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Title: Geometric morphometrics as a tool for improving the comparative study of behavioural postures

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**Abstract:** Describing postures has always been a central concern when studying behaviour. However, attempts to compare postures objectively at phylogenetical, populational, inter- or intra-individual levels generally either rely upon a few key elements or remain highly subjective. Here we propose a novel approach, based on well-established geometric morphometrics, to describe and to analyse postures globally (i.e. considering the animal's body posture in its entirety rather than focusing only on a few salient elements, such as head or tail position). Geometric morphometrics is concerned with describing and comparing variation and changes in the form (size and shape) of organisms using the coordinates of a series of homologous landmarks (i.e. positioned in relation to skeletal or muscular cues that are the same for different species for every variety of form and function and that have derived from a common ancestor, i.e. they have a common evolutionary ancestry, e.g. neck, wings, flipper /hand). We applied this approach to horses, using global postures 1) to characterise behaviours that correspond to different arousal levels, 2) to test potential impact of environmental changes on postures. Our application of geometric morphometrics to horse postures showed that this method can be used to characterise behavioural categories, to evaluate the impact of environmental factors (here human actions) and to compare individuals and groups. Beyond its application to horses, this promising approach could be applied to all questions involving the analysis of postures (evolution of displays, expression of emotions, stress and welfare, behavioural repertoires...) and could lead to a whole new line of research.

Response to Reviewers: Dear Dr Thatje,

Thank you for considering the submission of our manuscript "Geometric morphometrics as a tool for improving the comparative study of behavioural postures" in Naturwissenschaften.

Please find a revised version in the attached file. We have taken into account all your comments, adding information to the legend to figure 1, adding the "Material and Methods" header and removing phone/fax numbers from affiliations.

Yours sincerely,

Carole Fureix

1 **Geometric morphometrics as a tool for improving the comparative study of behavioural**  
2 **postures**

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15 Describing postures has always been a central concern when studying behaviour. However,  
16 attempts to compare postures objectively at phylogenetical, populational, inter- or intra-  
17 individual levels generally either rely upon a few key elements or remain highly subjective.  
18 Here we propose a novel approach, based on well-established geometric morphometrics, to  
19 describe and to analyse postures globally (*i.e.* considering the animal's body posture in its  
20 entirety rather than focusing only on a few salient elements, such as head or tail position).  
21 Geometric morphometrics is concerned with describing and comparing variation and changes  
22 in the form (size and shape) of organisms using the coordinates of a series of homologous  
23 landmarks (*i.e.* positioned in relation to skeletal or muscular cues that are the same for  
24 different species for every variety of form and function and that have derived from a common  
25 ancestor, *i.e.* they have a common evolutionary ancestry, *e.g.* neck, wings, flipper /hand). We  
26 applied this approach to horses, using global postures 1) to characterise behaviours that  
27 correspond to different arousal levels, 2) to test potential impact of environmental changes on  
28 postures. Our application of geometric morphometrics to horse postures showed that this  
29 method can be used to characterise behavioural categories, to evaluate the impact of  
30 environmental factors (here human actions) and to compare individuals and groups. Beyond  
31 its application to horses, this promising approach could be applied to all questions involving  
32 the analysis of postures (evolution of displays, expression of emotions, stress and welfare,  
33 behavioural repertoires...) and could lead to a whole new line of research.

34  
35 **Keywords:** posture analysis; geometric morphometrics; innovative methodological  
36 application ; horses; ethology

39       **INTRODUCTION**

1  
2 40       From Darwin (1872) to Platon (Frere 1998), descriptions of animal behaviour have always  
3  
4 41       been based on postures. Because body expression is one way for animals to convey emotions,  
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6 42       Darwin based his concept of continuity of behaviour between species on the continuum of  
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8 43       postural expressions of emotions. All through the history of animal behaviour research,  
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10 44       description of postures has been central (Guyomarc'h et al. 1987) and fundamental for  
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12 45       defining behavioural repertoires (Baerends 1972), evaluating individual or population  
13  
14 46       differences or the evolution of behaviour (Wickler 1967). More recently, postures have again  
15  
16 47       been considered as a major tool for evaluating stress and emotional states (*e.g.* Beerda et al.  
17  
18 48       1999; Reefmann et al. 2009) or detecting anxiety (Lepicard et al. 2003).

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20  
21 49       However, attempts to compare postures objectively at these different levels (definition of  
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23 50       behavioural repertoires, evolution of behaviour, impact of stress...) generally rely only upon a  
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25 51       few key elements, only salient parts of the body like the head and tail, and/or remain highly  
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27 52       subjective (*i.e.* mere visual evaluation with no quantifiable details). For instance, descriptions  
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29 53       of blue tits' (*Cyanistes caeruleus*) attack / flight postures (Stokes 1962) were based on 36  
30  
31 54       correlations between 9 elements such as wings, beak, and tail, whose positions were evaluated  
32  
33 55       arbitrarily. Baerends & van der Cingel (1962) compared the "snap display" of common  
34  
35 56       herons, *Ardea cinerea*, to displays of other species on the basis of measurements of neck  
36  
37 57       angle, head orientation, and tibio-tarsal angle, but such measurements were also focused on a  
38  
39 58       few salient elements and did not consider the animal's body posture in its entirety (*i.e.* did not  
40  
41 59       provide a global posture assessment).

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44 60       Current researchers are faced with the same difficulties when comparing postures.  
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46 61       Descriptions of individual profiles of male quail (*Coturnix japonica*) displays have used a  
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48 62       combination of elements (*e.g.* legs bent/ extended, head stretched and body or wings lowered,  
49  
50 63       Lumineau et al. 2005), which do not provide a global overall posture assessment. Estimation

64 of welfare and of anxiety are also based on a few salient elements, such as tail angle and trunk  
65 height in anxious mice (Lepicard et al. 2003). When used, global posture assessments are  
66 based on very coarse postural elements, *e.g.* the animal is merely recorded as lying or  
67 standing (Huzzey et al. 2005; Krawczel et al. 2008; Xin 1999), or remain subjective (*e.g.* the  
68 low posture in stressed dogs, where “the position of the tail is lowered (...) and the legs are  
69 bent” compared to “the breed specific posture shown by dogs under neutral conditions”,  
70 Beerda et al. 1999).

