and 23 and
402 43 contact whisking clips, respectively, for rat and opossum. Traces from the raw
403 behavioural data, shown in Figure 7(a,b), illustrate that during regular whisking bouts
404 opossums move their whiskers in a sinusoidal, back-and-forth, pattern similar to that in the
405 rat. In our previous analysis (Mitchinson et al., 2011) we found that rats whisk at a slightly
406 higher frequency, during regular whisking episodes, compared to opossum (mean 8.7hz
407 compared to 7.3hz) and with somewhat larger amplitudes (mean 43° compared to 36°). In

408 both species, the whiskers spread out more as they protract forward and bunch together as
409 they retract (Figure 7c,d), this is likely to be largely as a consequence of the morphology of
410 the system rather than due to active control (Grant et al., 2009).

411

412 Whereas the general form of whisking is similar in the two species during regular whisking
413 bouts, in rat, there is a substantial decrease in angular spread of the whiskers following and
414 during contact with an object (between ANOVA: $F(1,52)=5.473$, $p=0.023$), as shown in
415 Figure 8a. This is consistent with our earlier study (Grant et al., 2009) and has also been
416 noted in our laboratory to be a bilateral effect in this species (unpublished data). In
417 comparison (see Figure 8a), there is no evidence of any change in spread in the opossum
418 following contact with an object (between ANOVA: $F(1,93)=0.013$, $p=0.910$). Similarly, as
419 shown in Figure 8b, there is also a large decrease in retraction velocity following contact with
420 a vertical surface in rat (between ANOVA: $F(1,54)=4.813$, $p=0.033$) which is not matched in
421 opossums (between ANOVA: $F(1,95)=0.316$, $p=0.575$). There are no significant differences
422 in whisker protraction velocities (8c) and set-point (the average mean angular position, see
423 8d) between regular whisking and contact whisking in either species. Example digital video
424 frames, shown in Figure 9, further illustrate that whilst the rats reduce their whisker spread
425 following a contact, in opossums, whisker spread remains largely unchanged during object
426 exploration.

427

428 As shown in Figure 6, the genal whiskers, which can be clearly seen at the caudal edge of the
429 cheek in Figure 9(b,d), themselves have sling-like muscles with some similarities to those
430 that move the macrovibrissae. To evaluate the capacity for independent movement in these
431 whiskers we examined 12 exemplar clips of opossum regular whisking. During bouts of
432 whisking with the mystacial vibrissae typically also saw some lower-amplitude sinusoidal
433 motion in the tracked genal whisker as illustrated in Figure 10. Genal whisker motion was
434 largely synchronized with movement of the mystacial vibrissae, however, macrovibrissal
435 movement does not appear to entail movement of the genal whiskers as illustrated in Figure
436 10 where there are whisk cycles during which the macrovibrissae move sinusoidally but the
437 tracked genal whisker does not. This finding is consistent with the genal whisker motion
438 being driven, at least in part, by its own intrinsic muscle.

439

440 **Discussion**

441 The opossum mystacial pad has a grid-like follicle layout, with nasal and maxillary
442 compartments. There are fewer whisker rows than in rat, however, in both species the
443 whiskers are coupled together within rows by horizontal intrinsic muscles, a distinctive
444 pattern suggesting that a similar arrangement may have existed in an early mammalian
445 common ancestor. Similarly, opossums and rats also have generally similar extrinsic
446 musculature that can reshape the mystacial pad and drive whisker retraction and protraction
447 movements.

448

449 We also noted some interesting differences between the rat and opossum vibrissal
450 musculature. First, we found oblique intrinsic muscles in the opossum that have not been
451 described in any other species. Second, we found whiskers that are connected to each other
452 by a distinctive arrangement of intrinsic muscles, that we term inverted-straddlers, since this
453 reverses (rostrocaudally) a pattern seen in rats. The superficial extrinsic muscles were
454 generally similar to those observed in rats, but the deep retracting and extrinsic protracting
455 muscles appeared to be considerably less well-developed in the opossum, contributing to the
456 overall appearance of the mystacial pad as less substantial in opossum compared to rat.
457 Indeed, within the opossum mystacial pad, muscle territory is reduced, whilst collagen
458 representation is relatively enriched compared with the whisker pad in the rodent species.

459

460 Some of these differences in mystacial musculature seem likely to relate to qualitative
461 differences in the capacity of the animal to control their whiskers during active sensing
462 behaviours. In particular, analyses of rat whisking during exploration of objects, here and in
463 Grant et al. (2009), have shown that these animals have the capacity to reduce the angular
464 spread of their whiskers in a manner that increases whisker tip contact with surfaces of
465 interest. In the analysis of opossum whisking behaviour reported above we found no evidence
466 of a similar capacity in this species, suggesting that this may have evolved separately within
467 the lineage of modern rodents.

468

469 **Comparisons with other species**

470 Figure 11 summarises the arrangement of the opossum snout muscle system, we next discuss
471 some similarities and differences to other mammalian species in which the whiskers and
472 facial musculature have been investigated.

473

474 *Organisation of the facial vibrissae*

475 The layout of the opossum mystacial pad is quite similar to that of the wallaby (Waite et al.
476 2006) as there seem to be four rows of vibrissae in both species, with two nasal whiskers
477 present in the dorsal-most row. Weller (1993) also found two dorsal-most whiskers in brush-
478 tailed opossums, however, that study reported six rows of whiskers in total (or five if their
479 row A is labelled as nasal whiskers). It seems possible that the relatively large size of the
480 furry buccal pad whiskers might lead them to be confused with the mystacial whiskers,
481 indeed, Weller (1993) described the whiskers in the lower rows as “numerous and difficult to
482 count” (p. 21). By looking at the muscles of the pad it is possible to clearly distinguish the
483 mystacial whiskers from the nasal whiskers and furry buccal pad. If these whiskers have been
484 mis-labelled as mystacial pad whiskers in the Weller study then it would appear that the
485 opossum does have a very similar whisker layout to other marsupials in which the whiskers
486 have been investigated.

487

488 Compared to other mammals in which whisking has been recorded (see Mitchinson et al.,
489 2011) the opossum has relatively few whiskers, indeed far less than hamsters (Wineski 1985;
490 Haidarliu et al. 1997), rats (Haidarliu et al. 2010), mice (Dorfl, 1982) and shrews (Kulikov,
491 2011). Amongst rodents, the layout of the guinea pig mystacial pad is perhaps more similar to
492 the opossum (Haidarliu et al. 1997), containing a row of nasal whiskers and only two
493 whiskers in row A.

494

495 *Intrinsic musculature*

496 Intrinsic muscles that couple rostral and caudal whiskers within the same row have also been
497 described in mice (Dörfl 1982), hamsters (Wineski 1985), guinea pigs (Haidarliu et al. 1997),
498 rats (Haidarliu et al. 2010) and shrews (Yohro 1977), lending confidence to the view that this
499 may be a primitive mammalian trait. A new finding in the opossum is the layout of the
500 inverted-straddlers in the dorso-caudal area of the pad, and the presence of oblique intrinsics
501 muscles in the two dorsal whisker rows. In a study of big-clawed shrew, Yohro (1977) also
502 found differences in the dorsal and ventral whisker rows but with the ventral whiskers having
503 more muscles “straddling” between whisker rows. We might expect to find variations in this
504 part of the pad in other species, and in the future it might be interesting to attempt to relate
505 these differences to behavioural specialisations. Here, we also found differences between the
506 dorsal and ventral whisker layouts suggesting compartmentalisation of the pad.

507 Compartmentalisation in mice was described by Yamakado and Yohro (1979), who identified
508 a nasal compartment (rows A and B) and a maxillary compartment (ventral rows) of
509 whiskers that develop from different growth centres in embryo. Different growth centres are
510 also likely to be responsible for the nasal and maxillary divisions in the opossum pad too, and
511 so potentially this might be a primitive mammalian feature.

512

513 A further novel finding in opossum are the bundles of intrinsic muscles in row A and B that
514 originate from the ventral surface of the rostral whisker capsule and are inserted into the
515 dorsal surface of the neighbouring caudal whisker. The position and attachment of these
516 muscles suggest that they cause a torsional rotation of whisker rows A and B. Torsional
517 rotation of whiskers in rat was first described by Knutsen et al. (2008) who proposed that
518 torsional movements could allow ventral whisker rows to scan downwards whilst dorsal
519 whisker rows scan upwards. As we were only filming from above in the current study we are
520 unable to make estimates of the torsional rotation of whiskers, however, based on the muscle
521 layout in opossum it seems possible that the dorsal two rows may rotate more than the two
522 more ventral rows.

523

524 *Extrinsic musculature*

525 Superficial extrinsic muscles that drive retraction movements of the vibrissae, and similar to
526 those seen in opossum, have been described in hamsters (Wineski 1985), mice (Dorfl 1982,
527 Klingener 1964), rats (Haidarliu et al. 2010), jerboas (Klingener 1964), didelphid opossums
528 (Minkoff et al. 1979) and shrews (Yohro 1977). However, in the big-clawed shrew, the
529 striated *M. nasolabialis superficialis* is also associated with smooth muscle fibres just
530 beneath the corium (Yohro 1977), unlike the rat and opossum, suggesting that there are
531 variations in extrinsic musculature between different species.

532

533 The different components of the *M. nasolabialis profundus* muscle group have been
534 described in mouse (Dorfl 1982, Klingener 1964, Rinker 1954), hamster (Wineski 1985) and
535 rat (Haidarliu et al. 2010, Rinker 1954). The deep retracting muscles of the *M. nasolabialis*
536 *profundus* can be slightly different in placement or characteristics between rodent species
537 (Wineski 1985, Haidarliu et al. 2010), however, in general, these muscles pull the deep layers
538 of the whisker pad forward, so that the whiskers are retracted back. In rat, whisker retractions
539 are found to be faster than protraction movements, and both types of movement may be
540 actively controlled (Berg and Kleinfeld 2003). For instance, Grant et al. (2009) found that,

541 following a contact, whisker retraction velocities slowed down in the subsequent whisk cycle.
542 In opossum the equivalent deep retraction muscles are relatively reduced and attach rostrally
543 to collagen fibers. This difference in musculature possibly also explains the lack of retraction
544 velocity control when the opossum explores objects.

545

546 The extrinsic protracting muscles also vary between rodent species (Rinker 1954, Klingener
547 1964), in placement especially. In some rodents they have been described as having
548 characteristics similar to the superficial extrinsic muscles (Rinker 1954, Klingener 1964),
549 being flat and running in the same plane. However, Dorfl (1982) and Haidarliu et al (2010),
550 have found them to run in a different plane. Dorfl (1982) found the muscles to insert between
551 the five rows of follicles in mouse, whereas Haidarliu et al. (2010) found them to be present
552 in rat, between the five whisker rows and also dorsal and ventral to the whisker rows. In the
553 opossum we see that they run between rows A-B, B-C, C-D and also lie ventral to row D.
554 Haidarliu et al. (2010) used the term “focusing” to describe the extrinsic protracting muscles,
555 hypothesising that they help bring about the reduction in rostrocaudal spread of the whisker
556 field that has been described during foveal whisking (Berg and Kleinfeld 2003) and surface
557 investigation (Grant et al. 2009, 2012). That the opossum does not appear to reduce whisker
558 spread during object exploration could be due to a number of differences in these muscles.
559 First, the difference in attachment positions of the muscles between rodents and opossums
560 might cause changes in spread reduction behaviour, a comparison between the attachment
561 sites can be seen in Figure 12). Second, the muscle bundles in the opossum appear to be
562 weaker, or attached partially to other places, which could change the direction of the muscle
563 fibres. Overall this could reduce the capability to reshape the mystacial pad in a manner that
564 could be used to bring the whiskers closer together.

565

566 *Genal whiskers*

567 Many orders of mammals have genal whiskers, including marsupials (e.g. the gray short-
568 tailed and Virginia opossums and the Tasmanian devil), edenta (e.g. hairy armadillos),
569 insectivora (e.g. tenrecs), chiroptera (e.g. bats), primates (e.g. lemurs, aye ayes), carnivora
570 (e.g. jackals) and rodents (e.g. dormice) (Pocock 1914). However, their position on the face
571 and the number of whisker can vary immensely. In *Monodelphis domestica* we usually see
572 six genal whiskers positioned mid-way between the edge of the mouth and the bottom of the
573 ear, well below the eye. During macrovibrissal whisking we have seen evidence of smaller
574 amplitude movements in the genal whiskers largely synchronised with those of the

575 macrovibrissae. We believe this is the first report of sinoidal whisker motions in non-
576 mystacial whiskers. In the current study we have also shown that these whiskers have sling
577 muscles, similar to those involved in protracting the mystacial vibrissae, and suggesting that
578 genal whisker movements may be actively generated rather than simply being a consequence
579 of the movement of the extrinsic musculature of the nearby mystacial pad. Genal intrinsic
580 muscles attach to the skin rather to other whisker follicles. During evolution of the mystacial
581 whiskers it seems plausible that sling-like muscles, that originally anchored the whiskers to
582 the skin, may have adapted to attach themselves to neighbouring follicles along the principle
583 axis of whisker motion. Simulation results from Simony et al. (2010) show that contraction of
584 an intrinsic muscle in the rat causes movement of both attached whiskers. The evolutionary
585 step of chaining the intrinsic muscles would therefore have enhanced their effectiveness in
586 protracting the mystacial whiskers whilst also providing a stronger coupling of the movement
587 of adjacent vibrissae.

