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## Kinematic analysis of bimanual movements during food handling by head-fixed rats

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2

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6 **Running Head**

7 Kinematic analysis of bimanual movements in rats

8

9 **Author contributions**

10 M.I. and J.W. designed experiments. M.I. conducted experiments and drafted manuscript and figures.

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12

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17 **ABSTRACT**

18 Bimanual coordination – in which both hands work together to achieve a goal – is crucial for the  
19 basic needs of life, such as gathering and feeding. Such coordinated motor skill is highly developed  
20 in primates, where it has been most extensively studied. Rodents also exhibit remarkable dexterity  
21 and coordination of forelimbs during food handling and consumption. However, rodents have been  
22 less commonly used in the study of bimanual coordination because of limited quantitative measuring  
23 techniques. Here we describe a high-resolution tracking system that enables kinematic analysis of rat  
24 forelimb movement. The system is used to quantify forelimb movements bilaterally in head-fixed  
25 rats during food handling and consumption. Forelimb movements occurring naturally during feeding  
26 were encoded as continuous 3-D trajectories. The trajectories were then automatically segmented and  
27 analyzed, using a novel algorithm, according to the laterality of movement speed or the asymmetry  
28 of movement direction across the forelimbs. Bilateral forelimb movements were frequently observed  
29 during spontaneous food handling. Both symmetry and asymmetry in movement direction were  
30 frequently observed, with symmetric bilateral movements quantitatively more common. The  
31 proposed method overcomes a limitation in the precise quantification of bimanual coordination in  
32 rodents. This enables the use of powerful rodent-based research tools such as optogenetics and  
33 chemogenetics in the further investigation of neural mechanisms of bimanual coordination.

34

35 **Keywords**

36 bimanual coordination; head-fixed; motion capture technology; classification of behavior; kinematic  
37 analysis.

38 **New & Noteworthy**

39 We describe a new method for quantifying and classifying three-dimensional, bilateral forelimb  
40 trajectories in head-fixed rats. The method overcomes limits on quantifying bimanual coordination in  
41 rats. When applied to kinematic analysis of food handling behavior, continuous forelimb trajectories  
42 were automatically segmented and classified. Bilateral forelimb movements were observed more  
43 frequently than unilateral movements during spontaneous food handling. Both symmetry and  
44 asymmetry in movement direction were frequently observed. However, symmetric bilateral forelimb  
45 movements were more common.

46

## 47 INTRODUCTION

48 The ability to execute bimanual actions –involving the coordinated interplay of both limbs – is  
49 crucial not only for the most basic needs of daily life, such as gathering and feeding, but also for the  
50 heights of human creative achievement exhibited in art and music. Despite being computationally  
51 expensive, the ability to coordinate the limbs bilaterally has been advantageous and selected for in  
52 evolution. The neural mechanisms underlying bimanual movements have long been a focus of  
53 research in primates because of their significance for behavioral neuroscience, for the  
54 pathophysiology of movement disorders, and as a basis for rehabilitation or diagnosis (Ponsen et al.  
55 2006; Reinkensmeyer et al. 2016; Swinnen 2002; Swinnen and Wenderoth 2004; van Delden et al.  
56 2012; Wu et al. 2010). Many important advances toward understanding bimanual coordination have  
57 been made using human and non-human primates as the experimental subject (Swinnen 2002).  
58 Primates have many advantages because of their advanced capabilities and the availability of  
59 sophisticated analytical apparatus. However, primates are less well suited to invasive experimental  
60 manipulations, or the use of transgenic approaches to understand the neural mechanisms. In contrast,  
61 such manipulations are readily applied in rodent models, which are therefore advantageous for  
62 addressing neural mechanisms of the mammalian brain. Like primates, rodents also exhibit dexterous  
63 coordination of forelimbs to handle food objects when eating (Whishaw and Coles 1996). There is,  
64 therefore, much to be gained from further developing quantitative and qualitative measuring  
65 techniques suitable for use with rodent models in the study of bimanual coordination. The aim of the  
66 present investigation was to develop a method for quantifying bimanual movement in the rat.

67 Quantification of bimanual coordination during spontaneous food handling behavior has  
68 been reported in freely moving (Allred et al. 2008; Tennant et al. 2010; Whishaw and Coles 1996)  
69 and head-fixed rodents (Whishaw et al. 2017b). Evaluation of behavior in these reports has been  
70 based on investigator observation of action and postures of hands by off-line video analysis. In recent  
71 years, the emergence of kinematic analysis with 2-D lever and 3-D motion capture has enabled the  
72 documentation of qualitative measures such as tortuosity, oscillations, and variability in unimanual

73 motor control (Azim et al. 2014; Guo et al. 2015; Kawai et al. 2015; Palmér et al. 2012; Panigrahi et  
74 al. 2015). Implementation of these analytical techniques has furthered our fundamental  
75 understanding of rodent motor behavior. However, it is important to extend these methods to the  
76 problems of classification of bimanual movements during natural action sequences.

77         Here we report an imaging system for measuring bimanual coordination in rats. The system  
78 uses a pair of high-speed cameras to capture 3-D forelimb position during bimanual food handling.  
79 In the system, rats are head-fixed in order to provide a reference frame for recording. A semi-  
80 automated tracking program generates trajectories of forelimb position in animal egocentric 3-D  
81 space. Trajectories are transformed into kinematic parameters such as speed, velocity, or movement  
82 direction. To show potential uses of kinematic data obtained with the system, we demonstrate  
83 segmentation and mathematical analysis of rat forelimb movements to measure laterality of  
84 movement speed and asymmetry of movement direction during food handling. Finally, using a  
85 classification algorithm, we demonstrate high-throughput of large amount of kinematic data from  
86 multiple rats to quantify spontaneous food handling behavior.

87 **MATERIALS AND METHODS**

88 *Animals.* Ten- to twelve-week-old Male Long Evan rats weighing 350-450 g were kept under a  
89 reversed 12 hrs light/dark cycle (10:00 am to 10:00 pm), constant temperature (25°C) and humidity.  
90 Rats were housed with *ad libitum* access to water and food before weight restriction. Animals were  
91 habituated to the experimenter for more than three days before the start of behavioral recording. All  
92 experiments were approved by the Committee for Care and Use of Animals at the Okinawa Institute  
93 of Science and Technology.

