Employing virtual reality to reveal individual
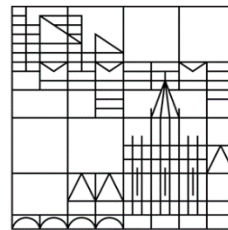locusts' decision-making
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Abstract

Animals are constantly faced with choices where to feed, move next, or search for night shelter. Appropriate choices maximize survival and reproductive success. Research on decision-making in animals has mainly focused on *which* choice animals make, rather than on *how* they choose. In collectives, the mechanism of decision-making has been studied extensively in the last twenty years, yet little is known about the underlying mechanism in the individual's brain. Sridhar *et al.* (in prep) propose a neuronal model that describes the underlying mechanism of an individual's decision-making process. It predicts animals to average the direction of presented targets, moving in the centroid direction. As the angle between targets increases, the animal bifurcates at a critical angle, and eventually turns towards one of the targets. Multi-choice situations are then broken down to binary choices, successively eliminating choices, and resulting in a multi-bifurcation pattern. Sridhar *et al*. validate the model experimentally in *Drosophila melanogaster*, yet with kinematic limitations. Here, I tested the model's spatial *and* kinematic predictions of decision-making in the desert locust *Schistocerca gregaria*. In a state-of-the-art virtual reality system, I presented freely walking individual locusts with equally attractive targets. In accord with the model's predictions, locusts made decisions in a (multi-)bifurcation pattern. These cross-species results suggest that the model represents a generic, species-unspecific algorithm, robust across scales and therefore applicable to both individual and collective decision-making.

Introduction

Animals must constantly make appropriate choices to maximize biological fitness. Correct decisions about where to forage, sleep, or whom to court influence survival and reproductive success (McFarland, 1977). Decision-making in animals has been studied extensively, but research has mainly focused on the outcome of decisions between different options in a non-spatial context (Ellis and Pearce, 1962; Halliday, 1977; Logan, 1965; Wallace, 1958). These studies on asymmetric decision-making investigate the natural situation animals are faced with, but do not give insight in the brain's decision-making process. To understand decision-making in the brain, it is necessary to investigate the symmetric case, i.e. choices made between equal options, in a spatial context.

Sridhar *et al.* (in prep) propose a neuronal model with a discrete algorithm that describes the underlying mechanism of the individuals' decision-making process. It describes spatial and kinematic properties of an animal's trajectory when approaching symmetric targets. In the brain, different groups of neurons encode different targets vectorially (Sarel *et al.*, 2017). When presented with two equally attractive targets, the animal averages the direction of the two targets, i.e. the vectors, and moves in the centroid direction - making a compromise. As the animal moves towards the targets, the angle between them increases. The animal is predicted to decelerate before it reaches a critical threshold angle between the two targets, then it turns randomly towards one of them, switching from compromise to a choice. This switch results from neurons reaching consensus, which is visible in the trajectories bifurcating. The prediction can be extended to an unlimited number of targets. The model predicts multi-choice situations to be broken down to binary choices. If an individual is presented with multiple targets, it averages all directions to a centroid and eliminates options successively when reaching the critical angle between targets. This then leads to a multi-bifurcation pattern. Moving in the centroid direction rather than straight towards one target comes with the cost of a longer trajectory but maximizes the animal's sensitivity to differences in quality between targets, as the animal's susceptibility peaks close to the bifurcation (Sridhar *et al.*, in prep). Thus, the animal is more likely to make the best choice.

The model successfully predicts spatial properties of decision-making in the fruit fly *Drosophila melanogaster* (Sridhar *et al.*, in prep). The trajectories of *Drosophila's* flight pattern follow the

predicted (multi-) bifurcation pattern. However, experiments with *Drosophila* rely on the assumption of constant speed. Any prediction of kinematic properties, e.g. stopping at the bifurcation, cannot be tested in this setup. It is necessary to understand the kinematics as well as spatial behavior to predict animal movement precisely. To fill this gap, I studied decision-making in the desert locust *Schistocerca gregaria* in a state-of-the-art virtual reality setup.

