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Mystacial whisker layout and musculature in the guinea pig (Cavia porcellus): a social, diurnal mammal.

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1 **Mystacial whisker layout and musculature in the guinea pig (*Cavia porcellus*): a social,**

2 **diurnal mammal.**

3 Running title: Guinea pig whisker layout and musculature

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1

26   **Abstract**

27   All mammals (apart from apes and humans) have whiskers that make use of a similar muscle

28   architecture. Whisker specialists, such as rats and mice, tend to be nocturnal and arboreal,

29   relying on their whisker sense of touch to guide exploration around tree canopies at night. As

30   such, nocturnal arboreal rodents have many whiskers that are organised into a grid-like

31   pattern, and moved using a complex array of muscles. Indeed, most arboreal, nocturnal

32   mammals tend to have specialised whiskers, that are longer and arranged in a dense, regular

33   grid, compared to terrestrial, diurnal mammals. The guinea pig diverged early from murid

34   rodents (around 75 million years ago), and are ground-dwelling, diurnal animals. It would be

35   predicted that, as a terrestrial mammal, they may have less whiskers and a reduced muscle

36   architecture compared to arboreal, nocturnal rodents. We examined the mystacial whisker

37   layout, musculature and movement capacity of Guinea pig (*Cavia porcellus*) whiskers and

38   found that they did indeed have a disorganized whisker layout, with a fortification around the

39   eye area. In addition, there was a reduction in musculature, especially in the intrinsic muscles.

40   Despite guinea pigs not cyclically moving their whiskers, the mystacial musculature was still

41   very similar to that of murid rodents. We suggest that the conserved presence of whisker

42   layout and musculature, even in visual mammals such as primates and guinea pigs, may

43   indicate that whiskers still play an important role in these animals, including protecting the

44   eyes and being involved in tactile social behaviours.

45

46   **Introduction**

47   All mammals (apart from apes and humans) have facial whiskers (vibrissae) (Ahl, 1976).

48   Whisker specialists, such as mice, rats and hamsters are able to move their whiskers to

49   perform large, quick, cyclic sweeps (termed whisking), which is amongst the fastest

50   movements that mammals can make (occurring at around 25Hz in mice) (Welker, 1964;

51   Wineski, 1983; Jin et al., 2004). The fast and precise positioning of the whiskers are enabled

52   by a specialist whisker musculature, a complicated architecture of intrinsic and extrinsic

53   muscles (Dörfl, 1982), which are represented mainly by fast muscle fibres (Jin et al., 2004).

54   Perhaps the most well-studied muscle group is that of the intrinsic muscles, represented by

55   sling-like muscles that link around the base of each whisker follicle, causing the whiskers to

56   protract forward (Dörfl, 1982). The layout of the intrinsic muscles has been found to be

57   largely preserved from marsupials (Grant et al., 2013) to rodents (Dörfl, 1982), to nocturnal

58   arboreal primates (Muchlinski et al., 2013). That intrinsic whisker muscles are preserved

59   between marsupials and rodents, even though their last common ancestor occurred around

60   160 million years ago (Luo et al., 2011), suggests that the common ancestor of extant

61   mammals may well have had moveable whiskers involved in active touch sensing. While

62   intrinsic muscles are largely preserved, the extrinsic muscles of the mystacial pad can largely

63   vary between species (Yohro, 1977). In the marsupial *Monodelphis domestica* (Grant et al.,

64   2013), for example, some of the extrinsic muscles are so reduced that vibrissal control is

65   limited, and whisker spread and velocity cannot be controlled during object exploration.

66

67   The number, layout and musculature of the mystacial vibrissae are all closely linked to the

68   function and movement abilities of the whiskers. Small, social, nocturnal and arboreal

69   mammals have been found to have longer vibrissae with a more densely packed vibrissal

70   field than that of ground-dwelling and burrowing mammals (Pocock, 1914; Lyne, 1959; Ahl,

71   1986; Muchlinski et al., 2013). Exceptions to this include semi-aquatic (i.e. Australian water

72   rat (Dehnhardt et al., 1999)) and aquatic mammals (such as pinnipeds and sirenians

73   (Dehnhardt, 2002)), that have long and densely-arranged whiskers, despite them being large,

74   diurnal animals; indeed the California sea lion has the longest whiskers of all mammals. In

75   these animals, the whiskers are a likely adaptation for an aquatic lifestyle, and are used for

76   navigation and prey capture in a dark, underwater environment (Grant & Arkley, 2016).

77   Arboreal, nocturnal rodents actively position their whiskers for use in a variety of functions,

78   including navigation, locomotion, exploration, hunting and social touch (Grant & Arkley,

3

79 2016). Extensive studies in arboreal, nocturnal mice and rats have revealed that they possess

80 three groups of whisking muscles (protractors, retractors, and vertical vibrissae deflectors)

81 leading to a range of whisker movements and vast control abilities (Haidarliu et al., 2010).

82 However, diurnal mammals, such as some primates, lack organized vibrissae, have very thin

83 whiskers and a reduced whisker follicle without intrinsic muscles (Muchlinski et al., 2013).

84

85 Guinea pigs, and other Hystricomorphs, diverged from the murid rodents before the

86 artiodactyls and primates, and are often thought of as a separate order from rodentia (Graur et

87 al., 1991). They represent an early divergence in eutherian evolution, and as such often have

88 rather anomalous characteristics compared to other mammals, such as their facial bone

89 structure (Muchlinski, 2008) and body muscles (Potter et al., 1957). The guinea pig, and

90 other Histricomorphs, have a unique facial anatomy, in that the media masseter (mastication)

91 muscle passes through the infraorbital foramen (IOF) of the skull, which makes it particularly

92 large, compared to the IOF of other rodents (Muchlinski, 2008). While some aspects of the

93 maxilliary facial musculature has been described in guinea pigs (Muchlinski 2008), that of

94 the mystacial pad has yet to be considered, despite guinea pigs being able to generate fast and

95 large amplitude whisker movements; however, these movements do not tend to be cyclic and

96 usually occur in isolation (Jin et al., 2004). Due to their early divergence, we might expect the

97 guinea pig to have a whisker layout and musculature more similar to the marsupial, than the

98 rodent tactile specialists that evolved later, such as mice and rats. Moreover, we might expect

99 the guinea pig whisker system to be even further reduced and disorganized, due to them being

100 diurnal, ground-living mammals.

101

102 The aim of this study is to describe the muscle architecture of the mystacial pad in the guinea

103 pig anatomically, by cutting the mystacial pads of the guinea pig in the tangential plane and

104 staining consecutive slices for cytochrome c oxidase activity and Masson's Trichrome.

105 Because of the differences in proposed up-to-date schemes of whisker layouts in the guinea

106 pig mystacial pad (Sikich et al., 1986; Haidarliu and Ahissar, 1997), we also re-examine here

107 the layout of the mystacial pad *in situ*. All results will be compared in detail with those of rat,

108 and also to other animals such as opossums and shrews. We will go on to consider the impact

109 of diurnality on the whisker pad muscles and layout.

110

111

112   **Materials and Methods**

113   *Pad removal*

114   Eight female adult Dunkin-Hartley guinea pigs were used in the anatomy section of this

115   study, each weighing between 350-400 g. The guinea pigs were euthanized via an overdose

116   of anaesthetic. The mystacial pads were removed by cutting down the skin in the sagittal

117   plane and around each pad (around 2mm on either side of the pad). They were placed into a

118   solution of fixative (4% paraformaldehyde in 0.1 M phosphate buffer) and left for one hour.