71 Ethologists try to cope with the limitations of subjective categorisation by using inter-  
72 subjective agreement, asking different observers to categorise the same items. Yet these  
73 procedures do not guarantee the reproducibility of the measures, for instance between  
74 laboratories or groups of animals. Moreover, despite the importance of “postural behaviour as  
75 an integrated biological sensor” (Xin 1999), no satisfying global representation, leading to  
76 appropriate statistical comparisons of body postures in their entirety, has yet emerged. Here  
77 we argue that systematic quantitative analyses, using clear anatomical landmarks, would both  
78 1) allow one to study posture as a whole and 2) improve the objectivity and reproducibility of  
79 postural measures by quantifying the amplitude of their variation rather than recording their  
80 mere occurrence. Automated behaviour and movement detection has been used in animals, as  
81 in kinematic studies using markers stuck or painted on animals, or surgically implanted (*e.g.*  
82 in horses: Licka and Peham 1998, Faber et al. 2000, Haussler and Erb 2006, Peham and  
83 Schobesberger 2006, Hobbs et al. 2010; in ferrets: Kafkafi and Golani 1998). However, one  
84 of the major limitations of this methodology is that animals are tested in highly artificial  
85 situations, moving in front of fixed cameras in a calibrated environment since fixed  
86 “positions” defined beforehand in the environment are needed to obtain coordinates to use as  
87 a reference frame for measurements in the tracking program. Here we propose a novel  
88 approach to describe and to analyse global body postures on the basis of geometric

89 morphometrics (hereafter GM) recognised in biology for its descriptive power and its high  
90 statistical power (Adams et al. 2004). We applied GM for the first time to the study of posture  
91 in domestic horses *Equus caballus*, testing whether GM could be used for detecting different  
92 body posture. The horse is a highly appropriate model to study the application of GM tools to  
93 ethological questions for several reasons. First, visible postures associated with different  
94 activities or behaviour have been described previously by Kiley-Worthington (1976), but only  
95 head and tail positions were considered. Here we predicted that GM would allow us to  
96 describe and to analyse global body posture variation as a function of behaviour. Second,  
97 horses have recently been shown to be sensitive to subtle cues humans display while  
98 interacting with animals, for instance in relation to their attentional (Proops & McComb 2010;  
99 Takimoto & Fujita 2008) and emotional states (Keeling et al. 2009; Hama et al. 1996). Such a  
100 high sensitivity to humans allows one to predict that horses' posture could vary according to  
101 the presence of people (*i.e.* while a horse is interacting with humans, *e.g.* being led) or their  
102 absence (*i.e.* while a horse is performing spontaneous behaviour, *e.g.* locomotion in a  
103 pasture). Finally, working conditions such as being ridden may lead to undesirable postures at  
104 work (neck height and curve) leading to the same long-term negative effects, such as the  
105 occurrence of chronic back problems (Lesimple et al. 2010). This may influence posture, as  
106 horses with back problems have been reported to present a flat and rigid whole back (Cauvin  
107 1997; Faber et al. 2000). Thus, horses provide an interesting model to test the impact of living  
108 conditions (*e.g.* working conditions) on posture by comparing domestic horses kept under  
109 natural conditions (optimal for their welfare) and horses from riding schools, kept under  
110 conditions known to have some negative impact on their welfare (*e.g.* poor working  
111 conditions leading to potential vertebral problems, social isolation in boxes, time-restricted  
112 feeding practices..., Mc Greevy et al. 1995; Cooper et al. 2000; Lesimple et al. 2010). We thus  
113 predicted that horses' global posture would vary according to living conditions.

114 **INTEREST AND CONDITIONS OF APPLICATIONS OF GEOMETRIC MORPHOMETRICS**

1  
2 115 Usually GM analyses morphological differences and changes in organisms (*e.g.* in  
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5 116 proportions and respective positions of skeletal features) using the coordinates of a series of  
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7 117 homologous reference points (“landmarks”) that can then be used to compare specimens. In  
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10 118 addition to statistical analysis of morphological changes, GM also provides a way to draw  
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12 119 pictures of morphological transformations, *i.e.* to *visualize* one morphology transforming into  
13  
14 120 another by gradually moving a cursor from one morphology to another in the software.  
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17 121 Through analyses of shape disparity (*i.e.* variety within a group of species as the outcome of  
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19 122 evolutionary processes) and variation within a single population, GM can tackle different  
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22 123 types of questions related to the skeleton such as taxonomic affiliation (*i.e.* whether  
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24 124 populations are drawn from multiple species, and, if so, by what morphological variable(s)  
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26 125 they are most effectively discriminated), phylogenetic relationships among taxa or  
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29 126 evolutionary issues. However, GM has been used only in a limited number of posture-related  
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32 127 studies: morphological variations in the seahorse vertebral system (Bruner & Bartolino 2008);  
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34 128 geometrical analysis of footprints in addition to baropodometrical analysis (Bruner et al.  
35  
36 129 2009). To our knowledge, no study using GM has been directly performed on the postures of  
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39 130 living animals.

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41 131 Zelditch et al. (2004) explained the rationale and applications of these methods in detail, so  
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44 132 we only summarise below some basic principles. The theory of shape underlying GM enables  
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46 133 a clear distinction between the notions of shape and of scale (size): the calculation of  
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49 134 “centroid size” (the sum of distances between every landmark and their centroid) provides a  
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51 135 measure of shape independent of size. Two objects of different size are considered similar in  
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54 136 shape when they appear identical after filtering out effects of location in space, rotation and  
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56 137 scale (*e.g.* with generalised least squares Procrustes superimposition), meaning in our case  
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138 that shapes of living individuals differing in size (*e.g.* breed-related differences) can be  
139 compared easily.

140 It is of interest that the geometry of a biological structure can thus be analysed as a whole  
141 using landmark coordinates. Patterns emerge from the analyses, allowing analysis of the  
142 overall posture rather than focusing independently only on salient elements (*e.g.* identifying  
143 variations that could occur in different parts of an individual, such as the back and the croup,  
144 rather than focusing on the angle between head and body). The analysis itself helps to reveal  
145 which variables (distances, relative positions or proportions) are meaningful for the biological  
146 questions addressed, potentially highlighting unsuspected subtle postural variations.

147 Criteria for choosing landmarks for GM are: (1) homologous anatomical loci, (2) consistent  
148 relative topological positions, (3) adequate morphological coverage to take into account shape  
149 variations, for instance at least three landmarks are required to study a bend, (4) reliable  
150 repeatability and (5) all landmarks in the same two dimensional plane (Zelditch et al. 2004). A  
151 problem related to these criteria is that complete sets of homologous landmarks are usually  
152 required for comparison, meaning that only sufficiently morphologically similar organisms  
153 (allowing similar sets of landmarks) can be used in interspecific comparisons. Emerging GM  
154 approaches now enable comparisons including 2D and 3D non-homologous landmarks  
155 (Bookstein 1997; Gunz et al. 2005). For instance, a specific posture associated with a given  
156 environment (*e.g.* head and back position when standing under non friendly-to-welfare  
157 breeding conditions) could be investigated across large mammals (*e.g.* horses, cows, ...) but  
158 not on highly morphologically different organisms such as mammals and poultry.

