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

Running title: Guinea pig whisker layout and musculature

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Abstract

All mammals (apart from apes and humans) have whiskers that make use of a similar muscle architecture. Whisker specialists, such as rats and mice, tend to be nocturnal and arboreal, relying on their whisker sense of touch to guide exploration around tree canopies at night. As such, nocturnal arboreal rodents have many whiskers that are organised into a grid-like pattern, and moved using a complex array of muscles. Indeed, most arboreal, nocturnal mammals tend to have specialised whiskers, that are longer and arranged in a dense, regular grid, compared to terrestrial, diurnal mammals. The guinea pig diverged early from murid rodents (around 75 million years ago), and are ground-dwelling, diurnal animals. It would be predicted that, as a terrestrial mammal, they may have less whiskers and a reduced muscle architecture compared to arboreal, nocturnal rodents. We examined the mystacial whisker layout, musculature and movement capacity of Guinea pig (*Cavia porcellus*) whiskers and found that they did indeed have a disorganized whisker layout, with a fortification around the eye area. In addition, there was a reduction in musculature, especially in the intrinsic muscles. Despite guinea pigs not cyclically moving their whiskers, the mystacial musculature was still very similar to that of murid rodents. We suggest that the conserved presence of whisker layout and musculature, even in visual mammals such as primates and guinea pigs, may indicate that whiskers still play an important role in these animals, including protecting the eyes and being involved in tactile social behaviours.

Introduction

All mammals (apart from apes and humans) have facial whiskers (vibrissae) (Ahl, 1976). Whisker specialists, such as mice, rats and hamsters are able to move their whiskers to perform large, quick, cyclic sweeps (termed whisking), which is amongst the fastest movements that mammals can make (occurring at around 25Hz in mice) (Welker, 1964; Wineski, 1983; Jin et al., 2004). The fast and precise positioning of the whiskers are enabled by a specialist whisker musculature, a complicated architecture of intrinsic and extrinsic muscles (Dörfl, 1982), which are represented mainly by fast muscle fibres (Jin et al., 2004). Perhaps the most well-studied muscle group is that of the intrinsic muscles, represented by sling-like muscles that link around the base of each whisker follicle, causing the whiskers to protract forward (Dörfl, 1982). The layout of the intrinsic muscles has been found to be largely preserved from marsupials (Grant et al., 2013) to rodents (Dörfl, 1982), to nocturnal arboreal primates (Muchlinski et al., 2013). That intrinsic whisker muscles are preserved between marsupials and rodents, even though their last common ancestor occurred around 160 million years ago (Luo et al., 2011), suggests that the common ancestor of extant mammals may well have had moveable whiskers involved in active touch sensing. While intrinsic muscles are largely preserved, the extrinsic muscles of the mystacial pad can largely vary between species (Yohro, 1977). In the marsupial *Monodelphis domestica* (Grant et al., 2013), for example, some of the extrinsic muscles are so reduced that vibrissal control is limited, and whisker spread and velocity cannot be controlled during object exploration.

The number, layout and musculature of the mystacial vibrissae are all closely linked to the function and movement abilities of the whiskers. Small, social, nocturnal and arboreal mammals have been found to have longer vibrissae with a more densely packed vibrissal field than that of ground-dwelling and burrowing mammals (Pocock, 1914; Lyne, 1959; Ahl, 1986; Muchlinski et al., 2013). Exceptions to this include semi-aquatic (i.e. Australian water rat (Dehnhardt et al., 1999)) and aquatic mammals (such as pinnipeds and sirenians (Dehnhardt, 2002)), that have long and densely-arranged whiskers, despite them being large, diurnal animals; indeed the California sea lion has the longest whiskers of all mammals. In these animals, the whiskers are a likely adaptation for an aquatic lifestyle, and are used for navigation and prey capture in a dark, underwater environment (Grant & Arkley, 2016). Arboreal, nocturnal rodents actively position their whiskers for use in a variety of functions, including navigation, locomotion, exploration, hunting and social touch (Grant & Arkley,

2016). Extensive studies in arboreal, nocturnal mice and rats have revealed that they possess three groups of whisking muscles (protractors, retractors, and vertical vibrissae deflectors) leading to a range of whisker movements and vast control abilities (Haidarliu et al., 2010). However, diurnal mammals, such as some primates, lack organized vibrissae, have very thin whiskers and a reduced whisker follicle without intrinsic muscles (Muchlinski et al., 2013).

Guinea pigs, and other Hystricomorphs, diverged from the murid rodents before the artiodactyls and primates, and are often thought of as a separate order from rodentia (Graur et al., 1991). They represent an early divergence in eutherian evolution, and as such often have rather anomalous characteristics compared to other mammals, such as their facial bone structure (Muchlinski, 2008) and body muscles (Potter et al., 1957). The guinea pig, and other Histricomorphs, have a unique facial anatomy, in that the media masseter (mastication) muscle passes through the infraorbital foramen (IOF) of the skull, which makes it particularly large, compared to the IOF of other rodents (Muchlinski, 2008). While some aspects of the maxilliary facial musculature has been described in guinea pigs (Muchlinski 2008), that of the mystacial pad has yet to be considered, despite guinea pigs being able to generate fast and large amplitude whisker movements; however, these movements do not tend to be cyclic and usually occur in isolation (Jin et al., 2004). Due to their early divergence, we might expect the guinea pig to have a whisker layout and musculature more similar to the marsupial, than the rodent tactile specialists that evolved later, such as mice and rats. Moreover, we might expect the guinea pig whisker system to be even further reduced and disorganized, due to them being diurnal, ground-living mammals.

The aim of this study is to describe the muscle architecture of the mystacial pad in the guinea pig anatomically, by cutting the mystacial pads of the guinea pig in the tangential plane and staining consecutive slices for cytochrome c oxidase activity and Masson's Trichrome. Because of the differences in proposed up-to-date schemes of whisker layouts in the guinea pig mystacial pad (Sikich et al., 1986; Haidarliu and Ahissar, 1997), we also re-examine here the layout of the mystacial pad *in situ*. All results will be compared in detail with those of rat, and also to other animals such as opossums and shrews. We will go on to consider the impact of diurnality on the whisker pad muscles and layout.