119   They were then straightened by placing them into histology cassettes (Medex Supply,

120   Monsey, NY, USA) packed with high-density foam. Twelve of the pads were then placed

121   into fixative solution enriched with sucrose up to 30% for twenty four hours, and then

122   sectioned on the freezing microtome for staining for cytochrome oxidase activity. The

123   remaining four pads were kept flattened for two weeks, then subjected to dehydration and

124   clearing, and mounted in paraffin wax to slice and stain with Mason's Trichrome.

125   *Staining for cytochrome oxidase activity*

126   After fixing, each of the pads was sectioned using a freezing microtome (Leica CM 1800)

127   into 60 μm thick slices in the tangential plane. All slices were stained for cytochrome oxidase

128   activity following the method developed by Wong-Riley (1979) and modified by Haidarliu et

129   al. (2010). Slices were floated in an oxygenated solution of 0.02% cytochrome c (0.75mg),

130   catalase solution (40μl), and 0.05% diaminobenzidine (5mg) in 0.1 M phosphate buffer. The

131   slices were incubated at room temperature on a shaking platform until the stain developed

132   (approximately 1-3 hours), and a clear differentiation between non-reactive and highly

133   reactive tissue structures could be determined. Slices were then rinsed with 0.1 M phosphate

134   buffer. Stained slices were mounted on microscope slides and left to air dry overnight. The

135   slices were then coverslipped with DPX.

136   The cross-sectional diameter of the intrinsic muscles was manually measured, perpendicular

137   to the follicle, on the C-row whisker follicles of one slide (Fig. 3B) using the image analysis

138   software Tracker (Brown 2015), and compared to an equivalent slice in rat.

139   *Staining with Masson's Trichrome*

140   Four of the pads were placed into empty histology cassettes and transferred to a tissue

141   processor (Shandon Citadel 2000), where tissues were dehydrated through a series of graded

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142　IMS baths (70%, 80%, 90%, 100%), and then immersed in xylene and paraffin wax. This

143　process took around 20 hours. The samples were then mounted in a block of paraffin wax and

144　sliced on an automatic rotary microtome (Thermos Scientific microtome HM355S) into 10

145　μm thick slices that were floated in a 35-37°C bath. Two mystacial pads were sliced

146　tangentially, and two were sliced horizontally to visualise the C-row follicles. Slices were

147　mounted onto slides and left to dry at 38°C overnight.

148　Slides were put in a fixative solution (4% paraformaldehyde in 0.1 M phosphate buffer) for 1

149　hour, and introduced to Bouin's Solution for 4 hours. They were then cleared with xylene,

150　rehydrated with ethyl alcohol (100%, 90%, 80%, 70%) and moved through a sequence of

151　solutions for the Masson's Trichrome staining (Biebrich Scarlet Acid, Phosphotungstic and

152　Phosphomolybdic Acids, Aniline Blue and Acidified Water), with multiple washes of

153　distilled water in-between each stage. The slices were then dehydrated with ethyl alcohol

154　(70%, 80%, 90%, 100%) and xylene, towel dried and cover-slipped using DPX. All slices

155　were visualised using a Zeiss Stereo Lumar V12 light microscope. Figures were captured

156　using Zeiss Axiovision, version 4.8. Occasional adjustments to exposure and white balance

157　were made.

158　*Behavioural Filming*

159　Nine adult female guinea pigs, of mixed strains, were used for filming. They were placed

160　individually into a transparent, Perspex, rectangular arena (20 x 50 x 15 cm) (Fig. 1A), which

161　was lit from below by an infrared light box (PHLOX LEDIR-BL-200/200-SLLUB-Q-1R-

162　24V). Each guinea pig was filmed from above using a digital high-speed video camera

163　(Phantom Miro ex2) recording at 500 frames per second with a shutter-velocity of 1 ms and a

164　resolution of 640x480 pixels. Multiple 1.5-s video clips were collected opportunistically (by

165　manual trigger) when the animal moved in the cameras field of view. Approximately 12 clips

166　were collected from each animal. Two-three clips from each guinea pig were selected based

167　on to the following selection criteria: i) the guinea pig was clearly in frame; ii) both sides of

168　the face were visible; iii) the head was level with the floor (no extreme pitch or yaw); and iv)

169　the whiskers were not in contact with a vertical wall. Twenty two clips in total were tracked

170　using the BIOTACT Whisker Tracking Tool (Perkon et al., 2011). The tracker semi-

171　automatically finds the orientation and position of the snout, and the angular position (relative

172　to the midline of the head) of each identified whisker. Tracking was validated by manually

173　inspecting the tracking annotations overlaid on to the video frames (Fig. 1B, inset).

174   The movement of the entire whisker field was determined from the unsmoothed mean of all

175   the tracked whisker angular positions for each side frame by frame (Grant et al., 2013), which

176   can be seen in Fig. 1B and is termed *naïve mean angle (nma)*. The *offset* was calculated as the

177   mean nma, and an average was taken between the two whisker sides. To estimate the

178   *amplitude,* the *offset* was removed from the whisking angle time series and the root mean

179   square value was computed to give the root-mean-square (RMS) whisking amplitude and was

180   estimated by multiplying the RMS whisking amplitude by $2\sqrt{2}$ (Chatfield, 2003). Whisk

181   *frequency* was not calculated as the *nma* did not often contain clear whisks, rather they were

182   more asymmetric movements that oriented the whisker field.

183   All work in this study conformed to UK Home Office Regulations and was approved by local

184   ethics committees.

185

186   **Results**

187   *Mystacial Pad Layout*

188   The layout of the guinea pig whiskers indicates that there are five rather irregular rows of

189   whiskers within the mystacial pad (Fig. 2). Dorsal to these five rows of mystacial vibrissae

190   and to the nostril, a row of five-to-six arcwise arranged nasal vibrissae passes in the rostro-

191   dorsal direction. The most dorsal row of the mystacial vibrissae, row A, is made of only two

192   whiskers. Row B comprises of usually three, but sometimes four vibrissae. Rows A and B are

193   caudally straddled by a straddler (α). Each of the rows C, D, and E contains usually five

194   vibrissae. Rows D and E are positioned more rostral, such that vibrissae D1 and E1 align with

195   vibrissae A2, B2 and C2. The misalignment of the rows D and E with rows A – C reveals the

196   guinea pig to have a more irregular whisker pad than other rodents, for example rat (compare

197   with Fig. 3A).

198   The straddler whiskers of the guinea pig also have a complex arrangement. Straddlers γ and

199   δ sit ventro-caudal to the rest of the whisker pad, and it is not clear from just looking at the

200   layout in Fig. 2, which whisker rows they are associated with. Straddler α sits caudal to rows

201   A and B. Straddler β straddles rows B and C. Muscle fibers from the ventral side of the

202   follicle C1, the dorsal side of the follicle D1 and straddler δ reach the follicle of the straddler

203   γ which is positioned more caudal to them (Fig. 3B). This is a rather irregular straddler

7

204 layout, compared to the rat (Fig. 3A) where straddler whiskers straddle consecutive rows A-

205 B, B-C, C-D, and D-E.

206 *Intrinsic Muscles*

207 The guinea pig whisker pad contains sling-like intrinsic muscles (Figs. 3B-D) that form a

208 sling around the rostral areas of each follicle and attach to the caudal follicle in the same row.