159 Finally, an important general concern is the choice of the reference points: what are  
160 *relevant* GM landmarks? Blind use of any accessible landmark risks introducing noise and  
161 possibly biasing elements in the analysis, via traits correlated with uncontrolled parameters.  
162 Conversely, and except for questions already analysed in depth and for which relevant

163 parameters have been accurately documented, restrictive use of a few landmarks focusing on  
164 some part of an animal's body could overlook relevant morphological information.  
165 Exploratory approaches must use an extended series of landmarks, which can be restricted (or  
166 not) later after analysing each landmark's contribution to the shape.

## 168 **THE STUDY ON HORSES: MATERIAL AND METHODS**

### 169 **Subjects and behaviour**

170 Experiments complied with current French laws (Centre National de la Recherche  
171 Scientifique) related to animal experimentation and were in accordance with the European  
172 directive 86/609/CEE. No licence / permit / institutional ethical approval were needed.  
173 Animal husbandry and care were under the management of a private owner (study 1) or the  
174 riding school staff (study 2). This experiment involved only horses in the "field" (no  
175 laboratory animals).

176 We studied two samples of horses kept under different conditions (natural conditions in  
177 study 1, horses from riding schools in study 2), allowing us to characterise postures in relation  
178 to categories of spontaneous behaviour and to evaluate the impact of environmental factors  
179 (human actions, general living conditions: housing, feeding and working conditions).

### 181 **Study 1**

182 This study aimed, by applying GM tools to horses' postures, to characterise postures in  
183 relation to categories of spontaneous behaviour and to evaluate the impact of environmental  
184 factors, here human actions (*i.e.* being led: walking and standing). This study included 6  
185 domestic horses kept under natural conditions for more than 10 years, stable social groups  
186 year-round in 1-2ha natural pastures, fed grass and hay *ad libitum* during winter (no industrial  
187 pellets) and not regularly exercised (2 geldings, 4 stallions; 13-20 years old; French

188 Saddlebred cross, Haflinger and mixed breeds). Horses were observed performing various  
189 spontaneous activities known to be related to different arousal levels (locomotion: slow  
190 exploration and sustained walk; motionless behaviour: rest and observation, Table 1) and  
191 while interacting with an experimenter (being led: walking and standing motionless near an  
192 experimenter; the same 2.6 m long and 600g lead rope was used in all interactions). The  
193 experimenter, with whom the horse was not familiar beforehand, did not talk to the horse,  
194 stayed on the horse's left side and held the lead rope slackly at a predefined distance from the  
195 horse's head (1m), so that the experimenter never pulled the rope nor the horse's head.

196

## 197 **Study 2**

198 This study aimed to give a first evaluation of the impact of general living conditions  
199 (housing/feeding/working conditions) on horses' postures, again by applying GM tools. In  
200 order to address this issue, 63 horses from three riding schools were observed in addition to  
201 the study 1 horses in order to compare their postures. The horses from riding schools  
202 comprised 46 geldings and 17 mares, 5-20 years old, kept singly in 3 m \* 3 m individual  
203 straw-bedded boxes, fed industrial pellets (mainly composed of wheat bran: 30%, barley:  
204 28%, flour of alfalfa: 10%, palm kernel: 10%, soya bean: 10%, oats: 6%; treacle, corn,  
205 calcium carbonate, sodium chloride, vitamin A, D, E; copper sulphate) 3 times a day and hay  
206 once a day, exercised in riding lessons for 4-12 hours per week with at least one free day.  
207 Sixty-seven percent of the horses were French Saddlebreds, equally distributed among  
208 schools. The other horses belonged to various breeds or were unregistered animals. These  
209 riding school horses were included in a larger project evaluating horses' welfare using a  
210 multidimensional approach, involving health-related (*e.g.* vertebral state assessment),  
211 physiological, behavioural but also postural measures of the animal's welfare state. As  
212 previously described, data concerning horses being led (walk and stand, same lead rope as in

213 study 1) were recorded. Spontaneous activities (walks, rest...) could not be evaluated here  
214 because the horses were confined in boxes, thus preventing the experimenter from taking  
215 pictures perpendicularly and far away enough from the horse (cf. data recording).

### 217 **Data recording**

218 Eight landmarks (self-adhesive red felt discs, 34 mm in diameter, visible on all coat  
219 colours) were stuck onto the horse's right side. The landmarks were placed in a sagittal plane  
220 in relation to skeletal or muscular cues (thus enabling consistent reproduction of positioning)  
221 from head to croup along the spine (Fig. 1). Landmarks were placed on: the nasal bone under  
222 the eye, 2 cm in front of the zygomatic process (landmark 1); the temporo-maxillary joint  
223 (landmark 2); the atlas (landmark 3); the trapezium cervical ligament (landmark 4); the  
224 cervico-thoracic (landmark 5); the thoraco-lumbar (landmark 6) and the lumbo-sacral  
225 (landmark 7) junctions; and the first coccygeal vertebra (landmark 8) (Fig. 1).

226 Following Huard (2007) arguing that "only the animal's figurative body should be analysed  
227 due to limb mobility" when he applied GM tools to representations of Equidae in cave  
228 paintings, we voluntarily excluded limb positions recording. Indeed, limb movements inherent  
229 to locomotion could introduce too much noise into the analysis. Horses' postures were  
230 recorded using photographs taken perpendicularly 10m ± 1m from the horse (digital camera  
231 Canon EOS 20D, zoom lens 50mm to limit perspective distortions). All data were recorded by  
232 the same experimenters (E.S. – taking pictures and C.F. – leading the horses; see below). Data  
233 recording took place between 08.00 a.m. and 06.00 p.m. during a 3-week period for private  
234 owners' horses or a 2-day period at each riding school (in all schools during quiet periods,  
235 with no riding lessons). Horses kept under natural conditions were photographed 20 times in  
236 slow exploration walk, 20 times in sustained walk, 20 times while walking being led by an  
237 experimenter, 10 times while resting, 10 times while observing and 10 times while standing

238 held motionless near an experimenter. Thus each animal was photographed 20\*3 times in  
239 locomotion and 10\*3 times while performing motionless behaviours (yielding 90 photographs  
240 for each horse). In this preliminary approach, we took more photographs of locomotive than  
241 of static behaviours, as we assumed that intra-individual postural variations would be greater  
242 for locomotor behaviours. At the riding schools, horses were photographed on average 4.5 ( $\pm$   
243 0.9) times while walking led by an experimenter and 2.4 ( $\pm$  0.8) times while standing  
244 motionless near an experimenter. Riding school horses were not always available for  
245 observation, yielding different numbers of photographs per horse in this first approach  
246 (walking: from 2 to 7, and standing: from 2 to 5 per horse).