Materials and Methods

Pad removal

Eight female adult Dunkin-Hartley guinea pigs were used in the anatomy section of this study, each weighing between 350-400 g. The guinea pigs were euthanized via an overdose of anaesthetic. The mystacial pads were removed by cutting down the skin in the sagittal plane and around each pad (around 2mm on either side of the pad). They were placed into a solution of fixative (4% paraformaldehyde in 0.1 M phosphate buffer) and left for one hour. They were then straightened by placing them into histology cassettes (Medex Supply, Monsey, NY, USA) packed with high-density foam. Twelve of the pads were then placed into fixative solution enriched with sucrose up to 30% for twenty four hours, and then sectioned on the freezing microtome for staining for cytochrome oxidase activity. The remaining four pads were kept flattened for two weeks, then subjected to dehydration and clearing, and mounted in paraffin wax to slice and stain with Mason's Trichrome.

Staining for cytochrome oxidase activity

After fixing, each of the pads was sectioned using a freezing microtome (Leica CM 1800) into 60 µm thick slices in the tangential plane. All slices were stained for cytochrome oxidase activity following the method developed by Wong-Riley (1979) and modified by Haidarliu et al. (2010). Slices were floated in an oxygenated solution of 0.02% cytochrome c (0.75mg), catalase solution (40µl), and 0.05% diaminobenzidine (5mg) in 0.1 M phosphate buffer. The slices were incubated at room temperature on a shaking platform until the stain developed (approximately 1-3 hours), and a clear differentiation between non-reactive and highly reactive tissue structures could be determined. Slices were then rinsed with 0.1 M phosphate buffer. Stained slices were mounted on microscope slides and left to air dry overnight. The slices were then coverslipped with DPX.

The cross-sectional diameter of the intrinsic muscles was manually measured, perpendicular to the follicle, on the C-row whisker follicles of one slide (Fig. 3B) using the image analysis software Tracker (Brown 2015), and compared to an equivalent slice in rat.

Staining with Masson's Trichrome

Four of the pads were placed into empty histology cassettes and transferred to a tissue processor (Shandon Citadel 2000), where tissues were dehydrated through a series of graded

IMS baths (70%, 80%, 90%, 100%), and then immersed in xylene and paraffin wax. This process took around 20 hours. The samples were then mounted in a block of paraffin wax and sliced on an automatic rotary microtome (Thermos Scientific microtome HM355S) into 10 µm thick slices that were floated in a 35-37°C bath. Two mystacial pads were sliced tangentially, and two were sliced horizontally to visualise the C-row follicles. Slices were mounted onto slides and left to dry at 38°C overnight.

Slides were put in a fixative solution (4% paraformaldehyde in 0.1 M phosphate buffer) for 1 hour, and introduced to Bouin's Solution for 4 hours. They were then cleared with xylene, rehydrated with ethyl alcohol (100%, 90%, 80%, 70%) and moved through a sequence of solutions for the Masson's Trichrome staining (Biebrich Scarlet Acid, Phosphotungstic and Phosphomolybdic Acids, Aniline Blue and Acidified Water), with multiple washes of distilled water in-between each stage. The slices were then dehydrated with ethyl alcohol (70%, 80%, 90%, 100%) and xylene, towel dried and cover-slipped using DPX. All slices were visualised using a Zeiss Stereo Lumar V12 light microscope. Figures were captured using Zeiss Axiovision, version 4.8. Occasional adjustments to exposure and white balance were made.

Behavioural Filming

Nine adult female guinea pigs, of mixed strains, were used for filming. They were placed individually into a transparent, Perspex, rectangular arena (20 x 50 x 15 cm) (Fig. 1A), which was lit from below by an infrared light box (PHLOX LEDIR-BL-200/200-SLLUB-Q-1R-24V). Each guinea pig was filmed from above using a digital high-speed video camera (Phantom Miro ex2) recording at 500 frames per second with a shutter-velocity of 1 ms and a resolution of 640x480 pixels. Multiple 1.5-s video clips were collected opportunistically (by manual trigger) when the animal moved in the cameras field of view. Approximately 12 clips were collected from each animal. Two-three clips from each guinea pig were selected based on to the following selection criteria: i) the guinea pig was clearly in frame; ii) both sides of the face were visible; iii) the head was level with the floor (no extreme pitch or yaw); and iv) the whiskers were not in contact with a vertical wall. Twenty two clips in total were tracked using the BIOTACT Whisker Tracking Tool (Perkon et al., 2011). The tracker semi-automatically finds the orientation and position of the snout, and the angular position (relative to the midline of the head) of each identified whisker. Tracking was validated by manually inspecting the tracking annotations overlaid on to the video frames (Fig. 1B, inset).

The movement of the entire whisker field was determined from the unsmoothed mean of all the tracked whisker angular positions for each side frame by frame (Grant et al., 2013), which can be seen in Fig. 1B and is termed *naïve mean angle (nma)*. The *offset* was calculated as the mean nma, and an average was taken between the two whisker sides. To estimate the *amplitude*, the *offset* was removed from the whisking angle time series and the root mean square value was computed to give the root-mean-square (RMS) whisking amplitude and was estimated by multiplying the RMS whisking amplitude by $2\sqrt{2}$ (Chatfield, 2003). Whisk *frequency* was not calculated as the *nma* did not often contain clear whisks, rather they were more asymmetric movements that oriented the whisker field.