588

589 **Broader implications for understanding mammalian evolution**

590 The presence of whisking in both rodents and marsupials, shown in our earlier study
591 (Mitchinson et al. 2011) implies that a common ancestor to all modern therians (marsupial
592 and placental mammals) may have also employed active vibrissal sensing. The current study
593 provides compelling new evidence that whisking in rodents and marsupials uses similar
594 underlying mechanisms and is therefore unlikely to be convergent but an inherited trait, in
595 other words, that early mammals were also whisking animals.

596 If active vibrissal sensing was present at an early stage in the evolution of modern mammals
597 then it will have had greater impact on the broader course of mammalian evolution than has
598 hitherto been realized. Indeed, if early mammals actively moved their vibrissae, then this has
599 important implications for many other aspects of mammalian evolution. For instance, the
600 evolution of whisking musculature will have strongly shaped the broader evolution of the
601 muscles of the face (see Huber, 1930). Further, the need to control the movement of the
602 vibrissae will have brought about changes in sensorimotor circuits in the brain. Interestingly,
603 the opossum cortex lacks a distinct motor area, and the only area of the body in which
604 movement can be elicited by direct cortical microstimulation appears to be the vibrissal region
605 around the snout (Frost, et al., 2000). If this represents an ancestral condition it suggests that
606 the vibrissae musculature may have been one of the first, if not the first, area of the motor
607 system to have come under cortical control. Cortical influence on movement generally arises

608 where more sophisticated patterns of control are needed than can be realised by brainstem or
609 spinal circuits. Our results suggest the intriguing possibility that active vibrissal sensing may
610 therefore have been a key driver for the evolution of cortical motor control in early mammals.

611

612 Opossums are generalists, having excellent eye-sight and hearing, whereas rats are more
613 specialised towards tactile sensing having a comparatively poor visual sense. In progressing
614 to become more of a specialist in vibrissal sensing, rodents such as the rat appear to have
615 evolved a modified configuration of the snout and whiskers (compare e.g. Figures 9a and 9b)
616 in which, in addition to an increased number of whiskers and new degrees of freedom of
617 whisker movement (spread control), the snout has become visibly shorter and blunter.
618 Simulation and robotic studies (e.g. Prescott et al., 2009; Pearson et al., 2011; Towal et al.,
619 2011) have shown that the morphology of the snout and vibrissal array shapes a sensory
620 surface formed by the whisker tips and so helps determine the nature of the information that
621 can be obtained by animal. Moving from a more conical snout, as seen in the opossum, to the
622 rounder shape of the rat, and adding additional macro- and microvibrissae, alters the shape of
623 this surface and, in particular, substantially enhances the capacity of the animal to explore,
624 through vibrissal touch, the area in front of the head and around the snout tip.

625

626 **Conclusion**

627 The gray short-tailed opossum, *Monodelphis domestica*, engages in active vibrissal sensing
628 behaviours that have also been described in mice and rats. We found that the mystacial
629 muscle system of the opossum is very similar to that of rodent whisker specialists, containing
630 the four key muscle groups: the intrinsics, the superficial extrinsics, the deep retracting and
631 the extrinsic protracting muscles. Similarities in whisking behaviour, and mystacial pad
632 morphology and musculature, between these distantly related species lends confidence to the
633 view that a common ancestor of modern therian mammals possessed an active vibrissal
634 sensing system.

635

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796

797

798

799 FIGURE LEGENDS

800

801 **Figure 1. The layout of the *Monodelphis domestica* mystacial pad in comparison to that of the**
802 **rat.** (a) Photograph of the possum head, the mystacial pad (whiskers removed) is highlighted in the
803 black box. (b) Photograph of the rat head, mystacial whiskers and pad highlighted in the black box.
804 (c) A map of the macrovibrissal follicles revealed in ethanol/xylene-cleared entire snout preparation in
805 the opossum. The furry buccal pad (FBP) is indicated. (d) A map of the macrovibrissal follicles

806 revealed in ethanol/xylene-cleared entire snout preparation in the rat (adapted from Haidarliu et al.
807 2010). NV is nasal vibrissae, NS in nostril. (e) A slice of the opossum mystacial pad, stained for
808 cytochrome oxidase activity and revealing the sling-like intrinsic musculature. (f) A slice of the rat
809 mystacial pad, stained for cytochrome oxidase activity (adapted from Haidarliu et al. 2010), N is
810 nostril. (g) Close-up of the opossum whisker layout from the front (h) Close-up of the rat whisker
811 layout from the front.

812 **Figure 2. Opossum intrinsic musculature.** (a) General layout of the intrinsic muscles. (b) A close-
813 up of the α and β “inverted-straddlers” whisker B1 can be seen to be straddling α and β (see also panel
814 c). (c) Oblique intrinsic muscles—as well as the horizontal fascicles (HF) of the intrinsic muscles,
815 there are also the oblique fascicles (OF) that connect, for example, the ventral capsular surface of A1
816 with the dorsal capsular surface of A0. Scale bars are 1mm.

817 **Figure 3. Opossum superficial muscles *M. nasolabialis* (NL) and *M. maxillolabialis* (ML)** (a)
818 Attachment of NL to the mystacial pad. (b) Close-up of the striated NL muscle. (c) NL and ML can
819 both be seen in a more superficial slice than that in panel b. (d) Close-up of the striated ML muscle
820 fibres.

821 **Figure 4. Deep retracting vibrissal muscles in the opossum.** (b) View of the of the mystacial pad
822 around the nose, showing the deep retracting muscles *Pars interna Profunda* (PIP), *Pars maxillaris*
823 *profunda* (MP) and *Pars maxillaris superficialis* (MS). (a, c-d) Close-ups at different levels through
824 the pad of PIP, MS and MP, respectively, attached to their corresponding collagen fibres. (e) Close-up
825 of the collagen bundle attaching to the PIP muscle by rosette-like endings prepared using blue
826 collagen autofluorescence. (f) Muscle attachment to collagen bundles between the rows a-b, b-c and
827 c-d shown using blue collagen autofluorescence. (g) PIP collagen fibers and the MS collagen fibres
828 inserting into the collagen mat. These fibres attach to the muscles and insert into the deep collagen
829 fibrous mat so that the whole pad is moved when the muscles protract.

830 **Figure 5. Opossum extrinsic protracting muscles.** (c) Picture of an intermediate tangential layer of
831 the pad showing *Pars media inferior* (PMI) and *Pars media superior* (PMS). PMS intercepts between
832 rows A-B and B-C. PMI intercepts between rows B-C and C-D. a): PMS muscle attachment; b): PMI
833 muscle attachment.

834 **Figure 6. Opossum genal whiskers.** (a) Genal whisker arrangement from tangential slices. The genal
835 whisker area can be seen clearly in Figure 1a. (b) Ethanol/xylene-cleared preparation showing genal
836 whiskers arranged in an approximately vertical row. (c) Close-up of the genal follicle showing a
837 ringwulst and ring sinus. (d) Sling muscles on the genal whiskers, of which the extremities are
838 inserted dorsocaudally into the corium.

839 **Figure 7. Examples of rat and opossum whisker movements during movement across a smooth**
840 **surface (regular whisking).** (a, b) Mean angular position of the left (blue) and right (green) whisker
841 fields over the course of three whisk cycles. (c, d) Changes in angular spread of the whiskers over the
842 course of the same three whisk cycles. In both species, spread is strongly correlated to angular
843 position such that whiskers spread out as they are protract forward.

844 **Figure 8. Comparing whisking behaviour in regular and contacting whisking bouts in rats and**
845 **opossums.** (a) Mean angular spread (estimated by standard deviation of whisker angular positions) of
846 the rat and opossum whiskers, in episodes of regular whisking and contact whisking. Rats
847 significantly reduce their spread when they contact a surface, whereas there is no significant
848 difference in possums. (b) Mean retraction velocity is also significantly decreased during contact in
849 rat, but not in opossum, suggesting that differential control of whisker velocity may underlie the
850 change in whisker spread. (c, d) Mean whisker protraction velocity and set-point (average of the mean
851 whisker position) are not altered significantly between regular and contact whisking in either species.

852 **Figure 9. Effect of surface contact on whisker spread in rat (left) and opossum (right).** These
853 digital video frames were selected to show the whiskers at maximum protraction during movement
854 across a smooth floor (a, b), or after contact with a vertical surface (c, d). In rats, there is a visible
855 reduction in whisker spread following contact with surfaces suggesting that the whiskers are brought
856 closer together in order to increase the number of surface contacts. In opossum there was no evidence
857 of a similar reduction in whisker spread, suggesting that these animals lack the capability to control
858 the whiskers in this fashion. Note that head movement can cause changes in apparent spread viewed
859 from an overhead camera, in Grant et al. (2009) changes in whisker spread in rat were shown to be
860 significant after controlling for changes in head tilt. This figure also illustrates the difference in
861 vibrissal morphology between the rat and opossum, with the rat lacking the prominent genal vibrissae
862 seen in the opossum but possessing a rounder, blunter snout that can provide better coverage, by the
863 whiskers, of the region in front of the animal (see Discussion).

