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Demonstrating a measurement protocol for studying comparative whisker movements with implications for the evolution of behaviour

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ABSTRACT

Background: Studying natural, complex behaviours over a range of different species provides insights into the evolution of the brain and behaviour. Whisker movements reveal complex behaviours; however, there does not yet exist a protocol that is able to capture whisker movements and behaviours in a range of different species.
New method: We develop a new protocol and make recommendations for measuring comparative whisker movements and behaviours. Using two set-ups – an enclosure camera set-up and a high-speed video set-up - we capture and measure the whisker movements of sixteen different captive mammal species from four different animal collections.
Results: We demonstrate the ability to describe whisker movements and behaviours across a wide range of mammalian species. We describe whisker movements in European hedgehog, Cape porcupine, domestic rabbit, domestic ferret, weasel, European otter and red fox for the first time. We observe whisker movements in all the species we tested, although movement, positions and behaviours vary in a species-specific way.
Comparison with existing method(s): The high-speed video set-up is based on the protocols of previous studies. The addition of an enclosure video set-up is entirely new, and allows us to include more species, especially large and shy species that cannot be moved into a high-speed filming arena.
Conclusions: We make recommendations for comparative whisker behaviour studies, particularly incorporating individual and species-specific considerations. We believe that flexible, comparative behavioural protocols have wide-ranging applications, specifically to better understand links between the brain and complex behaviours.

1. Introduction

The brain is a product of evolution; however, evolutionary neuroscience is relatively understudied compared to other areas, such as behaviour, anatomy and physiology (Cisek and Hayden, 2022). A recent review article by Cisek and Hayden (2022) argues for the importance of evolutionary studies to enhance the study of the brain. One of the first places to start would be to incorporate more species into neuroscience studies. While early studies in neuroscience adopted a range of animal models (Laurent, 2020), more recently only a handful of standard animal models, including rats, mice, zebra fish and aplysia, are employed, often to specifically address questions about the human brain (Cisek and Hayden, 2022; Yartsev, 2017). A comparative approach, making use of new phylogenetic and genetic techniques, will give fresh insights into neuroscience (Bryer et al., 2022; Yartsev, 2017). While this might be relatively straightforward for some aspects of neuroscience, such as anatomy, comparing behavioural capacities across species is challenging (Cisek and Hayden, 2022) since behaviours are flexible and complex (Pessoa et al., 2022).

A recent study (Bryer et al., 2022) made use of phylogenetic techniques to model quantity discrimination in 33 bird and mammal species. Bryer et al. (2022) developed a model to account for variation in species, individuals and tasks between studies, in order to investigate the effect of relatedness (phylogeny) and brain morphology on a species' ability to discriminate between different numbers. However, this approach would not lend itself to all types of behaviour, especially types that are less constrained and cannot be tested using psychophysics. Indeed, it is these other types of complex, natural behaviours, such as navigation (Dennis et al., 2021) or foraging (Rudebeck and Izquierdo, 2021), that are of great interest to neuroscientists, since brain function is ultimately aimed at controlling our interactions with the world, and is especially linked with these elements of survival (Cisek and Hayden, 2022).

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Complex behaviours are those which are made up of multiple elements of movement, and also incorporate elements of learning, memory and sensing (Byrne, 1993; Miller, 1999). One such possible complex, natural behaviour is that of whisker movements, which are closely linked with both navigation and foraging (Grant and Goss, 2022), as well as sensing, motor control, attention and cognition (Arkley et al., 2014; Grant and Goss, 2022; Mitchinson and Prescott, 2013; Simanaviciute et al., 2020). Whiskers are an established model of sensory processing in neuroscience, but much of what we know is constrained to only a few species, including laboratory rats and mice, and some zoo species, such as pinnipeds and sirenians (Grant and Goss, 2022). Muchlinski et al. (2020) categorised whether 205 mammalian species moved their whiskers in a behaviour called whisking – the to-and-fro cyclic movement that some animals can make with their whiskers - and suggested that whisking was not the ancestral state of mammals. However, whisking only captures symmetrical and cyclic movements of the whiskers, rather than all possible whisker movements. It is not possible to infer whether the ancestors of mammals had moveable whiskers without observing more varied whisker behaviours across mammalian species.

Indeed, whisker behaviours are much more varied than simple cyclic whisking. For example, many mammals, such as rodents and shrews, have been found to engage in active touch behaviours in response to a whisker contact (Grant et al., 2018). These can include: i) reducing whisker spread, or the span of the whisker field (Fig. 1b), to enable more whisker contacts with an object (Grant et al., 2018, 2009), and ii) contact-induced asymmetry (Fig. 1c), which enables whiskers to touch lightly against an object on the side ipsilateral to contact (by pushing whiskers back), and increasing the number of whisker contacts on the side contralateral to the object (by pushing whiskers forward) (Mitchinson et al., 2011, 2007). These behaviours are controlled by an array of mystacial muscles (Grant et al., 2017, 2013a, 2013b; Haidarliu et al., 2010), and often occur in anticipation of object contact (Grant et al., 2009). Whisker positions and movements are also thought to be closely linked to attention (Mitchinson and Prescott, 2013); for example the yellow areas in Fig. 1 may indicate the focus of attention of an animal (as suggested by Mitchinson and Prescott (2013)). Changing whisker positions and movements in response to an object contact is likely to increase the quality of information gained from touch, for example, by increasing the number of contacts, and controlling the force and acceleration of the whisker against a surface (Grant et al., 2009; Milne et al., 2021; Mitchinson et al., 2007). This will improve the efficiency of touch sensing tasks, such as for identifying objects (Lederman and Klatzky, 1987; Milne et al., 2021), guiding foraging and hunting (Adachi et al., 2022; Anjum et al., 2006; Milne and Grant, 2014) and locomotion (Arkley et al., 2017, 2014; Grant et al., 2018). Indeed, whisker control is likely to be as varied and complex as that of human fingertip movements. Whiskers are as sensitive as fingertips (Dehnhardt et al., 1998), and can engage in task-specific movements, making sweeping movement across textures and feeling around the edges of shapes, much like human fingertips (Milne et al., 2021). The fact that whisker control behaviours: i) are made up of multiple movements, ii) appear later in development (perhaps showing evidence of learning (Grant et al., 2012)) and iii) are associated with cognition and perception, suggests that whisker movements are a good example of a complex behaviour.

Many set-ups exist for measuring whisker movements, but these are usually constrained to studies in laboratory rodents for sensory neuroscience studies (Diamond et al., 2008; Evans et al., 2019). They mainly consist of constrained, well-lit, laboratory arenas with high-speed video cameras employed to capture the fast moving whiskers (Knutsen et al., 2005; Petersen et al., 2020; Ritt, 2012; Simanaviciute et al., 2020), that can move at frequencies of up to 25 Hz in mice (Mitchinson et al., 2011). The whiskers from the video footage collected in these set-ups can be tracked automatically using custom whisker trackers such as WhiskerMan (Petersen et al., 2020), ART v2 (Gillespie et al., 2019; Hewitt et al., 2018), BWTT (Mitchinson et al., 2011), Whisk (Clack et al., 2012), WhiskEras (Betting et al., 2020) or DeepLabCut (Sehara et al., 2021), to extract measurements of whisker angles, speeds and frequency of movement. These set-ups can be employed to measure the whiskers of other small mammalian species too, including species of rodent, marsupial and shrew (Anjum et al., 2006; Arkley et al., 2017; Grant et al., 2013a, 2013b; Mitchinson et al., 2011). For example, a study of eleven small, quadrupedal mammal species employed a high-speed camera set-up with automated tracking (Grant et al., 2018) to find

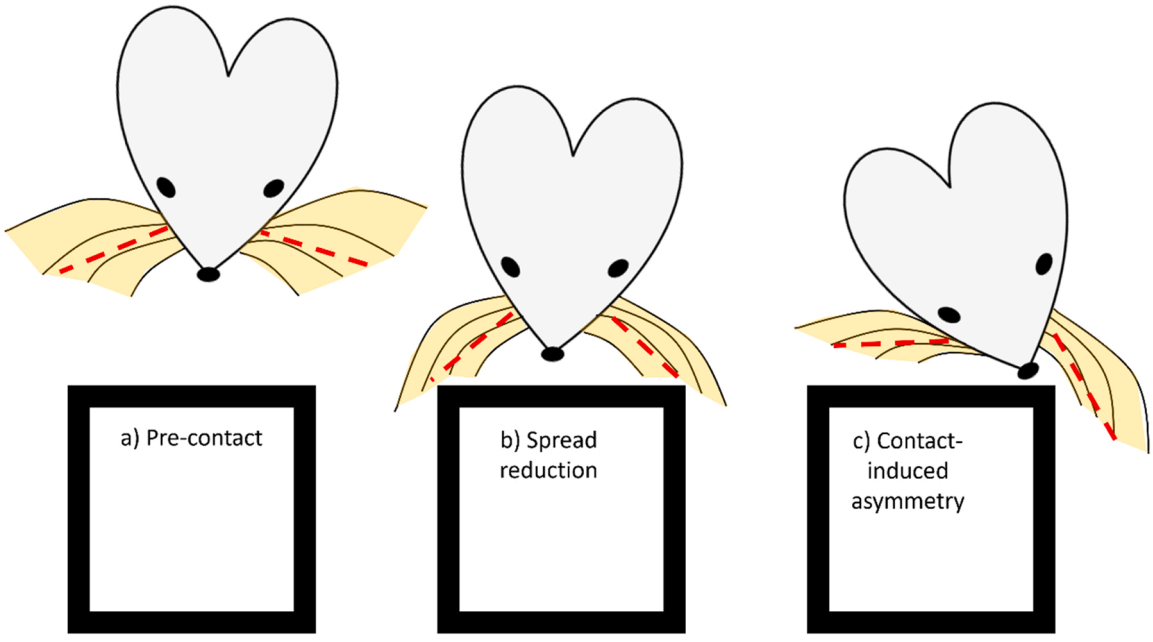

Fig. 1. Example contact-related whisker behaviours. Prior to a contact (a) the whiskers are fairly symmetrical (compare red-dotted lines on each side) and spread out (see yellow area, which is especially large at the whisker tips). Following a contact, an animal can reduce their whisker spread (panel b shows smaller yellow area, with whiskers bunched tighter together, compared to panel a); and/or preform contact-induced asymmetry, where one whisker side is more forward than another (i.e. compare the position of the red dotted lines in panel c).

that all species moved their whiskers. Many species also revealed elements of active control associated with increasing whisker contact with objects of interest, which included behaviours such as asymmetric whisking and reduction of spread upon whisker contact (Grant et al., 2018, 2009; Mitchinson et al., 2007) that had previously only been shown in laboratory animals (Grant et al., 2018). However, set-ups of this kind can only be used for small species that can be easily moved and handled into a box. The automated trackers are also not general enough to use in different set-ups or with different species. Furthermore, some species do not have fast moving whiskers and simply do not require being filmed in high speed (e.g. Pinnipeds; Grant et al., 2013; Milne et al., 2021, 2020). Therefore, enclosure-based filming set-ups are also useful. These are likely to be less-invasive (since animals do not need to be moved to a separate arena) and are especially useful for filming larger animals with bigger whiskers that move slowly, as well as shy animals, or those that might experience stress from handling.