Locusts are well suited for this experimental setup. Presentation of simple visual stimuli is sufficient to attract locusts (Wallace, 1958) and motivate them to make decisions; no complex landscape is required. They are an established model organism in behavioral biology and physical parameters for experimental conditions are well known. Furthermore, locusts are an ecologically important species, as their foraging on crops causes food shortages (Uvarov, 1977). Understanding the locusts' decision-making may help to predict the marching patterns of wingless juveniles and help to contain locust swarms early (Enserink, 2004).

Several kinematic properties of locusts are already known, yet not in the context of decision-making. Locusts show intermittent walking behavior (Bazazi *et al.*, 2012). During these frequent stops, locusts often move their head horizontally, a behavior known as peering (Kennedy, 1945). Wallace (1959) analyzes peering and associates it with motion parallax to estimate distance. The animal evaluates distance from an object by changing its own angular alignment to it. For objects that are close, there is a strong angular shift, whereas distant objects remain at the same angular position relative to the observer (Sobel, 1990). Manipulating locusts' motion parallax shows that they use peering to estimate distance (Collett, 1978; Sobel, 1990). In addition, stops are related to turns (Bazazi *et al.*, 2012), but it is unknown whether these are linked to directed reorientation.

To better understand the nature of decision-making, I investigated these kinematic properties in the context of decision-making in a virtual reality system. The locust was walking freely and physically centered on a rotatable sphere as a two-dimensional treadmill. I presented the animal with two or three visual stimuli at different angles and with a single stimulus as a control. The animal was tracked with a computer vision camera which allowed the precise observation of spatial and kinematic properties in the context of decision-making. Real-time tracking enabled the presentation of a real-time perspective corrected stimulus (closed-loop method) to the animal, according to where it was moving. Appropriate presentation and

perspective of stimuli created a realistic illusion for the animal. Closed-loop experiments made interaction between animal behavior and stimulus possible, e.g. increase of size of the stimulus when the animal was approaching it virtually. The VR method allowed observation and restart of experimental trials without intervention during the experiment.

First, I wanted to test if the decision-making model by Sridhar *et al.* can be applied to locusts and whether its predictions hold true in a system without speed assumptions. I hypothesized (i) locusts make choices in a bifurcation pattern when approaching targets and to break down multiple choices to binary choices, as predicted by Sridhar's model. Second, (ii) I hypothesized that the stopping behavior of locusts should be linked to reorientation i.e. correcting their angular direction towards a target. This led to my final hypothesis (iii), that if (i) and (ii) are true, locusts should stop preferentially at the bifurcation point where the decision is made. Stopping there would allow the locust to re-estimate distance and orientation and enable it to make an informed decision.