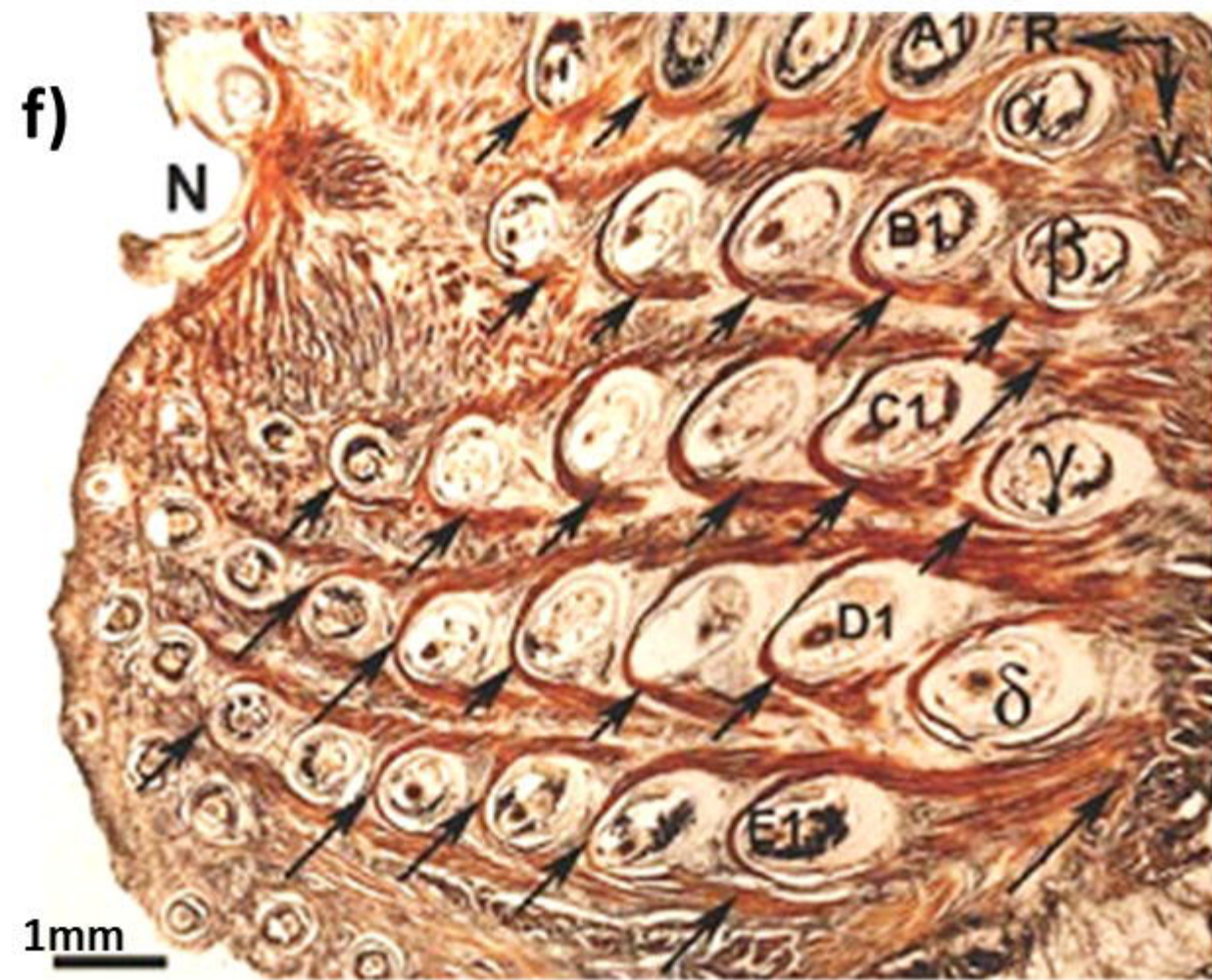
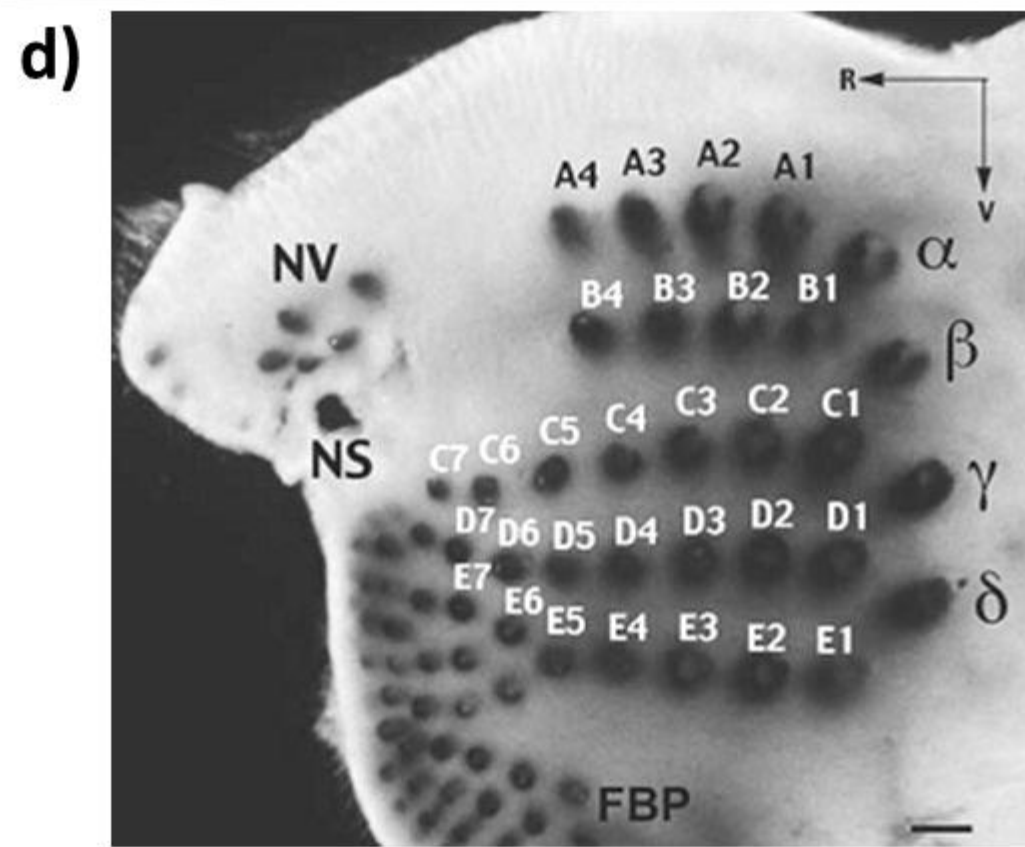
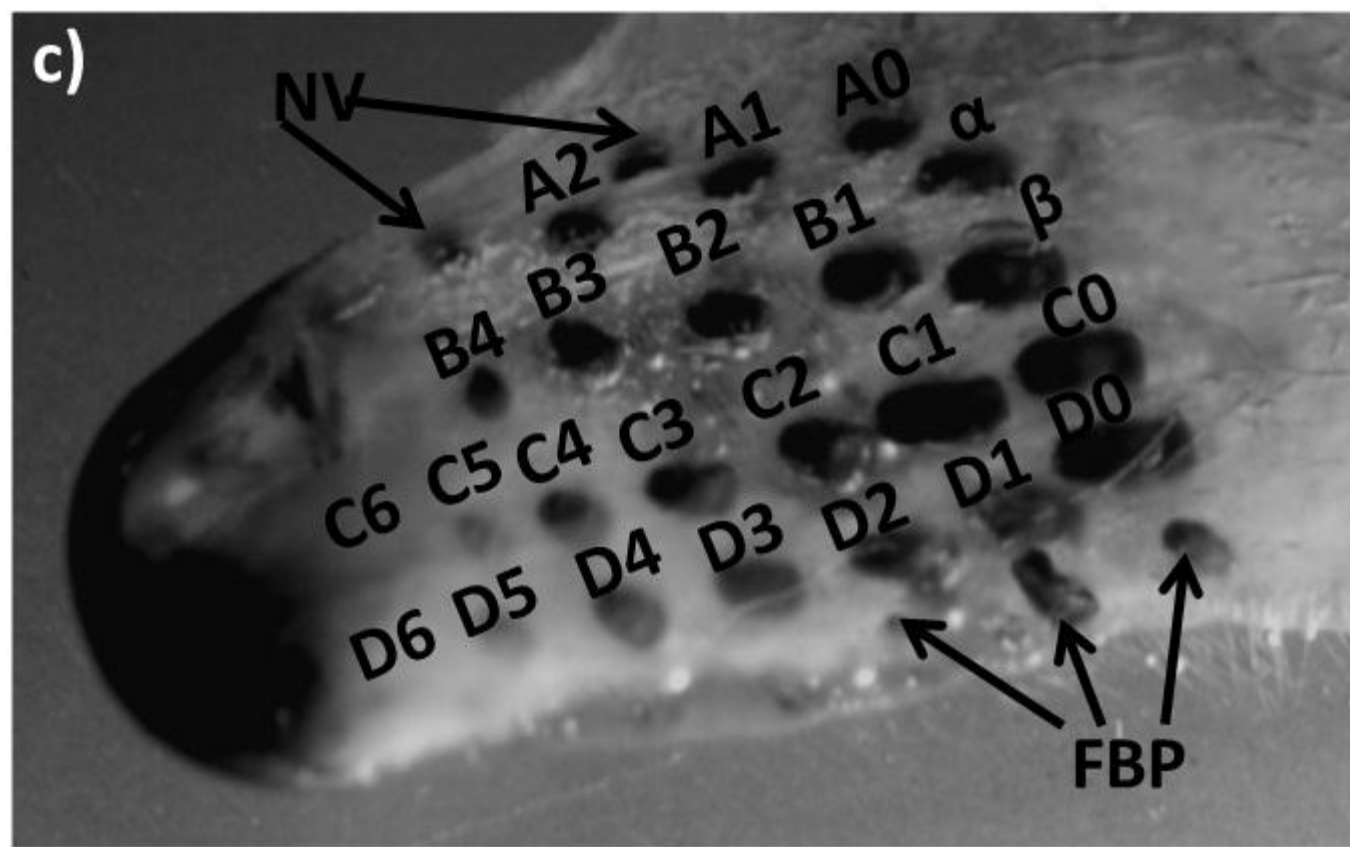
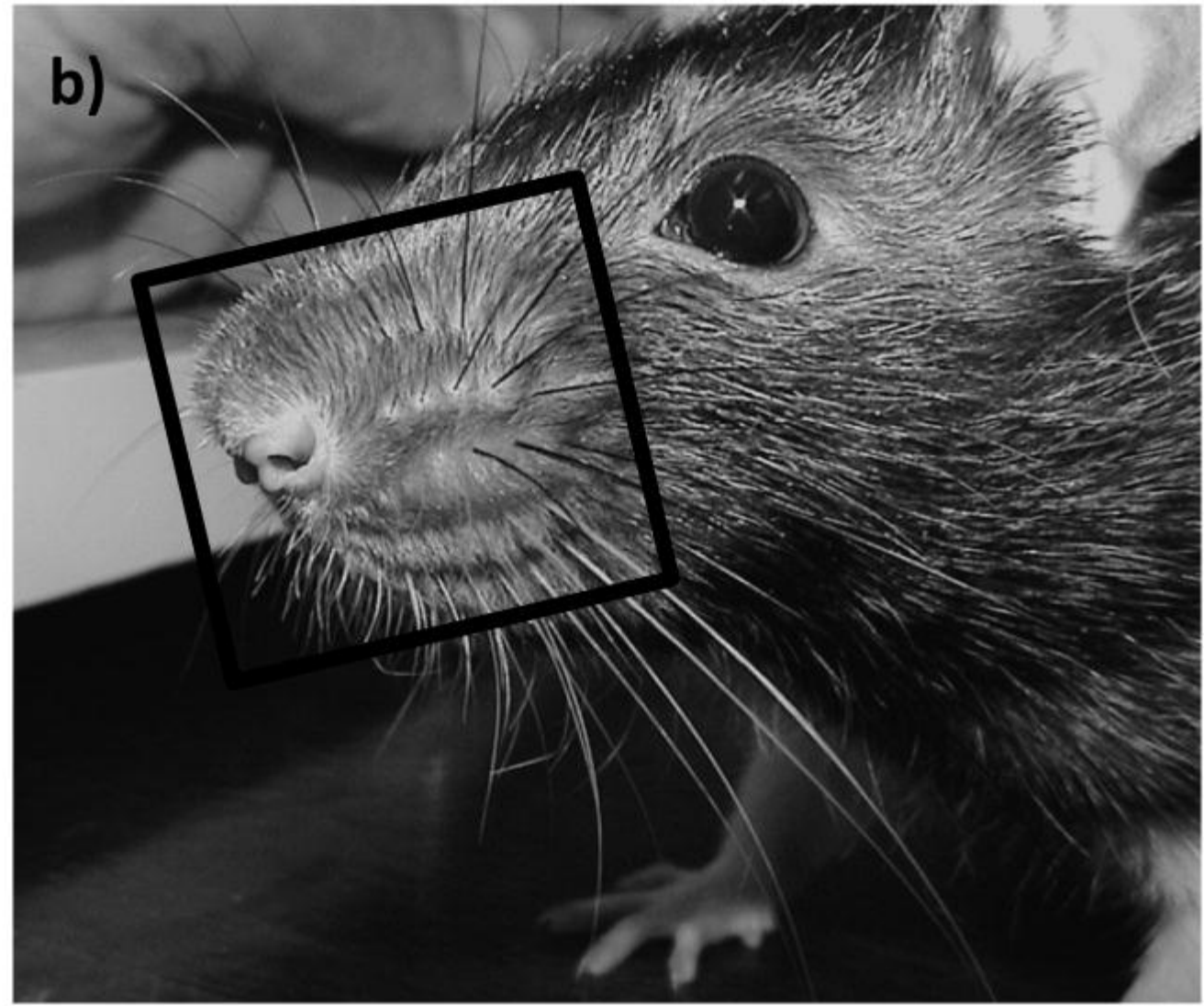
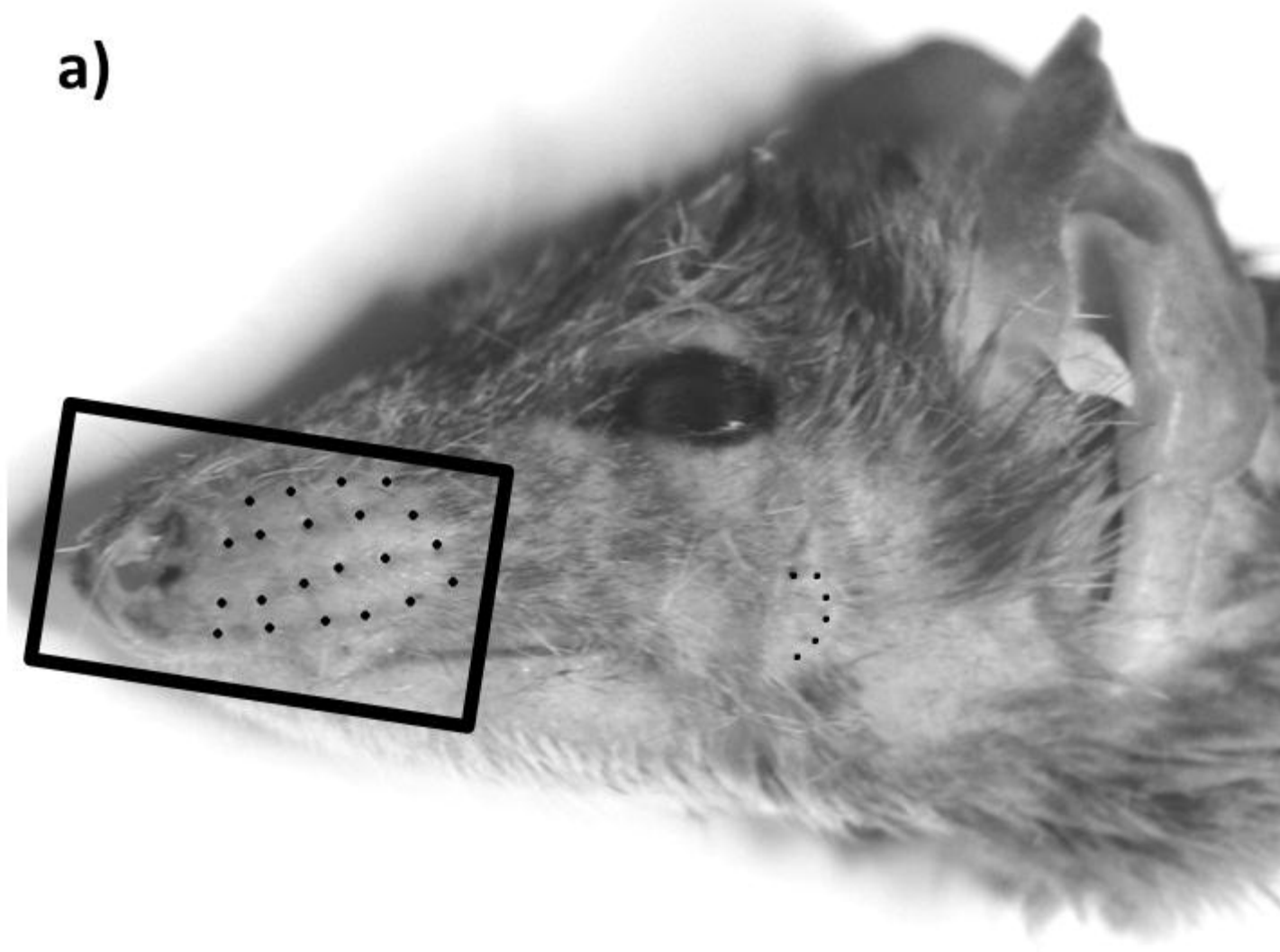
864 **Figure 10. Movement of the opossum genal whiskers.** Two manual tracking examples (a and b).
865 Two macrovibrissae (blue—left whisker field, green—right whisker field) and one genal whisker
866 (red) during two episodes of regular whisking. The genal whisker moves synchronously with the
867 macrovibrissae, but at a lower amplitude, during some, but not all, whisk cycles.