One such study adopted an action camera during an active feeding task, to film the whisker movements of three species of Pinniped (harbour seal, California sea lion and Pacific walrus) in their enclosures (Milne et al., 2020). Due to the variability of the enclosure footage, especially the complex background of the footage, the whiskers could only be tracked manually to extract metrics of whisker movements and positions (MWA Hewitt et al., 2016), which revealed the importance of whisker movements in Pinnipeds to orient the head towards food (Milne et al., 2020). This exact method using a feeding task can only be applied to large, trained mammals and has only been demonstrated in Pinnipeds so far. A simpler enclosure set-up that can enable the measurement of whisker behaviours across a range of different species without training experiences, is therefore needed.

While we suggest that studying whisker movements comparatively can give us insights into the evolution of mammalian sensing, behaviour and neuroscience, there does not yet exist a methodological protocol that is able to capture whisker movements from a range of different mammalian species that differ in size, training capabilities, handling experience and their speed of whisker movements. Therefore, the aim of this study is to make recommendations for a whisker behaviour protocol that is able to be rolled out to different animal collections and species. Species-specific, and even individual-specific, adjustments will have to be made based on animal size, training, handling, personality, whisker size and the speed of whisker movements; therefore, we will develop within this study a series of recommendations for measuring whisker movements across species.

2. Methods

2.1. Animals

Sixteen different mammalian species were included in this study, spanning four orders and eleven families (Table 1). Shrews (Anjum et al., 2006; Grant et al., 2018), murid rodents (Arkley et al., 2017; Grant et al., 2018; Mitchinson et al., 2011) and pinnipeds (Milne et al., 2020; Milne and Grant, 2014) are well-represented in other whisker behaviour studies, therefore, this study incorporated representative species of all these groups. These species were also chosen since they were accessible and available for us to study in UK zoos. Indeed, all individuals were housed in captivity in UK zoos. Four zoo collections were used for data collection, including Rhyl SeaQuarium (for harbour seals), Reaseheath Zoo (for Cape porcupine), Williamson Park Zoo (for domestic guinea pig and ferret) and the Wildwood Trust (for all other species). One to three adult individuals were tested for each species, and, where possible, both sexes were tested (Table 1). All experimental protocols were approved by the ethical committee at Manchester Metropolitan University (ID:6009), as well as the local ethical committees at each collaborating zoo. Introducing such enrichment stimuli and filming are all part of normal zoo husbandry procedures, therefore our work did not require additional UK Home Office licenses.

Table 1
Animals used within the study, including sixteen different species from four different orders.

Species	Common name	Order	Family	Animal numbers
Neomys fodiens	Eurasian water shrew	Eulipotyphla	Soricidae	1 male
Erinaceus europaeus	European hedgehog	Eulipotyphla	Erinaceidae	1 male
Hystrix africaeaustralis	Cape porcupine	Rodentia	Hystricidae	1 male, 2 females
Cavia porcellus	Domestic guinea pig	Rodentia	Caviidae	2 females
Arvicola amphibius	European water vole	Rodentia	Cricetidae	2 males, 1 female
Apodemus sylvaticus	Wood mouse	Rodentia	Muridae	1 male, 2 females
Micromys minutus	Harvest mouse	Rodentia	Muridae	1 male, 2 females
Mus musculus	House mouse	Rodentia	Muridae	1 male, 2 females
Rattus norvegicus	Brown rat	Rodentia	Muridae	1 male, 2 females
Muscardinus avellanarius	Hazel dormouse	Rodentia	Gliridae	1 male, 2 females
Oryctolagus cuniculus domesticus	Domestic rabbit	Lagomorpha	Leporidae	2 females
Mustela furo	Domestic ferret	Carnivora	Mustelidae	2 females
Mustela nivalis	Weasel	Carnivora	Mustelidae	1 female
Lutra lutra	European otter	Carnivora	Mustelidae	1 male, 1 female
Vulpes vulpes	Red fox	Carnivora	Canidae	2 males
Phoca vitulina	Harbour seal	Carnivora	Phocidae	3 females

2.2. Experimental apparatus

Some species-specific adjustments were needed in order to clearly image the whiskers, while ensuring the least disruption to individuals. Therefore, we designed two experimental set-ups, a high-speed camera set-up and an enclosure camera set-up (Fig. 2). We also developed a decision tree to structure our choice of when to use each set-up (Fig. 3). Small mammals tend to have smaller, thinner whiskers that move faster (Grant et al., 2018). Therefore, small mammals, required a filming set-up with a high-speed video camera (Phantom ex-2, 500 fps) (Figs. 2a, 3). Filming in high-speed requires good lighting conditions to precisely image the whiskers with a good depth of field. Therefore, we used an infrared light slate to illuminate the whiskers (LEDW-BL-400/200-SL-LUB-Q-1R-24V, PHLOX) (Fig. 2a). Since many of the small mammals were nocturnal, it also meant that these were being tested in the dark, which is thought to be less stressful for them. Due to the controlled nature of high-speed filming and imaging over a light slate, in the high-speed camera set-up, animals were filmed in a Perspex arena (30 × 50 × 15 cm) (Fig. 2a).

However, some species could not be handled, as they had no previous human contact and/or were nervous (i.e. *Mustela nivalis*) (Fig. 3). Or they were physically too big to be moved around the zoo (i.e. *Phoca vitulina*) (Fig. 3). Futhermore, larger animals tend to have larger whiskers that do not move as fast; therefore, a high-speed camera is not needed in these species. Not having a high-speed camera reduces the need for wiring and laptops on site and meant that we could develop an enclosure camera set-up. The enclosure camera set-up had an action camera (Go Pro Hero 4, 240 fps) held on a clamp stand, that could easily be placed within an animal's enclosure and was especially non-invasive (Fig. 2b). We have not yet observed a large mammal with whiskers that move too fast or are too small for an action camera at 240 fps. If such a species was found, it would be possible to introduce a high-speed camera to an enclosure, as long as there is sufficient lighting, probably with a mixture of sunlight and spotlights. Since the enclosure flooring varied

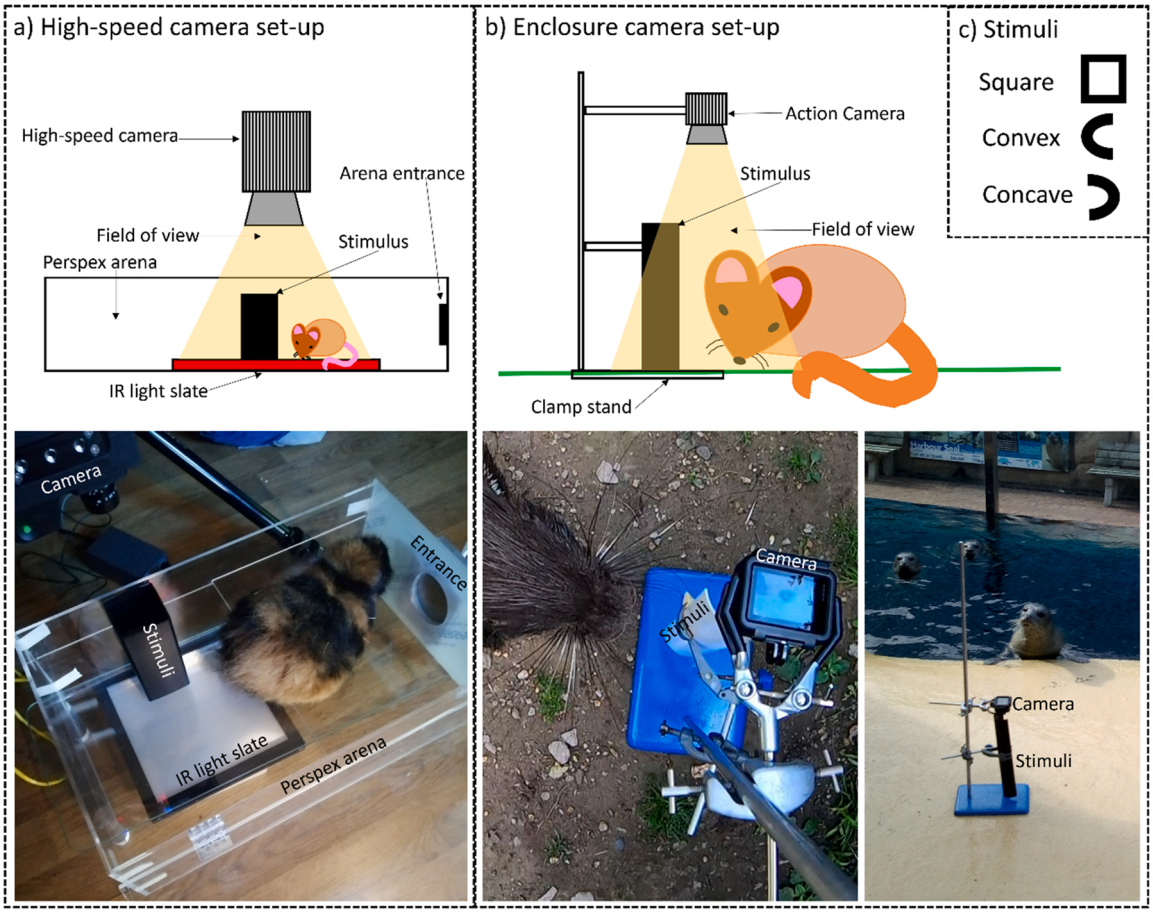

Fig. 2. Two experimental set-ups including: a) an infra-red filming high-speed camera set-up with Perspex arena (featuring *Cavia porcellus*); and b) an enclosure set-up using an action camera (featuring *Hystrix africaeaustralis* (left) and *Phoca vitulina* (right)). Diagrams of the experimental set-ups are shown in the top images, and photographs in the bottom images. c) shows the three stimuli as diagrams.