### 248 **Data and statistical analyses**

249 In all cases, data of landmark coordinates were extracted from photographs using Tps  
250 software (TpsDig2, TpsUtil) and analysed by Generalized Procrustes Analyses using R2.9.2  
251 and TpsRelW free software (R libraries: scatterplot3d, shapes and ade4, F3class command for  
252 the graphics). Briefly, landmarks were digitised by only one experimenter (ES, previously  
253 trained to this specific set of landmarks) from the photographs using tpsDig software, and  
254 then files were loaded from the tpsDig program into another tps software (tpsUtil) to define  
255 the sliders (*i.e.* the links between landmarks, creating the shape) and to save the sliders file.  
256 Then both files from the tpsDig program and the sliders file were loaded into the tpsRelw and  
257 R2.9.2 software to start shape analysis. Thus, Generalized Procrustes Analyses (allowing  
258 comparisons of shapes after filtering out effects of location in space, rotation and scale, for  
259 more statistical details see Zelditch et al. 2004) and Principal Component Analysis were  
260 conducted to identify postures in relation to behaviour (study 1) or to groups of horses  
261 according to their general living conditions (study 2). Data on aligned specimens filtering out  
262 effects of location in space, rotation and scale were also extracted from tpsRelW and R2.9.2

263 to conduct a Multivariate Analysis of Variance (MANOVA). MANOVA allows statistical  
1  
2 264 discrimination of postures in relation to behaviour or to groups of horses, taking into account  
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4 265 all the landmark coordinates (*i.e.* the global shape of the horse). MANOVAs were conducted  
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6  
7 266 using Statistica© 7.1 software (accepted *P* level at 0.05). Some slight postural variations  
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10 267 appeared for a given horse performing a given behaviour, probably due to the transitional  
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12 268 characteristic inherent to behavioural responses (compared with bones). However, it could be  
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14 269 overcome by taking several pictures of a horse performing the behaviour and do not prevent  
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16  
17 270 for statistically identifying inter-individual and inter-group variations (see results). Gender  
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19 271 differences in postures investigated in study 2 revealed no significant difference between  
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22 272 mares and geldings (MANOVA,  $F_1 = 1.86$ ,  $p > 0.05$ ). The difference stallions / geldings  
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24 273 (study 1) could not be statistically investigated here due to low number of geldings ( $n = 2$ ),  
25  
26  
27 274 but no sexual shape dimorphism was apparent between these two groups.  
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## 29 275

### 31 276 **THE STUDY ON HORSES: RESULTS**

#### 32 277 *Postures in Relation to Behaviour*

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34 277  
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36 278 The generalised Procrustes analyses allowed us to characterise postures associated with  
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39 279 given behaviours at the individual and group levels. Multivariate analysis (PCA) identified  
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41 280 specific postures in relation to behaviour for each individual horse (MANOVA for each horse:  
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43  
44 281  $F_{10, 168} = 19.44$  to  $38.94$ ,  $p < 0.001$  in all cases). These inter-behavioural postural variations  
45  
46 282 mainly concerned horses' neck height: horses' necks were highest when they were standing  
47  
48  
49 283 observing and lowest for exploratory walking (Fig. 2a and b). Low arousal behaviour postures  
50  
51 284 (standing observing, standing near a human, resting) showed some similarities but were  
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53  
54 285 distinct (on the left side of the graph Fig. 2a) and they clearly differed from active behaviour  
55  
56 286 postures (walking spontaneously, being led – on the right side of the graph Fig2a).  
57  
58 287 Exploratory walk posture, characterised by lower neck and wide head-neck angle, clearly  
59

288 differed from all the other postures. It is of interest that the horse's posture when being led by  
289 an experimenter differed from spontaneous locomotion (e.g. with a higher neck while walking  
290 spontaneously, Fig. 2a and b). Moreover, jaw-neck angles appeared narrower in spontaneous  
291 behaviour postures than when led by an experimenter.

292 Individual postural differences emerged for given behaviours (Fig.3), showing that this  
293 method could be used for analysing subtle individual postural variations. Nevertheless, each  
294 behavioural category could still be discriminated at the group level as inter-individual  
295 variability was lower than inter-behavioural variability (MANOVA,  $F_{60, 2452} = 18.48$ ,  $P <$   
296  $0.001$ ).

297 As the neck is the horses' most mobile area, a generalised Procrustes analysis was also  
298 conducted independently on back landmarks only (landmarks 5 to 8) to test for a so-called  
299 "Pinocchio effect" whereby one prominent feature can invalidate generalised Procrustes  
300 approaches and give misleading false positives. However, postures (without neck data,  
301 leading to less "biological" meaning about the horse posture, but necessary to control for a  
302 Pinocchio effect due to the prominent horses' neck mobility), could still be associated with  
303 different behaviours, both at the individual and the group levels (MANOVA,  $F_{60, 2452} = 25.10$ ,  
304  $P < 0.001$ ).

#### 306 *Impact of Environmental Factors on Postures*

307 Postures of horses kept under natural conditions clearly differed from postures of riding-  
308 school horses (MANOVA, standing motionless:  $F_{36, 570} = 22.51$ , walking:  $F_{36, 1150} = 45.40$ ,  $P$   
309  $< 0.001$  in both cases) even though the same behaviours were considered. Inter-group postural  
310 variations mainly occurred in terms of horses' neck and back roundness (Fig. 4a and b). Thus,  
311 horses kept under natural conditions held their necks higher and their backs were rounder than  
312 those of horses from riding schools. It is of interest that excluding horses kept under natural

313 conditions, postures could still be discriminated between riding schools (MANOVA, standing  
1 motionless:  $F_{24, 268} = 7.45$ , walking:  $F_{24, 540} = 11.69$ ,  $P < 0.001$  in both cases): horses from  
2 314 riding school B had on average straighter and flatter postures than horses from the other riding  
3  
4 315 schools (Fig. 4a and b).  
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## 11 318 **DISCUSSION**