209 These muscles are striated and made up of red, pink and white striated muscle fibers (Fig.

210 3D). The intrinsic muscles look much reduced, are much thinner, and are not as striking as

211 those seen in the rat (compare Figs. 3A and 3B). Indeed, the cross-sectional diameter of the

212 intrinsic muscles (measured at the point of the arrows on Figs. 3A and 3B) show that the C

213 row intrinsic muscle diameter is 0.080 ± 0.01 mm in rat and 0.061 ± 0.004 mm in guinea pig,

214 despite the guinea pig being slightly bigger overall.

215 In addition, the intrinsic muscles are, on the whole, more irregular in the guinea pig. In the

216 rat, the intrinsic muscles connect each consecutive follicle within the same row, forming a

217 regular, chain-like architecture (Figure 3A). However, in the guinea pig, oblique intrinsic

218 muscles pass both between and within vibrissal rows. Figure 3C shows an oblique intrinsic

219 muscle passing between follicles in different rows, from the ventral part of the B1 follicle

220 attaching to the dorsal part of the C1 follicle. Figure 3E shows an oblique intrinsic muscle

221 passing within vibrissal rows, from the ventral part of A2 crossing to the dorsal part of A1.

222 These oblique intrinsic muscles are not observed in rat.

223 *Whisker Follicles*

224 The intrinsic muscles can also be observed in Fig. 4, which shows a slice containing the C-

225 row of whiskers. The muscles (in red) can be clearly seen linking the bottom of a more rostral

226 follicle to the distal end of a more caudal follicle (C4-C3, C3-C2, C2-C1). In addition, the

227 whisker C2 (the second whisker follicle from the right) contains a clear follicle sinus and

228 ringwulst. The sinus can also clearly be seen in the follicles in Fig. 3B and C.

229 *Extrinsic Muscles*

230 The superficial extrinsic muscles, M. nasolabialis and M. maxillolabialis, are both present.

231 They insert into the caudal areas of the mystacial pad, and merge rostrally between the rows

232 of vibrissae (Fig. 5A and B). The bundles of the Mm. maxillolabialis and nasolabialis fan

233 rostrally, each forming a thin layer, so that they can usually be seen clearly in different slices

8

234   (Fig. 5A and B). Another superficial extrinsic muscle that participates in vertical vibrissa

235   spreading (Pars orbicularis oris of the M. buccinatorius) can also be seen in Fig. 2.

236   The deep vibrissa retracting muscles are part of the *M. nasolabialis profundus*. The Pars

237   interna profunda (PIP) occupies the most dorsal position in the rostral segment of the

238   mystacial pad. Its origin is represented by a number of tapered ends of muscle fibres that are

239   attached to the nasal cartilage. Muscle fibres fan and run toward rows A and B (Fig. 6A).

240   Guinea pigs possess a single Pars maxillaris that originates from a large area of the nasal

241   cartilage ventral to the PIP origin. It is not divided into two parts (superficialis et profunda),

242   as in many other rodents, and runs through and around rows C – E. The separation of the

243   deep vibrissa retracting muscles in to two groups, those targeting A and B rows and those

244   targeting rows C-E, may reflect compartmentalization of the guinea pig mystacial pad into

245   nasal and maxillary parts. The nasal and maxilliary compartments have been labelled on Figs.

246   3A and B in rat and guinea pig, and are also reflected in the higher density grouping of the

247   follicles in rows C-E, compared to A and B. The deep vibrissa retracting muscles submerge

248   near the proximal ends of the five vibrissal rows and insert into the deep fibrous mat that is

249   represented, similar to rats, by thick collagenous bundles (Fig. 7A, C). The collagenous

250   nature of these bundles was confirmed by their autofluorescence (Fig.7D).

251   The deep vibrissa protracting muscles can be seen in the mystacial pad slices as two groups

252   of densely arranged muscle bundles, that correspond to the Partes mediae superior et inferior

253   in other rodents (Fig. 7A). Their origins are not seen in tangential slices of the mystacial pad

254   because the nose of the guinea pig contains larger cartilages and well developed soft tissues,

255   compared with whisking rodents. Muscle bundles are cut transversally and contain three

256   types of muscle fibres (Fig. 8B and C), similar to those of the rat.

257

258   *Behaviour*

259   Behavioural data from the guinea pigs show that the whiskers are not moved in continuous

260   cycles; rather, they remain stationary, until a large head rotation or forward movement

261   occurs. Some cyclic movements (whisking) can be seen, but these only occur in short bouts

262   (Fig. 1B, right whisker in blue). Most whisker movements are in isolation, asymmetric and do

263   not show clear whisking (Fig. 8C). The whiskers were positioned at mean offset values of

264   98 ± 12.5 degrees, and moved with mean amplitudes of 44 ± 25.9 degrees (Fig. 8A and B).

9

265

266 **Discussion**

267 The guinea pig is a ground-dwelling, diurnal mammal of the group Histricomorpha. As such,

268 we would expect to see a reduction in the number of whiskers and mystacial muscles,

269 compared to climbing, nocturnal rodents, such as rats and mice. We see here that the number

270 of whiskers are not only reduced in number, but also more irregularly distributed through the

271 pad. While the intrinsic and extrinsic mystacial musculature is largely conserved between

272 guinea pigs and rats, it is more irregular and somewhat reduced in the guinea pig. This has

273 implications for behaviour, with the guinea pig moving their whiskers in isolation and

274 asymmetrically, compared to the cyclic and almost continuous movements of whiskers

275 observed in rats and mice.

276 *Whisker layout and follicles*

277 The guinea pig mystacial pad has around 23 whiskers arranged in a grid-like layout. It

278 contains five rows of whiskers, which is the same as in rats and mice (Haidarliu et al., 2010).

279 However, each row in the guinea pig contains fewer whiskers, especially the most dorsal row

280 A, which only contains two whiskers (Fig. 2). Indeed, the guinea pig has much fewer

281 whiskers than hamsters (23 whiskers, Wineski, 1985; Haidarliu and Ahissar, 1997), rats (33

282 whiskers, Haidarliu et al., 2010), mice (33 whiskers, Dörfl, 1982), and even shrews (around

283 40 whiskers Kulikov, 2011; Brecht et al. 2011) who have a much earlier evolutionary lineage

284 than guinea pigs. This reduction in whisker number in the guinea pig is, therefore, likely to be

285 associated with a diurnal, visual lifestyle, rather than simply being more primitive than rats

286 and mice.

287 As well as there being fewer whiskers in guinea pig, compared to rats and mice, the whiskers

288 are also more irregularly positioned (compare Fig. 3A and 3B). In rats, the straddler whiskers

289 are caudal to the main whisker rows, and sit between them in a regular fashion (Haidarliu et

290 al., 2010). In the guinea pig, straddlers α and β sit fairly uniformly and are caudal and dorsal

291 to row B and C, respectively; however, γ and δ do not align well with rows D and E (Fig. 2).

292 Whisker rows D and E are also displaced rostrally in the pad, such that D2 and E2 whisker

293 follicles are aligned with B3 and C3 (Fig. 2). The irregular organization of the whisker

294 follicles is also associated with a similar topographic disorganization of barrel structures in

295 the somatosensory cortex (Woolsey et al., 1975; Haidarliu et al., 1997).