94

95 *Surgery for head-fixation.* Carprofen (Rimadyl, Pfizer, 50 mg ml<sup>-1</sup>, sc) was administered  
96 immediately before surgery. Rats were anesthetized with isoflurane (3 - 4% induction, 1.5 - 2.5 % for  
97 maintenance), and placed on a stereotaxic frame for chronic experiments (SR-10R-HT, Narishige,  
98 Japan). Body temperature was monitored and maintained at 36.5 - 37.5°C with a heating pad. The  
99 skull was exposed and carefully cleaned with saline and cotton swabs. Super-Bond Green Activator  
100 (Sun Medical Inc., Japan) was judiciously applied to the skull, left for 20 sec, and then removed by  
101 saline. After the surface preparation, eight anchor screws (M 1 × 2) were drilled into the skull. The  
102 screws were then covered with a layer of dental cement (Super-Bond, Sun Medical Inc., Japan). A  
103 chamber frame (CFR-1, Narishige, Japan) was positioned above the skull and secured by additional  
104 layers of dental cement. Antibiotic was intraperitoneally administered after the surgery. A dietary  
105 supplement with Carprofen (Medigel CPF; Clear H<sub>2</sub>O, ME., US.) was given during post-op recovery  
106 for 5 days.

107

108 *Behavioral apparatus and recording setup.* A custom made stereotaxic frame for chronic  
109 experiments (SR-10R-HT, Narishige, Japan) was used for head-fixed behavioral experiments. A 3-D  
110 printed passive linear treadmill (80 mm wide and 130 mm long; Fig. 1A) was used to minimize  
111 animals' stress by allowing hindlimb movement. The treadmill was placed above a transparent  
112 acrylic base plate, and two high-speed cameras (HAS-L1, f = 6mm, DITECT, Japan) with infrared

113 LEDs were positioned 45 cm below the base to monitor forelimbs (Fig. 1 B and C). The two cameras  
114 were placed 130 mm apart and directed at an angle of 30° to each other. The accuracy of depth  
115 reconstruction was confirmed by using the MATLAB Stereo Camera Calibrator application. The  
116 mean reprojection error (the mean distance between the detected marker position and the reprojected  
117 points in the reconstructed model space) was 0.77 pixels (Hartley and Zisserman 2003).

118

119 *Habituation to head-fixation and pre-training.* Rats were food restricted prior to behavioral  
120 training. Body weight was maintained between 80 % and 90 % of the original weight. Animals were  
121 then habituated to the head-fixed apparatus. Habituation was based on the procedures previously  
122 reported (Isomura et al. 2009; Ollerenshaw et al. 2012; Schwarz et al. 2010), but modified for food  
123 restriction. Briefly, rats underwent the following steps: (1) Rats were placed in the behavioral  
124 chamber with *ad libitum* access to food for 20 min for 2 days. (2) The experimenter guided the rats  
125 into the half-cylindrical tunnel by providing a sweet jelly reward (Purin mix, House foods, Japan)  
126 using a stainless reward spout connected to a 50 mL syringe. The experimenter controlled the  
127 position of reward spout to induce animals to slide the chamber frame into the head attachment  
128 clamp. 10 – 20 ml of reward was provided in a day. (3) The experimenter held the rat's chamber with  
129 gentle force while providing reward. Initially, some rats tried to escape, and it took 2 - 3 attempts for  
130 the rats to retrieve 10 - 20 ml of sweet jelly reward. (4) Pre-training. Immediately after head-fixation,  
131 the experimenter gave a food reward cut into an annular shape (20 mm outer diameter, 10 mm inner  
132 diameter, 5 mm thickness, Fish Sausage, Marudai Food Co., Ltd, Japan) instead of the jelly reward.  
133 The pre-training continued until rats could retrieve, without dropping, five rewards for three  
134 consecutive days.

135

136 *Behavioral task and recording.* The reflective markers were handmade by covering a 3 mm  
137 diameter plastic half sphere with reflective tape (DITECT, Japan). On the day of the behavioral task,  
138 the experimenter gently held the forelimbs while the half-spherical markers were attached to the



139 lower side of the wrists using double-sided tape. The marker could be removed easily after  
140 behavioral testing. Rats did not try to remove the marker during the behavioral task. Trials started  
141 with bimanual grasping of food offered by the experimenter (Fig. 1E), and ended when the last piece  
142 of food was brought to the mouth. Rats underwent 16 - 21 trials in three days (5 - 7 trials per a day).  
143 Cases where rats showed unusual behavior, such as crossing two forelimbs or adopting a tripod  
144 stance during eating, were excluded from further analysis. In the present study, only two cases out of  
145 79 recorded session across five rats were excluded. All trials were recorded at 200 frames per second  
146 (1/500s exposure time and 600x800 pixel) and stored to hard disk.

147

148 *High-speed cameras and 3-D reconstruction.* The positions of reflective markers were traced  
149 using an in-house program assembled from a MATLAB toolbox (Computer Vision System Toolbox  
150 release 2016b, The MathWorks, Inc., MA., USA). Tracking was automatic except for adjustments to  
151 tracking parameters such as threshold which were required in response to the changes in reflection  
152 caused by marker angle. The program produced  $x$  and  $y$  coordinates of the marker position from 2-D  
153 video frames of Camera #1 and Camera #2. Depth reconstruction of the marker point was estimated  
154 by triangulation of the paired points on the 2D plane from Camera #1 and Camera #2 and the camera  
155 geometries. The resulting 3-D positions of the reflective marker in the camera coordinate system  
156 were represented as a time series data  $(x_t, y_t, z_t)$ . Depth reconstruction of marker position and  
157 calibration of camera position were conducted using the stereo camera calibrator package of the  
158 *MATLAB Computer Vision System Toolbox*. The reference frame defining the egocentric coordinate  
159 axes was included in the field of view of the cameras (Fig. 2A, B). Using this reference, the 3-D  
160 position data  $(x_t, y_t, z_t)$  was transformed into the egocentric coordinate system  $(lr_t, ap_t, dv_t)$  which  
161 represented time series data of forelimb position in left-right (lr) axis, anterior-posterior (ap) axis,  
162 and dorsal-ventral (dv) axis (Fig. 2 C). All data were filtered through a 20-Hz low-pass finite impulse  
163 response filter.