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

Results

Mystacial Pad Layout

The layout of the guinea pig whiskers indicates that there are five rather irregular rows of whiskers within the mystacial pad (Fig. 2). Dorsal to these five rows of mystacial vibrissae and to the nostril, a row of five-to-six arcwise arranged nasal vibrissae passes in the rostro-dorsal direction. The most dorsal row of the mystacial vibrissae, row A, is made of only two whiskers. Row B comprises of usually three, but sometimes four vibrissae. Rows A and B are caudally straddled by a straddler (α). Each of the rows C, D, and E contains usually five vibrissae. Rows D and E are positioned more rostral, such that vibrissae D1 and E1 align with vibrissae A2, B2 and C2. The misalignment of the rows D and E with rows A – C reveals the guinea pig to have a more irregular whisker pad than other rodents, for example rat (compare with Fig. 3A).

The straddler whiskers of the guinea pig also have a complex arrangement. Straddlers γ and δ sit ventro-caudal to the rest of the whisker pad, and it is not clear from just looking at the layout in Fig. 2, which whisker rows they are associated with. Straddler α sits caudal to rows A and B. Straddler β straddles rows B and C. Muscle fibers from the ventral side of the follicle C1, the dorsal side of the follicle D1 and straddler δ reach the follicle of the straddler γ which is positioned more caudal to them (Fig. 3B). This is a rather irregular straddler

layout, compared to the rat (Fig. 3A) where straddler whiskers straddle consecutive rows A-B, B-C, C-D, and D-E.

Intrinsic Muscles

The guinea pig whisker pad contains sling-like intrinsic muscles (Figs. 3B-D) that form a sling around the rostral areas of each follicle and attach to the caudal follicle in the same row. These muscles are striated and made up of red, pink and white striated muscle fibers (Fig. 3D). The intrinsic muscles look much reduced, are much thinner, and are not as striking as those seen in the rat (compare Figs. 3A and 3B). Indeed, the cross-sectional diameter of the intrinsic muscles (measured at the point of the arrows on Figs. 3A and 3B) show that the C row intrinsic muscle diameter is 0.080±0.01 mm in rat and 0.061±0.004 mm in guinea pig, despite the guinea pig being slightly bigger overall.

In addition, the intrinsic muscles are, on the whole, more irregular in the guinea pig. In the rat, the intrinsic muscles connect each consecutive follicle within the same row, forming a regular, chain-like architecture (Figure 3A). However, in the guinea pig, oblique intrinsic muscles pass both between and within vibrissal rows. Figure 3C shows an oblique intrinsic muscle passing between follicles in different rows, from the ventral part of the B1 follicle attaching to the dorsal part of the C1 follicle. Figure 3E shows an oblique intrinsic muscle passing within vibrissal rows, from the ventral part of A2 crossing to the dorsal part of A1. These oblique intrinsic muscles are not observed in rat.

Whisker Follicles

The intrinsic muscles can also be observed in Fig. 4, which shows a slice containing the C-row of whiskers. The muscles (in red) can be clearly seen linking the bottom of a more rostral follicle to the distal end of a more caudal follicle (C4-C3, C3-C2, C2-C1). In addition, the whisker C2 (the second whisker follicle from the right) contains a clear follicle sinus and ringwulst. The sinus can also clearly be seen in the follicles in Fig. 3B and C.

Extrinsic Muscles

The superficial extrinsic muscles, M. nasolabialis and M. maxillolabialis, are both present. They insert into the caudal areas of the mystacial pad, and merge rostrally between the rows of vibrissae (Fig. 5A and B). The bundles of the Mm. maxillolabialis and nasolabialis fan rostrally, each forming a thin layer, so that they can usually be seen clearly in different slices

(Fig. 5A and B). Another superficial extrinsic muscle that participates in vertical vibrissa spreading (Pars orbicularis oris of the M. buccinatorius) can also be seen in Fig. 2.

The deep vibrissa retracting muscles are part of the *M. nasolabialis profundus.* The Pars interna profunda (PIP) occupies the most dorsal position in the rostral segment of the mystacial pad. Its origin is represented by a number of tapered ends of muscle fibres that are attached to the nasal cartilage. Muscle fibres fan and run toward rows A and B (Fig. 6A). Guinea pigs possess a single Pars maxillaris that originates from a large area of the nasal cartilage ventral to the PIP origin. It is not divided into two parts (superficialis et profunda), as in many other rodents, and runs through and around rows C – E. The separation of the deep vibrissa retracting muscles in to two groups, those targeting A and B rows and those targeting rows C-E, may reflect compartmentalization of the guinea pig mystacial pad into nasal and maxillary parts. The nasal and maxilliary compartments have been labelled on Figs. 3A and B in rat and guinea pig, and are also reflected in the higher density grouping of the follicles in rows C-E, compared to A and B. The deep vibrissa retracting muscles submerge near the proximal ends of the five vibrissal rows and insert into the deep fibrous mat that is represented, similar to rats, by thick collagenous bundles (Fig. 7A, C). The collagenous nature of these bundles was confirmed by their autofluorescence (Fig.7D).

The deep vibrissa protracting muscles can be seen in the mystacial pad slices as two groups of densely arranged muscle bundles, that correspond to the Partes mediae superior et inferior in other rodents (Fig. 7A). Their origins are not seen in tangential slices of the mystacial pad because the nose of the guinea pig contains larger cartilages and well developed soft tissues, compared with whisking rodents. Muscle bundles are cut transversally and contain three types of muscle fibres (Fig. 8B and C), similar to those of the rat.

Behaviour

Behavioural data from the guinea pigs show that the whiskers are not moved in continuous cycles; rather, they remain stationary, until a large head rotation or forward movement occurs. Some cyclic movements (whisking) can be seen, but these only occur in short bouts (Fig. 1B, right whisker in blue). Most whisker movements are in isolation, asymmetric and do not show clear whisking (Fig. 8C). The whiskers were positioned at mean offset values of 98±12.5 degrees, and moved with mean amplitudes of 44±25.9 degrees (Fig. 8A and B).