296   Individual whisker follicles in the guinea pig are large, and contain a clear follicle sinus and

297   ringwulst, similar to rats and opossums (Grant et al., 2013). This agrees with observations

298   from Rice et al. (1986), who found that guinea pig follicles were of a similar structure to

299   hamsters, mice, rats, gerbils, rabbits, guinea pigs and cats. Rice et al. (1986) measured the

300   degree of innervation in the guinea pig follicle, approximated by the number of axons in the

301   deep vibrissal nerve, and found it to be comparable to all these animals. However,

302   innervation of the inner conical body (the deep area of the follicle), in particular, was

303   decreased in the guinea pig and cat, compared to whisking animals such as the hamster,

304   mouse, rat and gerbil (Rice et al., 1986). This variation of innervation in the guinea pig

305   between the inner conical body and other areas of the follicle sinus complex (such as the

306   cavernous sinus and the ring sinus) caused the authors to conclude that innervation of the

307   guinea pig follicle was disorganized through the structure.

308   *Musculature*

309   The guinea pig mystacial pad contains intrinsic whisker muscles. This is relatively

310   unsurprising as intrinsic muscles have also been described in mice (Dörfl, 1982), hamsters

311   (Wineski, 1985), opossums (Grant et al., 2013), rats (Haidarliu et al., 2010), shrews (Yohro,

312   1977) and even nocturnal primates (Muchlinski et al., 2013), lending confidence to the view

313   that this is a primitive mammalian trait. The intrinsic muscles in guinea pigs are thinner than

314   those in rats (Fig. 3A and B) by around 0.02 mm, despite guinea pigs being slightly larger

315   than the rats overall. In addition, the intrinsic muscles are also more irregular. For example,

316   two types of oblique intrinsic muscles occur in guinea pig; those that pass between follicles in

317   different rows, and those that connect follicles the same row (Fig. 3). Oblique intrinsic

318   muscles that connect follicles in neighboring rows (i.e. between B and C in Fig. 3C) are

319   relatively rare, and as yet have only been observed in the more ventral rows of the mystacial

320   pad in the big-clawed shrew ("straddling" muscles) (Yohro, 1977). The oblique intrinsic

321   muscles that connect follicles in the same row can be observed in the guinea pig in row A

322   (Fig. 3D). The position and attachment of these oblique intrinsic muscles in row A suggests

323   that they may cause a torsional rotation of the most dorsal whiskers, enabling the A row to

324   rotate during protraction. This type of oblique intrinsic muscle has only been observed before

325   in the opossum, *Monodelphis domestica*, which contains oblique intrinsic muscles in both the

326   A and B rows (Grant et al., 2013). In the opossum, the oblique intrinsic muscles were thought

327   to fortify the eye area (Grant et al., 2013), perhaps moving the whiskers in front of eye for

328   protection against collisions. The presence of these oblique intrinsic muscles in both the

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329   opossum and guinea pig may not, therefore, simply be representative of a disorganization of

330   the pad, but also lends support for the idea that whiskers could have a possible function in

331   protecting the eye area.

332   Superficial extrinsic muscles, that drive retraction movements of the vibrissae, are present in

333   the guinea pig (Fig. 5A and C), and have previously been described in hamsters (Wineski,

334   1985), mice (Dörfl, 1982; Klingener, 1964), rats (Haidarliu et al., 2010), jerboas (Klingener,

335   1964), opossums (Minkoff et al., 1979; Grant et al., 2013) and shrews (Yohro, 1977). There

336   is some variation between species, for example in the big-clawed shrew, the striated *M.*

337   *nasolabialis superficialis* is also associated with smooth muscle fibres just beneath the

338   corium (Yohro, 1977). In the guinea pig, these muscles look to be striated throughout, much

339   like in the rat and opossum (Grant et al., 2013; Haidarliu et al., 2010).

340   The guinea pig has deep vibrissa retracting muscles that are parts of the *M. nasolabialis*

341   *profundus.* They originate around the nose, run down most of the length of the mystacial pad

342   and pull the deep layers of the whisker pad forward, enabling the whiskers to retract back. In

343   mice and rats, these muscles belong to the bipennate type, indicating that their origins are

344   tendinous, and their attachment is limited by a small area of the nasal cartilage to which the

345   tendon is attached (Haidarliu et al., 2010, 2015). In the guinea pig, these muscles belong to a

346   divergent type; their origins are represented by multiple tapered ends of muscle fibres that

347   occupy a considerably larger surface of the nasal cartilage (Fig. 6). The fibres of such

348   muscles are long, and they fan in such a manner that their insertion sites are spread over a

349   large area reaching the deep fibrous mat of the mystacial pad. Similar fanning architecture of

350   the subunits of the *M. nasolabialis profundus*, and a single Pars maxillaris were also observed

351   in hamsters (Wineski, 1985).

352   Aspects of the deep retracting muscles have previously been described in mice (Dörfl, 1982;

353   Haidarliu et al., 2015; Klingener, 1964; Rinker, 1954), hamsters (Wineski, 1985), opossums

354   (Grant et al., 2013) and rats (Haidarliu et al., 2010; Rinker, 1954). In the opossum,

355   *Monodelphis domestica*, these muscles are greatly reduced, so much so the animal cannot

356   control retraction movements during contact (Grant et al., 2013). That these muscles are

357   almost absent in the opossum, but present in the guinea pig indicates that the deep retracting

358   muscles might have become more established in a common ancestor of guinea pigs and rats,

359   about 75 million years ago (Adkins et al., 2001).

360   The most dorsal deep retracting vibrissae muscle (PIP) submerges under the rows A and B

361   and is separated by a few hundred microns from the Pars maxillaris that runs toward rows C –

362   E. Such separation may reflect compartmentalization of the guinea pig mystacial pad into the

363   nasal and maxillary parts that has not yet been described in guinea pigs.

364   Compartmentalization of the mystacial pad has been already observed in mice (Yamakado

365   and Yohro, 1979) and opossums (Grant et al., 2013), and it has been shown that nasal and

366   maxillary compartments of the mystacial pad develop from different growth centres in

367   embryo (Yamakado and Yohro, 1979).

368   In the guinea pig mystacial pad, the Partes media superior and inferior of the *M. nasolabialis*

369   *profundus* differ significantly from those in rats and mice. In rats and mice, these deep

370   protracting muscles are organized in to groups and can be observed between vibrissae rows

371   along the whole length of the mystacial pad (Haidarliu et al., 2010). They act on the more

372   caudal vibrissae especially, pulling them rostrally to reduce the spread of the whiskers overall

373   during protraction. In the guinea pig, a number of discrete bundles of muscle fibers can be

374   seen sliced transversally (in Fig. 7); however, these are only observed in the most rostral area

375   of the mystacial pad. We therefore conclude that guinea pigs do not have extrinsic protracting

376   muscles that would be analogous to those described in mice and rats.

377   *Behaviour*

378   The guinea pig moves its whiskers with a mean amplitude of 44±25.9 degrees, which is

379   comparable to rats (43.19±7.65 degrees), but even larger than mice (31.25±11.64 degrees)

380   and opossums (36.04±9.53 degrees) (Mitchinson et al., 2011). The guinea pig positions its

381   whiskers with a mean offset angle of 98±12.5 degrees, which is similar to the rat

382   (100.63±9.21 degrees) and opossum (94.42±9.01 degrees), but set slightly further back than

383   the mouse (112.53±6.85 degrees) (Mitchinson et al., 2011). While the range and position of

384   the whisker movements is fairly comparable to rats and mice, the movements themselves are

385   really rather different. The movements are rarely cyclic, and whisking is often absent, or only

386   occurs in short bouts of around three or four whisks and usually only unilaterally (Fig. 1B,

387   Fig. 9C), which agrees with previous observations of guinea pig whisker movements (Jin et

388   al., 2004). Indeed, guinea pig whisker movements are often asymmetric, occurring with head

389   rotations, and do not resemble the whisking motions observed in rats, mice and opossums.