164

165 *Discretization of time series data.* Continuous position data were discretized into 50 ms  
 166 duration segments,  $s_t$  (Equation 1), with 5 ms shifts (Fig. 2D, E). The segment  $s_t$  was defined as the  
 167 array of 3-D position data of right and left forelimbs in a 50 ms time window.

168

$$s_t = \begin{bmatrix} lr_t & \cdots & lr_{t+50ms} \\ ap_t & \cdots & ap_{t+50ms} \\ dv_t & \cdots & dv_{t+50ms} \end{bmatrix} \#(1)$$

169

170 All segments of the right and left forelimb,  $s_{Rt}$  and  $s_{Lt}$ , were stored as the set  $S \ni \begin{bmatrix} s_{Rt} \\ s_{Lt} \end{bmatrix}_{1,2,\dots,N}$ ,  
 171 where  $s_{Rt}$  and  $s_{Lt}$  are vertically stacked. The sets,  $S$ , from different trials were horizontally  
 172 concatenated. Therefore, the total number of segments  $N$  depend on the time of each trial and  
 173 number of trials used for analysis. All segments in the set  $S$  were evaluated by classification scoring  
 174 methods.

175

176 *Qualitative measure of forelimb movements and classification.* All segments of behavioral data  
 177 were analyzed by the following three steps (Fig. 3):

178

179 (1) Extraction of moving segments. We first defined the maximum speed function  $\max(\bar{V}_R, \bar{V}_L)$ ,  
 180 where  $\bar{V}_R$  and  $\bar{V}_L$  are mean speed in a segment  $s_R$  and  $s_L$ . The function returns the value of  
 181 maximum speed among right or left forelimb in a segment. We defined the moving segment as  
 182  $\max(\bar{V}_R, \bar{V}_L) \geq 40$  ( $mm/sec$ ). That is, if mean speed of either left or right forelimb exceeded a  
 183 threshold, the segment was classified as a moving segment. Conversely, resting segments were  
 184 defined as  $\max(\bar{V}_R, \bar{V}_L) < 40$  ( $mm/sec$ ). The all moving segments were then analyzed by  
 185 following two metrics: (2) Speed Ratio, and (3) Asymmetry index.

186

187 (2) Speed Ratio Function. Bilateral forelimb movement was considered to occur when

188 movement amplitude across limbs remained uniform within a set limit; conversely, unilateral  
189 forelimb movement was considered to occur when there was significantly biased movement  
190 amplitude across limbs. To formalize the definition of bilateral and unilateral forelimb  
191 movement, the speed ratio of forelimbs was used (Equation 2).

192

$$SpeedRatio = \frac{\min\{\bar{V}_R, \bar{V}_L\}}{\max\{\bar{V}_R, \bar{V}_L\}} \#(2)$$

193

194 The *SpeedRatio* function is a measure of laterality of speed across both forelimbs, where 1  
195 indicates equal movement amplitude across the two forelimbs.

196 Movements were classified as bilateral or unilateral. A criterion of  $SpeedRatio \geq 0.5$  was  
197 used to isolate bilateral movements across two forelimbs, indicating when one forelimb was  
198 moving at no more than twice the speed of the other forelimb. Conversely,  $SpeedRatio < 0.5$   
199 was used to define unilateral movements, indicating when one forelimb moved at least twice as  
200 fast as the other forelimb in that segment. The boundary value was set to the half-maximum of  
201 *SpeedRatio* which is 0.5.

202

203 (3) Asymmetry index. Symmetric movements are also called mirror movements in cases where  
204 one limb moves as a mirrored copy of the contralateral limb. Physiologically, symmetry implies  
205 synchronized activation of homologous muscle groups, and asymmetry implies activation of  
206 non-homologous muscle groups. This definition is embedded in the egocentric framework  
207 discussed by Swinnen et al. (1998, 2001), in which movement is related to the longitudinal axis  
208 of the body and the coordination of corresponding limbs. The alternative, allocentric framework  
209 was not used in the present study because the limb movements were referenced to the body  
210 rather than the surrounding space. We defined symmetric movements as movement in the similar  
211 movement direction by both forelimb. Conversely, asymmetry index  $\theta$  (Equation 3), is the angle

212 between movement direction of the velocity vector of the left forelimb  $v_L$  (Equation 4) and the  
213 mirror transformed velocity vector of right forelimb  $v_{R_M}$  (Equation 5), estimated by the inverse  
214 cosine similarity function.

215

$$\theta = \cos^{-1} \left( \frac{v_{R_M} \cdot v_L}{|v_{R_M}| |v_L|} \right) \#(3)$$

216

$$v_L = \left( \frac{\Delta l r_L}{\Delta t}, \frac{\Delta a p_L}{\Delta t}, \frac{\Delta d v_L}{\Delta t} \right) \#(4)$$

217

$$v_{R_M} = \left( -\frac{\Delta l r_R}{\Delta t}, \frac{\Delta a p_R}{\Delta t}, \frac{\Delta d v_R}{\Delta t} \right) \#(5)$$

218

219 The mean asymmetry index of a segment  $\bar{\theta}$  was calculated from the mean of  $\theta$  in a 50 ms  
220 time window.

221 With the estimated  $\bar{\theta}$ , symmetric and asymmetric movements were classified. A segment was  
222 defined as symmetric movement if  $\bar{\theta} < \pi/4$  where the angle of movement direction of the left  
223 forelimb and mirrored right forelimb remained less than 45 degrees. Conversely, a segment was  
224 defined as asymmetric movement if  $\bar{\theta} \geq \pi/4$ , indicating that the angle between the two velocity  
225 vectors was greater than or equal to 45 degrees (Fig.3). The boundary value was based on the  
226 previous literature in which orthogonal lever press of two hands was defined as asymmetric  
227 bimanual movement (Cardoso de Oliveira et al. 2001). The present study used the value  $\pi/4$   
228 which is intermediate between perfect symmetric movement (0 degrees) and orthogonal  
229 asymmetric movements (90 degrees).

230

231

232 **RESULTS**

233 *Behavior in the apparatus during training.* After habituation to head-fixation, all rats spontaneously  
234 entered the treadmill. Some rats were able to slide the chamber frame into the head attachment clamp  
235 without the experimenter's guidance. All rats were able to perform food handling while in the head-  
236 fixed position. The rats rarely dropped food during bimanual food handling, showing a mean success  
237 rate (food consumption without dropping) of  $97.89 \pm 2.90\%$ , and a mean consumption time of  $27.92$   
238  $\pm 2.77$  sec per trial ( $n = 5$ ). Overall, head-fixation did not impede spontaneous food manipulation.