Results

Locusts showed a bifurcation pattern in two-choice experiments

I presented desert locusts with two equally attractive stimuli (vertical black posts, 30° or 45° apart) to investigate the manner of displacement in decision-making. Locusts initially compromised, walking towards the centroid vector of the directional vectors of the two posts and then chose one of the posts randomly (for trajectories see Fig. S1). The heatmap of all locusts' trajectories (Figure 1) illustrates the bifurcation pattern. I fit a piecewise phase transition function to calculate the critical angle at which locusts switched from compromise to choice behavior. The bifurcation angle was 73° in the 30° setup (Fig. S1b) and 89° in 45° experiments (Fig. S1c). Two-choice experiments revealed trajectories as predicted. Simulated trajectories (Fig. 1b) matched experimental data.
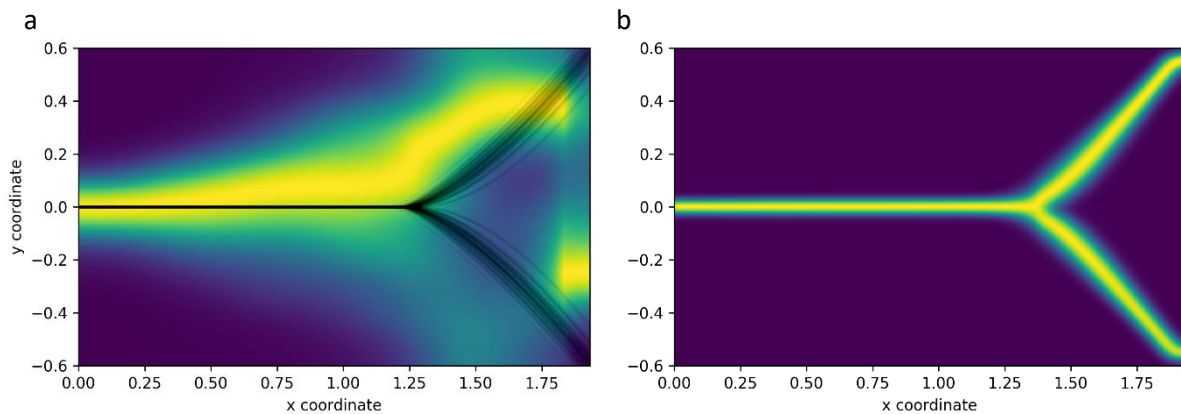

Figure 1. (a) Two-choice locust trajectories as density heat map and (b) the respective simulation. (a) Locusts showed a bifurcation pattern when approaching two posts. The piecewise phase transition function calculating the critical bifurcation angle is depicted in black. (b) Simulation of trajectories according to Sridhar *et al.'s* model matches experimental data.

Most locusts preferred one of the targets. There was a left bias in trajectories in both 30° and 45° setups. Most trajectories ended at the left target, the probability for animals turning randomly to the left target is low (p = 0.037 for 30° and p = 0.035 for 45° setups). At the individual level, there was no preference for the left target, though. Fourteen individuals strongly preferred (reaching the target > 70 %) the left target, and twelve individuals preferred the right one (p-value = 0.422). Six individuals showed no preference, another seventeen individuals were excluded as they reached any target only once.

Multi-bifurcation pattern in multi-choice experiments

Preliminary three-choice experiments revealed that locusts averaged their walking direction to the centroid of all three presented posts (for trajectories see Fig. S2). Locusts eliminated one of three choices when they reached a critical angle between all posts. After they passed the critical angle, they walked in the centroid direction of the two remaining posts. This resulted in a sequential bifurcation pattern (Fig. 2a). The pattern matches the model's prediction (Sridhar *et al*., in prep); Figure 2b shows the simulation of locusts' multi-bifurcation pattern.
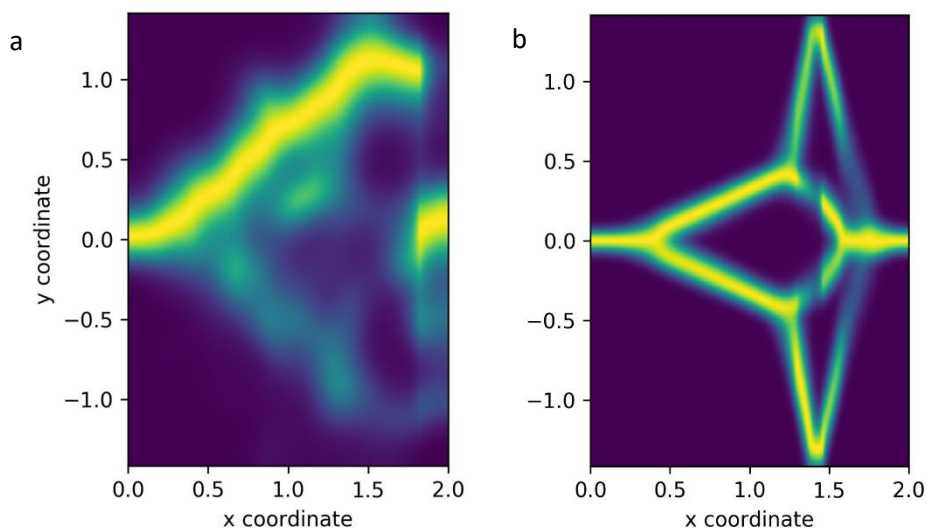

Figure 2. (a) Three-choice locust trajectories (45° between posts) represented as heat map and (b) the respective simulation. Locusts showed a multi-bifurcation pattern when approaching three posts. The experimental trajectories are consistent with simulations according to Sridhar's model.