12 319 Application of GM analysis to horse postures showed that this method can be used to  
13  
14 320 characterise behavioural categories for intra- and inter-individual comparisons, to evaluate the  
15  
16 321 impact of environmental factors and to compare individuals, groups or populations.  
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22 322 At the group level, postural variation occurred in respect of neck and back roundness that  
23  
24 323 were higher in horses kept under natural conditions than in horses from riding schools, both  
25  
26 324 for static and locomotor behaviours. Several factors could partly explain this inter-group  
27  
28 325 variation, such as breed, sex (although no sexual shape dimorphism was apparent in this  
29  
30 326 study) and living conditions (living in box / in pasture, socially isolated / in a group, regularly  
31  
32 327 exercised / not regularly exercised). We propose that exercise (*i.e.* riding) is likely to be a  
33  
34 328 variable of major interest. Repeated exercise is known to impact the physical state of the  
35  
36 329 horse, modifying its kinematics and muscular development (*e.g.* Ödberg & Bouissou 1999;  
37  
38 330 Biau & Barrey 2004; von Borstel et al. 2009), which is likely to influence the horses' posture.  
39  
40 331 In addition, incorrect riding techniques may be a potential source of back problems in horses  
41  
42 332 (*e.g.* Cauvin 1997, Lesimple et al. 2010). Manual examination of vertebral states, based on  
43  
44 333 bony and soft tissue manual palpation of localised regions of vertebral stiffness based on  
45  
46 334 spinal mobilisation and palpable areas of muscle hypertonicity (details in Lesimple et al.  
47  
48 335 2010; Fureix et al. 2010) had been carried out on the same sample of horses. This previous  
49  
50 336 examination showed that most riding school horses (73%) were severely affected by vertebral  
51  
52 337 problems, while only 27% of the horses could be considered either totally unaffected (15%) or  
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338 slightly affected (*i.e.* one slightly affected vertebra, 12%) (Fureix et al. 2010). Conversely,  
339 horses kept under natural conditions were exempt from such problems (Fureix et al.  
340 unpublished data). This suggests that vertebral problems may also be involved in inter-group  
341 postural variations. It has been reported that horses with back problems present gait anomalies  
342 when their whole back appears flat and rigid (Cauvin 1997; Faber et al. 2000). This  
343 hypothesis could also be supported by the fact that postures could still be discriminated  
344 between horses from different riding schools (with similar sex ratios and breeds). Indeed,  
345 horses from riding schools appear to differ in relation to the occurrence of vertebral problems  
346 (Lesimple et al. 2010) and health-related parameters (Fureix and Hausberger unpublished  
347 data). Accordingly, the horses from the riding school with the highest rates of vertebral and  
348 health-related problems also presented the straightest and flattest postures. In this exploratory  
349 study, statistical correlations between vertebral problems and postural data were not tested  
350 further due to the number of other variables (housing, feeding conditions, sex...etc.) which  
351 could explain inter-group postural variation in addition to the inter-group differences in  
352 vertebral states. However, our results here raise new questions about the potential impact of  
353 vertebral problems on postures, which are currently under investigation in a large-scale study  
354 (Seneque et al. in prep).

355 Beyond their application to horses, these promising results show that this method can be  
356 used to discriminate groups of animals, even though methodological improvements need be  
357 added before drawing conclusions concerning the impact of environmental factors (horses  
358 kept under natural conditions and coming from riding schools were not equally represented in  
359 this exploratory study) and individual characteristics (such as age, sex and breed) on postures.  
360 On-going studies are currently using this method on more balanced samples to address  
361 questions concerning welfare state and vertebral problem impact (Seneque et al. in prep),  
362 emotional level and impact of human positioning on horses' behaviour (Fureix et al. in prep).

363 Note that the experimenter was not allowed to pull on the rope when leading the horses, so  
1  
2 364 that she was not likely to influence posture by a direct action on the horse's head.  
3  
4 365 Nevertheless, one could address the question of the weight of the lead rope *per se*, which  
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6  
7 366 could partly explain the lower neck when horses are led compared to spontaneous walk, for  
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9 367 instance by fitting free-ranging horses with the same equipment. However even if the relative  
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11 368 impacts of human / lead rope presence *per se* on horses' postures remains to be investigated,  
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13 369 GM appears to be effective for detecting different body postures as a function of different  
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15 370 contexts.  
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19 371 Markers stuck on animals have been used in kinematic studies (*e.g.* horses: Licka and  
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21 372 Peham 1998, Faber et al. 2000, Haussler and Erb 2006, Peham and Schobesberger 2006,  
22  
23 373 Hobbs et al. 2010; ferrets: Kafkafi and Golani 1998). However, a major limitation of this  
24  
25 374 method is that animals must move in front of fixed cameras in a calibrated environment (*i.e.*  
26  
27 375 need for fixed "positions" defined beforehand in the environment to obtain coordinates to use  
28  
29 376 as a reference frame for measurements in the tracking program). Consequently, it is difficult  
30  
31 377 to study free-ranging or slightly constrained subjects, as the animal may move out of the  
32  
33 378 reference frame. This could be overcome in a small arena equipped with several cameras, but  
34  
35 379 prevents observation in a large space, such as the usual pasture for horses or other stock.  
36  
37 380 Insomuch as animals can be fitted before hand with landmarks (as in kinematic  
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39 381 investigations), the protocol used here, *i.e.* taking pictures orthogonally sideways, appears to  
40  
41 382 be easier to implement in the field, as it does not require a calibrated space, provided that  
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43 383 there are no visual obstacles between the photographer and the subject. Procrustes adjustment  
44  
45 384 corrects for moderate variations in camera/horse distance, as it allows comparisons of shapes  
46  
47 385 after filtering out effects of location in space, rotation and scale (Zelditch et al. 2004). Thus  
48  
49 386 the experimenter could take pictures from various distances (*e.g.* here  $10\text{m} \pm 1\text{m}$ ), so as not to  
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51 387 interfere with the animal's spontaneous behaviour (as long as the pictures were taken  
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388 orthogonally sideways, which is facilitated by the possibility for the experimenter to move  
389 freely from a calibrated environment). Thus this protocol allows free-roaming or slightly  
390 constrained subjects complete freedom of movement, a major advantage in studying posture  
391 in relation to spontaneous behaviours (outside the context of a human / animal interaction).

392 Beyond its application to horses, this approach adds an innovative way to standardise  
393 methods related to measuring and interpreting postures in relation to behaviour. This  
394 promising use of GM for ethology, and behavioural research in general, could open a broad  
395 field of investigation, adding a complementary tool, in fundamental ethology, physiology,  
396 behavioural ecology and evolutionary biology, involving inter-individual, inter-population or  
397 inter-specific comparisons in relation to context or internal state.

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503 **FIGURES CAPTIONS**

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2 504 **Fig 1** The eight landmarks. Landmarks were stuck onto the horse's right side and placed  
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5 505 in relation to skeletal or muscular cues on: the nasal bone under the eye, 2 cm in front of the  
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7 506 zygomatic process (landmark 1); the temporo-maxillary joint (2); the atlas (3); the trapezium  
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9 507 cervical ligament (4); the cervico-thoracic (5); the thoraco-lumbar (6) and the lumbo-sacral  
10  
11 508 (7) junctions and the first coccygeal vertebra (8). Photographs were taken perpendicularly  
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14 509 from the horse performing various behaviours, and data of landmark coordinates were  
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16 510 extracted from photographs using GM software and analysed by Generalized Procrustes  
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19 511 Analyses, allowing to describe and to analyse global body posture variation (*e.g.* as a function  
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21 512 of behaviour).