239

240 During food consumption, rats made periodic transitions between resting and moving. In the  
241 resting state, rats held the food item in a low position, and chewed on it. During movement, rats  
242 brought the food item to a higher position and dynamically manipulated it, changing the holding  
243 position and rotating the object. Frequently observed behaviors are shown in single video frames in  
244 Fig. 4A-D. Some of these behaviors have been reported by Whishaw and colleagues (Whishaw and  
245 Coles 1996; Whishaw et al. 2017b). Rats exhibited bimanual downward and upward reaching  
246 behaviors at different times. On first exposure to the food item, reaching down, grasping, and upward  
247 movement of the forelimbs occurred to bring the food item toward and against the mouth. These  
248 movements often punctuated the transition between resting and active states. In some cases, rats used  
249 the downward reaching behaviour to break the food item by tearing with the teeth. The bimanual and  
250 unimanual displacements involved in releasing and holding the food item were usually seen when  
251 rats changed their grasping position (Fig. 4C, D).

252

253 To interpret the 3-D trajectories of typical forelimb actions, we compared the video frames of  
254 representative manually identified behaviors with the corresponding 3-D scatter plots of wrist  
255 position marker coordinates (Fig. 4, right column). We found that the sequence of wrist positions as  
256 represented in the scatter plots clearly illustrated bimanual and unimanual behaviors. For example,  
257 upward bimanual reach was evident in the sequence of points indicating the position of each wrist as

258 they shifted towards the ventral side (Fig. 4A). In unimanual movements the separation between the  
259 points corresponding to the moving wrist indicated larger displacements, contrasting with the closely  
260 spaced points corresponding to the other, relatively stationary forelimb (Fig. 4D). These observations  
261 illustrate the potential of analyzing the transition of wrist positions for quantifying several types of  
262 active forelimb states.

263

264 *Full 3-D reconstruction of position of wrists.* To provide a basic data set of the entire action  
265 sequence of an eating behavior, time series data of wrist positions in egocentric coordinates were  
266 generated from 77 trials across 5 rats. Representative trajectories of both wrists in the egocentric  
267 coordinate space are shown in Fig. 5. On the ventral side, forelimbs followed an arc-shaped  
268 relatively convergent trajectory, whereas on the dorsal side the trajectories diverged and became  
269 intermingled. The intermingled structure suggests that food manipulation consists of highly variable  
270 action patterns, such as symmetric/asymmetric bimanual movements and unimanual movements. To  
271 categorize forelimb use during continuous action sequences, we segmented the continuous time  
272 series data of wrist positions using a 50 ms sliding time window (Fig. 2D, E). The segments were  
273 generated from all data sets across 5 rats, and subjected to analysis in order to score and classify  
274 them into subtypes of unimanual and bimanual movements.

275

276 *Extraction of Moving segments.* We considered movement to be occurring whenever the speed of  
277 one limb exceeded a threshold of 40 mm/sec (Fig. 6A-E). This non-zero criterion for “movement”  
278 was chosen because, even in the resting state, some physiological activity such as chewing,  
279 breathing, or sniffing, causes jittering of the forelimb position. The probability distribution of  
280 forelimbs speed showed a natural dip around 40mm/sec (Fig. 6A). The natural dip was also seen in  
281 the probability distribution of the processed speed variable which is maximum speed function (Fig.  
282 6B). Thus, the thresholding process removed physiological movement artefacts. According to this  
283 criterion the portion of time spent moving was  $0.29 \pm 0.05$  (Fig. 6C,  $n = 5$ ).

284

285         *Bilateral movements vs unilateral movements.* The extracted moving segments described above  
286 included both bilateral and unilateral forelimb movements. We analyzed these movements, based on  
287 the laterality of movement speed across two forelimbs by applying the speed ratio function (Fig. 7).  
288 The speed ratio was defined as the ratio of mean speeds between two forelimbs in a segment, based  
289 on the idea that both forelimbs move at similar amplitude for bilateral movements, while one  
290 forelimb moves faster than the other in unilateral movements (Fig. 7A-B). We found that the mean  
291 probability distribution of the speed ratio was biased towards 1, suggesting that the majority of  
292 forelimb movements during food handling were bilateral; conversely, unilateral forelimb movements  
293 were less frequent (Fig. 7B). Some representative segments of bilateral or unilateral forelimb  
294 movements based on the boundary value of 0.5 are shown in Fig. 7C, D.

295

296         *Symmetric vs asymmetric movement.* One of the main purposes of the kinematic analysis of  
297 forelimb movements is to determine the relative amounts of symmetric and asymmetric movement  
298 during the natural sequence of food handling behavior (Fig 8A-D). Symmetric bimanual movements  
299 are a subset of bimanual movements generated by the activation of homologous muscle groups  
300 across two limbs. Conversely, an asymmetric bimanual movement is caused by different (non-  
301 homologous) muscle groups. In the present study, symmetric bimanual movements were defined as  
302 those in which the movement direction of a forelimb mirrors the other forelimb with respect to the  
303 sagittal plane of the body (Fig. 8A). The asymmetry index  $\theta$  is found by subtracting the angle of  
304 movement direction of a forelimb from the mirrored angle of the contralateral forelimb movement.

305

306         We assigned the asymmetry index  $\theta$  to each segment, and calculated the probability density  
307 function of the asymmetry index. The probability distribution was significantly biased in the less  
308 asymmetric direction suggesting that the symmetric state predominates (Fig. 8B). Representative  
309 symmetric or asymmetric movements based on the boundary value of  $\pi/4$  are shown in Fig. 8C, D.

310

311 *High-throughput analysis of kinematic data for quantification of forelimb movements.* Finally,  
312 many (more than 430,000) segments from 77 trials across 5 rats were subjected to automatic analysis  
313 and classification. The speed ratio and asymmetry index of all moving segments were measured (Fig.  
314 9). The classification algorithm (Fig. 3) was applied to all segments to illustrate the time series of the  
315 following motor behaviors: bilateral movement, unilateral movement, symmetric movement, or  
316 asymmetric movement. The time series data of those categories of motor behavior revealed the  
317 frequent transition of movement mode during feeding behavior (Fig.9A and B). The transition of  
318 movement mode was visualized by overlaying the colored movement categories on the continuous  
319 3D trajectories of forelimb position (Fig. 9C). The time fraction of unilateral versus bilateral and  
320 symmetric versus asymmetric movements were quantified. Relative frequency of each mode of  
321 forelimb movements revealed the organization of bimanual motor behavior during the natural  
322 sequence of eating (Fig. 9D). The mean percentages of forelimb use in respect of movement  
323 amplitude were 89% of bilateral movements and 11% of unilateral movements. The mean  
324 percentages of forelimb use in respect of movement direction were 41% of asymmetric movement  
325 and 59% of symmetric movement.