Locusts corrected direction by stopping

Locusts showed an intermittent walking pattern (for stop frame classification see Fig. S3). In the one-post (control) setup, stopping was generally associated with a median angular shift of 38.2° (Fig. S4a), whereas walking only showed median shifts of 9.2° (Fig. S4b). Stopping was also linked with angular correction towards the post. I compared the angular deviation of the locust to the post before and after stops. The mean deviation from the post was 61° before a stop, and 39.5° after, resulting in a mean improvement of 21°. Angular improvement was significant (Fig. 3a). Locusts were unlikely to turn away from the post ($p < 10^{-13}$). During walking periods, locusts did not improve their directedness towards posts (Fig. 3b).
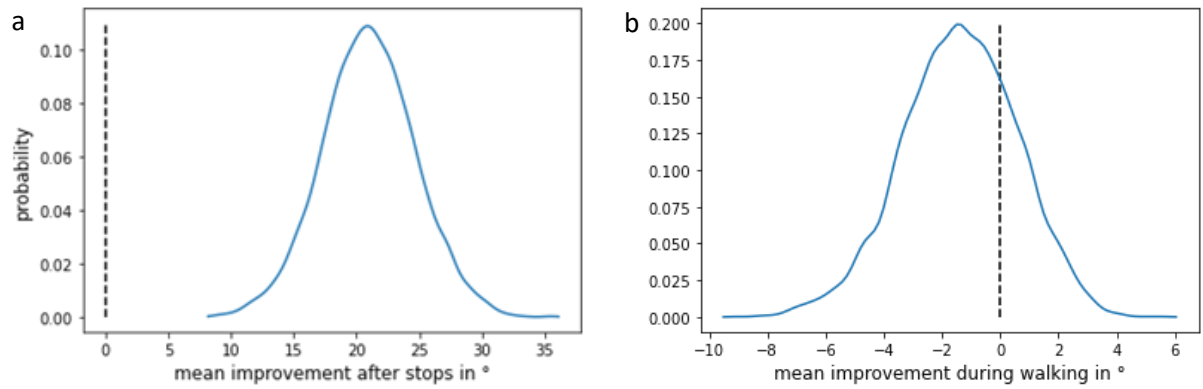
Figure 3. After stops, locusts improved their mean angular directedness towards the post. Means of bootstrapped data of the one post control are depicted. (a) The graph shows the mean angular improvement after stops towards the target. The dashed line indicates the probability (p = 1.03 $*10^{-13}$) for locusts turning away from the post. (b) The graph shows the mean improvement during 150-frame-longwalking periods (equal to mean length of stops). During walking, locusts did not improve their directedness towards the post. The dashed line indicates the probability of locusts turning away from the post.