Discussion

The guinea pig is a ground-dwelling, diurnal mammal of the group Histricomorpha. As such, we would expect to see a reduction in the number of whiskers and mystacial muscles, compared to climbing, nocturnal rodents, such as rats and mice. We see here that the number of whiskers are not only reduced in number, but also more irregularly distributed through the pad. While the intrinsic and extrinsic mystacial musculature is largely conserved between guinea pigs and rats, it is more irregular and somewhat reduced in the guinea pig. This has implications for behaviour, with the guinea pig moving their whiskers in isolation and asymmetrically, compared to the cyclic and almost continuous movements of whiskers observed in rats and mice.

Whisker layout and follicles

The guinea pig mystacial pad has around 23 whiskers arranged in a grid-like layout. It contains five rows of whiskers, which is the same as in rats and mice (Haidarliu et al., 2010). However, each row in the guinea pig contains fewer whiskers, especially the most dorsal row A, which only contains two whiskers (Fig. 2). Indeed, the guinea pig has much fewer whiskers than hamsters (23 whiskers, Wineski, 1985; Haidarliu and Ahissar, 1997), rats (33 whiskers, Haidarliu et al., 2010), mice (33 whiskers, Dörfl, 1982), and even shrews (around 40 whiskers Kulikov, 2011; Brecht et al. 2011) who have a much earlier evolutionary lineage than guinea pigs. This reduction in whisker number in the guinea pig is, therefore, likely to be associated with a diurnal, visual lifestyle, rather than simply being more primitive than rats and mice.

As well as there being fewer whiskers in guinea pig, compared to rats and mice, the whiskers are also more irregularly positioned (compare Fig. 3A and 3B). In rats, the straddler whiskers are caudal to the main whisker rows, and sit between them in a regular fashion (Haidarliu et al., 2010). In the guinea pig, straddlers α and β sit fairly uniformly and are caudal and dorsal to row B and C, respectively; however, γ and δ do not align well with rows D and E (Fig. 2). Whisker rows D and E are also displaced rostrally in the pad, such that D2 and E2 whisker follicles are aligned with B3 and C3 (Fig. 2). The irregular organization of the whisker follicles is also associated with a similar topographic disorganization of barrel structures in the somatosensory cortex (Woolsey et al., 1975; Haidarliu et al., 1997).

Individual whisker follicles in the guinea pig are large, and contain a clear follicle sinus and ringwulst, similar to rats and opossums (Grant et al., 2013). This agrees with observations from Rice et al. (1986), who found that guinea pig follicles were of a similar structure to hamsters, mice, rats, gerbils, rabbits, guinea pigs and cats. Rice et al. (1986) measured the degree of innervation in the guinea pig follicle, approximated by the number of axons in the deep vibrissal nerve, and found it to be comparable to all these animals. However, innervation of the inner conical body (the deep area of the follicle), in particular, was decreased in the guinea pig and cat, compared to whisking animals such as the hamster, mouse, rat and gerbil (Rice et al., 1986). This variation of innervation in the guinea pig between the inner conical body and other areas of the follicle sinus complex (such as the cavernous sinus and the ring sinus) caused the authors to conclude that innervation of the guinea pig follicle was disorganized through the structure.

Musculature

The guinea pig mystacial pad contains intrinsic whisker muscles. This is relatively unsurprising as intrinsic muscles have also been described in mice (Dörfl, 1982), hamsters (Wineski, 1985), opossums (Grant et al., 2013), rats (Haidarliu et al., 2010), shrews (Yohro, 1977) and even nocturnal primates (Muchlinski et al., 2013), lending confidence to the view that this is a primitive mammalian trait. The intrinsic muscles in guinea pigs are thinner than those in rats (Fig. 3A and B) by around 0.02 mm, despite guinea pigs being slightly larger than the rats overall. In addition, the intrinsic muscles are also more irregular. For example, two types of oblique intrinsic muscles occur in guinea pig; those that pass between follicles in different rows, and those that connect follicles the same row (Fig. 3). Oblique intrinsic muscles that connect follicles in neighboring rows (i.e. between B and C in Fig. 3C) are relatively rare, and as yet have only been observed in the more ventral rows of the mystacial pad in the big-clawed shrew ("straddling" muscles) (Yohro, 1977). The oblique intrinsic muscles that connect follicles in the same row can be observed in the guinea pig in row A (Fig. 3D). The position and attachment of these oblique intrinsic muscles in row A suggests that they may cause a torsional rotation of the most dorsal whiskers, enabling the A row to rotate during protraction. This type of oblique intrinsic muscle has only been observed before in the opossum, *Monodelphis domestica*, which contains oblique intrinsic muscles in both the A and B rows (Grant et al., 2013). In the opossum, the oblique intrinsic muscles were thought to fortify the eye area (Grant et al., 2013), perhaps moving the whiskers in front of eye for protection against collisions. The presence of these oblique intrinsic muscles in both the

opossum and guinea pig may not, therefore, simply be representative of a disorganization of the pad, but also lends support for the idea that whiskers could have a possible function in protecting the eye area.

Superficial extrinsic muscles, that drive retraction movements of the vibrissae, are present in the guinea pig (Fig. 5A and C), and have previously been described in hamsters (Wineski, 1985), mice (Dörfl, 1982; Klingener, 1964), rats (Haidarliu et al., 2010), jerboas (Klingener, 1964), opossums (Minkoff et al., 1979; Grant et al., 2013) and shrews (Yohro, 1977). There is some variation between species, for example in the big-clawed shrew, the striated *M. nasolabialis superficialis* is also associated with smooth muscle fibres just beneath the corium (Yohro, 1977). In the guinea pig, these muscles look to be striated throughout, much like in the rat and opossum (Grant et al., 2013; Haidarliu et al., 2010).