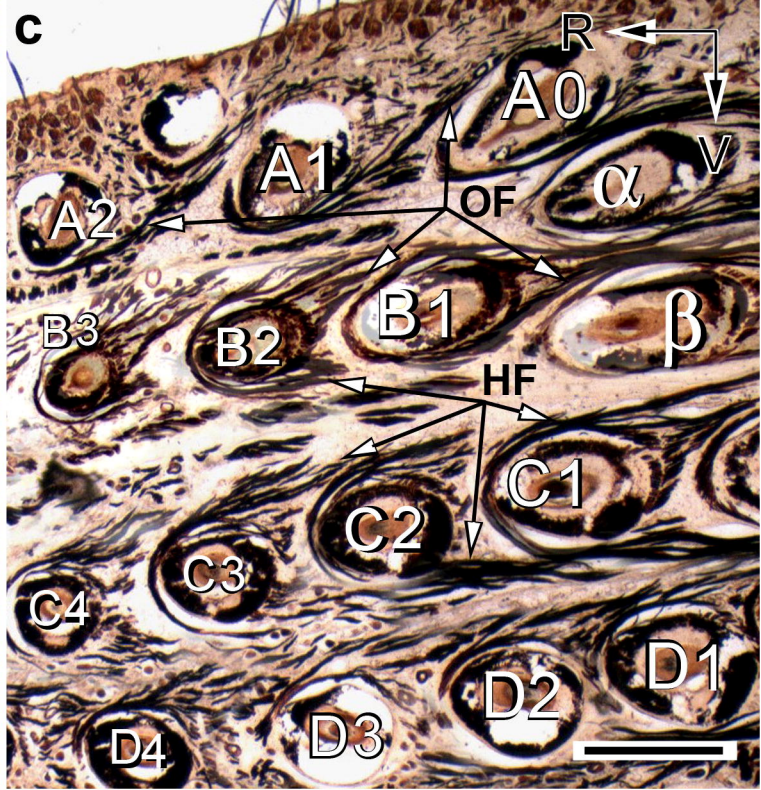
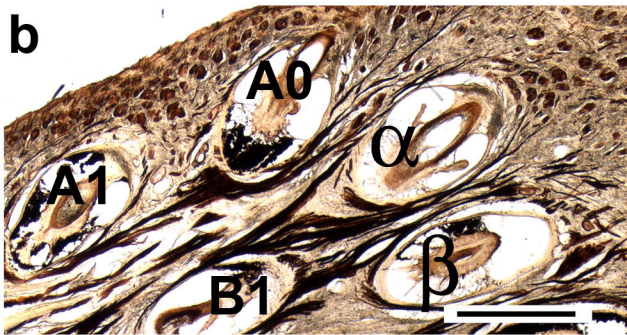
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869 **Figure 11. Schematic representation of the mystacial pad muscles in the opossum *Monodelphis***
870 ***domestica*.** (a) Intrinsic muscles. FBP, furry buccal pad; NV, nasal vibrissae; OI, and RI, oblique and
871 regular intrinsic muscles, respectively; marked black circles represent mystacial vibrissae. (b)
872 Extrinsic muscles. FB, fibrous bundles passing between vibrissal rows; FE, fibrous extensions
873 (branches) connecting FB with vibrissal follicles; ML, *M. maxillolabialis*; MP, MS and PIP, *Partes*

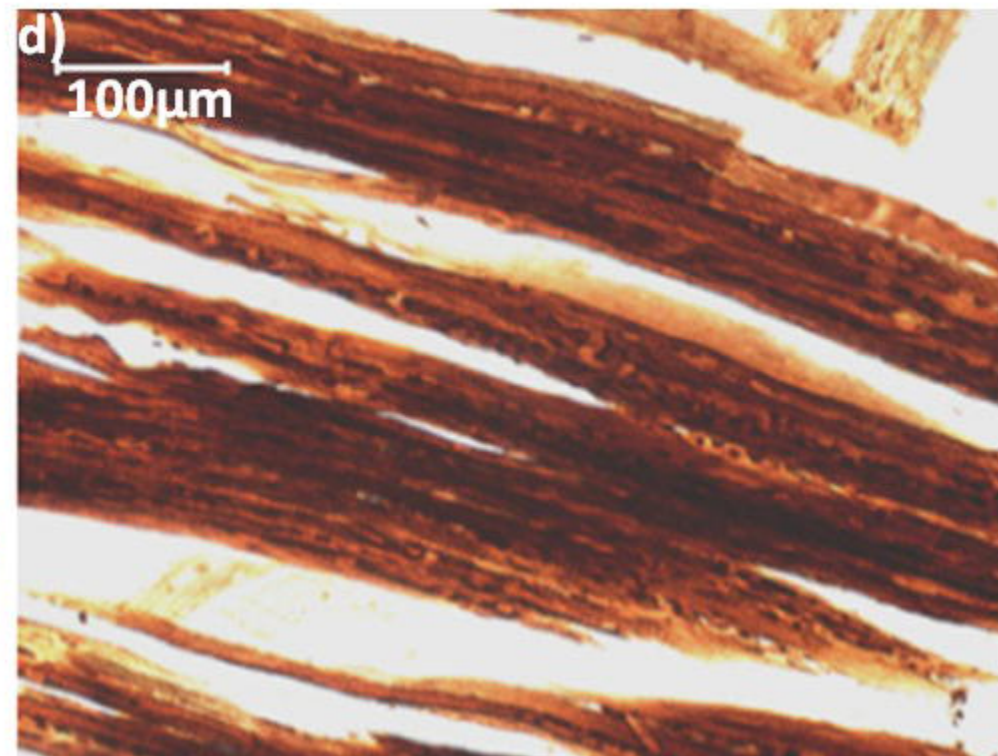
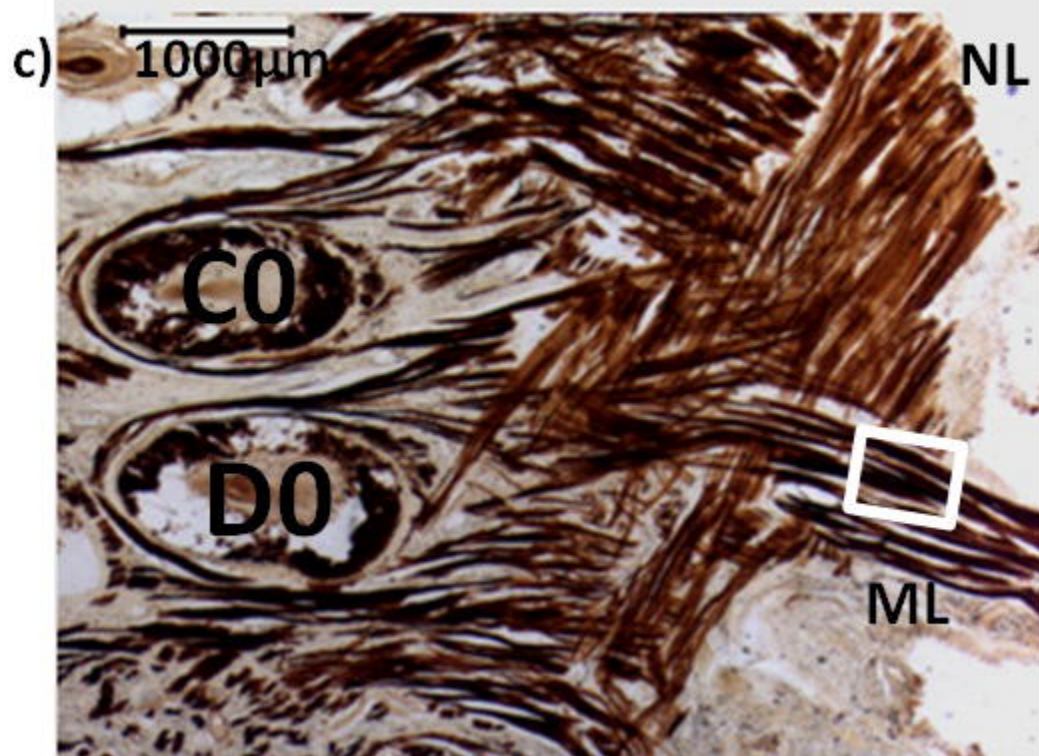
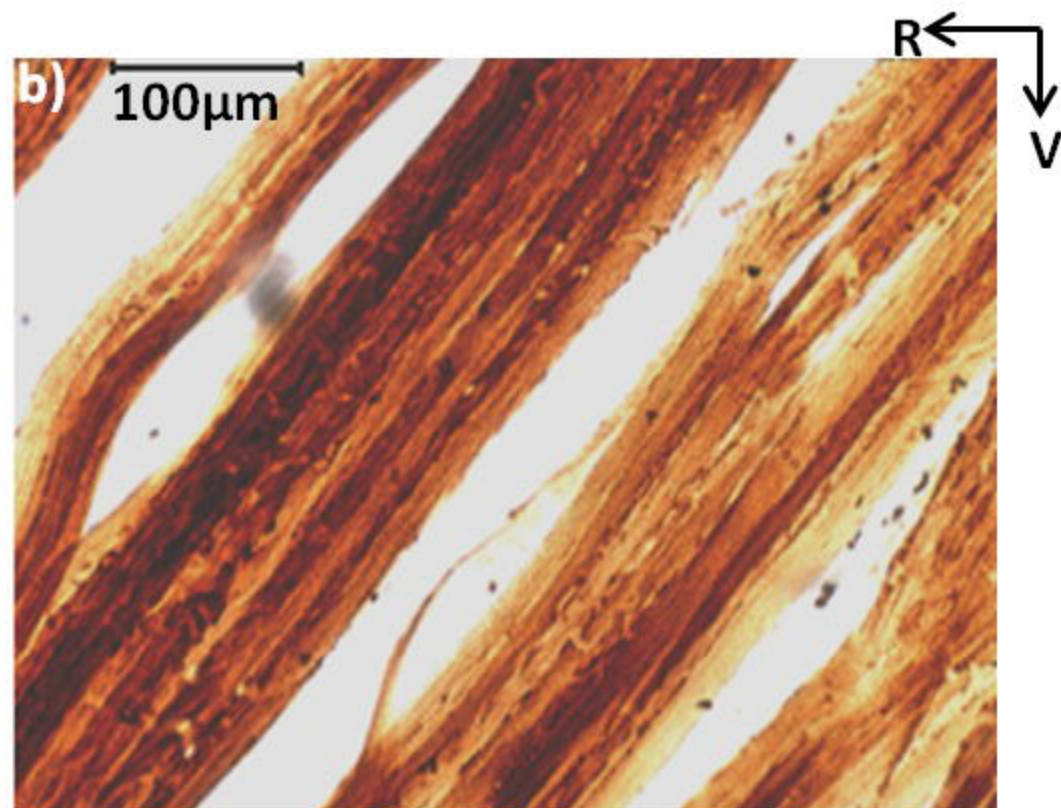
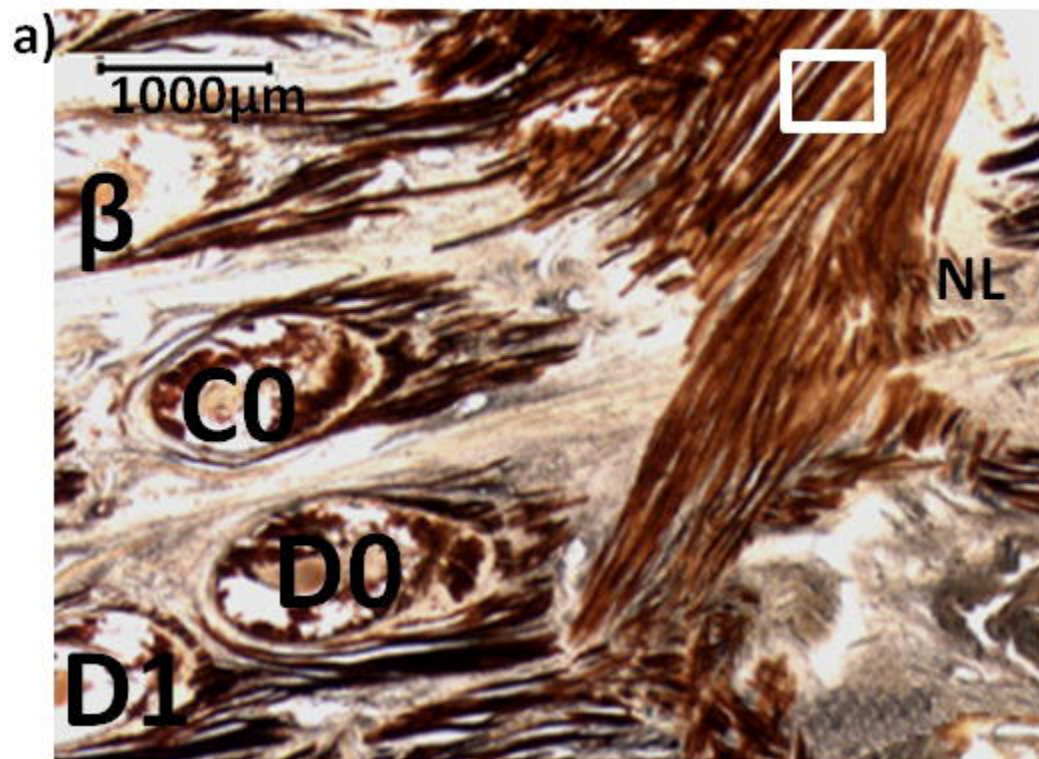
874 *maxillares profunda* and *superficialis*, and *Pars interna profunda* of the *M. nasolabialis profundus*,
875 respectively; NL, *M. nasolabialis*; SA, sites of attachment of ML and NL muscle fibre ends to the
876 corium of the mystacial pad; other figure labels as in a).