326 **DISCUSSION**

327 We report on the development of a high-resolution tracking system for kinematic analysis of rat  
328 forelimb movements and its application to the study of bimanual coordination. The system uses  
329 optical motion tracking to obtain 3-D bimanual wrist movement trajectories from natural action  
330 sequences. The 3-D trajectories were used in the kinematic analysis of coordination of forelimb  
331 movements in head-fixed rats during food handling and consumption. Movement laterality and  
332 asymmetry across forelimbs was quantified in movement segments automatically extracted from the  
333 continuous action sequence. Results showed that the speed of forelimb movement during eating  
334 behavior were highly balanced bilaterally. Symmetry in movement direction was more frequently  
335 observed than asymmetry. However, a considerable amount of asymmetry in movement direction  
336 was also observed. To our knowledge, this is the first application of this method to visualizing  
337 bilateral forelimb trajectories during spontaneous food handling behavior in rodents, extending  
338 previous studies of food handling behavior.

339

340 Limb use in spontaneous food handling behavior was first reported as a method of motor  
341 assessment in rats by Whishaw and Coles (1996). Since then, this quantitative method has been  
342 widely used to assess motor function in research on movement disorders and motor control (Allred et  
343 al. 2008; Brown and Teskey 2014; Manfré et al. 2017; Tennant et al. 2010; Wang et al. 2017; Xu et  
344 al. 2009). In these earlier studies, assessment of forelimb motor skills relied on manual identification.  
345 Grasping pattern, position of forelimb, timing of adjustment as well as global scores such as  
346 consumption time and drop rate required manual observation of video frames. The present study  
347 extends this method of assessment of motor skill in food handling behavior by using a kinematic  
348 tracking system. The sub-second kinematic information obtained from this system enables detection  
349 of subtle changes in behavior, such as changes in the ratio of symmetric to asymmetric activity  
350 during bimanual movement. Such measurements are difficult to obtain by manual observation of  
351 video frames.

352

353           Quantifying the incidence of specific motor patterns during natural action sequences is  
354 challenging. To make quantitative analysis feasible, the study of motor control often focuses on  
355 trained, repetitive, uniform action sequences such as skilled reaching, and lever pressing tasks (Guo  
356 et al. 2015; Hira et al. 2013; Isomura et al. 2009; Kawai et al. 2015; Palmér et al. 2012). Measures of  
357 such motor patterns are low dimensional, not requiring extensive data processing, unlike more  
358 natural sequences. In contrast to these more uniform action sequences, food handling behavior  
359 involves highly variable action sequences of forelimb movements. While the analysis of such  
360 sequences is more demanding, they provide good examples of naturally occurring bimanual  
361 coordination.

362

363           To quantify natural action sequences, it is necessary to identify specific behaviors when they  
364 occur. Segmentation with a sliding window, as used in the present study, is commonly used to detect  
365 behavioral events in time series data sets such as moving pictures and multi-point body kinematic  
366 information, in human as well as animal behavior (Burgos-Artizzu et al. 2012; Liu et al. 2009). Once  
367 the analytical criteria for a specific behavior – the “*detector*” – has been defined, the behavior can be  
368 identified in the continuous sequence dataset. Our mathematical definitions of bimanual movements  
369 were used to detect bilateral versus unilateral, and symmetric versus asymmetric forelimb  
370 movements within the natural action sequence data (Fig.9).

371

372           In the present study, we demonstrated the use of a movement asymmetry index and speed  
373 ratio for quantifying asymmetry of movement direction and laterality of movement speed. A  
374 limitation of this strategy is that each class of movement includes actions which may be mediated by  
375 different neuromotor channels (Whishaw et al. 2017a). For example, movements classified as  
376 symmetric bilateral forelimb movements in the present study involve movement symmetry with  
377 bimanual holding (Fig.4A) and bimanual release (Fig.4C). Specific motor behaviors such as

378 movement of hand to mouth, or reaching, may require a distinct movement detection algorithm. For  
379 instance, the distance between mouth position and forelimb position could be useful in defining hand  
380 to mouth movements. Another limitation is that the present movement detection has the threshold of  
381 40mm/sec. The threshold would not permit the detection of slow bilateral forelimb movements such  
382 as the moment during transition from resting to upward reach. To study, in particular, slow upward  
383 motion arising from rest, another definition of movement onset based on position, such departure  
384 from a delineated area defined as resting position, might be useful. Recently, dimensionality  
385 reduction algorithms and machine learning approaches have captured action repertoires from natural  
386 action sequences (Berman et al. 2014; Robie et al. 2017). Further development is needed for  
387 exploring bimanual action patterns from bimanual food handling behavior.

388

389 The proposed mathematical definitions of symmetric and asymmetric bimanual movement  
390 were based on movements of the forelimb markers. Physiologically, however, symmetric versus  
391 asymmetric bimanual movements are distinguished by the pattern of activated muscle groups across  
392 limbs. For instance, simultaneous activation of homologous muscle groups generates symmetric  
393 bimanual movements. In contrast, activating different muscle groups with the same timing causes  
394 asymmetric bimanual movements. The present definition of the asymmetry, index  $\theta$ , is the  
395 directional error between movement vectors of forelimbs calculated by the cosine similarity function.  
396 It is based on the idea that the activation of identical muscle groups across forelimbs results in  
397 mirror-image endpoint trajectories. This implicitly assumes that movement and muscle activity are  
398 measures of the same thing. However, it should be noted that significant physical perturbations may  
399 occur and cause, in response, changed muscle activation patterns even though the trajectory of the  
400 forelimb marker is unchanged. Thus, in the proposed system, perturbations such as bumping a part of  
401 the head-fixing device, should be excluded from the analysis.