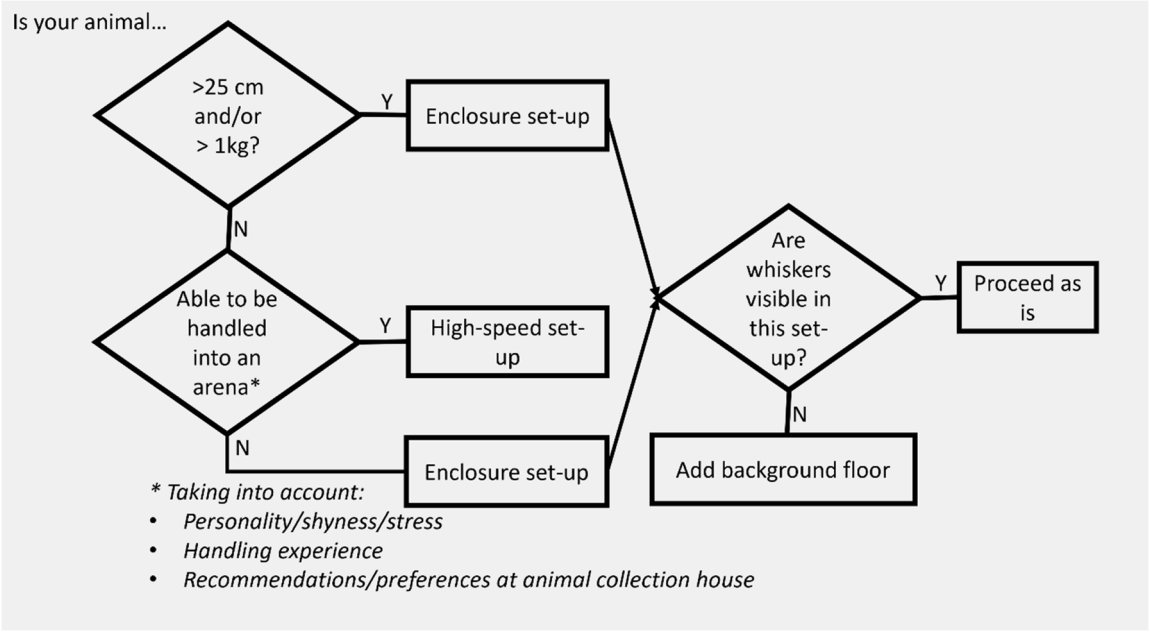

Fig. 3. Decision tree to structure the selection of experimental set-ups. Large species, and individuals who were shy with less handling experience were filmed in the enclosure camera set-up, whereas small species that could be handled into an arena were filmed in the highspeed camera enclosure. We have not observed a large species with whiskers that are too fast or too small for an action camera at 240 fps. If such a species was found, it would be possible to introduce a high-speed camera to an enclosure lit with a mixture of sunlight and spotlights.

between enclosures (Fig. 2b), and there were species (and even individual) differences in whisker colour, black or white foam flooring tiles were placed under the camera to make the whiskers more visible against the background (see Supplementary video, Fig. 3).

To encourage whisker movements, stimuli were added below the cameras in both set-ups. Stimuli consisted of square and semi-circular guttering pipe, cut to lengths of 15 cm (for high-speed set-up) and 30 cm (for enclosure set-up), based on the camera heights. These different stimuli were used to encourage exploration, since animals are likely to inspect novel objects. Indeed, these stimuli were entirely new to all individuals, and were thought to be equally non-ethologically relevant to each species. In our previous investigations, we have observed that a simple, novel object is sufficient to increase an animal's interest in the task, and encourage whisker movements (i.e. whisking) as well as control behaviours, such as asymmetry and spread reduction (Grant et al., 2020, 2018; Simanaviciute et al., 2020). The semi-circular guttering could also be rotated to be a concave or convex stimulus. We did consider whether to have different widths of stimuli depending on the size of the species, and their whisker lengths. However, whisker lengths did not vary greatly between different species (i.e. in agreement with Table 1 in Dougill et al., 2020), therefore, we kept the stimuli consistently the same between species. Some of the species required additional encouragement to approach and explore the stimulus, which included beckoning and coaxing with a hand, putting food around the stimulus and introducing other positive scents (such as salmon oil for the Domestic ferrets) (Table 2).

2.3. Experimental protocol

Each individual was filmed over one to three sessions (Table 2). If an individual was filmed over multiple sessions then there was always at least a day free between filming sessions to reduce stress. For the high-speed video set-up (Table 2), individuals were caught in their enclosure in a cardboard tube. The tube was then inserted into the arena and the animal emerged. All species were introduced via a tube to the high-speed arena, apart from the European hedgehog and domestic guinea pigs, which were introduced by hand as they were used to handling. Individual one-second clips were collected opportunistically and manually, as the animal passed under the camera and explored the stimulus. Clips were collected on each stimulus (square, concave and convex), with 6 – 30 clips collected per individual, over a period of approximately 10 min per session. Most species actively investigated the stimulus without further encouragement, except for the domestic guinea pigs, which did not explore much, and were encouraged using additional food around the stimulus.

For the enclosure set-up, the camera recorded continuously throughout each session. Unlike in the high-speed camera set-up, individuals were not removed from their enclosures, but were filmed alongside their conspecifics within their enclosures over a period of 10–25 min per session. Some species required additional encouragement to explore the stimulus in the enclosure, which is documented in Table 2.

2.4. Video processing and tracking

Once the video footage had been collected, it was reviewed. Individual video clips were reviewed for the high-speed footage, and individual stimulus interactions were extracted from the enclosure footage. Each individual interaction with the stimulus – defined as an approach and whisker contact with the stimulus – was reviewed by eye and analysed further, but only if: i) whiskers were contacting the object; ii) whiskers were clearly visible; and iii) the individual's head was fairly horizontal, with no extreme pitch (i.e. up or down head tilting) or roll (i.e. head rotations). Making sure the head was fairly flat to the ground in the video footage was important to ensure accurate imaging and measurement of the whiskers, since we only image the whiskers in two-dimensions. 8 – 31 stimulus interactions were included for further analysis per species (0–15 per individual).

Whiskers were tracked either manually (using the Manual Whisker Annotator (MWA), Hewitt et al., 2016) or automatically (using the Automated Rodent Tracker (ART) V2, Gillespie et al., 2019) (Fig. 4). Footage from all species in the high-speed camera set-up was tracked using ARTV2 (Fig. 4a-f), and each clip was reviewed by eye to ensure accurate tracking. In some species (the water vole (Fig. 4g), European hedgehog (Fig. 4h) and domestic guinea pig (Fig. 4i), ARTV2 did not track, probably due to the head shape of these species being significantly different from mice and rats, so ARTV2 did not recognise them (Hewitt et al., 2018). In these species, the Tracker recognised the rear or tail of the animal as the head, and no whiskers were tracked at all. Therefore, these species' whiskers were manually tracked, along with all the species filmed using the enclosure camera set-up (Fig. 4g-p). This involved manually identifying the whisker base and shaft (a point around two-thirds of the way along the shaft towards the tip) on three whiskers on each side of the face, in every frame, as well as the nose and mid-head (Fig. 4g-p). ARTV2 was not able to track the enclosure clips, since it was only developed for rodents in back-lit enclosure set-ups, and the enclosure camera did not have the correct lighting and had inconsistent backgrounds.

In order to validate the tracking, MWA and ARTV2 tracking was compared in the Brown rat footage (*Rattus norvegicus*) (Fig. 5). Manual

Table 2
Video set-up and footage collected from each species. Na is not applicable, since the enclosure camera recorded continuously throughout a session, rather than in one second clips as per the high-speed camera. Occasions filmed refers to the number of times (occasions) all of the individuals of that species were filmed (i.e. All three porcupines were filmed on one occasion).

Species	Common name	Set-up	Extra stimulus	Occasions filmed	Clips collected	Clips/interactions analysed
Neomys fodiens	Eurasian water shrew	High-speed cam	–	1	14	8
Erinaceus europaeus	European hedgehog	High-speed cam	–	1	19	9
Hystrix africaeaustralis	Cape porcupine	Enclosure-cam	Peanuts and fruit	1	Na	17
Cavia porcellus	Domestic guinea pig	High-speed cam	Dry food and carrots	1	Na	10
Arvicola amphibius	European water vole	High-speed cam	–	3	29	9
Apodemus sylvaticus	Wood mouse	High-speed cam	–	2	71	18
Micromys minutus	Harvest mouse	High-speed cam	–	2	90	22
Mus musculus	House mouse	High-speed cam	–	1	26	19
Rattus norvegicus	Brown rat	High-speed cam	–	1	34	12
Muscardinus avellanarius	Hazel dormouse	High-speed cam	–	3	87	14
Oryctolagus cuniculus domesticus	Domestic rabbit	Enclosure-cam	–	1	Na	10
Mustela furo	Domestic ferret	Enclosure-cam	Salmon oil	2	Na	21
Mustela nivalis	Weasel	Enclosure-cam	–	2	Na	7
Lutra lutra	European otter	Enclosure-cam	Fish	2	Na	15
Vulpes vulpes	Red fox	Enclosure-cam	Meat and hand coaxing	3	Na	11
Phoca vitulina	Harbour seal	Enclosure-cam	Fish	1	Na	31