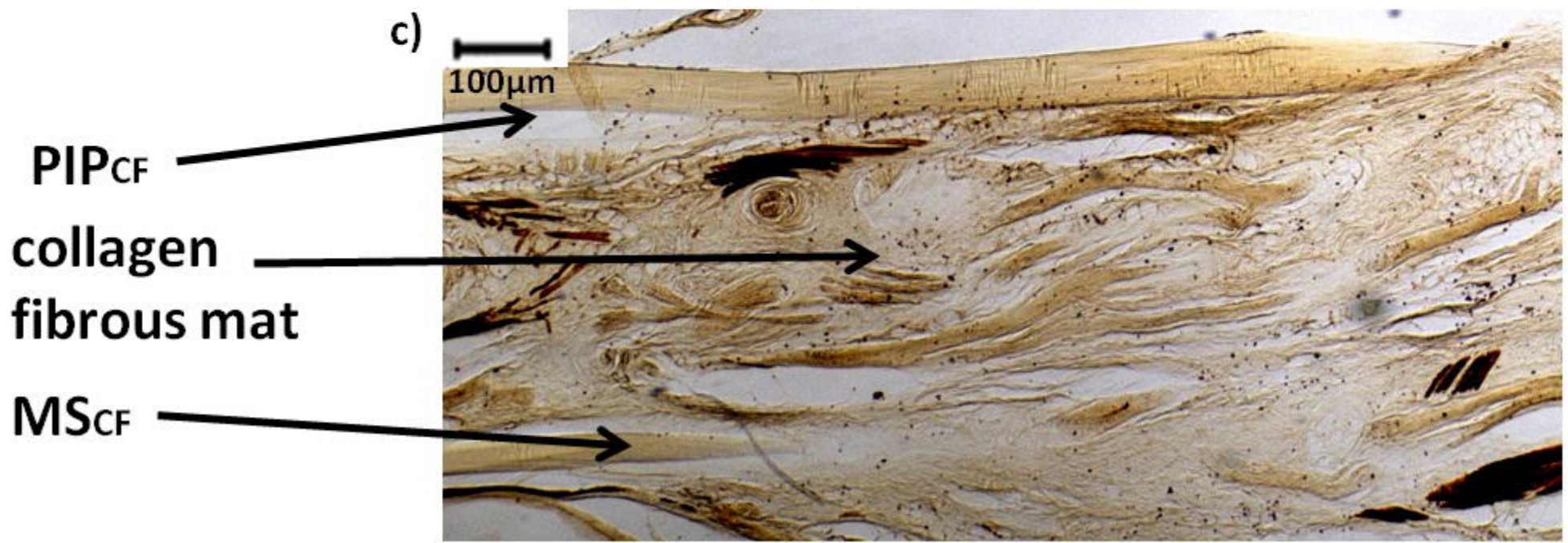
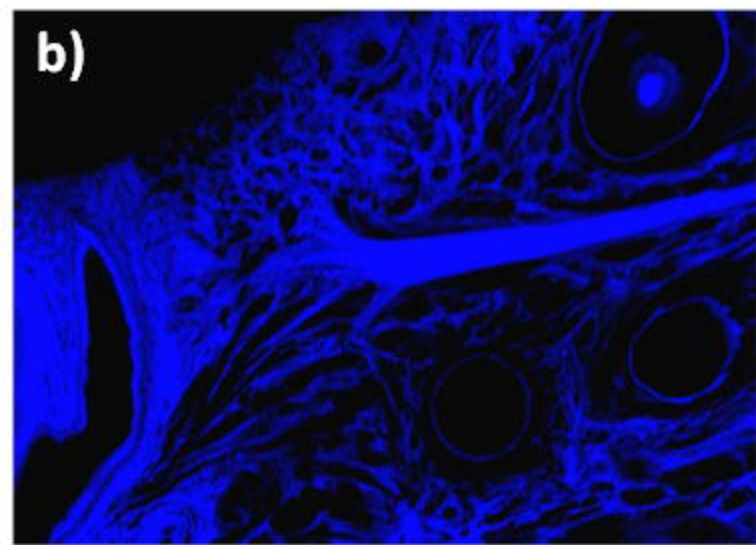
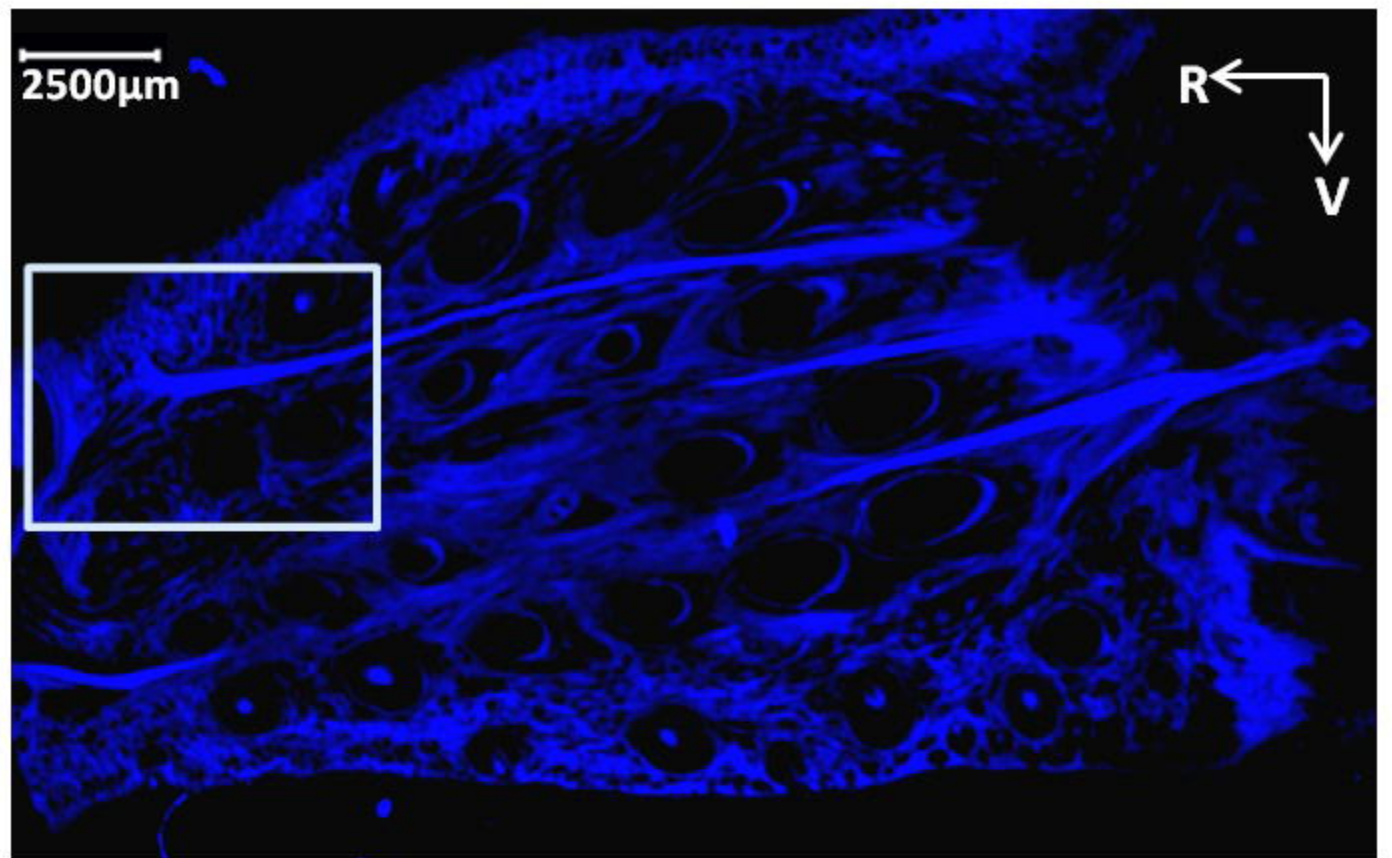
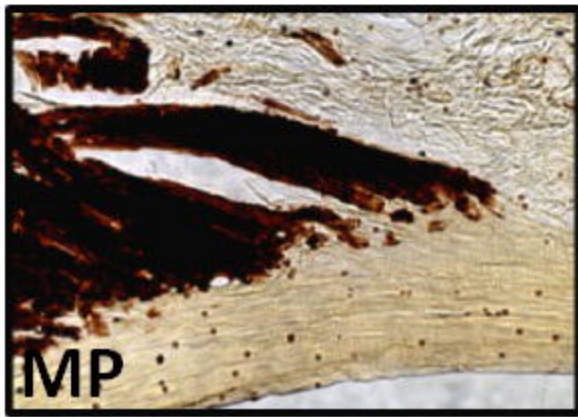
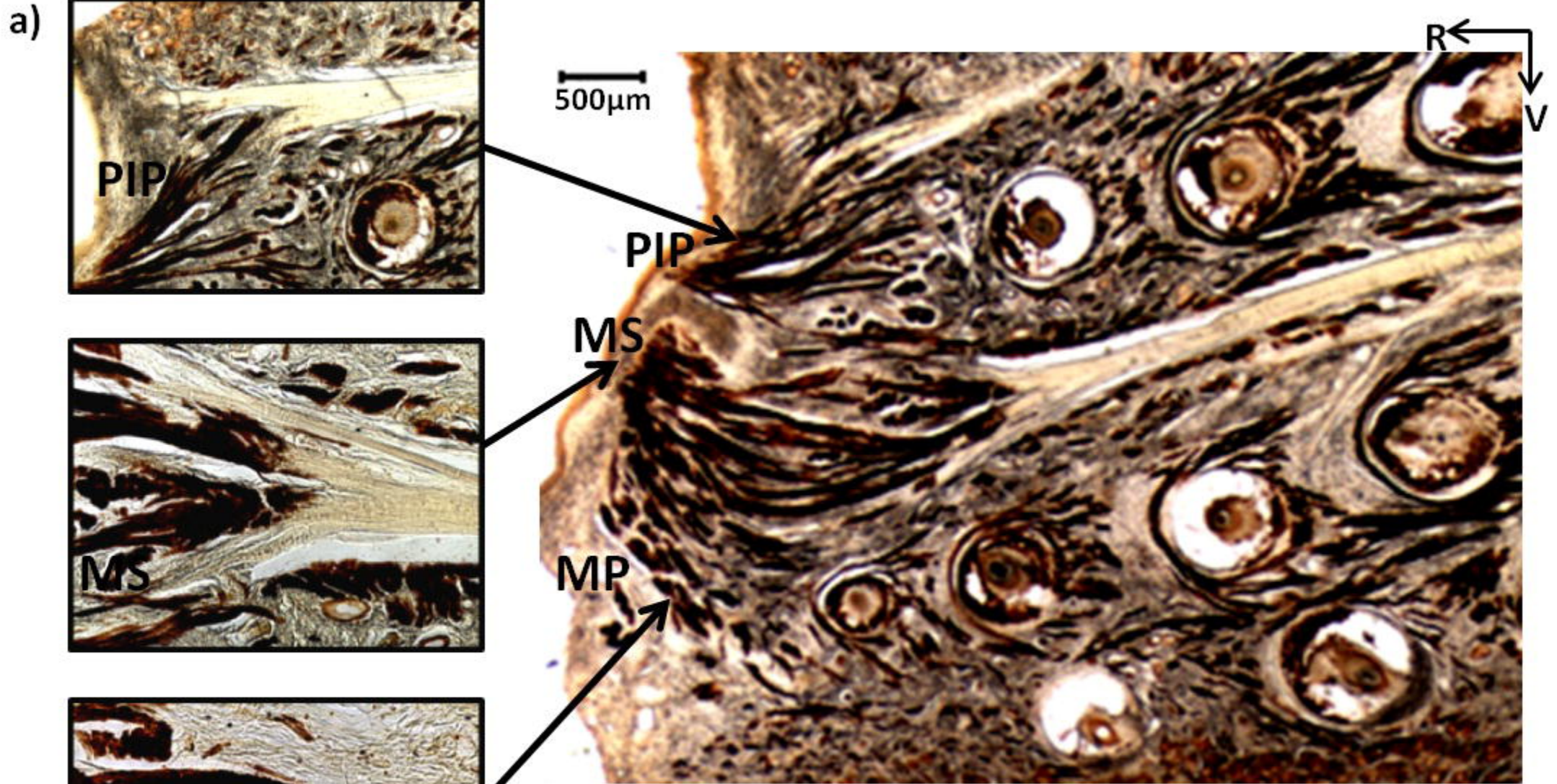
877 **Figure 12. Rostral skull sections of the skull (a) in rat and (b) in opossum.** PM is premaxilla and
878 A is the attachment place of Partes mediae superior et inferior. (b) is based on Figure 1a in Wible
879 (2003) pg. 72. The difference in the positioning of the attachment sites of PMS and PMI in opossum,
880 compared to rat, could explain the absence of the spread reduction behaviour.