402

403 Using the proposed system in the present study led to the finding that both asymmetric and

404 symmetric bilateral movements occur in food handling behavior, with symmetric bilateral forelimb  
405 movements quantitatively more commonly observed. In rodents, previous studies of forelimb use  
406 have observed laterality in grasping (a release to regrasp movements against food object) and holding  
407 position asymmetry in food handling (Allred et al. 2008; Whishaw and Coles 1996; Whishaw et al.  
408 2017b). The present study further extended the previous results by adding that asymmetry was  
409 observed in dynamic bilateral forelimb use in rats. Our results suggest that symmetric bilateral  
410 forelimb movements were more frequently observed than asymmetric bilateral forelimb movements  
411 during handling of donut-shaped food reward. The shape of the food may have been a factor in the  
412 symmetry of the hand to mouth movements in feeding, functionally linking the forelimbs together  
413 when they were used to bring the food item to the mouth (Fig. 4A). Another possible interpretation is  
414 that the animal has natural tendency towards symmetric movements, which has been reported in  
415 various experimental conditions in humans (Swinnen 2002). Symmetric forelimb movements might  
416 be a fundamental mode of bilateral forelimb movements in rats, however, this idea needed to be  
417 investigated further.

418

419         The use of awake head-fixed rats under food restriction is less frequently reported than their  
420 use with water restriction, with the most recent report more than 10 years ago (Heck et al. 2007).  
421 Technical aspects of shaping behaviour by food reward may be a factor in the less frequent use of  
422 food restriction. The training of head-fixation in rats used graded exposure methods (Schwarz et al.  
423 2010), based on compensating restraint anxiety with reward. In many experimental paradigms, water  
424 reward is easy to provide with a spout while animals remain restrained. In contrast, providing solid  
425 food items prompts rats to return from the restrainer by backward locomotion, making it difficult for  
426 the animal to associate the reward and environment. We, therefore, delivered jelly reward via  
427 stainless spout to guide rats to the head-fixed position, instead of providing pieces of solid food. In  
428 addition, a linear passive treadmill that we implemented significantly buffered backward locomotion  
429 reducing restraint stress on the animal. The two approaches synergistically improved training

430 efficiency.

431

432 Bimanual coordination deficits are observed in neurodegenerative disorders such as  
433 Parkinson's disease (Almeida et al. 2002; Johnson et al. 1998; Vercruysse et al. 2014), Huntington's  
434 disease (Brown et al. 1993; Johnson et al. 2000; Verbessem et al. 2002), Alzheimer's disease (Martin  
435 et al. 2017), and traumatic brain injury (Caeyenberghs et al. 2011; Gooijers et al. 2016). Parkinsonian  
436 patients have difficulty in asymmetric bimanual coordination (Almeida et al. 2002; Ponsen et al.  
437 2006; Stelmach and Worringham 1988). Recent evidence suggests that recovery from hypokinesia in  
438 Parkinson's disease is not necessarily correlated with improvement in coordinated bimanual  
439 movements (Almeida and Brown 2013; Daneault et al. 2016; Igarashi et al. 2015). The decline in  
440 bimanual motor performance is also seen in healthy aging (Serbruyns et al. 2015). The unique  
441 mechanisms of bimanually coordinated movement need to be further studied to advance  
442 understanding of physiological mechanisms of neurodegenerative disorders and aging. We suggest  
443 that the presented measurement will illuminate bimanual coordination as a target of investigation by  
444 new – but almost exclusively rodent-based – research tools such as optogenetics, chemogenetics, *in*  
445 *vivo* electrophysiology, and multi-photon imaging.

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449

450 **GRANTS**

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452 **FIGURE LEGENDS**

453 Fig. 1. Schematics of apparatus and imaging setup. (A) Illustration of the passive treadmill for the  
454 head-fixed behavioral device. Inset shows the assembled apparatus. (B and C) Schematic diagrams  
455 of the configuration of the rat positioned in the apparatus, from the front (B) and the side (C). The rat  
456 is shown on the passive treadmill holding a retrieved donut shaped food item. Two high-speed  
457 cameras are placed 45 cm below the transparent floor to monitor the reflective markers on the wrists.  
458 (D) View from camera 1 and camera 2 (inset). Note reflective markers attached to rat's wrists for  
459 semi-automatic tracing. (E) Timeline of the sequence of a trial of food handling and consumption.

460

461 Fig. 2. Egocentric coordinate reference frame in the recording frame, and segmentation of forelimb  
462 trajectory. (A-C) Forelimb position was projected on the egocentric coordinate system based on the  
463 reference marker. (A) Example of 3D printed reference frame of egocentric coordinates. Four  
464 triangularly placed reflective markers indicate the origin, posterior to anterior axis (P- A), left to right  
465 axis (L - R) and dorsal to ventral (D - V) axis of the rat (B). (C) Example of 3-D forelimb trajectories  
466 projected on the egocentric coordinate space. (D) Example 50 ms time window for data  
467 segmentation. Note the speed of the right and the left forelimb increased over time indicating  
468 bilateral movement initiation. (E) Representative segments of forelimb trajectories in each time  
469 window in (D). D, dorsal; V, ventral; A, anterior; P, posterior; R, right; L, left. Numbers in 3-D plots  
470 are expressed in millimeters.

471

472 Fig. 3. Decision tree for classification of segments.

473

474 Fig. 4. Representative behavior under head-fixed conditions. Frames in the left three columns show  
475 four different behaviors. Scatter plots in the right column illustrate corresponding 3-D forelimb  
476 trajectory. The color scale indicates the normalized time. Note that the duration of each behavior is  
477 variable. (A) Bimanual upward reach. Both forelimb simultaneously move toward the anterodorsal

478 side. (B) Bimanual downward reach. Both forelimb simultaneously move toward the posteroventral  
479 side. (C) Bimanual release. Both hand simultaneously release the food item and regrasp it to change  
480 the position of the hands. (D) Unimanual release. One hand release and regrasp of the food object  
481 with support of other hand. Abbreviations: D, dorsal; V, ventral; A, anterior; P, posterior; R, right; L,  
482 left. Numbers in 3-D plots are expressed in millimeters.

483

484 Fig. 5. Reconstruction of the whole sequence of forelimb movement during spontaneous food  
485 handling behavior. Positions of the right (blue) and the left (orange) forelimbs were captured by  
486 camera. Frames are shown rotated in 30° steps. Abbreviations: D, dorsal; V, ventral; A, anterior; P,  
487 posterior; R, right; L, left.