The guinea pig has deep vibrissa retracting muscles that are parts of the *M. nasolabialis profundus.* They originate around the nose, run down most of the length of the mystacial pad and pull the deep layers of the whisker pad forward, enabling the whiskers to retract back. In mice and rats, these muscles belong to the bipennate type, indicating that their origins are tendinous, and their attachment is limited by a small area of the nasal cartilage to which the tendon is attached (Haidarliu et al., 2010, 2015). In the guinea pig, these muscles belong to a divergent type; their origins are represented by multiple tapered ends of muscle fibres that occupy a considerably larger surface of the nasal cartilage (Fig. 6). The fibres of such muscles are long, and they fan in such a manner that their insertion sites are spread over a large area reaching the deep fibrous mat of the mystacial pad. Similar fanning architecture of the subunits of the *M. nasolabialis profundus*, and a single Pars maxillaris were also observed in hamsters (Wineski, 1985).

Aspects of the deep retracting muscles have previously been described in mice (Dörfl, 1982; Haidarliu et al., 2015; Klingener, 1964; Rinker, 1954), hamsters (Wineski, 1985), opossums (Grant et al., 2013) and rats (Haidarliu et al., 2010; Rinker, 1954). In the opossum, *Monodelphis domestica*, these muscles are greatly reduced, so much so the animal cannot control retraction movements during contact (Grant et al., 2013). That these muscles are almost absent in the opossum, but present in the guinea pig indicates that the deep retracting muscles might have become more established in a common ancestor of guinea pigs and rats, about 75 million years ago (Adkins et al., 2001).

The most dorsal deep retracting vibrissae muscle (PIP) submerges under the rows A and B and is separated by a few hundred microns from the Pars maxillaris that runs toward rows C – E. Such separation may reflect compartmentalization of the guinea pig mystacial pad into the nasal and maxillary parts that has not yet been described in guinea pigs. Compartmentalization of the mystacial pad has been already observed in mice (Yamakado and Yohro, 1979) and opossums (Grant et al., 2013), and it has been shown that nasal and maxillary compartments of the mystacial pad develop from different growth centres in embryo (Yamakado and Yohro, 1979).

In the guinea pig mystacial pad, the Partes media superior and inferior of the *M. nasolabialis profundus* differ significantly from those in rats and mice. In rats and mice, these deep protracting muscles are organized in to groups and can be observed between vibrissae rows along the whole length of the mystacial pad (Haidarliu et al., 2010). They act on the more caudal vibrissae especially, pulling them rostrally to reduce the spread of the whiskers overall during protraction. In the guinea pig, a number of discrete bundles of muscle fibers can be seen sliced transversally (in Fig. 7); however, these are only observed in the most rostral area of the mystacial pad. We therefore conclude that guinea pigs do not have extrinsic protracting muscles that would be analogous to those described in mice and rats.

Behaviour

The guinea pig moves its whiskers with a mean amplitude of 44±25.9 degrees, which is comparable to rats (43.19±7.65 degrees), but even larger than mice (31.25±11.64 degrees) and opossums (36.04±9.53 degrees) (Mitchinson et al., 2011). The guinea pig positions its whiskers with a mean offset angle of 98±12.5 degrees, which is similar to the rat (100.63±9.21 degrees) and opossum (94.42±9.01 degrees), but set slightly further back than the mouse (112.53±6.85 degrees) (Mitchinson et al., 2011). While the range and position of the whisker movements is fairly comparable to rats and mice, the movements themselves are really rather different. The movements are rarely cyclic, and whisking is often absent, or only occurs in short bouts of around three or four whisks and usually only unilaterally (Fig. 1B, Fig. 9C), which agrees with previous observations of guinea pig whisker movements (Jin et al., 2004). Indeed, guinea pig whisker movements are often asymmetric, occurring with head rotations, and do not resemble the whisking motions observed in rats, mice and opossums. The lack of whisking movements is probably associated with the thin and irregular intrinsic

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

Implications

The total number of whiskers are reduced in the guinea pig (at 23 whiskers), which is a more comparable amount to the marsupial opossum (23 whiskers), than to rats and mice (33 whiskers), despite them being closer related. Diurnal primates also have fewer whiskers (with a minimum of 7 whiskers) that tend to be especially thin, with smaller whisker follicles lacking in intrinsic muscles (Muchlinski 2010; Muchlinski et al., 2013), compared to nocturnal primates (who have a minimum of 11 whiskers). In addition, the layout of the whiskers tends to be disorganized in diurnal primates, who lack a clear grid-like arrangement (Muchlinski et al., 2013). These aspects can also be observed in the guinea pig, but to a slightly lesser extent, and might indicate common properties of a diurnal, visual lifestyle. While there were no differences in the whisker follicle appearance, it was fairly large and contained a sinus - the mystacial pad of the guinea pig was disorganized in terms of whisker layout, intrinsic musculature and even innervation of the follicle. It might, therefore, be that vibrissae organization, innervation distribution and whisker number are key predictors of whisker specialisation in mammals, with whisker specialists, such as mice and rats, having more whiskers that are better organized.

That the diurnal guinea pig still has large and sensitive whisker follicles, and can exert movement over the whiskers using a complex architecture of intrinsic and extrinsic muscles, indicates that the whiskers are functional in this animal, despite a greater reliance on vision. Overall, the guinea pig mystacial pad is remarkably similar to rats and mice, despite them moving their whiskers less and being ground-dwelling and diurnal. This might be due to these animals being relatively closely related or, more likely, that the whiskers maintain an important role for the guinea pig. Although being arboreal and nocturnal are important factors in predicting the presence of intrinsic muscles, aspects of body size and other lifestyle variables are also important influences (Mitchinson et al., 2011; Muchlinski et al., 2013), such as being small and living in social groups (Muchlinski et al., 2013). Guinea pigs are extremely social animals and live in large groups displaying quite complex social behaviours. While whisker touch is implicated in social behaviours (Barnett, 2007; Muchlinski et al., 2013; Wolfe et al., 2011) this has not yet been explored in guinea pigs. It does seem likely

that the whiskers could play an important role in aggressive and submissive interactions in the guinea pig (for example, see figures in Grant and Mackintosh 1963).