390   The lack of whisking movements is probably associated with the thin and irregular intrinsic

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391 whisker muscles, causing the whiskers to move less often, compared to those of rats and

392 mice.

393 *Implications*

394 The total number of whiskers are reduced in the guinea pig (at 23 whiskers), which is a more

395 comparable amount to the marsupial opossum (23 whiskers), than to rats and mice (33

396 whiskers), despite them being closer related. Diurnal primates also have fewer whiskers (with

397 a minimum of 7 whiskers) that tend to be especially thin, with smaller whisker follicles

398 lacking in intrinsic muscles (Muchlinski 2010; Muchlinski et al., 2013), compared to

399 nocturnal primates (who have a minimum of 11 whiskers). In addition, the layout of the

400 whiskers tends to be disorganized in diurnal primates, who lack a clear grid-like arrangement

401 (Muchlinski et al., 2013). These aspects can also be observed in the guinea pig, but to a

402 slightly lesser extent, and might indicate common properties of a diurnal, visual lifestyle.

403 While there were no differences in the whisker follicle appearance, it was fairly large and

404 contained a sinus - the mystacial pad of the guinea pig was disorganized in terms of whisker

405 layout, intrinsic musculature and even innervation of the follicle. It might, therefore, be that

406 vibrissae organization, innervation distribution and whisker number are key predictors of

407 whisker specialisation in mammals, with whisker specialists, such as mice and rats, having

408 more whiskers that are better organized.

409 That the diurnal guinea pig still has large and sensitive whisker follicles, and can exert

410 movement over the whiskers using a complex architecture of intrinsic and extrinsic muscles,

411 indicates that the whiskers are functional in this animal, despite a greater reliance on vision.

412 Overall, the guinea pig mystacial pad is remarkably similar to rats and mice, despite them

413 moving their whiskers less and being ground-dwelling and diurnal. This might be due to

414 these animals being relatively closely related or, more likely, that the whiskers maintain an

415 important role for the guinea pig. Although being arboreal and nocturnal are important factors

416 in predicting the presence of intrinsic muscles, aspects of body size and other lifestyle

417 variables are also important influences (Mitchinson et al., 2011; Muchlinski et al., 2013),

418 such as being small and living in social groups (Muchlinski et al., 2013). Guinea pigs are

419 extremely social animals and live in large groups displaying quite complex social behaviours.

420 While whisker touch is implicated in social behaviours (Barnett, 2007; Muchlinski et al.,

421 2013; Wolfe et al., 2011) this has not yet been explored in guinea pigs. It does seem likely

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422   that the whiskers could play an important role in aggressive and submissive interactions in

423   the guinea pig (for example, see figures in Grant and Mackintosh 1963).

424

425   *Conclusions*

426   In agreement with other studies on diurnal mammals, guinea pigs have fewer and less-

427   organized whiskers, than arboreal, nocturnal rodents. While the reduction in whisker number

428   and mystacial musculature suggests a larger reliance of the guinea pig on visual information,

429   overall, the mystacial pad is surprisingly similar to rat and mouse, indicating that the

430   whiskers may still play an important role in the life of the guinea pig. We suggest here that

431   protecting the eye and social touch behaviours are both roles that the whiskers might play in

432   guinea pig, and these will be important aspects of future research. Furthermore, we provide

433   evidence that vibrissae organization, in terms of mystacial musculature, follicle layout and

434   whisker number, is a key predictor of whisker specialisation in mammals.

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531   **Figure Legends**

532   **Figure 1. Recording and tracking guinea pig behaviour. A**. The experimental set-up. The

533   high-velocity video camera above the arena, which was illuminated from below by an

534   infrared light box. **B**. An example of recording of whisker angles (nma: naïve mean angle) of

535   the left (in red) and right (in blue) whisker fields. Inset is the tracked video footage showing

536   head and whisker traces.

537   **Figure 2. Layout of the mystacial vibrissae in a superficial tangential slice of the**

538   **mystacial pad of the guinea pig.** Staining for cytochrome oxidase activity. (A1 – E5)

539   Follicles of the mystacial vibrissae ; α – δ, straddler follicles; FBP, furry buccal pad; N,

540   nostril; N1 – N6, a row of follicles of the nasal (rhinal) vibrissae; POO, Pars orbicularis oris

541   of the M. buccinatorius; R, rostral; V, ventral. Scale bar = 1 mm.

542   **Figure 3. Intrinsic muscles in the rat (A) and guinea pig (B – E). A** and **B** show the layout

543   of the mystacial pad of the rat and guinea pig, intrinsic muscles of the C row vibrissae are

544   indicated by black arrows, although intrinsic muscles are present throughout, from row A to

545   E in both rat and guinea pig. (N) Nasal compartment. (M) Maxilliary compartment. **C**. A

546   tangential slice of the mystacial pad showing intrinsic muscles at higher magnification,

547   including a straddling oblique intrinsic muscle (arrow head); **D**. enlarged boxed area in **C**; **E**.

548   row A and oblique intrinsic muscle between follicles of the vibrissae A1 and A2 (arrow

549   head). (1) Follicle sinus. Scale bars in **A** and **B** = 1 mm, **C** and **E** = 0.5 mm and **D** = 0.1 mm.

550   All figure panels show tangential slices stained for cytochrome oxidase activity.

551   **Fig. 4. Guinea pig whisker follicles of the C-row**. A horizontal slice of the mystacial pad

552   stained with Masson's Trichrome. C1 – C4, whisker follicles; C, caudal, M, medial. (1) Ring

553   sinus; (2) ringwulst. Scale bar = 1 mm.

554   **Figure 5. Superficial vibrissa retracting extrinsic muscles of the guinea pig mystacial**

555   **pad.** Tangential slices of the mystacial pad stained for cytochrome oxidase activity.

556   (α, β, γ) Straddler follicles; B1, C1, vibrissal follicles; ML, M. maxillolabialis; NL, M.

557   nasolabialis; R, rostral; V, ventral. Scale bars = 0.5 mm

558   **Figure 6. Deep extrinsic vibrissa retracting muscles of the guinea pig.** A tangential slice

559   of the mystacial pad stained for cytochrome oxidase activity. These muscles are part of the

560   M. nasolabialis profundus. **A.** A deep tangential slice of the mystacial pad. **B.** Enlarged boxed

561   area in (**A**). (α, β, γ, δ) straddler follicles; (A1 – E2) vibrissa follicles. (1) Pars interna

562   profunda; (2) Pars maxillaris; (3) Pars anterior; (4, 5) tapered ends of the muscle fibres of the

563   Pars interna profunda and Pars maxillaris, respectively, that are attached to the nasal

564   cartilage; N, nostril; R, rostral; V, ventral. Scale bars = 1 mm in (A) and 0.5 mm in (B).