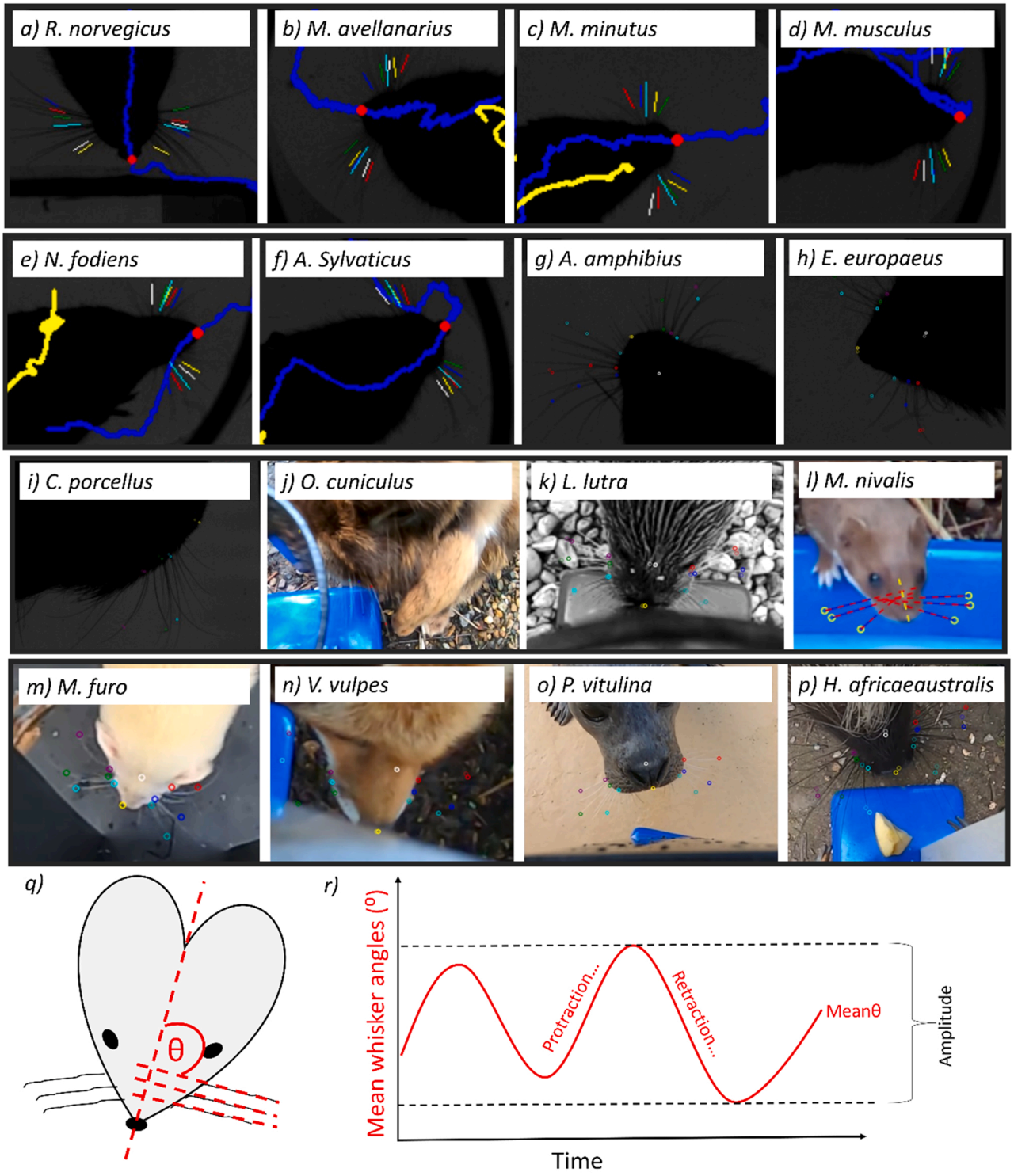

Fig. 4. Example tracking from each of the species investigated. Including high-speed set-up with automatic tracking (from panel a to f), high-speed set-up with manual tracking (from panel g to i), and enclosure set-up with manual tracking (from panel j to p). Panel q shows a diagram of the tracking whisker angles (θ), calculated as the angle that a whisker makes with the mid-line of the head (also shown on *M. nivalis* in panel l). This angle is then averaged for all whiskers, and each side, to give mean whisker angles, indicated in panel r, along with some metrics. Amplitude is the maximum amplitude of the signal and mean angular position is the mean of the mean whisker angles.

inspection of whisker video (Fig. 5a) and traces (Fig. 5b) showed good agreement between the tracking methods; and a Bland-Altmann plot constructed from ten tracked video clips also showed good agreement between the two methods (Fig. 5c). It was not possible to compare the manual and automatic trackers on the same whiskers, since whisker identity varied and was not maintained across frames in the automatic tracker, which is why mean whisker angles are used throughout. However, whisker metrics outputted from the two trackers are calculated in exactly the same way, since both trackers were developed in-house, using the same calculations (Gillespie et al., 2019; Hewitt et al., 2016). Therefore, when the same whisker was tracked in a frame by both the manual and automatic tracker (i.e. in Fig. 5a, indicated with a yellow

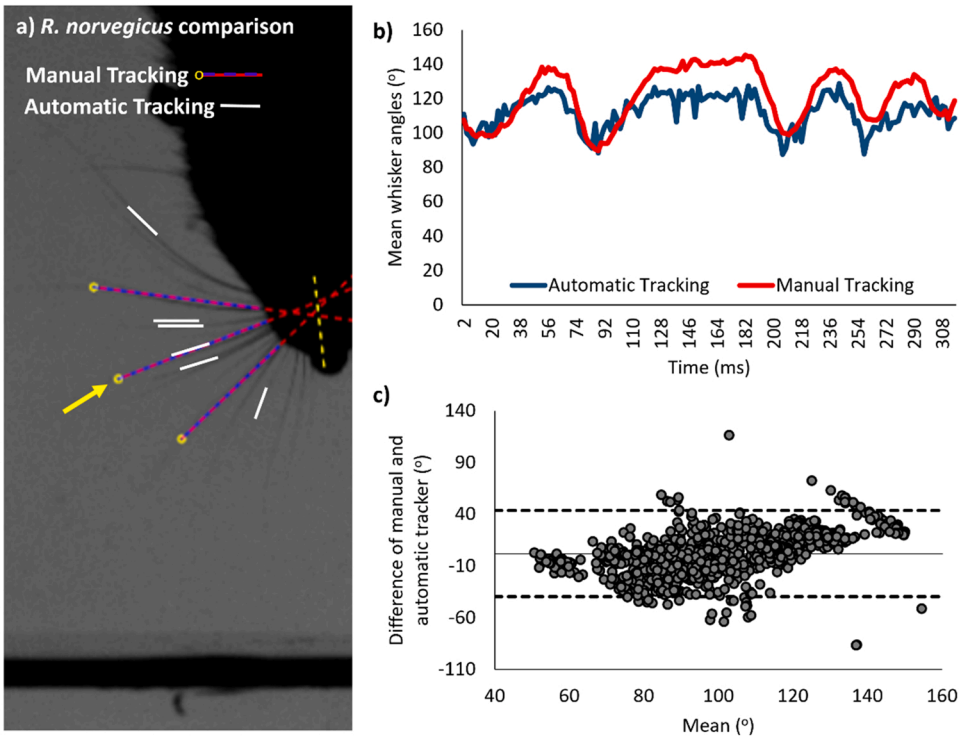

Fig. 5. Comparison of manual (Manual Whisker Annotator) and automatic (Automated Rodent Tracker V2) Whiskers Trackers in *Rattus norvegicus*. Screen shot from one frame showing example tracking from the manual (red and blue dashed lines) and automatic (white lines) whisker trackers. The yellow arrow points out a whisker that is tracked in both the manual and automatic tracker in this one frame. b) Example video clip of tracking of mean whisker angles for both manual and automatic tracking. c) Bland-Altmann plot showing per-frame datapoints for ten tracked clips; the mean (solid line at 2°) and confidence intervals (dashed lines) are shown on the plot, illustrating the difference in mean whisker angles between the manual and automatic whisker trackers.

arrow), the calculated angle value would be the same. Therefore, we were confident to use both trackers interchangeably in this way.

It would have been beneficial to compare filming set-ups, but this was not possible, since there was not a species that was suitable to swap between set-ups that would give good quality footage and behave well in both set-ups; hence the requirement for such species-specific considerations (Fig. 3). For example, the brown rats were filmed in the high-speed camera set-up, but their whiskers would be too small to image clearly in the enclosure set-up. Since we could clearly see the whiskers in both set-ups, and the trackers were fairly equivalent, we are relatively confident that the two set-ups are comparable.

All automated and manual tracking was checked for accuracy. A minimum of three tracked whiskers on each side were required to be present in every frame to be included in our analysis. This was achieved in all of our processed individual object interactions and example tracking can be seen in Fig. 4. Once the tracking was reviewed, metrics were extracted, including mean whisker angles (mean of all the whisker angles θ, averaged per side, in Fig. 4q). Whisker angles were calculated as the angle between whiskers and the mid-line of the head (Fig. 4 l and q), such that more protracted (forward) whiskers had larger angles, and retracted (more backward) whiskers had smaller angles. From the mean whisker angle traces (Fig. 4r), we could extract whisker angular position (the mean of the mean whisker angle traces) and whisker amplitude (the difference between the minimum and maximum whisker angle in the trace, Fig. 4r). These were calculated for the left and right side, and then a mean was taken, to give one measure per stimulus interaction. It is worth bearing in mind that these metrics were only calculated in two-dimensions from the one camera positioned above.

As well as quantitative metrics, qualitative behavioural descriptors were also allocated to each species. These were developed in Grant et al. (2018) and were slightly amended to easily score whisker control between different species and included: whisking (*Was cyclic, bilateral whisking present?*), contact-induced asymmetry (*Do ipsilateral whiskers have lower angular positions than contralateral whiskers?)* and spread reduction (*Do whiskers reduce spread, or bunch up, during contact?*).

2.5. Data analysis

There were no overall significant differences in mean whisker angular position or amplitude between the square, convex and concave stimuli (Between-ANOVA: Angular position: $F(2, 232) = 1.118$, $p = 0.329$; Amplitude: $F(2, 231) = 0.578$, $p = 0.562$). Therefore, the data from the stimuli were combined. Data from individuals and sexes were also combined, and the species were compared using a between-ANOVA. Summaries of each metric and behaviour can be seen in Table 3.

To demonstrate the applications of studying comparative whisker movements, the quantitative metrics (angular position and amplitude) were correlated against standardised IOF area (IOF area adjusted for skull size, as per Muchlinski et al., 2010). Values of standardised IOF area were extracted from the datasets provided in Muchlinski et al., (2010, 2020) and Milne et al. (2022). This was only possible for 10/16 of the species present here, since the other species (European hedgehog, Cape porcupine, domestic guinea pig, domestic ferret, weasel, European otter) were not in the datasets. Due to the small sample size, a Spearman's Rank Correlation Test was selected.

3. Results

This protocol enabled whisker movements to be described in some species for the first time, including European hedgehog, Cape porcupine, domestic rabbit, domestic ferret, weasel, European otter and red fox. We observed that all the species we studied moved their whiskers with amplitudes of ~21–53° (Table 3, Fig. 6). All the species positioned their whiskers with average angular positions of ~70–130°. However, both these values revealed species-specific differences (Between ANOVA: Angular position: $F(15, 234) = 65.046$, $p < 0.001$; Amplitude: $F(15, 234) = 26.972$, $p < 0.001$). For example, Cape porcupine, European water vole, brown rat, domestic rabbit, domestic ferret and European otter all had the largest amplitude movements (>40°) (Table 3, Fig. 6b). Asterisks on Fig. 6 indicate that many of the small quadrupedal species (water shrew, wood mouse, harvest mouse, house mouse and hazel dormouse) had similar angular positions (~70°−85°) and amplitude

Table 3
Summary results for each species. Quantitative metrics include angular position and amplitude (mean±S.D), and behavioural descriptors identify the presence of whisking, contact-induced asymmetry and spread reduction.