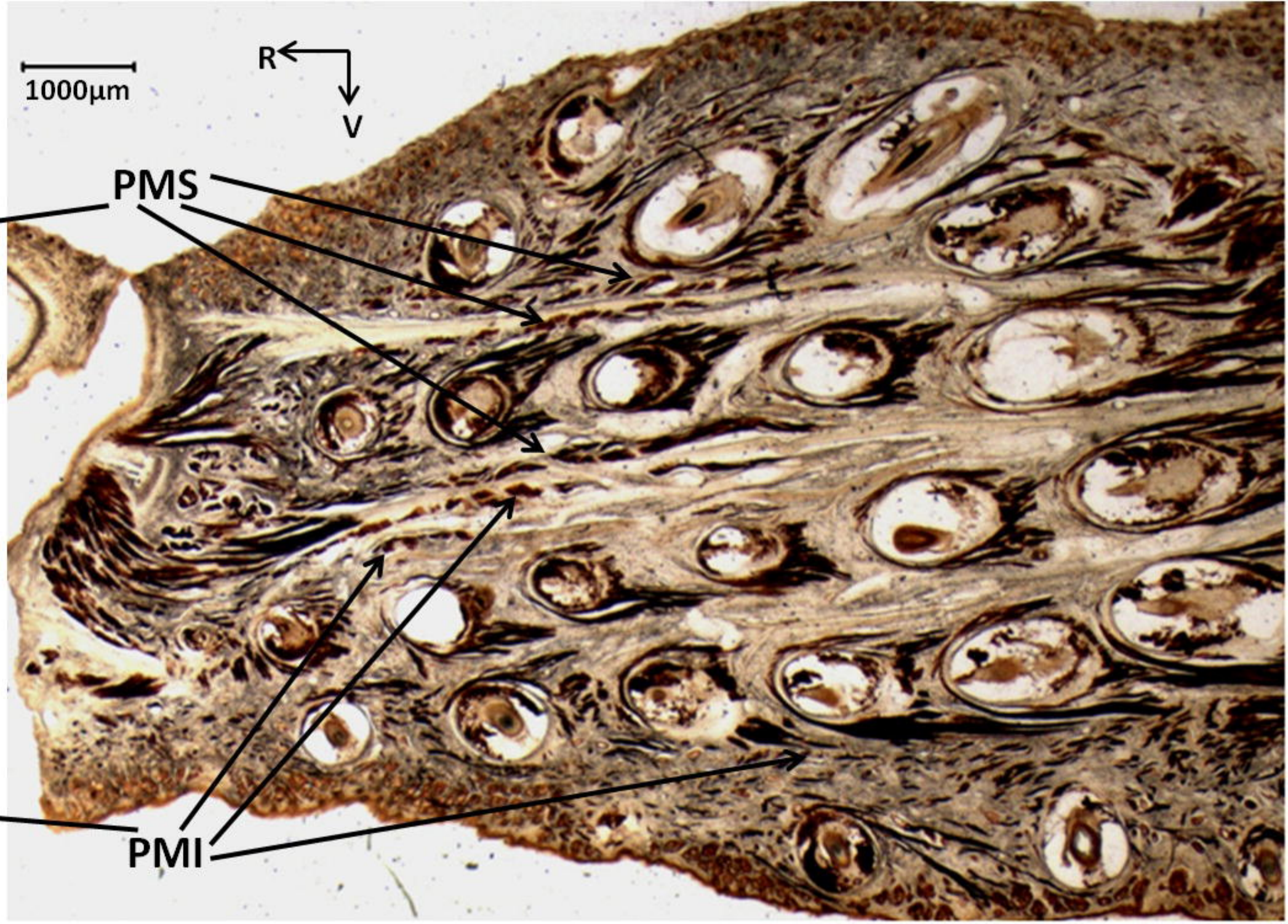
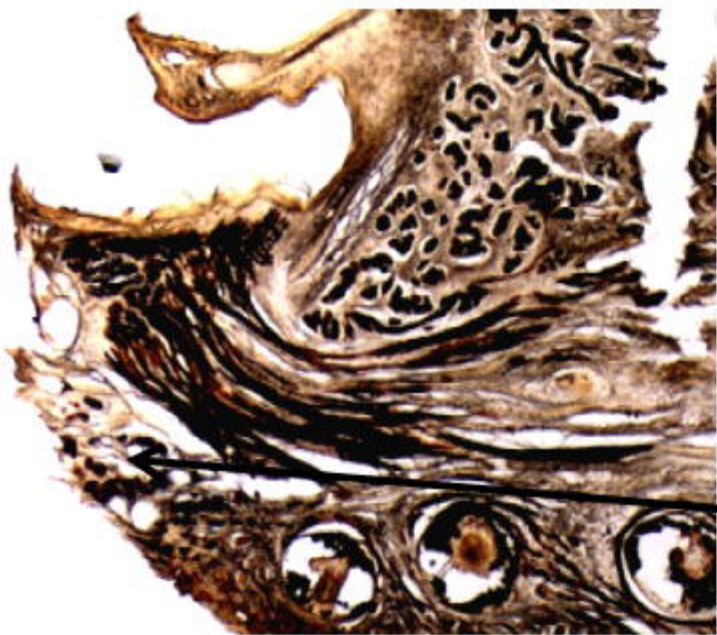
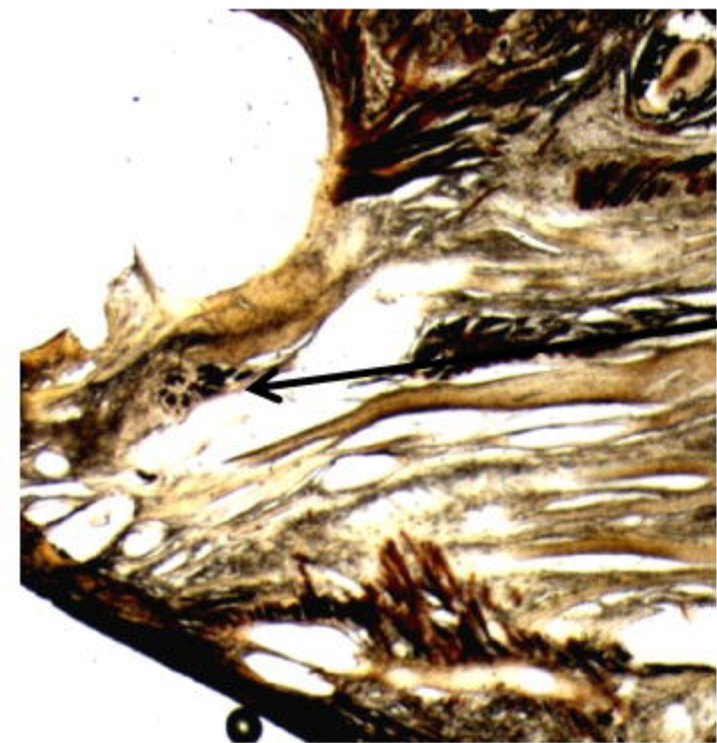
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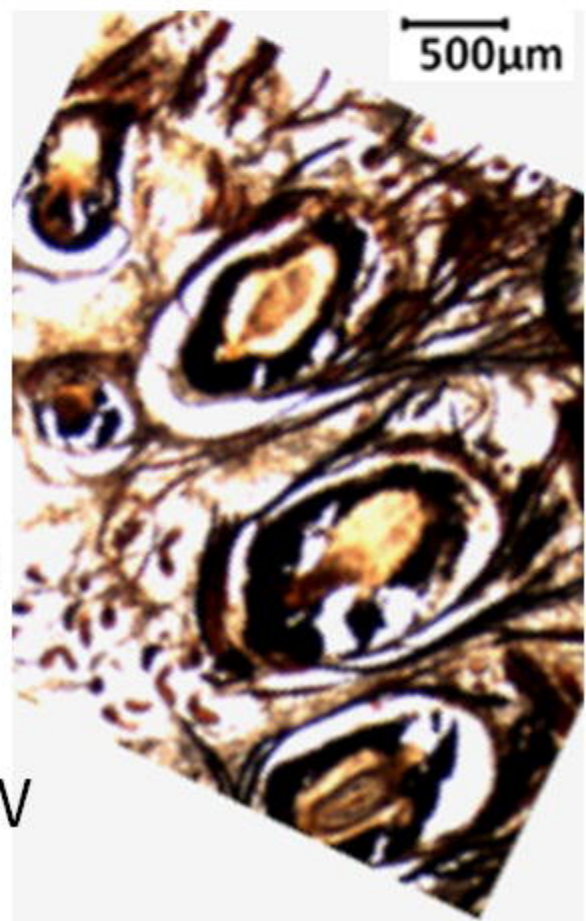
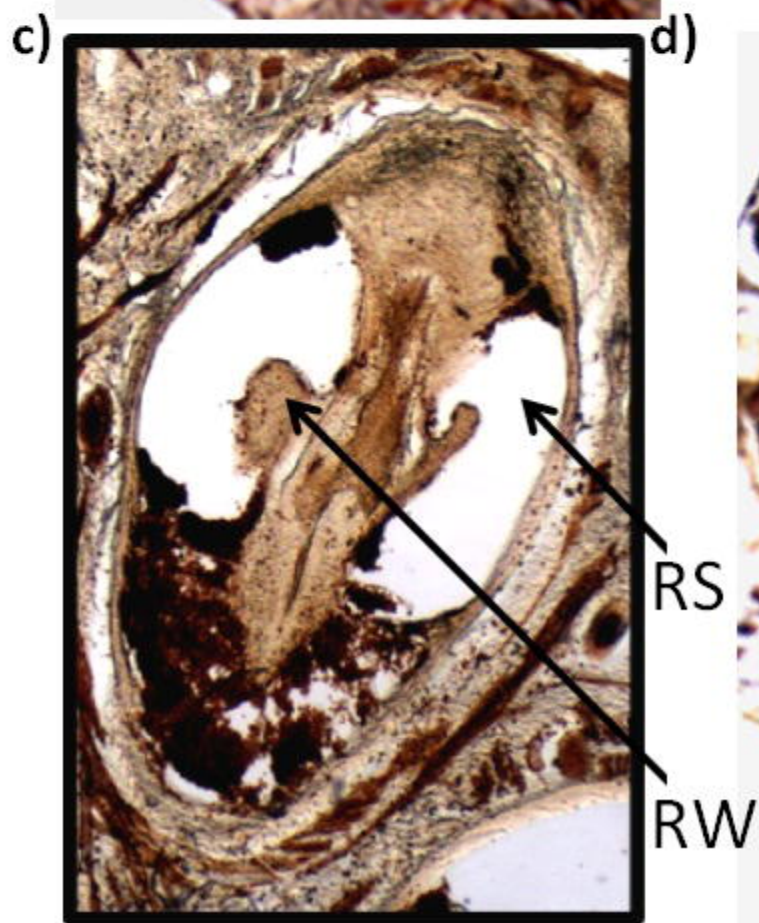
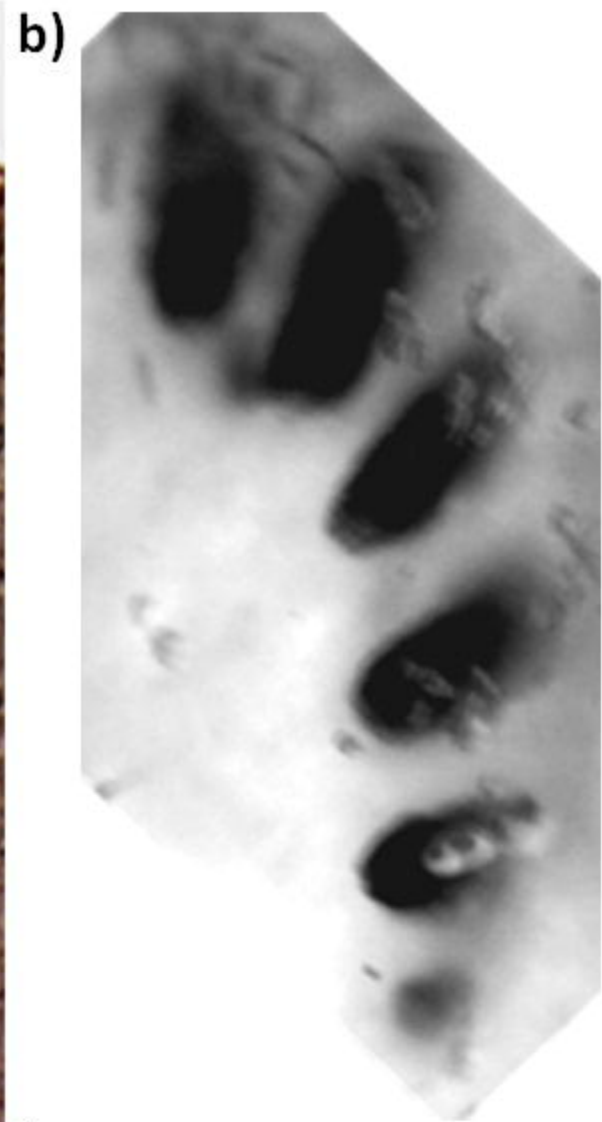
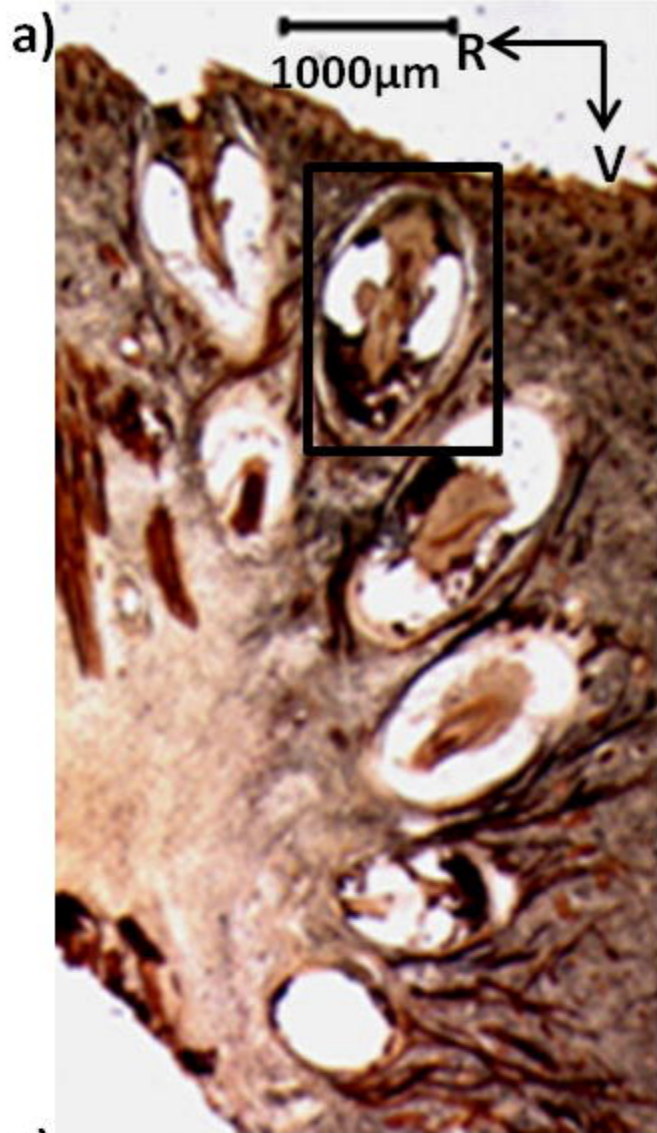