Locusts did not stop preferentially at the bifurcation point

Locusts made decisions in a bifurcation pattern and used stopping to correct their direction. I investigated if stopping occurred preferentially at the bifurcation point, as locusts changed their direction of walking there. I investigated stopping behavior in relation to multiple parameters; i.e. the x coordinate of trajectories (Fig. S5a, b), the distance to the closest post (Fig. S5 c, d) and the angle between posts perceived by the animal (Fig. 4). I found no evidence for an increase in stops at the bifurcation point (Figure 4).
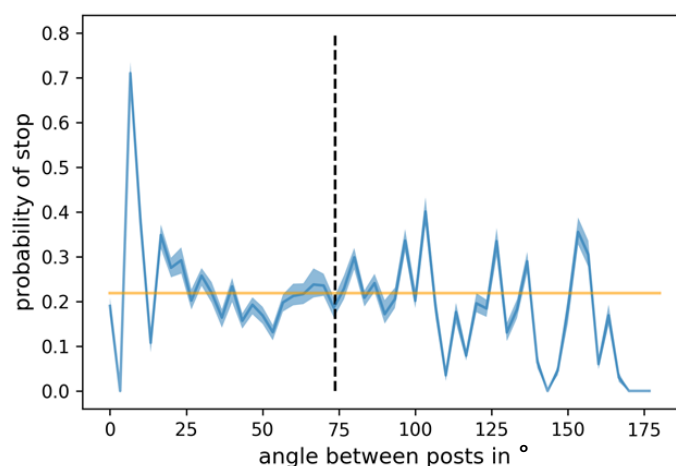

Figure 4. Locusts do not increase stopping at the bifurcation point. The probability of stop frames is depicted in relation to the angle between posts perceived by the locust. I analyzed bootstrapped data. The blue line depicts the probability of stop frames in relation to the angle between posts. Error regions are the light blue area. They show the 95 % interquantile range of the random samples of all frames. The dashed line indicates the bifurcation angle; horizontal lines represent mean probabilities of stops to occur at any angle of different samples.

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Discussion

Bifurcating increases susceptibility - handedness might ease decision-making

My results show that locusts performed decision-making from start to target in a bifurcation pattern. Locusts walked in the centroid direction of two posts until they reached a critical angle. At this point, they broke symmetry between equal choices and turned to one of the posts.

The model of Sridhar *et al.* (in prep) predicts averaged movement until a critical angle and a subsequent choice. By default, postponing the decision where to go incurs costs. The detour increased the distance travelled by 8 % for experiments with 45° between posts. Nevertheless, it is a trade-off that keeps both targets available as they are approached equally, and proximity enhances the perception of differences between targets. The model calculates an animal's susceptibility for differences to reach a maximum close to the bifurcation. The exact bifurcation point can change species and context dependently. The bifurcation point embodies a position for making a well-informed choice with a good ratio between costs (increased distance) to benefit (reliable information on differences).

Locusts' trajectories of decision-making were consistent with the model predictions of Sridhar *et al*. (in prep.) and previous experimental data. *Drosophila melanogaster* solves decision-making in a bifurcation pattern, too (Sridhar *et al*., in prep). The virtual reality setup for *Drosophila* is largely the same as for locusts, but the fly is tethered, and constant speed is assumed. Even if *Drosophila* was slowing down without changing direction, the experimental data outcome would have implied that the fly continued as before. Here, I showed that the model's prediction held true for a system without speed assumptions. Additionally, the cross-species results imply that the model of decision-making is valid for a wider range of species and possibly represents an algorithm that has evolved early.

Locusts showed preferences for one of the targets. The left target in both 30° and 45° experiments was reached more often. Handedness in locusts has been found to be context dependent and prevalent at the individual rather than at the population level (Bell and Niven, 2014). In accordance with Bell and Niven, I found handedness to be expressed on the individual level, as most locusts showed a strong preference for either the left or the right

target. The strong left bias on the single trajectory level arose from few individuals contributing many trials to the left target, rather than locusts having a left bias on the population level. Handedness in animals is widespread (Frasnelli *et al.*, 2012) and was shown to be generally advantageous in terms of computation effectiveness and better performance on tasks, extensively reviewed by Vallortigara and Rogers (2005). Lateralized locusts were shown to perform better in certain tasks than non-lateralized individuals (Bell and Niven, 2016). Another advantage of handedness could be that when confronted with two equally attractive choices animals would not become stuck with indecision at the bifurcation point, but it might ease their choice for one of the targets. Comparison of behavior of handed locusts versus locusts without preferences would be required to investigate this hypothesis.