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26 514 **Fig 2** Postures in relation to behaviours at the individual level (here horse E2)

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29 515 **a)** Principal component analysis (Thin plate spline, TPS, relative warp analysis) based on  
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31 516 TPS shape parameters. Barycentres of the observed postures (letters, see b for the  
32  
33 517 representation of corresponding postures) and distribution values (showing the range of  
34  
35 518 variation between observed postures for a given behaviour, represented on the graph by a  
36  
37 519 circle around letters) are represented for each behaviour: **E** = exploratory walk, **O** =  
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39 520 observation, **R** = rest, **S** = standing motionless near an experimenter, **W** = sustained walk, **We**  
40  
41 521 = walk led by the experimenter. **b)** Corresponding postures as depicted by TPS deformation  
42  
43 522 grids, showing the mean horse's posture for a given behaviour. For instance, the first grid (at  
44  
45 523 the top) represents the horse's mean posture while standing observing (localised by the letter  
46  
47 524 **O** on the left of the Principal Component Analysis representation). Axis 1 explained 78.60%  
48  
49 525 of postural variation. Inter-behavioural postural variation mainly occurred in terms of horses'  
50  
51 526 neck height that varied from the highest when a horse was standing observing (**O**, grid at the  
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53 527 top of the list) to the lowest in exploratory walking (**E**, lowest grid).



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1  
2 **Fig 3.** Postures in relation to individual horses (here for the behaviour sustained walk)

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4  
5 530 Principal component analysis (Thin plate spline, TPS, relative warp analysis) based on  
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7 531 TPS shape parameters. Barycentres of the observed postures (E1, E2, E3...) and distribution  
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9 532 values (showing the range of variation between observed postures for a given behaviour,  
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11 533 represented on the graph by a circle around letters) are represented for each horse.  
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14 534 Corresponding postures as depicted by TPS deformation grids, showing the mean individual  
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16 535 horse's posture when performing sustained walk. Axis 1 explained 52% of postural variation.  
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19 536 Inter-individual postural variation mainly occurred in terms of horses' neck and croup height  
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22 537 in relation to the back position, which varied from the highest for the horse E2 (left side) to  
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24 538 the lowest for the horse H1 (right side). Thus individual postural differences emerged for  
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26 539 given behaviours, showing that this method could be used for analysing subtle individual  
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29 540 postural variations. However, note that each behavioural category could still be statistically  
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31 541 discriminated as inter-individual variability was lower than inter-behavioural variability.

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36 **Fig 4** Postures in relation to behaviours at the group level.

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39 544 Principal component analysis (Thin plate spline, TPS, relative warp analysis, axes 2 and  
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41 545 3) based on TPS shape parameters and corresponding postures (black lines) as depicted by  
42  
43 546 deformation grids. Barycentres of the observed postures (letters) and distribution values  
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45  
46 547 (showing the range of variation between observed postures for the behaviour, represented on  
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48  
49 548 the graph by a circle around letters) are represented for **A**: horses kept under natural  
50  
51 549 conditions, **B, C and D**: horses from riding school B, riding school C and riding school D.  
52  
53 550 Mean postures (representation extracted from TPS deformation grids) are represented for each  
54  
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56 551 population of horses (from A to D) while **a**) standing motionless near the experimenter and **b**)  
57  
58 552 walking led by the experimenter.

553 Axis 1 (not shown) explained respectively 50.80 % of the postural variation when horses  
1  
2 554 stood motionless and 49.70% when horses walked (variation occurred in neck height). For  
3  
4 555 both behaviours considered, the inter-group postural variation occurred mainly in horses'  
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7 556 neck height and back roundness: horses kept under natural conditions (A) had higher necks  
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9 557 and back roundness than horses from riding schools (B, C and D). Distribution along axis 3  
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11 558 revealed that horses' postures also differed among riding schools: horses from riding school B  
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13 559 had on average straighter and flatter posture than those from riding schools C and D.  
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561 **TABLE**

562

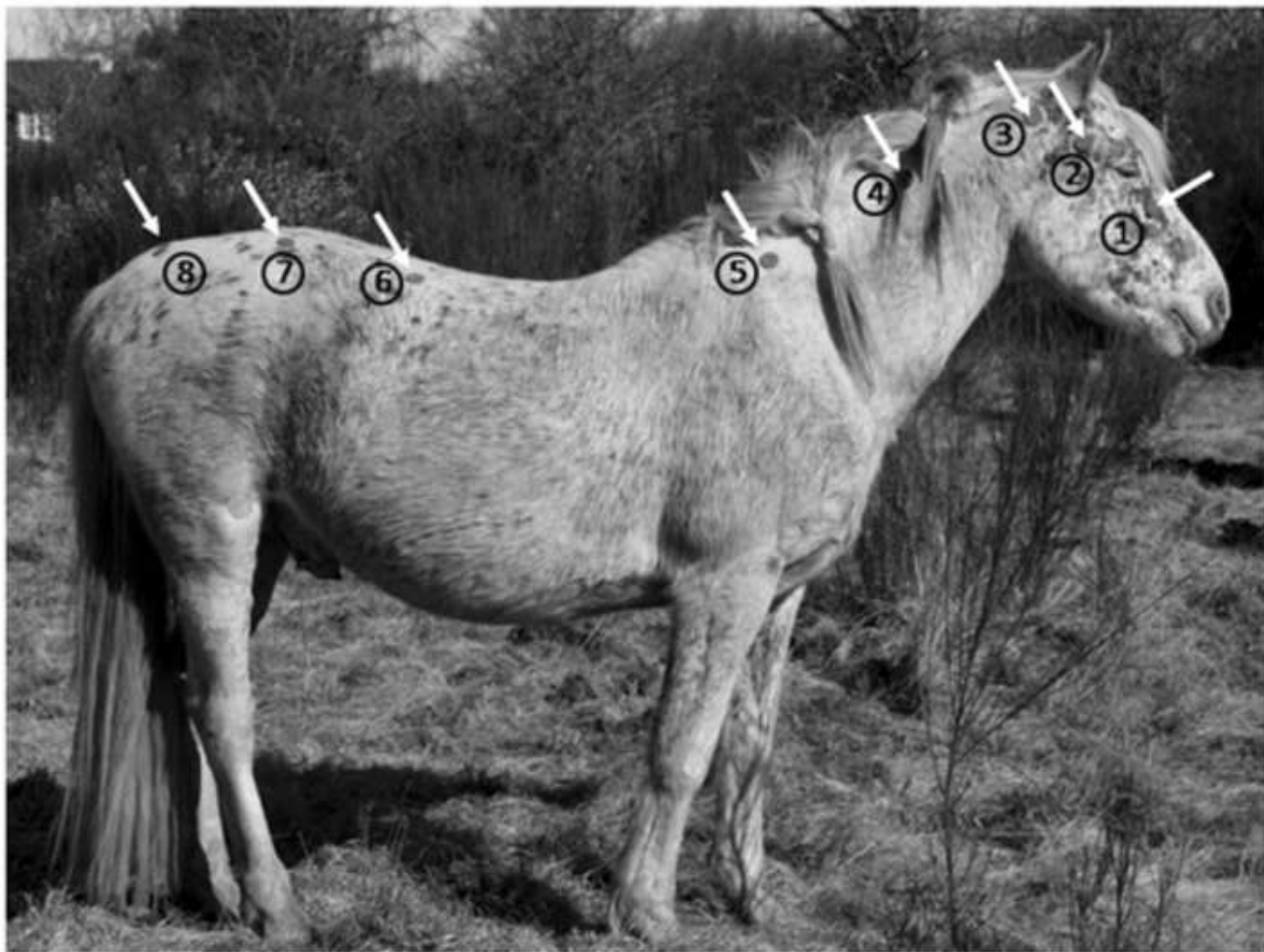
563 Table 1. Description of spontaneous activities known to be related to different arousal levels  
 564 (used here for assessing horses' postures according to behaviour, applying tools from  
 565 geometric morphometrics). Please note that these behaviours were only recorded for horses  
 566 kept under natural conditions (in stable social groups in pasture). Adapted from McDonnell  
 567 (2003)