565   **Figure 7. Deep extrinsic vibrissa protracting and retracting muscles of the guinea pig.** A

566   tangential slice of the mystacial pad stained for cytochrome oxidase activity. **A.** A very deep

567   tangential slice of the mystacial pad. **B** and **C.** Enlarged boxed areas in **A**, respectively. **D.**

568   Collagen autofluorescence in the area shown in **C**. (α) straddler follicle; CF, collagenous

569   bundles of the deep fibrous mat; MB, muscle bundles; MF, muscle fibres; N, nostril; N1, a

570   follicle of the nasal vibrissae; PM, Pars maxillaris; PMI, pars media inferior; PMS, Pars

571   media superior; R, rostral; V, ventral. Scale bars = 1 mm in (**A**), 0.1 mm in (**B**), and 0.5 mm

572   in (**C**) 247 and (**D**)

573   **Figure 8. Whisker movements in guinea pig**. **A.** A histogram of whisker offset, the mean

574   angular position of the whiskers; **B.** a histogram of whisker amplitude, the amount the

575   whiskers move; **C.** an example trace of mean whisker angular positions from the left (in red)

576   and right (in blue) whisker fields.

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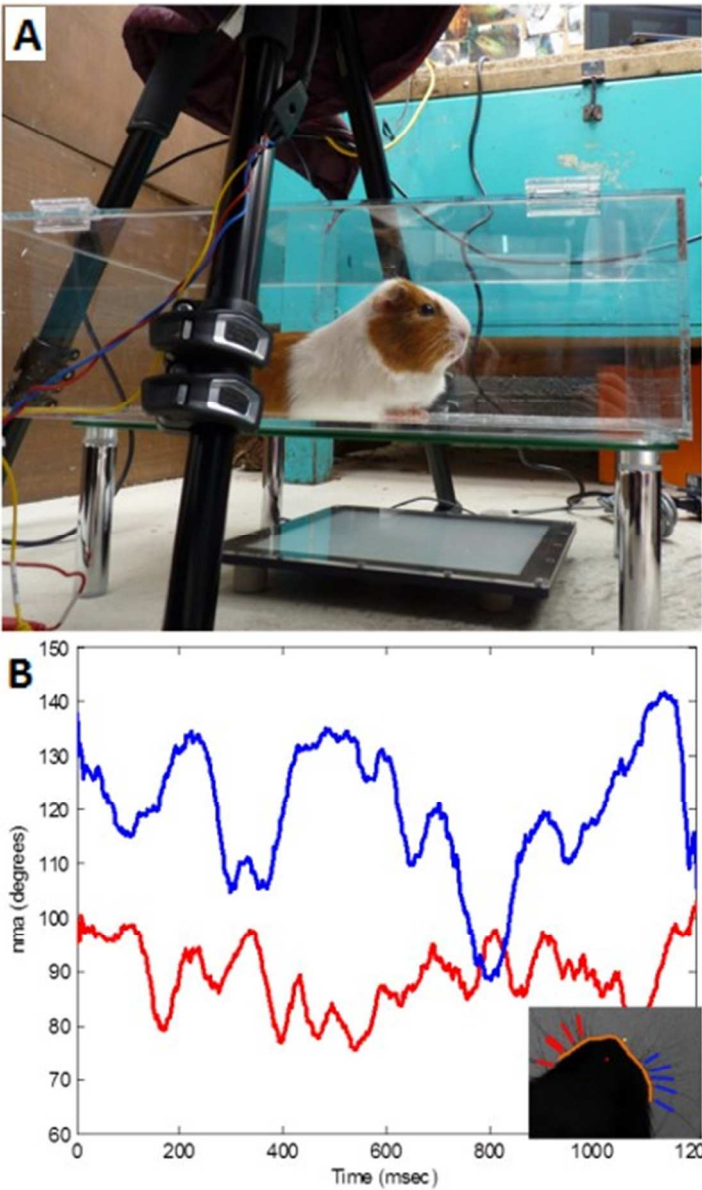48
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Figure 1. Recording and tracking guinea pig behaviour. A. The experimental set-up. The high-velocity video camera above the arena, which was illuminated from below by an infrared light box. B. An example of recording of whisker angles (nma: naïve mean angle) of the left (in red) and right (in blue) whisker fields. Inset is the tracked video footage showing head and whisker traces.
Figure 1
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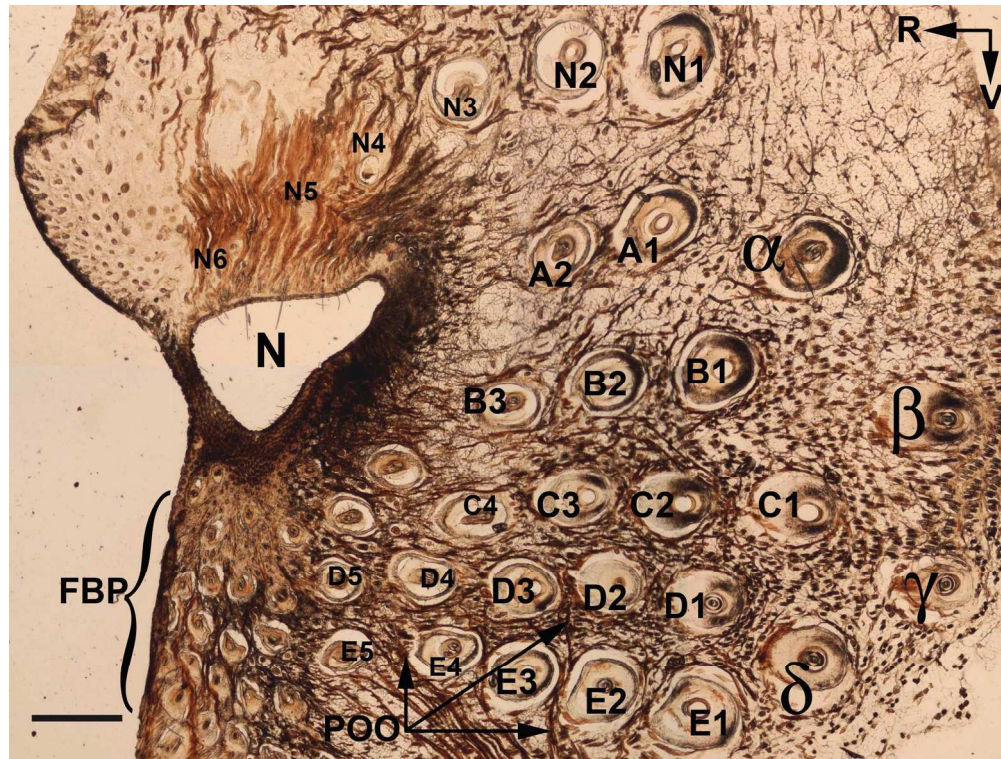
Figure 2. Layout of the mystacial vibrissae in a superficial tangential slice of the mystacial pad of the guinea pig. Staining for cytochrome oxidase activity. (A1 – E5) Follicles of the mystacial vibrissae; α-δ straddler follicles; FBP, furry buccal pad; N, nostril; N1 – N6, a row of follicles of the nasal (rhinal) vibrissae; POO, Pars orbicularis oris of the M. buccinatorius; R, rostral; V, ventral. Scale bar = 1 mm.
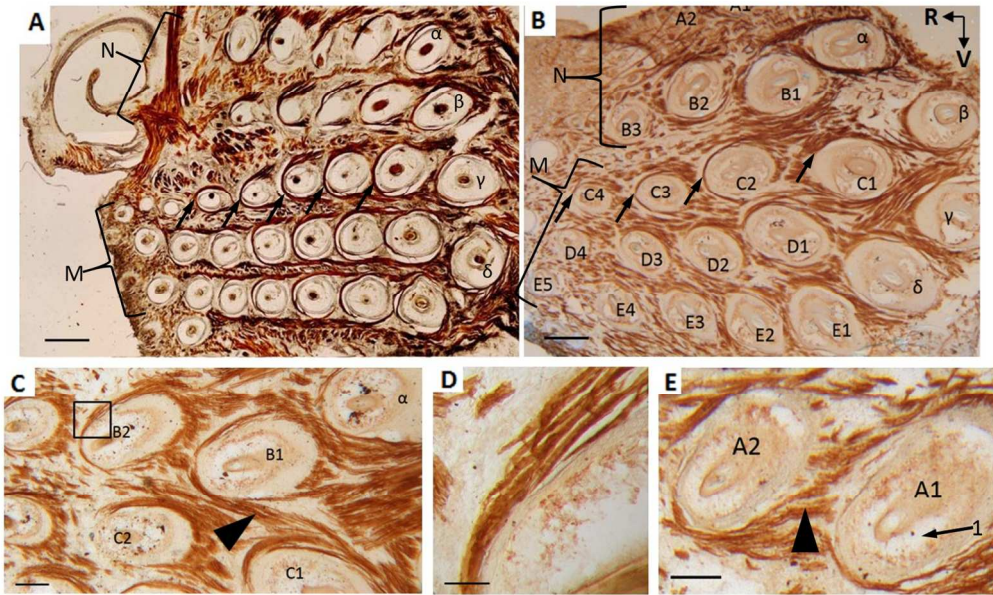
Figure 2
180x135mm (300 x 300 DPI)