488

489 Fig. 6. Moving segments were exclusively selected by maximum speed function. (A) Probability  
490 distribution of speed of right and left forelimb movements across 5 rats. Left panel, linear scale.  
491 Right panel, logarithmic scale. Note the dip of probability density at the threshold indicated by the  
492 dotted line (40 mm/sec). (B) Mean probability distribution of maximum speed function across 5 rats.  
493 (C) Mean proportion of moving segments in all segments. (D-E) Example trajectories of the  
494 segments in the resting state (D) and during movement (E). Abbreviations: D, dorsal; A, anterior; R,  
495 right; L, left. Numbers in 3-D plots are expressed in millimeters.

496

497 Fig. 7. Bilateral and unilateral forelimb movements during food handling. (A-C) Laterality of  
498 movement speed was quantified by speed ratio. (A) Graphical representation of speed ratio as a  
499 measure of laterality of left and right forelimb in speed. Each dot represents the mean speed of the  
500 right  $\bar{V}_R$  and left forelimb  $\bar{V}_L$  in a segment. The empty space at the left-bottom corner represents  
501 resting segments not included in the analysis. (B) Mean probability distribution of the speed ratio  
502 across 5 rats. (C-D) Example segments of bilateral and unilateral forelimb movements (C) and  
503 unimanual movements (D). Abbreviations: D, dorsal; A, anterior; R, right; L, left. Numbers in 3-D



504 plot are expressed in millimeters.

505

506 Fig. 8. Symmetric and asymmetry forelimb movements during food handling. (A-B) Asymmetry in  
507 movement direction was analyzed in terms of the error of movement vector direction between two  
508 forelimbs. (A) Graphical representation of mean angle of vector direction  $\bar{\theta}$ . Arrows indicates the  
509 example trajectory of left forelimb  $L$  and the mirrored right forelimb  $R_M$ . The asymmetry index  $\bar{\theta}$   
510 was calculated based on the error of movement vector direction between  $L$  and  $R_M$ . The dotted line  
511 illustrates the midline for the mirror transformation. (B) The probability distribution of the mean  
512 similarity of vector direction  $\bar{\theta}$ . (C-D) Examples of symmetric movement (C) and asymmetric  
513 movement (D). Abbreviations: D, dorsal; V, ventral; A, anterior; P, posterior; R, right; L, left.  
514 Numbers in 3-D plots are expressed in millimeters.

515

516 Fig. 9. Analytical pipeline enabling high-throughput of kinematic data for quantification of bilateral  
517 forelimb movements. (A) Pipeline of classification characterizes time course of behavioral states of  
518 spontaneous food handling behavior. The top two black traces indicate speed ratio and asymmetry  
519 indices respectively. The colored bars indicate the time of occurrence of each motor behavior defined  
520 by thresholding. The color code of each behavioral mode is shown in (B). Line graph indicates the  
521 speed of the right and left forelimb. (B) Magnified view of the shaded area in (A). (C) Corresponding  
522 actual trajectories of forelimbs. The color indicates the behavioral type shown in (B). Note that for  
523 clarity the color of unilateral movement overrides other categorizations when speed ratio exceeds the  
524 predefined threshold. (D) Quantitative analysis of behavioral types. Mean percentage of the  
525 behavioral type of movement classified in accordance with speed ratio (top) and asymmetry index  
526 (bottom). Abbreviations: Rest, resting; Bilat, bilateral forelimb movement; Unilat, unilateral forelimb  
527 movement; Sym, symmetric movement; Asym, asymmetric movement; D, dorsal; V, ventral; A,  
528 anterior; P, posterior; R, right; L, left.

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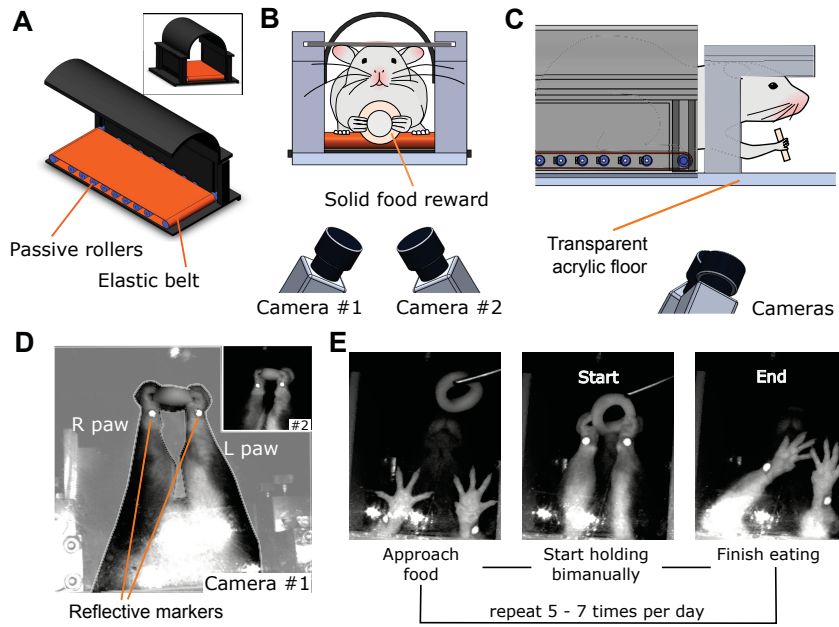