Multi-choices are broken down to binary choices

Drosophila melanogaster breaks down complex decisions to successive binary choices. When *Drosophila* approaches multiple posts, it averages all posts' directions to a centroid. When it reaches a critical angle between all stimuli, choices are eliminated successively. The flight trajectories to stimuli result in a multi-bifurcation pattern (Sridhar *et al.*, in prep). Locusts showed a tendency to a similar spatial movement pattern, when faced with multiple posts. To consolidate these results, a larger number of trajectories in three-choice experiments is required. As three-choice experiments offer the option of four different trajectories to locusts according to the model's prediction, at least twice as much data is needed as for two-choice experiments.

In Figure 2a, the first bifurcation is hardly detectable. The starting angle between the outer posts was already 90°. As I found the critical angle in two-choice experiments to be 89° (with 45° between posts), the first bifurcation was probably already exceeded before the start of the experiment. The critical threshold angle in *Drosophila* is 110° and thus I had expected locusts to bifurcate at a similar angle. To improve visibility of the multi-bifurcation pattern, the angle between posts should be decreased and the distance to the posts increased. A lower starting angle alone is not sufficient, as for the 30° setup the distance was probably too short and the trajectories therefore too noisy to detect a clear pattern. It is noteworthy, that in pilot experiments (cmp. Section S1) locusts seemed to lose interest in objects that were far away.

The loss of interest in seemingly very distant objects was an observation made by Sobel (1990), too. Distant objects in virtual reality systems pose another problem for locusts. I assume that locusts received two distance cues to posts during experiments. One distance cue was the increase of the size of the posts while they approached them. The other cue they used to measure distance was motion parallax. Regardless of virtual distance to a post, locusts might have estimated the distance to any presented post to be 0.4 m - the distance to the projection screen - when peering for motion parallax. As the VR system did not correct for motion parallax, locusts then received two incoherent distance cues. For greater distances, the gap between the two cues would increase continuously. Once the locust started walking, it would have expected the post size to increase rapidly. A distant post did not change in absolute projected size, though, leading to a strong conflict between the two distance cues. Locusts may have judged distance information on these distant objects as unreliable. This is a possible reason for locusts to lose interest in virtual objects at a rather long distance. A virtually close object would still have led to slightly incoherent distance information, but as object size still increased rapidly, it gave the impression of approaching a near target.

Therefore, I propose the implementation of motion parallax compensation to the VR system. This would require an enhanced tracking mechanism which does not only detect the displacement of the locust's center of mass from the sphere's center. It also needs head tracking and real time horizontal compensation for the locust's peering movement.

Locusts corrected direction by intermittent walking

Locusts walked intermittently. Bazazi *et al.* (2012) showed these stops to be related to turns between clockwise and anti-clockwise movement. Locusts moved in a round arena with a centered obstacle and therefore spatial movement options were limited, thus they only accounted for 180° turns. Instead, the present VR setup allowed locusts to walk freely in any direction, making it possible to evaluate any change in direction. Stopping was generally associated with a median angular shift of 38.2°, whereas walking was rather straight, shifting the angle of direction by only 9.2° (Fig. S4a). In former experiments, walking was not motivated by and directed to any particular target. That prompted me to ask if stopping was related to reorientation. The virtual reality setup allowed me to show that indeed the direction of

walking towards a post was significantly corrected after a stop, the mean angle of improvement towards a post was 21°. Mean divergence from the post after a stop was still at 39.5°. It might seem surprising that the divergence afterwards was not lower. Locusts are highly attracted to the edges of objects (Collett and Paterson, 1991). I calculated the deviation angle towards the center of the post and not to its borders. As locusts walk to edges, there should always be a remaining divergence from the center.

So far, it is known, that locusts change their direction during stops and that stops are used for motion parallax and therefore estimating distance. Here I could provide evidence, that locusts did not randomly change direction after a stop, e.g. for searching. Instead, they used it for reorientation and correcting their walking direction towards targets.