Species	Common name	Ang pos. (°)	Amp. (°)	Whisking	Asym	Spread red.
Neomys fodiens	Eurasian water shrew	71.81 ± 5.00	23.65 ± 3.54	Some, only retractions	Yes	No
Erinaceus europaeus	European hedgehog	124.39 ± 8.48	35.03 ± 12.62	No, Dabbing movements with head	No	No
Hystrix africaeaustralis	Cape porcupine	98.24 ± 19.96	53.45 ± 14.24	Yes	Yes	No
Cavia porcellus	Domestic guinea pig	114.52 ± 11.43	30.75 ± 8.82	Some, small unilateral bouts	Yes	No
Arvicola amphibius	European water vole	110.65 ± 8.37	48.53 ± 10.76	Yes	Yes	Yes
Apodemus sylvaticus	Wood mouse	86.06 ± 4.48	21.68 ± 2.57	Yes	Yes	Yes
Micromys minutus	Harvest mouse	85.46 ± 5.38	24.48 ± 1.78	Yes	Yes	Yes
Mus musculus	House mouse	86.17 ± 3.32	25.17 ± 2.26	Yes	Yes	Yes
Rattus norvegicus	Brown rat	80.76 ± 8.55	46.52 ± 7.90	Yes	Yes	Yes
Muscardinus avellanarius	Hazel dormouse	79.33 ± 6.02	21.42 ± 3.91	Yes	Yes	Yes
Oryctolagus cuniculus domesticus	Domestic rabbit	129.86 ± 7.02	49.88 ± 11.53	Yes	No	No
Mustela furo	Domestic ferret	104.44 ± 15.4	41.40 ± 14.24	Some bouts	Yes	No
Mustela nivalis	Weasel	109.43 ± 7.86	58.25 ± 20.89	No, dabbing movement of head	Yes	No
Lutra lutra	European otter	102.06 ± 10.91	43.04 ± 8.98	Yes, some small bouts	Yes	No
Vulpes vulpes	Red fox	83.19 ± 17.08	39.65 ± 11.00	Yes, some	No	No
Phoca vitulina	Harbour seal	119.59 ± 7.00	27.74 ± 8.56	No	Yes	No

(~21°–25°) whisker movements to each other, and somewhat different to the other species (apart from the angular positions of the brown rat, Fig. 6a).

Species-specific differences were also observed in the behavioural descriptors too. Cyclic, continuous whisking was observed in 7/16 species ('Yes' scores in Table 3), 6/16 species revealed some rhythmic whisker movements, and 3/16 revealed whisker protractions without any rhythmic whisker movements (Table 3). European hedgehog and weasel obtained some rhythmic movements by dabbing the head against a surface, rather than the whiskers. Contact-induced asymmetry was observed in the majority of species (13/16), but lacking in European hedgehog, domestic rabbit and red fox (Table 3). Spread reduction was not common and only present in 6 rodent species (Table 3).

Both whisker angular position and amplitude were not significantly correlated to standardised IOF area (Spearman's Rank Correlation: Angular position: $r = 0.018$, $p = 0.960$; Amplitude: $r = 0.030$, $p = 0.934$, Fig. 7). Although the rodents (red points) and harbour seal (labelled) had the larger standardised IOF areas (>0.15), compared to the other species.

4. Discussion

We present here a protocol that can be used to capture and describe whisker movements and behaviours in many species of mammals. No other protocol exists that is able to collect whisker movement footage from such a wide range of mammalian species that vary in size, as well as training and handling experience. Using this protocol, we can see that all species moved their whiskers, indicating the prevalence of whisker movements across mammals (Table 3, Fig. 6b). Species moved their whiskers with mean amplitude values ranging from around 20° to 55°. However, whisker positions and amplitudes varied significantly between species, as did other behavioural descriptors, including whisking, asymmetry and spread reduction (Table 3).

4.1. Implications of behavioural observations

Our protocol revealed whisker movements in all the species that we studied, many of which have never had whisker movements observed before. Whisker movements are thought to be driven by intrinsic whisker muscles, which form a sling around the base of each whisker and, when they contract, enable protraction of the whiskers forward (Dörfl, 1982; Haidarliu et al., 2010). Intrinsic muscles have previously documented in mice (Dörfl, 1982), rats (Haidarliu et al., 2010), hamsters (Wineski, 1985), guinea pigs (Grant et al., 2017; Jin et al., 2004), opossums (R. A. Grant et al., 2013), shrews (Yohro, 1977) and even nocturnal primates (Muchlinski et al., 2013). While intrinsic muscles have not yet been described in all of the species that we studied here, the presence of whisker movements in all these species, as well as the prevalence of intrinsic muscles across mammalian species, suggests that all the species we studied are likely to have intrinsic whisker muscles. In support of this, we present here unpublished mystacial pad images (Fig. 8), featuring intrinsic muscles (indicated by black arrows) in house mouse (Fig. 8a), as well as five other newly described species, including harvest mouse (Fig. 8b), weasel (Fig. 8c), domestic ferret (Fig. 8d), Asian short-clawed otter (Fig. 8e) and common shrew (Fig. 8f). The mustelids are an especially interesting group, since they have not had their whisker movements nor musculature described at all in the past. We observed that both the weasel and domestic ferret have particularly large and ordered intrinsic muscles (Fig. 8c and f, respectively), as well as relatively large whisker amplitudes (>40°, Fig. 6b). We are not able to make cross-sectional measurements of the muscles, since the orientation of the pad and muscles do vary somewhat between species. However, that the intrinsic muscles are present in all these species is an important finding. Since we observe intrinsic muscles and whisker movements from marsupials (Grant et al., 2013) to primates (Muchlinski et al., 2013), with many species in between, suggests that this muscle architecture might have been present in a common ancestor of therian mammals.

While all the species in this study moved their whiskers, we demonstrate here that they are moved through different angular positions and amplitudes (Fig. 6), and with varying periodicity (Table 3). All species of Rodentia whisked apart from the domestic guinea pig in agreement with Grant et al. (2017). This is also in agreement with other studies that have found whisking to be prevalent across rodents, with rodentia containing perhaps the most whisking species of any order (Grant et al., 2018; Muchlinski et al., 2020). The domestic rabbit also made full whisking movements in the videos, whereas more sporadic or smaller whisking episodes could be seen in the Eurasian water shrew, domestic guinea pig, domestic ferret, European otter and red fox. Whisker movements are likely to be an important aspect of touch sensing to help improve the sensation of tactile signals, such as the detection of changes in acceleration and force, which increases sensitivity (Hollins and Risner, 2000; Lederman, 1983). Even in species that did not whisk their whiskers, such as the European hedgehog and weasel, they tended to move their heads to dab their whiskers rhythmically against a surface. While we did not observe this behaviour in harbour seals, it has been previously been documented (Grant et al., 2013). Therefore, movement of the whiskers over surfaces is likely to be an important aspect of whisker sensing.

Bringing more whiskers onto a surface, by reducing the spread, or span, of the whiskers, is also likely to improve the amount of sensory information that the whiskers can gather (Grant et al., 2009). We only

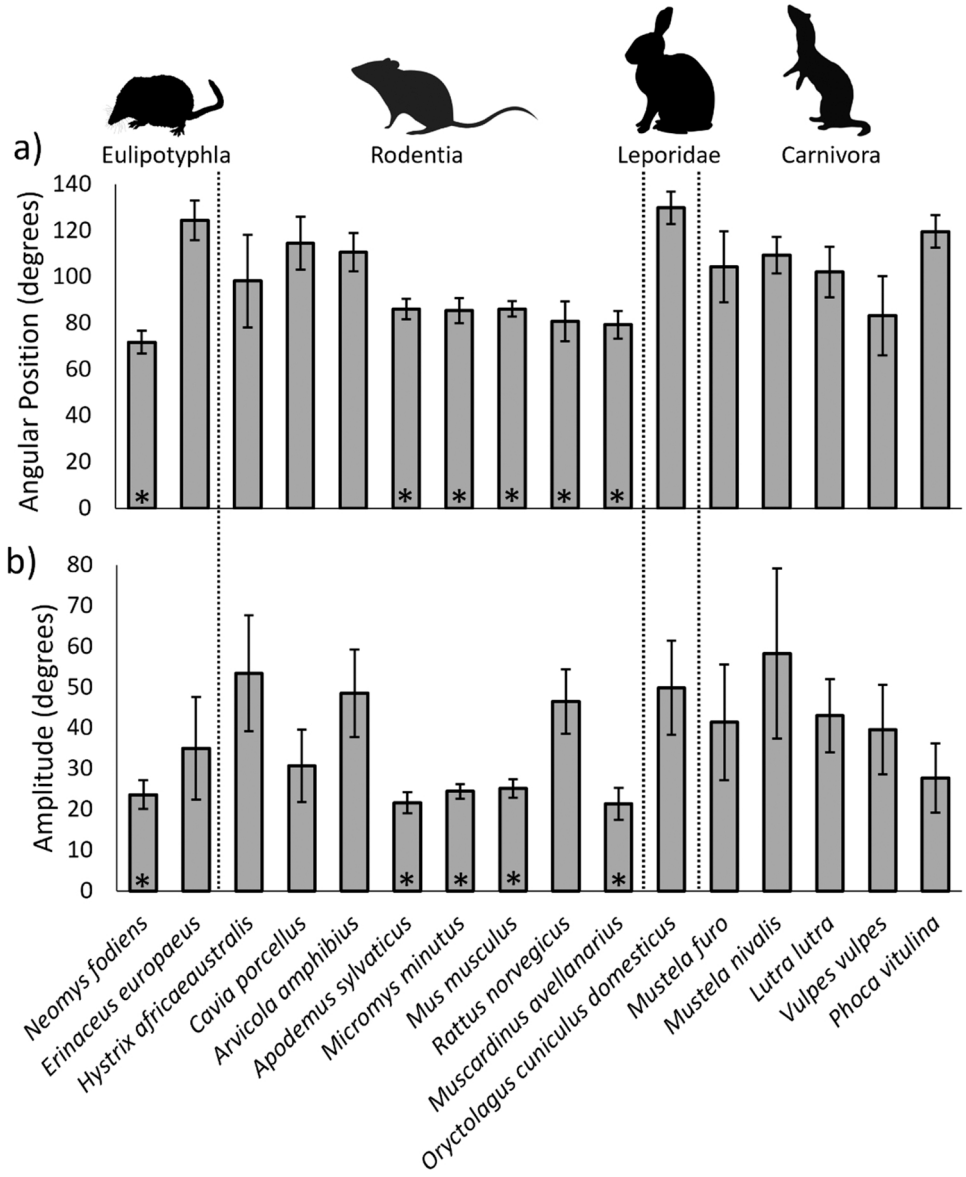

Fig. 6. Whisker position and movements in sixteen mammalian species. a) angular position; b) amplitude. Bars are mean values, with error bars being standard deviation values. Asterisks refer to a group of small mammals that have similar values of the same metric, that were, on the whole, significantly different to the other species.