Figure 3. Intrinsic muscles in the rat (A) and guinea pig (B – E). A and B show the layout of the mystacial pad of the rat and guinea pig, intrinsic muscles of the C row vibrissae are indicated by black arrows, although intrinsic muscles are present throughout, from row A to E in both rat and guinea pig. (N) Nasal compartment. (M) Maxilliary compartment. C. A tangential slice of the mystacial pad showing intrinsic muscles at higher magnification, including a straddling oblique intrinsic muscle (arrow head); D. enlarged boxed area in C; E. row A and oblique intrinsic muscle between follicles of the vibrissae A1 and A2 (arrow head). (1) Follicle sinus. Scale bars in A and B = 1 mm, C and E = 0.5 mm and D = 0.1 mm. All figure panels show tangential slices stained for cytochrome oxidase activity
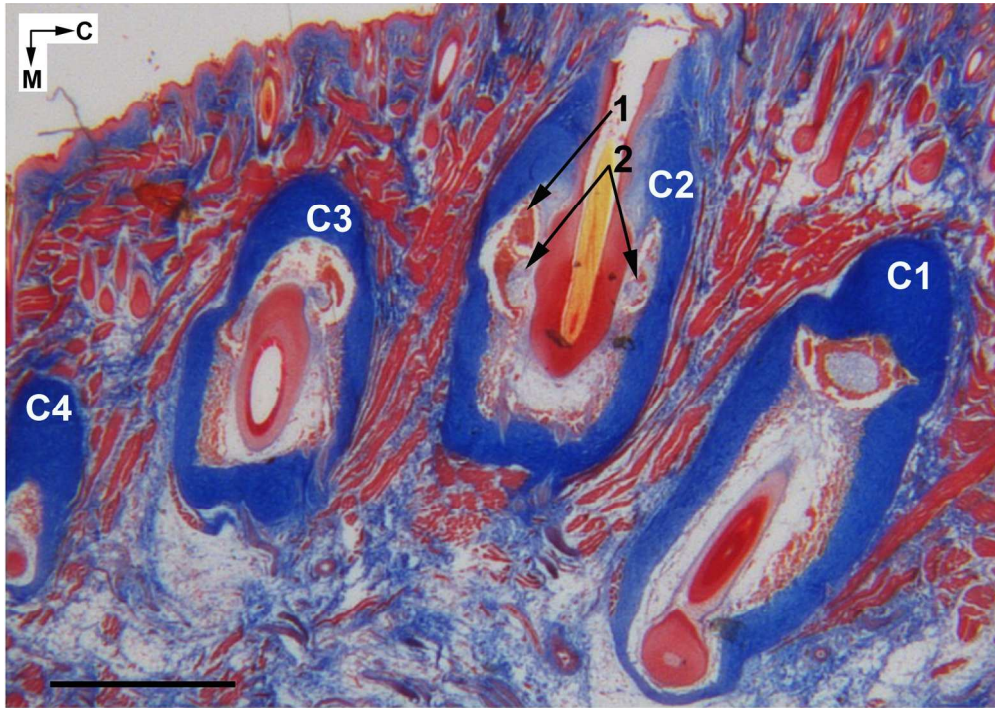Figure 3
272x164mm (150 x 150 DPI)

Fig. 4. Guinea pig whisker follicles of the C-row. A horizontal slice of the mystacial pad stained with Masson's Trichrome. C1 – C4, whisker follicles; C, caudal, M, medial. (1) Ring sinus; (2) ringwulst. Scale bar = 1 mm.
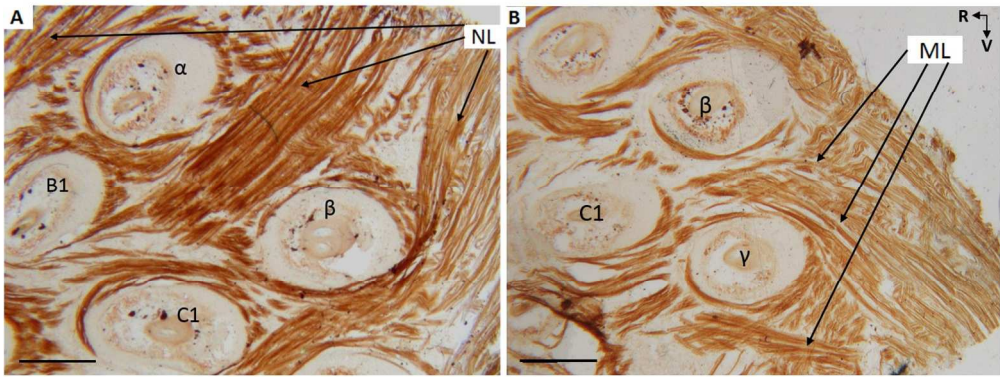Figure 4
170x120mm (300 x 300 DPI)