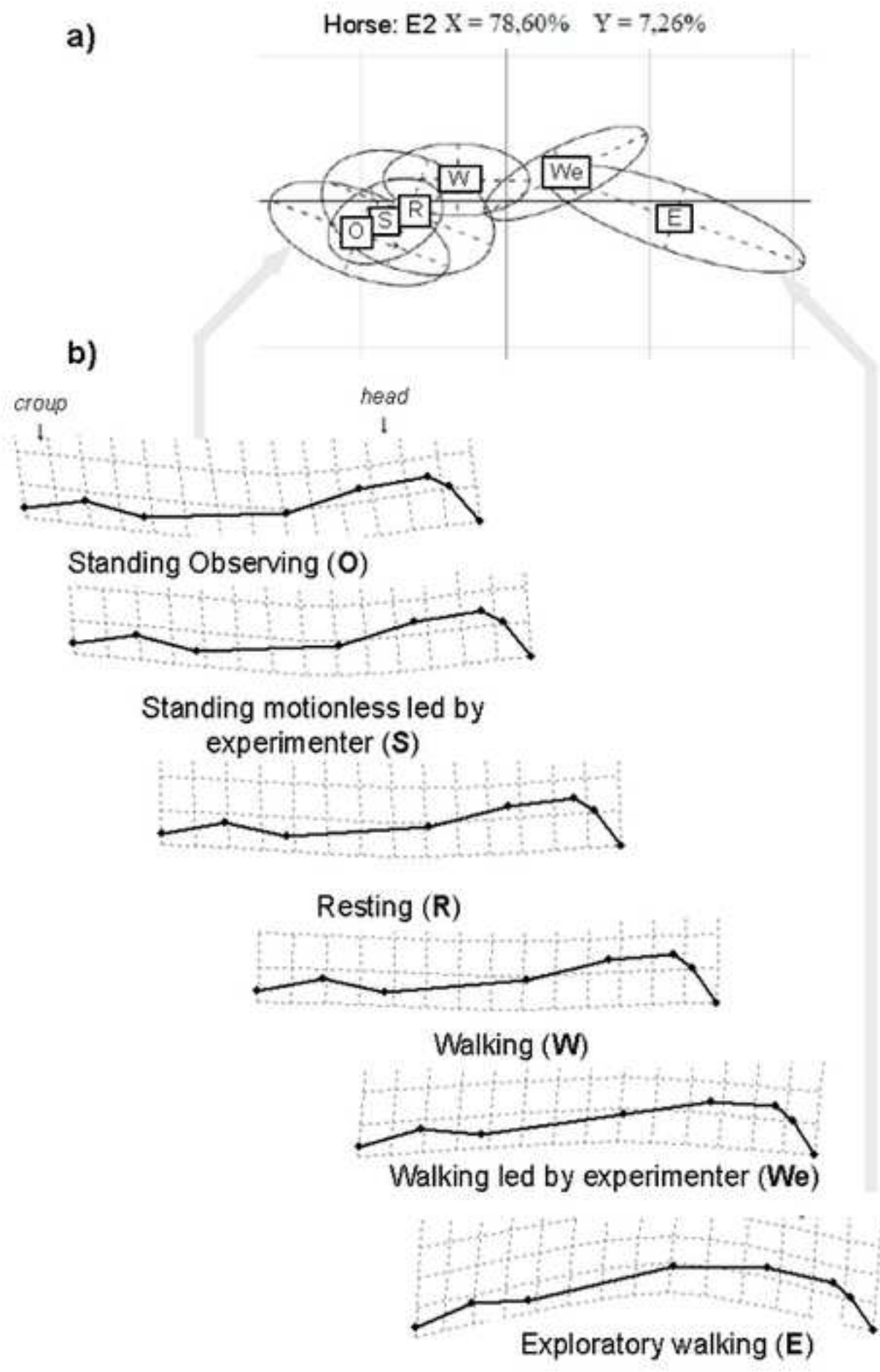
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Name	Description
Slow exploratory walk	The horse walks slowly with its neck horizontal or below the horizontal, ready to stop and sniff the ground or the wall. This is the characteristic slow walk of a quiet horse in a calm situation. There is no muscular tension.
Sustained walk	The horse walks energetically and looks forward or around.
Standing resting	When resting, the horse stands with its eyes at least partly closed. Its muscles relax and its lips can get droopy. The horse can be standing on only three legs.
Standing observing	The horse stands still, with head and ears oriented towards the object.