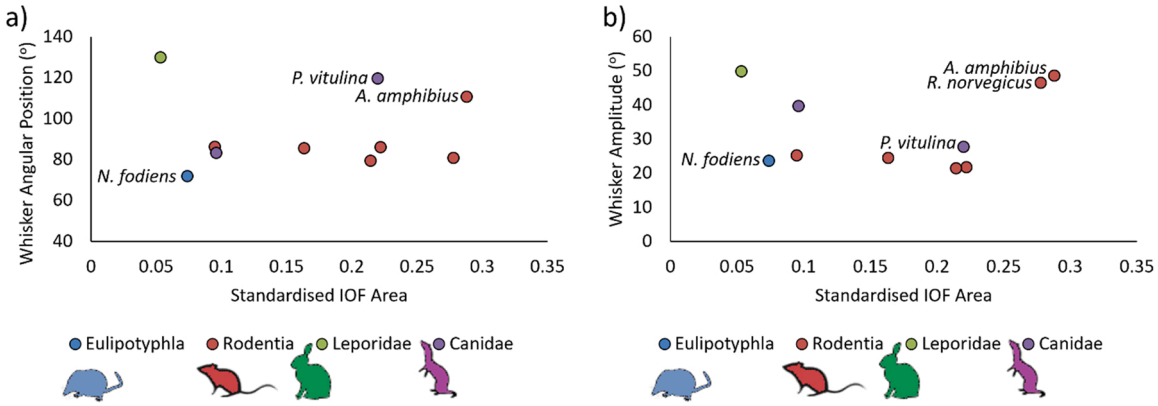

Fig. 7. Correlating whisker position (a) and movements (b) with standardised IOF area.

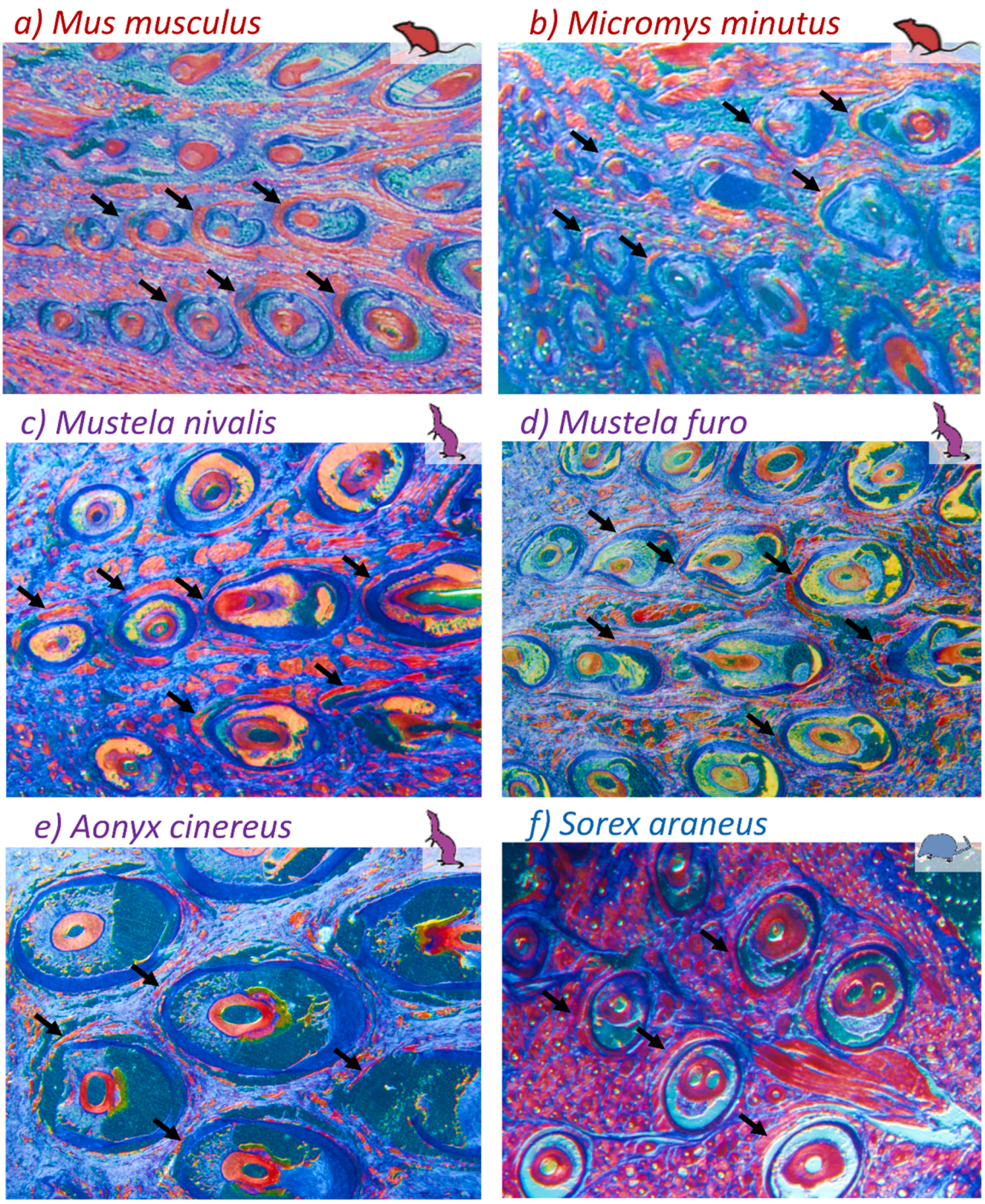

Fig. 8. Mystacial pad musculature images of six species of mammal. Mystacial (cheek) pads were dissected from cadavers, and then sliced (10 μm) and stained with Masson's trichrome. Species common names include: a) house mouse, b) harvest mouse, c) weasel, d) domestic ferret, e) Asian short-clawed otter, and f) common shrew. Coloured labelling indicates the orders Rodentia (red), Carnivora (mustelids) (purple) and Eulipotyphla (blue). Black arrows correspond to the intrinsic muscles around individual whisker follicles. Rostral whiskers are more leftwards, in each of the panels.

observed this whisker spread reduction in the Rodentia, including European water vole, murids (wood mouse, harvest mouse, house mouse and brown rat) and European dormouse. Indeed, this behaviour has only ever previously been observed in rodents (Grant et al., 2018, 2013). It has been found to be absent in both the grey short-tailed opossum, *Monodelphis domestica*, (Grant et al., 2013) and domestic guinea pig (Grant et al., 2017), which both had reduced Pars media superior and inferior muscles - the muscles responsible for pulling the caudal whiskers closer and reducing whisker spread (Grant et al., 2017; Haidarliu et al., 2010). The absence of spread reduction behaviour in many small mammals that whisk suggests that it may have evolved after whisking, and is likley to be accompanied by changes in the whisking musculature (Grant et al., 2013; Muchlinski et al., 2020), specifically the Pars media superior and inferior muscles.

Asymmetry, or more specifically Contact-induced asymmetry, often occurs following a unilateral contact and is characterised by the whiskers contralateral to the contact increasing in amplitude and the whiskers ipsilateral to the contact decreasing in amplitude (Mitchinson et al., 2007). Like the whisker behaviours of whisking and spread reduction, contact-induced asymmetry is also thought to improve touch sensing,

specifically by increasing the number of whisker contacts on the contralateral side, while ensuring light, precise touches on the ipsilateral side (Mitchinson et al., 2011, 2007). We observed aspects of this behaviour in all species apart from European hedgehog, domestic rabbit and red fox. Grant et al. (2018) suggested that the relationship between lifestyle (foraging and habitat preferences) and the ability to express different forms of contact-induced asymmetry may be worth investigating further in different mammalian species. This observation is further supported by our study, since the three species in which we did not observe the behaviour have a strong sense of smell, which may affect how the whiskers and whisker muscles may be employed. Indeed, whisker movements and sniffing are coupled together (Kleinfeld et al., 2015), and become decoupled depending on whether the animal is sampling olfactory or tactile cues. In more olfactory-focussed species, olfaction might perhaps take a precedent over asymmetric whisker behaviours. It would be interesting to investigate the prevalence of whisker control behaviours in more species, to make stronger predictions about evolution and functional associations.

It is worth bearing in mind that we developed this protocol to produce a simple task to elicit whisker movements in response to a novel object. Other tasks, such as feeding and object recognition, or tasks involving training, are likely to produce slightly different metrics to those we found, especially of whisker angle and amplitude, as the animals will be investigating a different object, with different properties, orientations etc. For example, a textured object may elicit more whisker stroking or dabbing movements, with larger whisker amplitudes, than those against smooth objects (Milne et al., 2021). It is difficult to ensure that other, more complex, tasks are equally relevant (or irrelevant) to each species, since animals may react differently to different stimuli, especially if they are ethologically relevant or associated with feeding. In such cases, other behaviours, such as licking, chewing or head movements may impact whisker movements or being able to view them for filming. Training specific object-recognition tasks is also an option; although challenging, since training is not possible with all individuals, not all collections engage in formal animal training, and training regimes are also likely to vary between collections.

4.2. Recommendations based on our method demonstration

The behavioural protocol that we have developed can easily be extended to include more species to investigate the evolution of behaviour using phylogenetic analysis as well as exploring behavioural associations with anatomical landmarks, such as demonstrated with the IOF area analysis in Fig. 7. While there was not a significant correlation between IOF area with the whisker metrics angular position or amplitude, it still showcases the ability to explore the association of whisker movements with metrics such as brain volume, nerve size, axon counts or muscle fibre counts, which will be useful for investigating comparative and evolutionary neuroscience. Explaining the association of whiskers with ecological traits is also important. Investigating whisker movements and behaviours, with habitat and foraging traits in more species using multivariate phylogenetic analyses, will give insights into the functional significances of these behaviours. Indeed, we believe that studying whisker movements and behaviours comparatively has the capacity to address many evolutionary questions in mammals, including those concerning the evolution of complex behaviours, facial anatomy, sensory neuroscience and ecology.

The protocol that we have developed here can also be used on genetically altered mammalian species to study the effect of altered neuronal circuitry or neurodegeneration on whisker movements (as per Garland et al., 2016; Grant et al., 2014; Landreth et al., 2021; Simanaviciute et al., 2020 in laboratory rodents). We believe that this protocol has wide-ranging applications in neuroscience and can be used to better understand the links between the brain and complex behaviours. Indeed, many neurophysiological recording systems have now been miniaturised and stabilised to allow measurement of the brain in freely moving animals, including for electrophysiology (Lee et al., 2006; Sharma et al., 2021) and imaging (e.g. calcium (Tang et al., 2022; Wirtshafter and Disterhoft, 2022) especially with two-photon imaging (Grienberger et al., 2022; Zong et al., 2022), and photoacoustics (Wang et al., 2021), that can even be used in tandem with optogenetic delivery systems (Sharma et al., 2021). These systems can be incorporated into our high-speed camera set-up with the animal in an enclosed arena. For larger, and more varied species, comparing whisker behaviour correlates with gross brain structure and nerve sizes from skull measurements or dissections would also be possible, as well as adopting less invasive neurophysiological recordings, such as functional magnetic resonance imaging (fMRI). Automating aspects of the protocol might also help to collect larger datasets in the future over longer periods of time. For example, by triggering cameras using beam breaks or machine vision cameras. Machine learning algorithms are constantly developing, which may also enable whisker tracking to be more automated in the future, even in a diverse group of species.