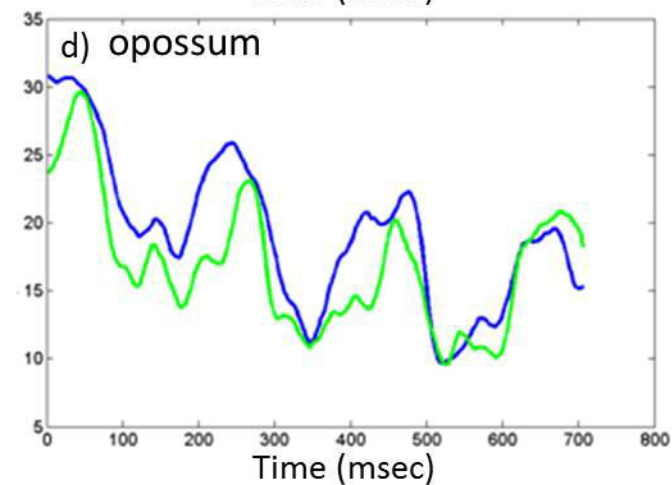
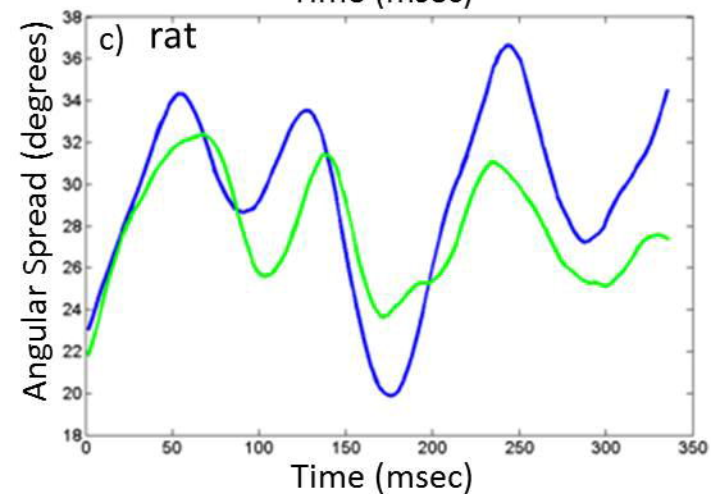
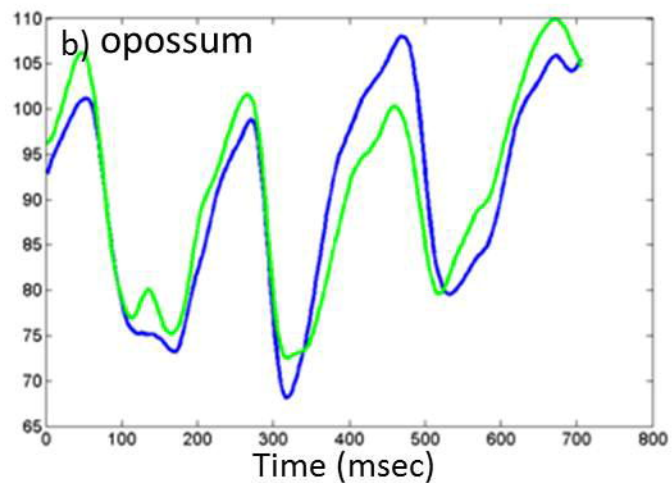
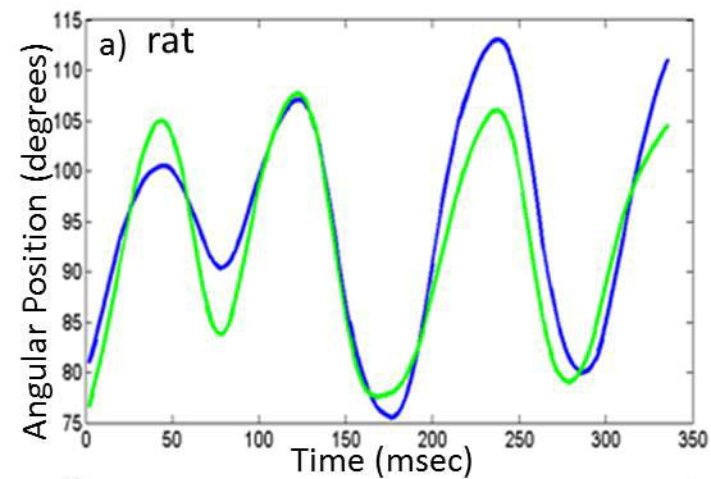