Conclusions

In agreement with other studies on diurnal mammals, guinea pigs have fewer and less-organized whiskers, than arboreal, nocturnal rodents. While the reduction in whisker number and mystacial musculature suggests a larger reliance of the guinea pig on visual information, overall, the mystacial pad is surprisingly similar to rat and mouse, indicating that the whiskers may still play an important role in the life of the guinea pig. We suggest here that protecting the eye and social touch behaviours are both roles that the whiskers might play in guinea pig, and these will be important aspects of future research. Furthermore, we provide evidence that vibrissae organization, in terms of mystacial musculature, follicle layout and whisker number, is a key predictor of whisker specialisation in mammals.

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Figure Legends

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 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 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.

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 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 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 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 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.

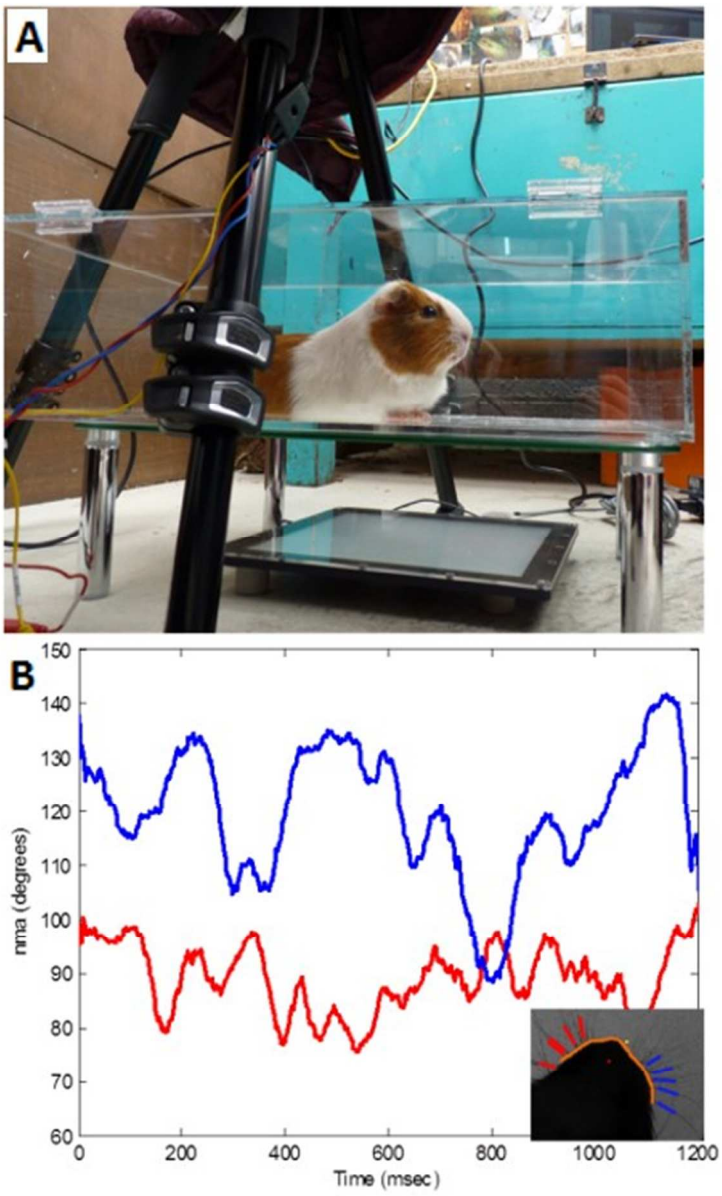

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Figure 1
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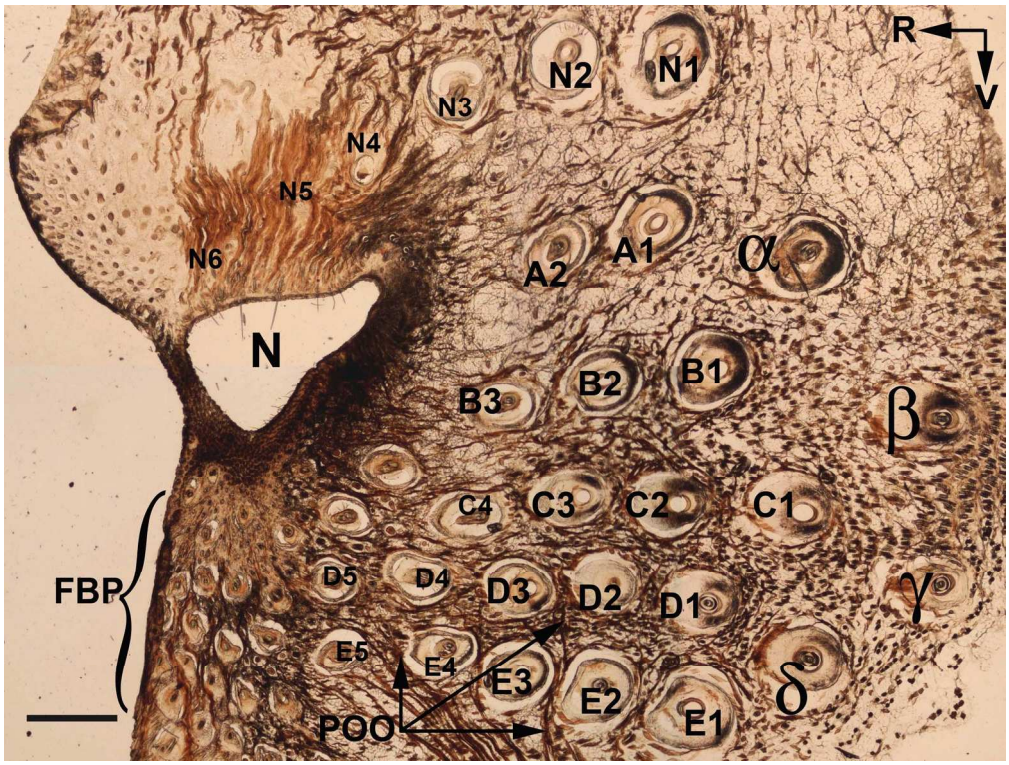