There are certainly challenges associated with working comparatively with many species, which include: i) accessing species; ii) working with multiple collections; iii) low sample numbers; iv) species-specific requirements and v) individual-specific requirements. These challenges are in no way restricted to whisker movement studies, but would apply to any large-scale comparative behaviour study. Firstly, accessing many species can be challenging. A large comparative behavioural study of this nature will involve working with many different animal collections (i.e. zoos), since it is unlikely that one collection will house all the species needed. Working across animal collections results in extra ethical approval stages, and certain adaptation of the methods to allow for the working practices of different collections. For example, some collections might not train or handle species and keepers might not enter animal enclosures at all (i.e. Wilson et al., 2015). These different working practices across collections might also affect the animals' behaviour (Bell et al., 2009; Melfi, 2013; Richter et al., 2009; Ward and Melfi, 2013), which may need to be taken into account at some point within the study. Accessing many species usually means that the sample numbers for each species is lower than those expected from other, more classic, behavioural neuroscience studies. Not only would accessing >10 individuals per species be time-consuming, it is unlikely that a researcher will be able to locate and access many individuals of the same species, and then they would likely be housed across multiple institutions. There are also species-specific differences in size, movement speed, diet, enclosure and husbandry requirements (i.e. access, training, safety) that may need to be taken into account during testing. Some of these might even change between different sexes of the same species (i.e. taking into account sex differences in aggression or size, such as in Pinnipeds). There may even be individual-differences in experience (i.e. of training or handling), as well as in personality, such as shyness or aggression, that may impact how a researcher engages with an animal. For comparative behavioural studies to work, it is important for researchers to work closely with animal collections and be flexible with their methodologies to take these challenges into account. We recommend to account for these challenges at the planning stage of each comparative behavioural study. Even with such a flexible and organic behavioural protocol, we believe that comparative studies can be important and useful scientific studies, especially to investigate the evolution of complex behaviours.

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CRediT authorship contribution

Robyn Grant: Conceptualization; Data curation; Formal analysis;

Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. **Hazel Ryan and Vicki Breakell:** Supervision; Resources; Writing - review & editing.

Declarations of interest

None.

Data availability

All species data is provided as mean and s.d in Table 3. Raw data is available on request too.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jneumeth.2022.109752.

References

Adachi, T., Naito, Y., Robinson, P.W., Costa, D.P., Hückstädt, L.A., Holser, R.R., Iwasaki, W., Takahashi, A., 2022. Whiskers as hydrodynamic prey sensors in foraging seals. Proc. Natl. Acad. Sci. U. S. A. 119, e2119502119 https://doi.org/10.1073/PNAS.2119502119/SUPPL_FILE/PNAS.2119502119.SM04.MP4.

Anjum, F., Turni, H., Mulder, P.G.H., Van Der Burg, J., Brecht, M., 2006. Tactile guidance of prey capture in Etruscan shrews. Proc. Natl. Acad. Sci. U. S. A. 103, 16544–16549. https://doi.org/10.1073/pnas.0605573103.

Arkley, K., Grant, R.A., Mitchinson, B., Prescott, T.J., 2014. Strategy change in vibrissal active sensing during rat locomotion. Curr. Biol. 24. https://doi.org/10.1016/j.cub.2014.05.036.

Arkley, K., Tiktak, G.P., Breakell, V., Prescott, T.J., Grant, R.A., 2017. Whisker touch guides canopy exploration in a nocturnal, arboreal rodent, the Hazel dormouse (Muscardinus avellanarius). J. Comp. Physiol. A Neuroethol. Sens., Neural, Behav. Physiol. 203. https://doi.org/10.1007/s00359-017-1146-z.

Bell, A.M., Hankison, S.J., Laskowski, K.L., 2009. The repeatability of behaviour: a meta-analysis. Anim. Behav. 77, 771–783. https://doi.org/10.1016/J.ANBEHAV.2008.12.022.

Betting, J.H.L.F., Romano, V., Al-Ars, Z., Bosman, L.W.J., Strydis, C., De Zeeuw, C.I., 2020. WhiskEras: a new algorithm for accurate whisker tracking. Front. Cell. Neurosci. 14, 372. https://doi.org/10.3389/FNCEL.2020.588445/BIBTEX.

Bryer, M.A.H., Koopman, S.E., Cantlon, J.F., Piantadosi, S.T., MacLean, E.L., Baker, J.M., Beran, M.J., Jones, S.M., Jordan, K.E., Mahamane, S., Nieder, A., Perdue, B.M., Range, F., Stevens, J.R., Tomonaga, M., Ujfalussy, D.J., Vonk, J., 2022. The evolution of quantitative sensitivity. Philos. Trans. R. Soc. B 377, 20200529. https://doi.org/10.1098/RSTB.2020.0529.

Byrne, R.W., 1993. A formal notation to aid analysis of complex behaviour: understanding the tactical deception of primates. Behaviour 127, 231–246. https://doi.org/10.1163/156853993X00038.

Cisek, P., Hayden, B.Y., 2022. Neuroscience needs evolution. Philos. Trans. R. Soc. B Biol. Sci. 377. https://doi.org/10.1098/RSTB.2020.0518.

Clack, N.G., O'Connor, D.H., Huber, D., Petreanu, L., Hires, A., Peron, S., Svoboda, K., Myers, E.W., 2012. Automated tracking of whiskers in videos of head fixed rodents. PLOS Comput. Biol. 8, e1002591 https://doi.org/10.1371/JOURNAL.PCBI.1002591.

Dehnhardt, G., Mauck, B., Hyvärinen, H., 1998. Ambient temperature does not affect the tactile sensitivity of mystacial vibrissae in harbour seals. J. Exp. Biol. 201, 3023–3029.

Dennis, E.J., Hady, A., El, Michaiel, A., Clemens, A., Gowan Tervo, D.R., Voigts, J., Datta, S.R., 2021. Systems neuroscience of natural behaviors in rodents. J. Neurosci. 41, 911–919. https://doi.org/10.1523/JNEUROSCI.1877-20.2020.

Diamond, M.E., Von Heimendahl, M., Knutsen, P.M., Kleinfeld, D., Ahissar, E., 2008. "Where" and "what" in the whisker sensorimotor system. Nat. Rev. Neurosci. https://doi.org/10.1038/nrn2411.

Dörfl, J., 1982. The musculature of the mystacial vibrissae of the white mouse. J. Anat. 135, 147–154.

Dougill, G., Starostin, E.L., Milne, A.O., van der Heijden, G.H.M., Goss, V.G.A., Grant, R. A., 2020. Ecomorphology reveals Euler spiral of mammalian whiskers. J. Morphol. jmor 21246. https://doi.org/10.1002/jmor.21246.

Evans, M.H., Loft, M.S.E., Campagner, D., Petersen, R.S., 2019. Sens. Environ. Whiskers. https://doi.org/10.1093/ACREFORE/9780190264086.013.226.

Garland, H., Wood, N.I., Skillings, E.A., Detloff, P.J., Morton, A.J., Grant, R.A., 2016. Characterisation of progressive motor deficits in whisker movements in R6/2, Q175 and Hdh knock-in mouse models of Huntington's disease. J. Neurosci. Methods. https://doi.org/10.1016/j.jneumeth.2017.04.020.

Gillespie, D., Yap, M.H.M.H., Hewitt, B.M.B.M., Driscoll, H., Simanaviciute, U., Hodson-Tole, E.F.E.F., Grant, R.A.R.A., 2019. Description and validation of the LocoWhisk system: Quantifying rodent exploratory, sensory and motor behaviours. J. Neurosci. Methods Elsevier B. V. https://doi.org/10.1016/j.jneumeth.2019.108440.

Grant, R., Wieskotten, S., Wengst, N., Prescott, T., Dehnhardt, G., 2013a. Vibrissal touch sensing in the harbor seal (Phoca vitulina): How do seals judge size? J. Comp. Physiol. A Neuroethol. Sens., Neural, Behav. Physiol. 199. https://doi.org/10.1007/s00359-013-0797-7.

Grant, R.A., Goss, V.G.A., 2022. What can whiskers tell us about mammalian evolution, behaviour, and ecology? Mamm. Rev. 52, 148–163. https://doi.org/10.1111/MAM.12253.

Grant, R.A., Mitchinson, B., Fox, C.W., Prescott, T.J., 2009. Active touch sensing in the rat: anticipatory and regulatory control of whisker movements during surface exploration. J. Neurophysiol. 101. https://doi.org/10.1152/jn.90783.2008.

Grant, R.A., Mitchinson, B., Prescott, T.J., 2012. The development of whisker control in rats in relation to locomotion. Dev. Psychobiol. 54. https://doi.org/10.1002/dev.20591.

Grant, R.A., Haidarliu, S., Kennerley, N.J., Prescott, T.J., 2013b. The evolution of active vibrissal sensing in mammals: evidence from vibrissal musculature and function in the marsupial possum Monodelphis domestica. J. Exp. Biol. 216. https://doi.org/10.1242/jeb.087452.

Grant, R.A., Delaunay, M.G., Haidarliu, S., 2017. Mystacial whisker layout and musculature in the Guinea Pig (Cavia porcellus): a social, diurnal mammal. Anat. Rec. 300. https://doi.org/10.1002/ar.23504.

Grant, R.A., Breakell, V., Prescott, T.J., 2018. Whisker touch sensing guides locomotion in small, quadrupedal mammals. Proc. R. Soc. B Biol. Sci. 285. https://doi.org/10.1098/rspb.2018.0592.

Grant, R.A., Wong, A.A., Fertan, E., Brown, R.E., 2020. Whisker exploration behaviours in the 5xFAD mouse are affected by sex and retinal degeneration. Genes, Brain Behav. 19. https://doi.org/10.1111/gbb.12532.

Grant, R.A.R.A., Sharp, P.S.P.S., Kennerley, A.J.A.J., Berwick, J., Grierson, A., Ramesh, T., Prescott, T.J.T.J., 2014. Abnormalities in whisking behaviour are associated with lesions in brain stem nuclei in a mouse model of amyotrophic lateral sclerosis. Behav. Brain Res. 259, 274–283. https://doi.org/10.1016/j.bbr.2013.11.002.

Grienberger, C., Giovannucci, A., Zeiger, W., Portera-Cailliau, C., 2022. Two-photon calcium imaging of neuronal activity, 2022 Nat. Rev. Methods Prim. 21 (2), 1–23. https://doi.org/10.1038/s43586-022-00147-1.

Haidarliu, S., Simony, E., Golomb, D., Ahissar, E., 2010. Muscle architecture in the mystacial pad of the rat. Anat. Rec. Adv. Integr. Anat. Evol. Biol. 293, 1192–1206. https://doi.org/10.1002/ar.21156.