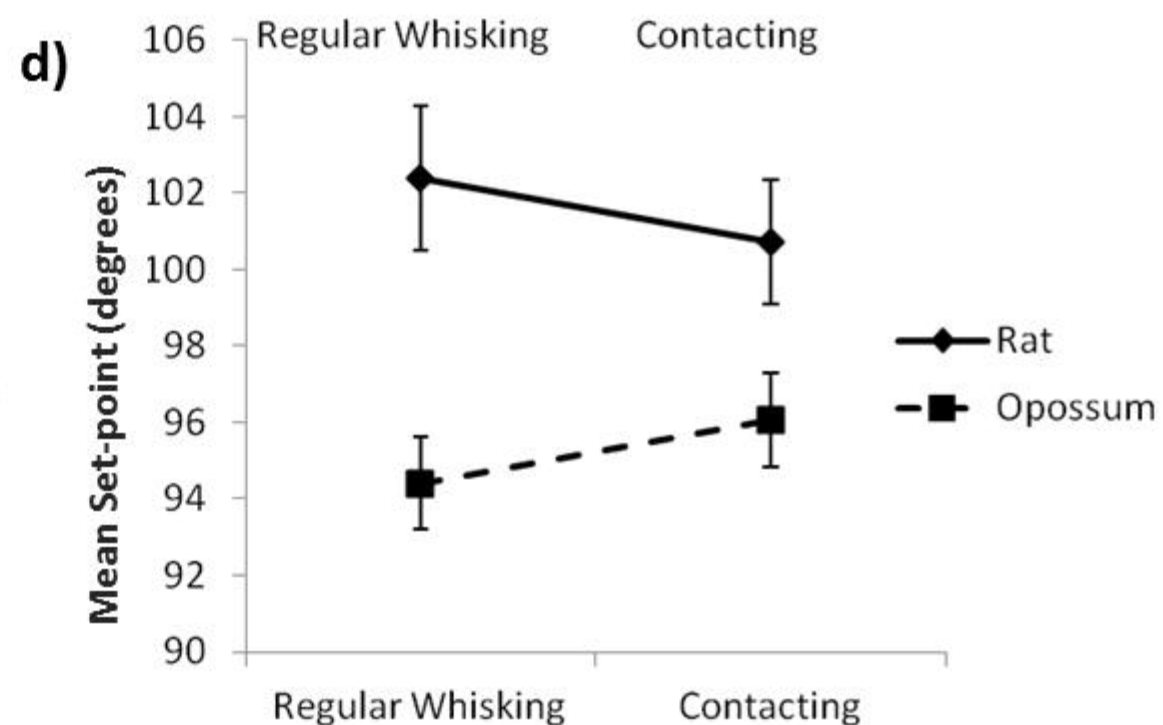
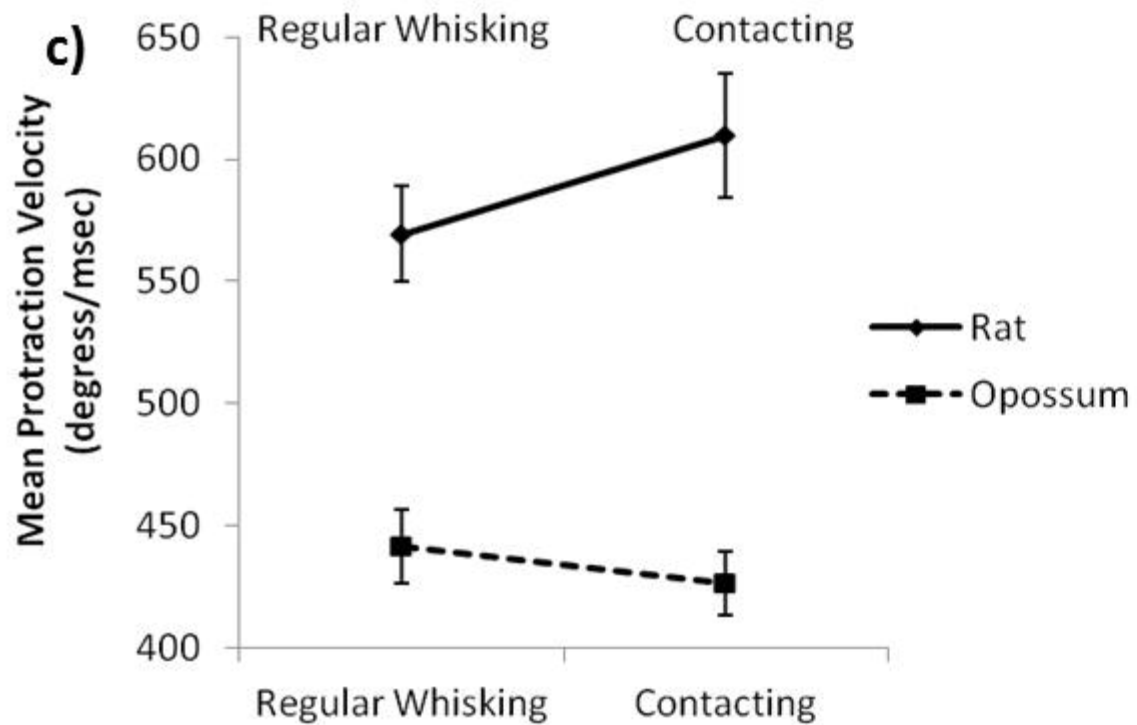
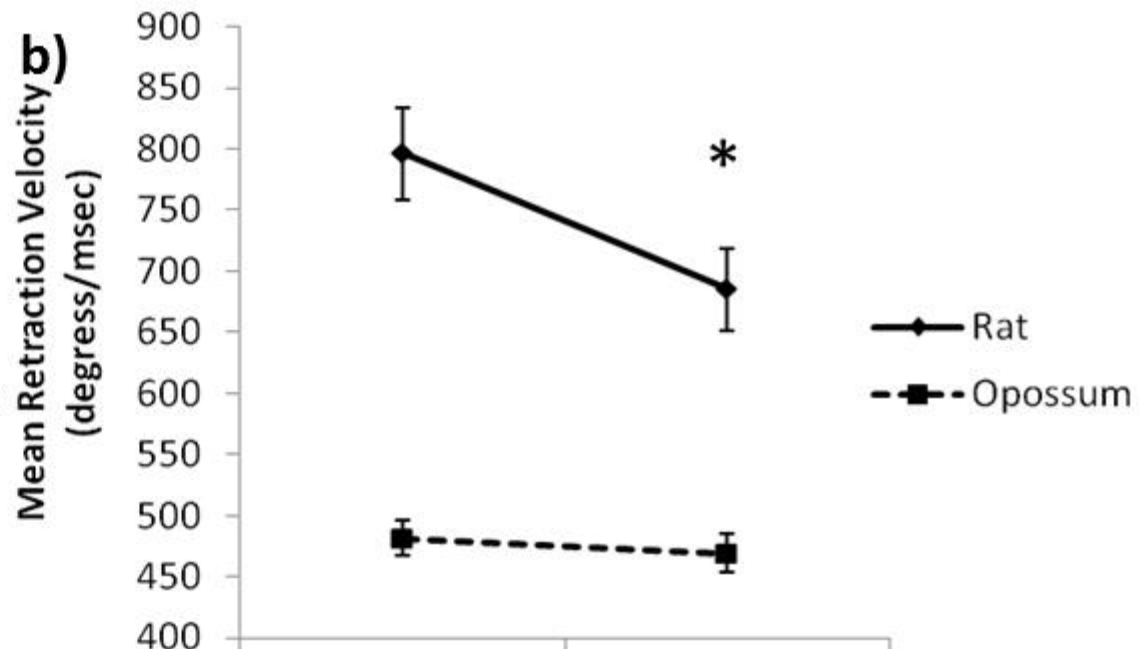
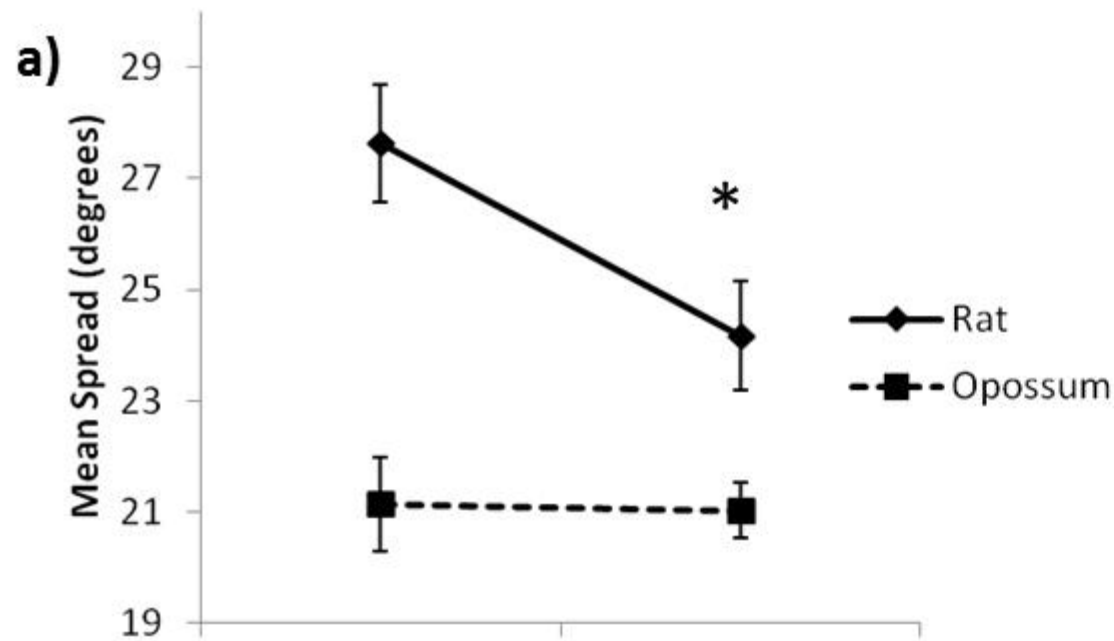




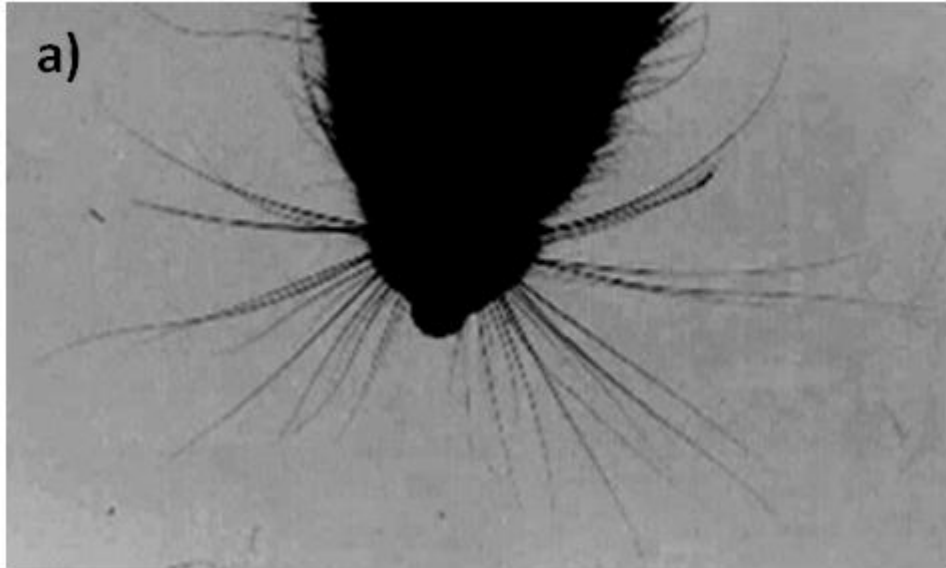




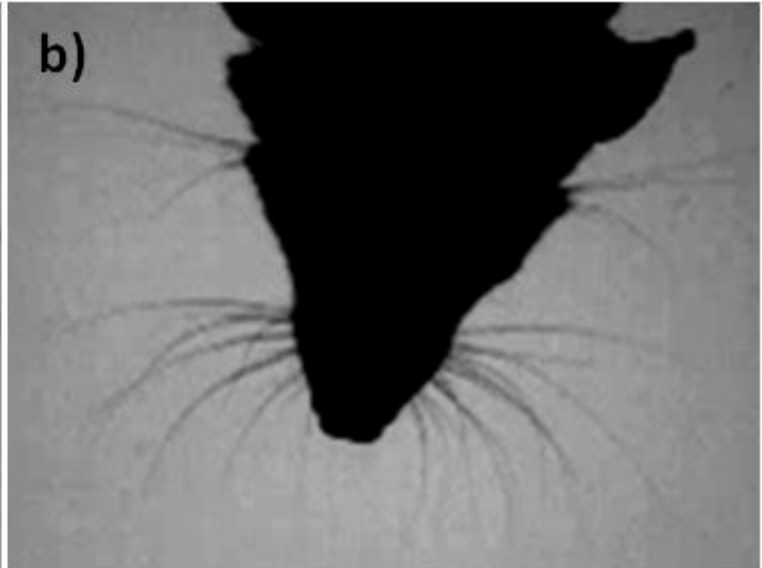




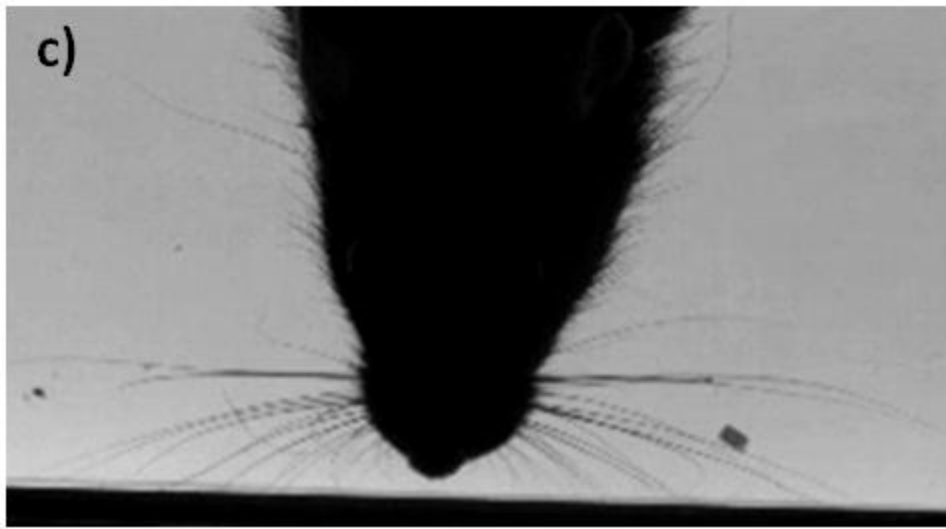
a)



b)



c)



d)