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Figure 2
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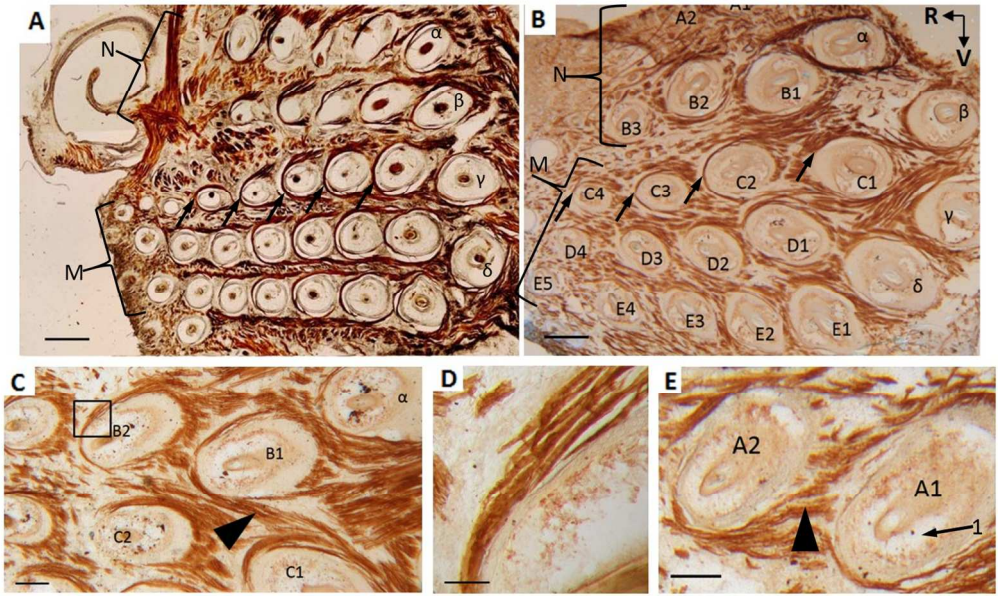

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Figure 3
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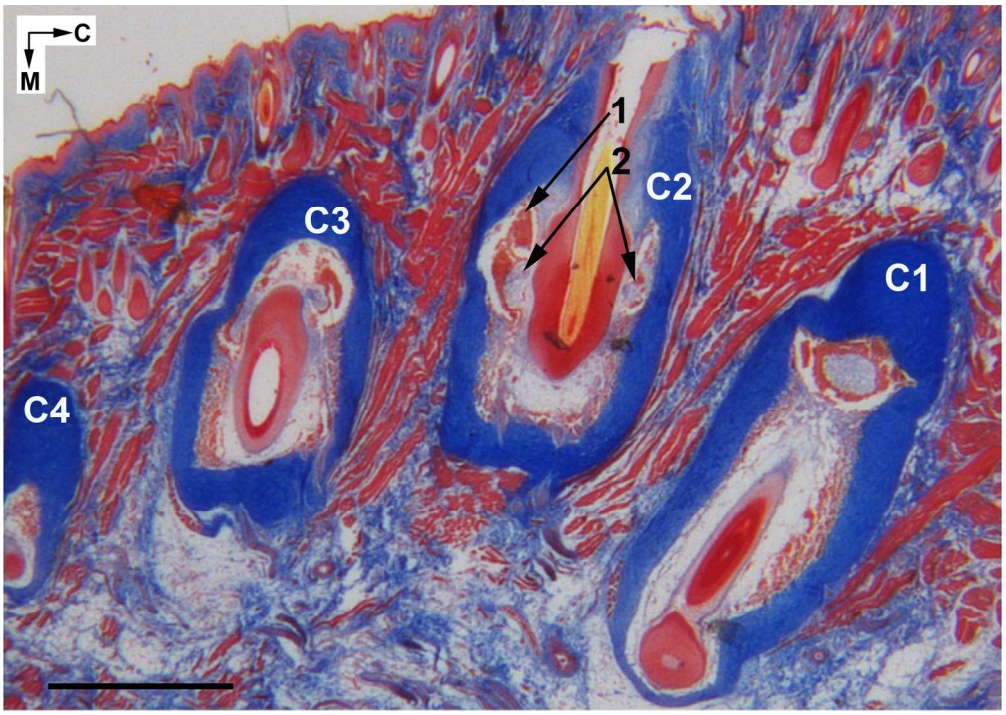

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Figure 4
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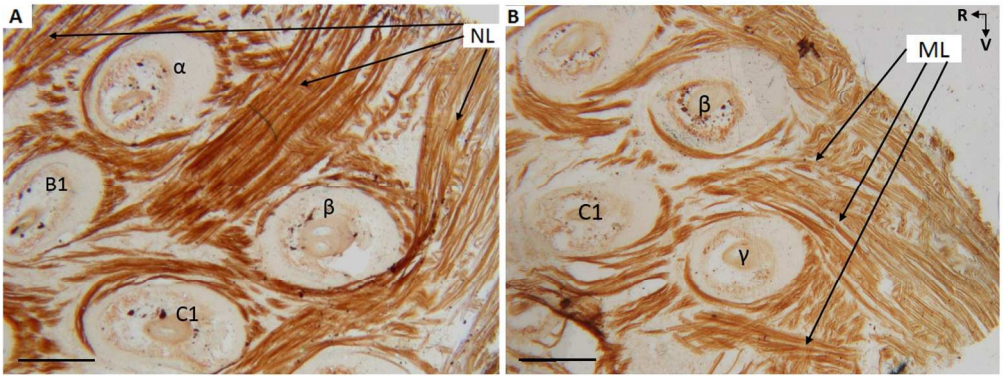