Hewitt, B.M., Yap, M.H., Grant, R.A., 2016. Manual whisker annotator (MWA): a modular open-source tool. J. Open Res. Softw. 4. https://doi.org/10.5334/jors.93.

Hewitt, B.M., Yap, M.H., Hodson-Tole, E.F., Kennerley, A.J., Sharp, P.S., Grant, R.A, 2018. A novel automated rodent tracker (ART), demonstrated in a mouse model of amyotrophic lateral sclerosis. J. Neurosci. Methods 300, 147–156. https://doi.org/10.1016/j.jneumeth.2017.04.006. https://doi.org/10.1016/j.jneumeth.2017.04.006.

Hollins, M., Risner, S.R., 2000. Evidence for the duplex theory of tactile texture perception. Percept. Psychophys. 62, 695–705. https://doi.org/10.3758/BF03206916.

Jin, T.E., Witzemann, V., Brecht, M., 2004. Fiber types of the intrinsic whisker muscle and whisking behavior. J. Neurosci. 24, 3386–3393. https://doi.org/10.1523/JNEUROSCI.5151-03.2004.

Kleinfeld, D., Deschênes, M., Moore, J.D., 2015. The central pattern generator for rhythmic whisking. In: Sensorimotor Integration in the Whisker System. Springer, New York, pp. 149–165. https://doi.org/10.1007/978-1-4939-2975-7_7.

Knutsen, P.M., Derdikman, D., Ahissar, E., 2005. Tracking whisker and head movements in unrestrained behaving rodents. J. Neurophysiol. 93, 2294–2301. https://doi.org/10.1152/JN.00718.2004/ASSET/IMAGES/LARGE/Z9K0040544990010.JPEG.

Landreth, K., Simanaviciute, U., Fletcher, J., Grayson, B., Grant, R.A., Harte, M.H., Gigg, J., 2021. Dissociating the effects of distraction and proactive interference on object memory through tests of novelty preference: https://doi.org/10.1177/23982128211003199.

Laurent, G., 2020. On the value of model diversity in neuroscience, 2020 Nat. Rev. Neurosci. 218 (21), 395–396. https://doi.org/10.1038/s41583-020-0323-1.

Lederman, S.J., 1983. Tactual roughness perception: Spatial and temporal determinants. Can. J. Psychol. Can. Psychol. 37, 498–511. https://doi.org/10.1037/h0080750.

Lederman, S.J., Klatzky, R.L., 1987. Hand movements: a window into haptic object recognition. Cogn. Psychol. 19, 342–368. https://doi.org/10.1016/0010-0285(87)90008-9.

Lee, A.K., Manns, I.D., Sakmann, B., Brecht, M., 2006. Whole-cell recordings in freely moving rats. Neuron 51, 399–407. https://doi.org/10.1016/J.NEURON.2006.07.004.

Melfi, V., 2013. Is training zoo animals enriching? Appl. Anim. Behav. Sci. 147, 299–305. https://doi.org/10.1016/J.APPLANIM.2013.04.011.

Miller, E.K., 1999. The prefrontal cortex: minireview complex neural properties for complex behavior. Neuron 22, 15–17.
Milne, A.O., Grant, R.A., 2014. Characterisation of whisker control in the California sea lion (Zalophus californianus) during a complex, dynamic sensorimotor task. J. Comp. Physiol. A Neuroethol. Sens., Neural, Behav. Physiol. 200. https://doi.org/10.1007/s00359-014-0931-1.
Milne, A.O., Smith, C., Orton, L.D., Sullivan, M.S., Grant, R.A., 2020. Pinnipeds orient and control their whiskers: a study on Pacific walrus, California sea lion and Harbor seal. J. Comp. Physiol. A Neuroethol. Sens., Neural, Behav. Physiol. https://doi.org/10.1007/s00359-020-01408-8.
Milne, A.O., Orton, L., Black, C.H., Jones, G.C., Sullivan, M., Grant, R.A., 2021. California sea lions employ task-specific strategies for active touch sensing. J. Exp. Biol. 224 https://doi.org/10.1242/JEB.243085/VIDEO-1.
Milne, A.O., Muchlinski, M.N., Orton, L.D., Sullivan, M.S., Grant, R.A., 2022. Comparing vibrissal morphology and infraorbital foramen area in pinnipeds. Anat. Rec. 305, 556–567. https://doi.org/10.1002/AR.24683.
Mitchinson, B., Prescott, T.J., 2013. Whisker movements reveal spatial attention: a unified computational model of active sensing control in the rat. PLoS Comput. Biol. 9, 1003236. https://doi.org/10.1371/journal.pcbi.1003236.
Mitchinson, B., Martin, C.J., Grant, R.A., Prescott, T.J., 2007. Feedback control in active sensing: Rat exploratory whisking is modulated by environmental contact. Proc. R. Soc. B Biol. Sci. 274. https://doi.org/10.1098/rspb.2006.0347.
Mitchinson, B., Grant, R.A., Arkley, K., Rankov, V., Perkon, I., Prescott, T.J., 2011a. Active vibrissal sensing in rodents and marsupials. Philos. Trans. R. Soc. B Biol. Sci. 366. https://doi.org/10.1098/rstb.2011.0156.
Muchlinski, M.N., Durham, E.L., Smith, T.D., Burrows, A.M., 2013. Comparative histomorphology of intrinsic vibrissa musculature among primates: implications for the evolution of sensory ecology and "face touch.". Am. J. Phys. Anthropol. 150, 301–312. https://doi.org/10.1002/ajpa.22206.
Muchlinski, M.N., Wible, J.R., Corfe, I., Sullivan, M., Grant, R.A., 2020. Good vibrations: the evolution of whisking in small mammals. Anat. Rec. 303, 89–99. https://doi.org/10.1002/ar.23989.
Pessoa, L., Medina, L., Desfilis, E., 2022. Refocusing neuroscience: moving away from mental categories and towards complex behaviours. Philos. Trans. R. Soc. B 377, 20200534. https://doi.org/10.1098/RSTB.2020.0534.
Petersen, R.S., Rodriguez, A.C., Evans, M.H., Campagner, D., Loft, M.S.E., 2020. A system for tracking whisker kinematics and whisker shape in three dimensions. PLoS Comput. Biol. 16. https://doi.org/10.1371/journal.pcbi.1007402.
Richter, S.H., Garner, J.P., Würbel, H., 2009. Environmental standardization: cure or cause of poor reproducibility in animal experiments?, 2009 Nat. Methods 64 (6), 257–261. https://doi.org/10.1038/nmeth.1312.
Ritt, J.T., 2012. High-speed videography of embodied active sensing in the rodent whisker system. Neuromethods 67, 283–302. https://doi.org/10.1007/7657_2011_9/FIGURES/3_9.
Rudebeck, P.H., Izquierdo, A., 2021. Foraging with the frontal cortex: a cross-species evaluation of reward-guided behavior, 2021 Neuropsychopharmacol 471 (47), 134–146. https://doi.org/10.1038/s41386-021-01140-0.
Sehara, K., Zimmer-Harwood, P., Larkum, M.E., Sachdev, R.N.S., 2021. Real-time closed-loop feedback in behavioral time scales using DeepLabCut. eNeuro 8. https://doi.org/10.1523/ENEURO.0415-20.2021.
Sharma, K., Jackel, Z., Schneider, A., Paul, O., Diester, I., Ruther, P., 2021. Multifunctional optrode for opsin delivery, optical stimulation, and electrophysiological recordings in freely moving rats. J. Neural Eng. 18, 066013 https://doi.org/10.1088/1741-2552/AC3206.
Simanaviciute, U., Ahmed, J., Brown, R.E., Connor-Robson, N., Farr, T.D., Fertan, E., Gambles, N., Garland, H., Morton, A.J., Staiger, J.F., Skillings, E.A., Trueman, R.C., Wade-Martins, R., Wood, N.I., Wong, A.A., Grant, R.A., 2020. Recommendations for measuring whisker movements and locomotion in mice with sensory, motor and cognitive deficits. J. Neurosci. Methods 331, 108532. https://doi.org/10.1016/j.jneumeth.2019.108532.
Tang, J., Xue, R., Wang, Y., Li, M., Jia, H., Pakan, J.M.P., Li, L., Chen, X., Li, X., 2022. Optical fiber-based recording of climbing fiber Ca2+ signals in freely behaving mice, 2022 Biol Vol. 11. https://doi.org/10.3390/BIOLOGY11060907.
Wang, Y., Chu, T.S., Lin, Y.R., Tsao, C.H., Tsai, C.H., Ger, T.R., Chen, L.T., Chang, W.S. W., Liao, L.De, 2021. Assessment of brain functional activity using a miniaturized head-mounted scanning photoacoustic imaging system in awake and freely moving rats, 429 11 Biosens Vol. 11, 429. https://doi.org/10.3390/BIOS11110429.
Ward, S.J., Melfi, V., 2013. The implications of husbandry training on zoo animal response rates. Appl. Anim. Behav. Sci. 147, 179–185. https://doi.org/10.1016/j.applanim.2013.05.008.
Wilson, M.L., Perdue, B.M., Bloomsmith, M.A., Maple, T.L., 2015. Rates of reinforcement and measures of compliance in free and protected contact elephant management systems. Zoo. Biol. 34, 431–437. https://doi.org/10.1002/ZOO.21229.
Wineski, L.E., 1985. Facial morphology and vibrissal movement in the golden hamster. J. Morphol. 183, 199–217. https://doi.org/10.1002/JMOR.1051830208.
Wirtshafter, H.S., Disterhoft, J.F., 2022. In vivo multi-day calcium imaging of CA1 hippocampus in freely moving rats reveals a high preponderance of place cells with consistent place fields. J. Neurosci. 42, 4538–4554. https://doi.org/10.1523/JNEUROSCI.1750-21.2022.
Yartsev, M.M., 2017. The emperor's new wardrobe: rebalancing diversity of animal models in neuroscience research. Science 358 (80-), 466–469. https://doi.org/10.1126/SCIENCE.AAN8865/ASSET/209CBD7D-DE54-4478-A058-EBA8ED2FDD2A/ASSETS/GRAPHIC/358_466_FA.JPEG.
Yohro, T., 1977. Arrangement and structure of sinus hair muscles in the big-clawed shrew, <i>Sorex unguiculatus <i>. J. Morphol. 153, 317–331.
Zong, W., Obenhaus, H.A., Skytøen, E.R., Eneqvist, H., de Jong, N.L., Vale, R., Jorge, M. R., Moser, M.B., Moser, E.I., 2022. Large-scale two-photon calcium imaging in freely moving mice. e30 Cell 185, 1240–1256. https://doi.org/10.1016/J.CELL.2022.02.017.