Figure 5. Superficial vibrissa retracting extrinsic muscles of the guinea pig mystacial pad. Tangential slices of the mystacial pad stained for cytochrome oxidase activity. (α, β, γ) Straddler follicles; B1, C1, vibrissal follicles; ML, M. maxillolabialis; NL, M. nasolabialis; R, rostral; V, ventral. Scale bars = 0.5 mm
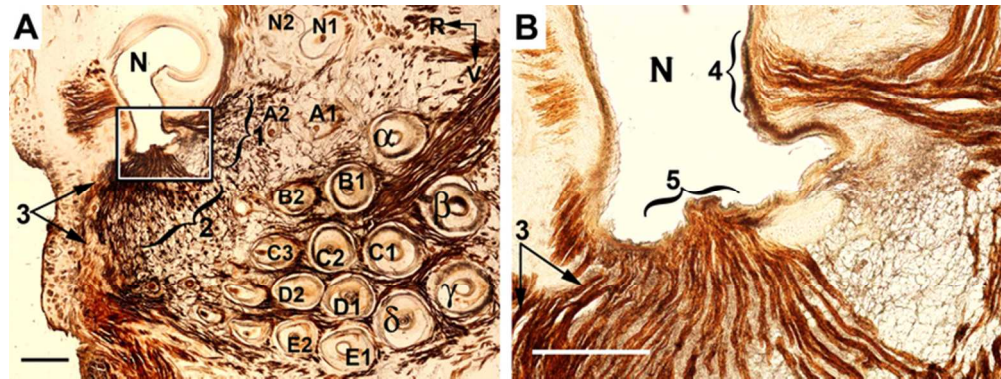
Figure 5
258x98mm (150 x 150 DPI)

Figure 6. Deep extrinsic vibrissa retracting muscles of the guinea pig. A tangential slice of the mystacial pad stained for cytochrome oxidase activity. These muscles are part of the M. nasolabialis profundus. A. A deep tangential slice of the mystacial pad. B. Enlarged boxed area in (A). (α, β, γ, δ) straddler follicles; (A1 – E2) vibrissa follicles. (1) Pars interna profunda; (2) Pars maxillaris; (3) Pars anterior; (4, 5) tapered ends of the muscle fibres of the Pars interna profunda and Pars maxillaris, respectively, that are attached to the nasal cartilage; N, nostril; R, rostral; V, ventral. Scale bars = 1 mm in (A) and 0.5 mm in (B).

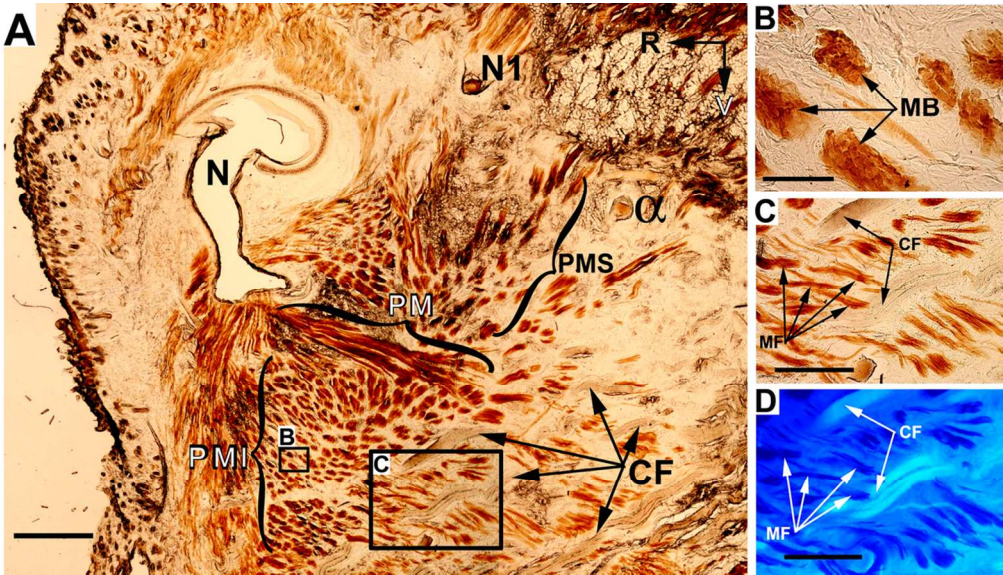Figure 6
68x25mm (300 x 300 DPI)

Figure 7. Deep extrinsic vibrissa protracting and retracting muscles of the guinea pig. A tangential slice of the mystacial pad stained for cytochrome oxidase activity. A. A very deep tangential slice of the mystacial pad. B and C. Enlarged boxed areas in A, respectively. D. Collagen autofluorescence in the area shown in C. (α) straddler follicle; CF, collagenous bundles of the deep fibrous mat; MB, muscle bundles; MF, muscle fibres; N, nostril; N1, a follicle of the nasal vibrissae; PM, Pars maxillaris; PMI, pars media inferior; PMS, Pars media superior; R, rostral; V, ventral. Scale bars = 1 mm in (A), 0.1 mm in (B), and 0.5 mm in (C) 247 and (D)
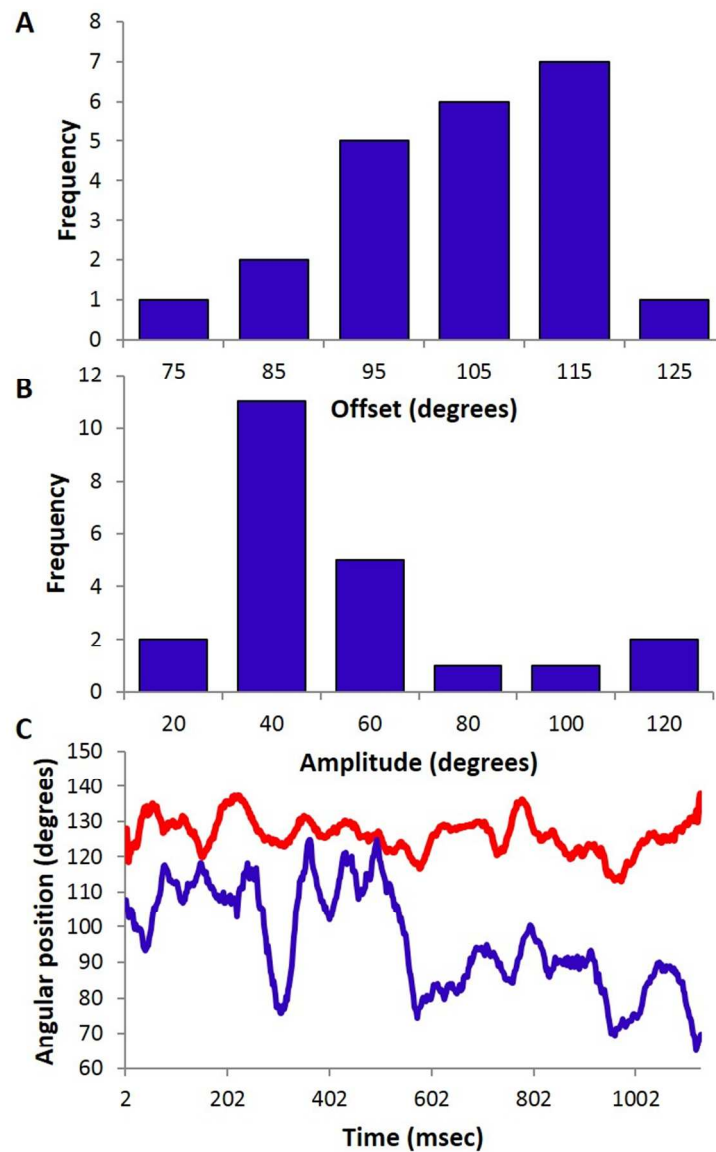
Figure 7

108x61mm (300 x 300 DPI)

Figure 8. Whisker movements in guinea pig. A. A histogram of whisker offset, the mean angular position of the whiskers; B. a histogram of whisker amplitude, the amount the whiskers move; C. an example trace of mean whisker angular positions from the left (in red) and right (in blue) whisker fields.

Figure 8
131x210mm (150 x 150 DPI)