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Figure 5
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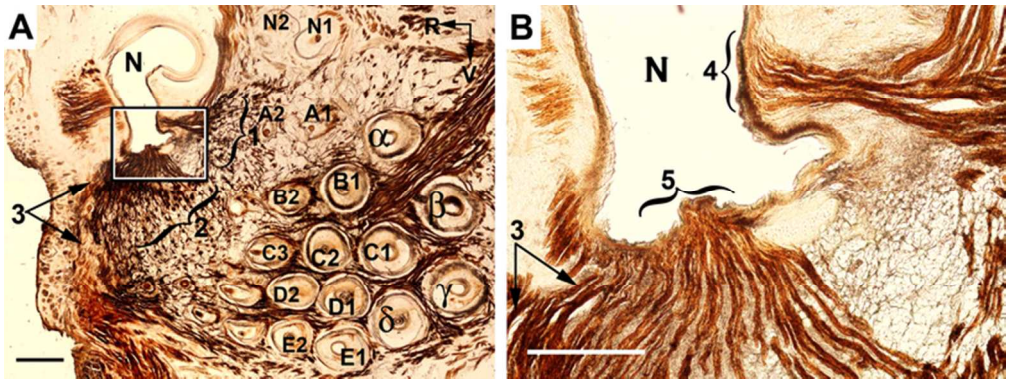

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Figure 6
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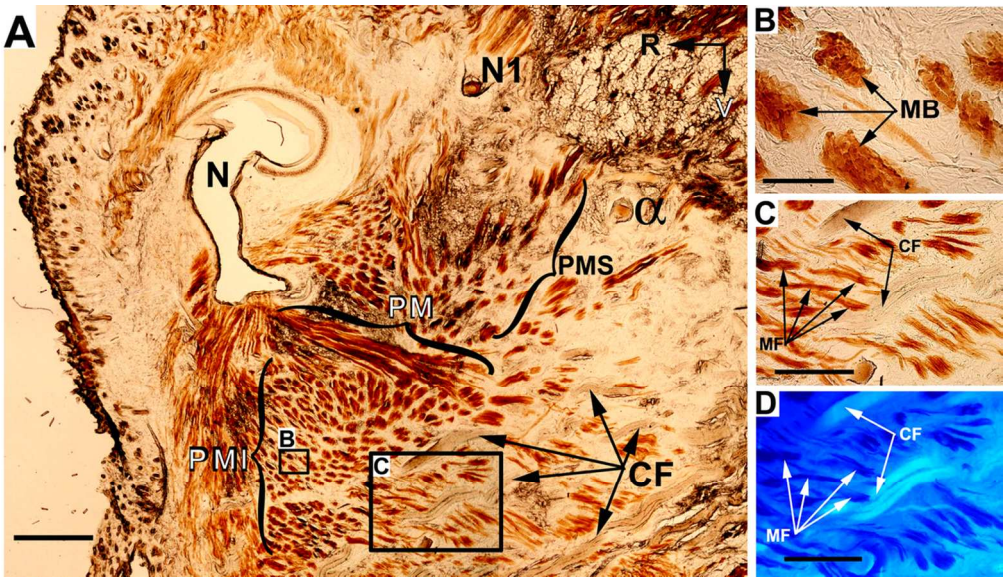

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Figure 7
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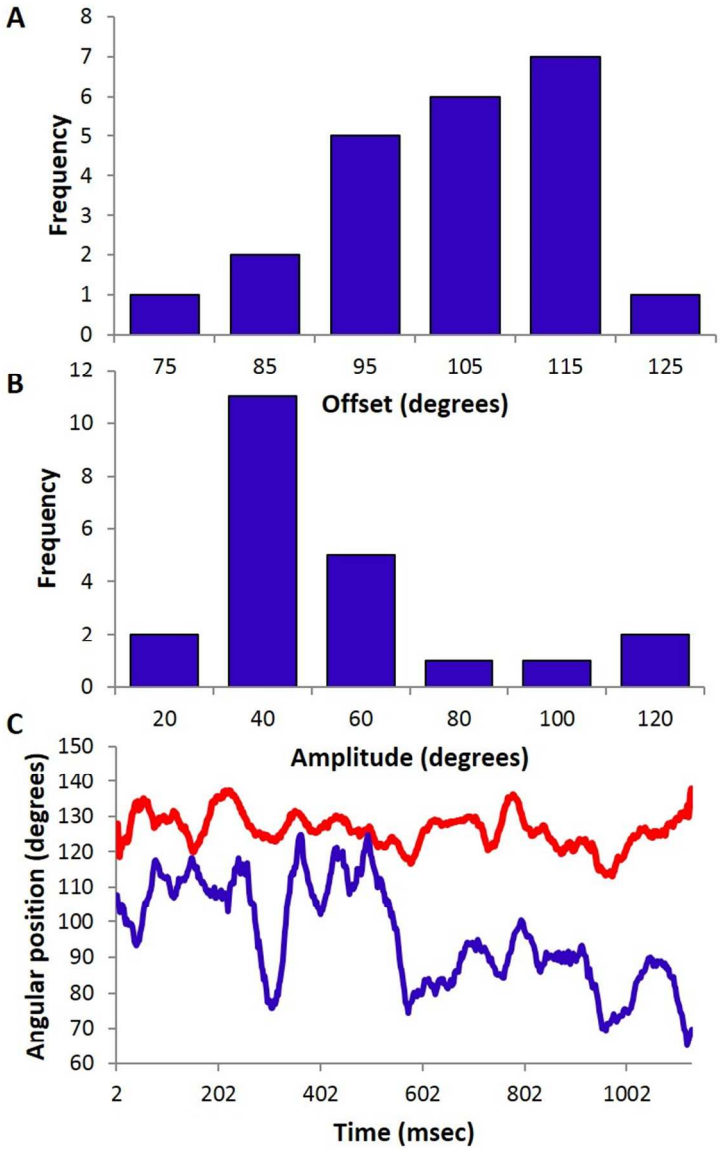

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Figure 8
131x210mm (150 x 150 DPI)