569

**Figure1**  
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**Figure3**  
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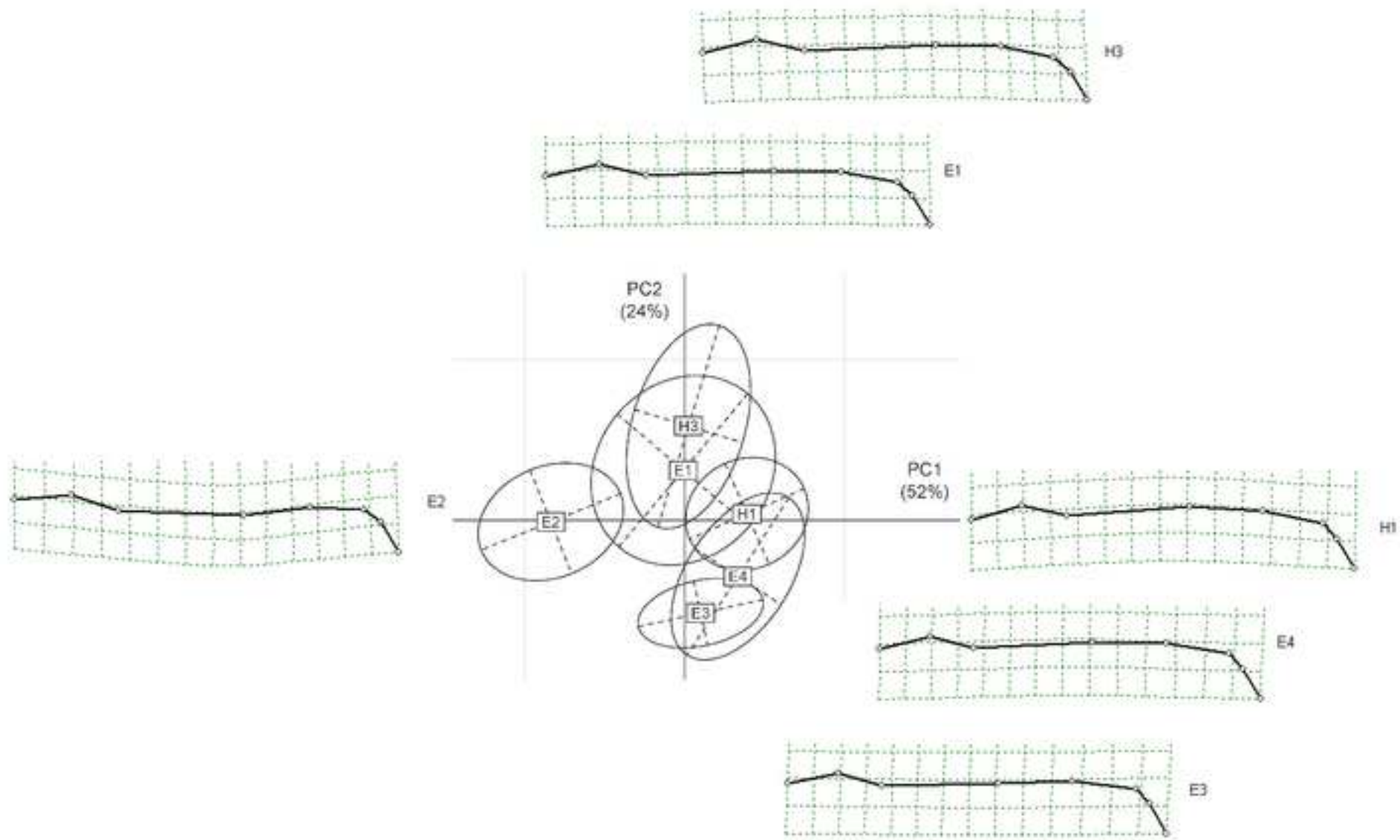
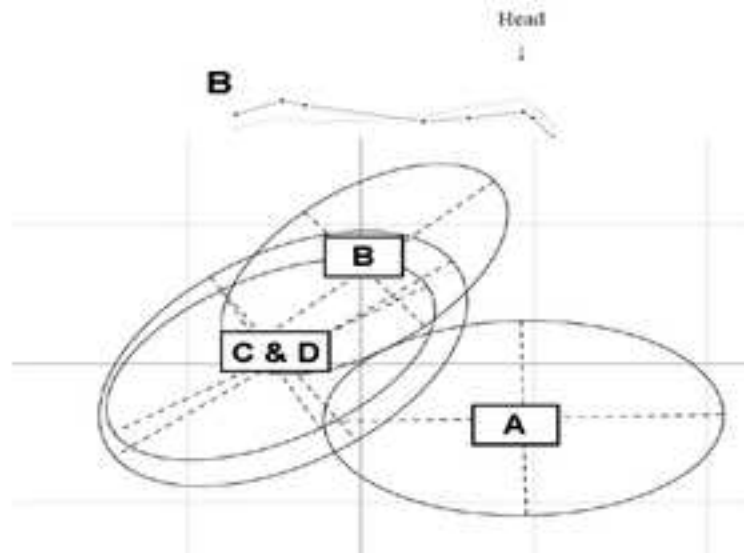
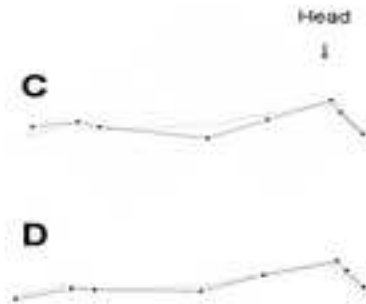


Figure4

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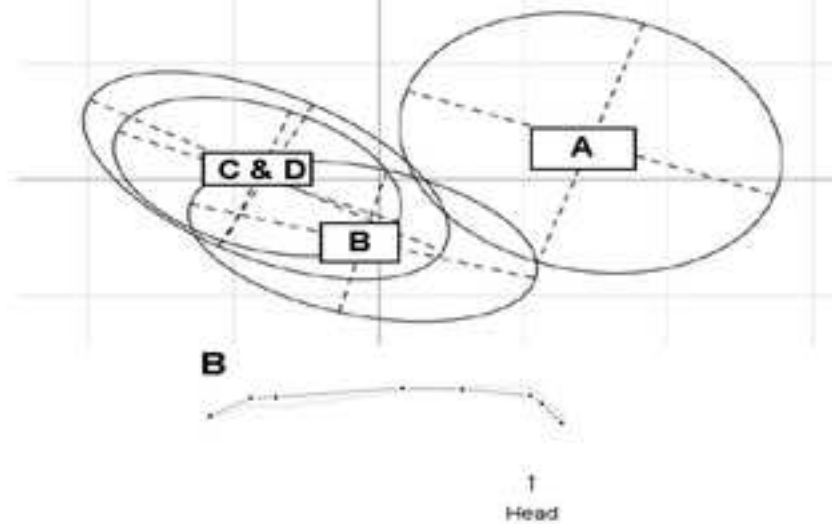
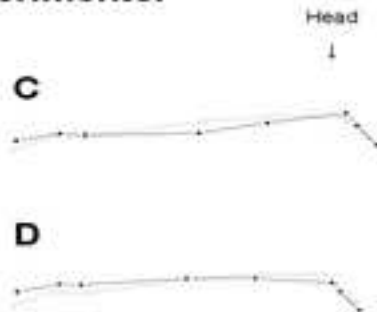
**(a) standing motionless near the experimenter**



X: 20%, Y: 9%



**(b) walking led by the experimenter**



X: 28%, Y: 8%