Figure 1

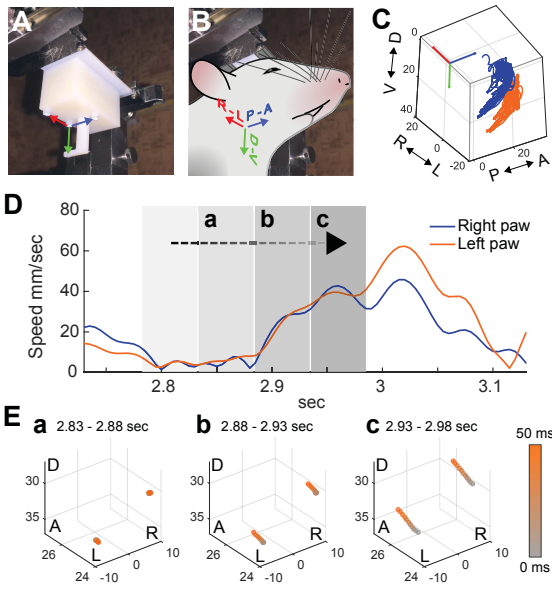


Figure 2

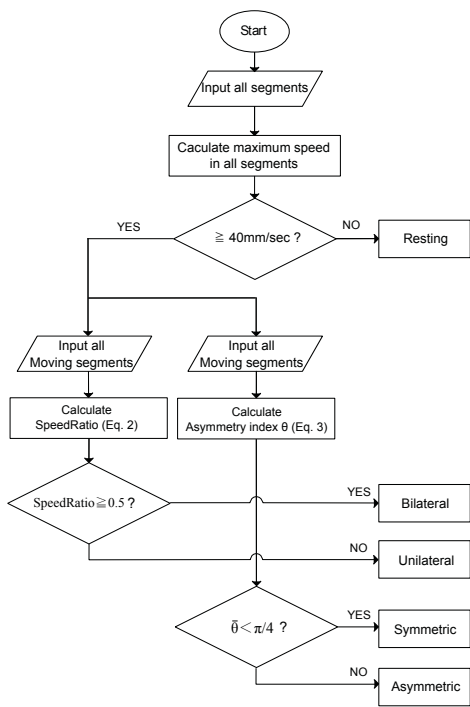


Figure 3



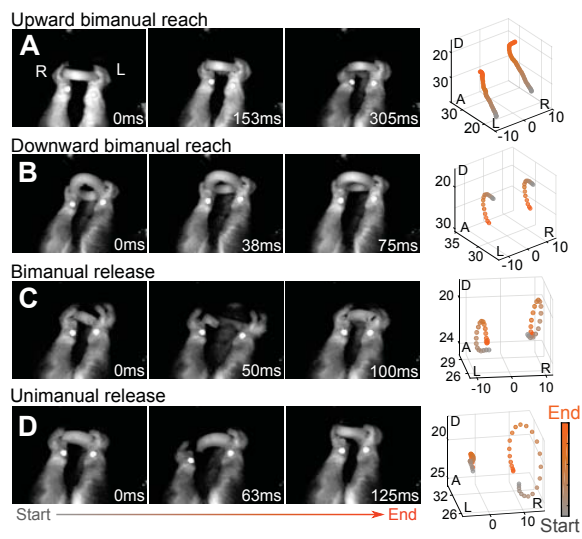


Figure 4

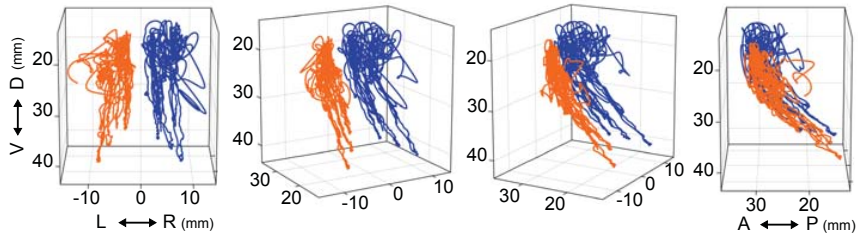


Figure 5

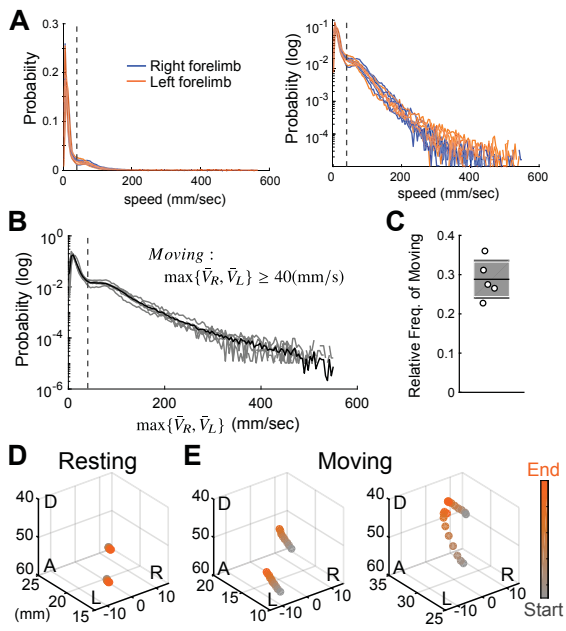


Figure 6

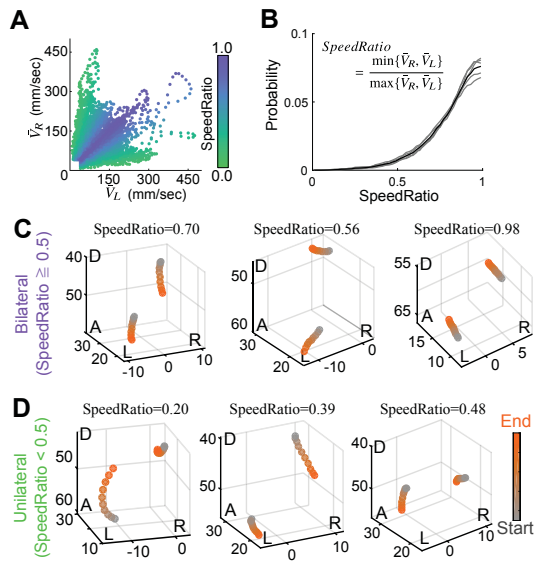


Figure 7

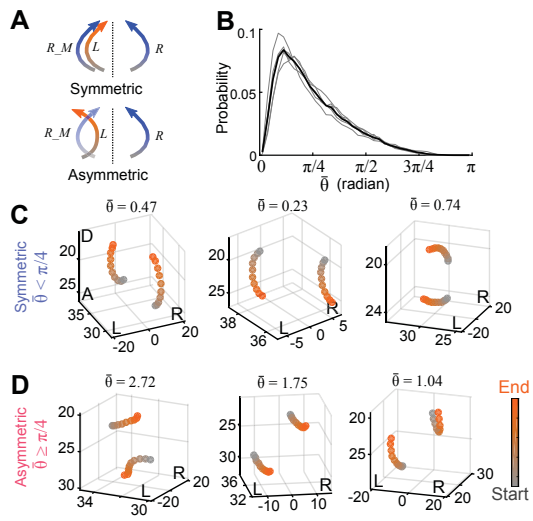


Figure 8