Stopping at the bifurcation point is not increased

I found locusts to use stopping to correct directedness towards posts and to show a bifurcation pattern when moving towards posts. Based on these results, I expected locusts to stop preferentially around the bifurcation. Here, locusts changed from target-averaged compromise to one-target-directed choice. Hence, locusts changed their walking direction at this point and consequently should stop to do so. Stopping at the bifurcation would allow the locust to absorb a maximum of information on the quality of different options and subsequently make an informed decision. In contrast to this expectation, I found no evidence for increased stopping behavior at the bifurcation. In experiments with posts at 30°, stopping in relation to the x coordinate in trajectories seemed to show a tendency of increased stopping frames at the bifurcation (Fig. S5a) as well as in relation to distance to the closest post (Fig. S5c). However, in experiments with posts at 45° there was no increase in stopping frames at the bifurcation for neither relation (Fig. S5b, S5d). Locusts stopped very often and irregularly. The resulting data on where they stop was largely distributed and noisy, so that an increased stopping at the bifurcation point is possibly hardly detectable.

The individual locust's decision-making may reveal its contribution to the collective. A collective does not consist of a mass of identical individuals, but individuality is partly maintained in the collective (Herbert-Read et al., 2013). If we understand the individual's influence with differing individual preferences on collective decision-making, we better understand the collective's movement.

The underlying mechanism of decision-making and consensus finding in collectives can be described with increasing accuracy by models of collective decision-making (Couzin *et al.*, 2005; Pinkoviezky *et al.*, 2018). Models are supported by experimental data of moving collectives, e.g. troops of baboons (Strandburg-Peshkin *et al.*, 2015). The models predict spatial properties of movement and a critical point at which a decision is made eventually. If initiators choose different directions where to move, remaining individuals will likely choose the average direction, demonstrating compromise behavior. As the angle between the diverging groups increases to a critical threshold angle, followers choose one of the directions over the other, switching from compromise to a consensus decision.

The similarity between the collective and the individual level arises the question, whether we can infer from knowledge of the individual to the collective level and vice versa. The potential inference across scales is supported by the similarity between levels and the generic algorithm of Sridhar *et al*. (in prep) which is applicable across scales. Locust pests could be contained early if decision-making of the animal and therefore the direction of movement was predictable precisely. Yet, we need to understand the extent of inference from the individual to the collective level.

Methods

Locusts

I obtained desert locusts *Schistocerca gregaria* at instar 5, the final juvenile unwinged instar, of both sexes from the Animal Research Facility of the University of Konstanz. I kept them at 25 - 27 °C at night and 31 - 33 °C during experiments with a relative humidity of 20 - 22 % based to pilot experiments. I used completely intact locusts for experiments only once. I conducted experiments the day of or day after delivery and did not feed locusts in the meanwhile (cmp. Section S1).

Virtual reality system setup

The virtual reality (VR) system for terrestrial insects (loopbio GmbH) is based on the FreemoVR engine (Stowers *et al.*, 2017). It consists of three components depicted in Figure 5: (a) the locomotion compensator, (b) the Motif Recording system with closed-loop extension and (c) the FreemoVR VR system.

The locomotion compensator (a) serves as a two-dimensional treadmill. It consists of a hollow polyethylene sphere with a diameter of 60 cm and a high friction surface to enable animals to walk on it. Two servo motors with rotary encoders turn the sphere to compensate for locomotion of the animal. This allows the animal to move infinitely on the sphere's center. Mechanics are adapted from Kaupert *et al.* (2017). The Motif Recording system with closed-loop extension (b) is the tracking and feedback-loop component of the system. Closed-loop experiments enable interaction between the animal and the stimulus. Tracking of the animal allows giving feedback on the direction of movement to the locomotion compensator to keep the animal centered. It consists of a Motif recording unit, i.e. a 100-fps infrared computer vision camera and a LED spotlight at 850 nm (infrared). Optical tracking is performed with a contrast-based method. The optical center of mass of the animal is detected and its deviation from the center of the sphere is converted into a compensation response of the motors. To achieve real-time updated stimulus presentation in the VR according to animal locomotion, a feedback instance is required. This is the closed-loop extension software that
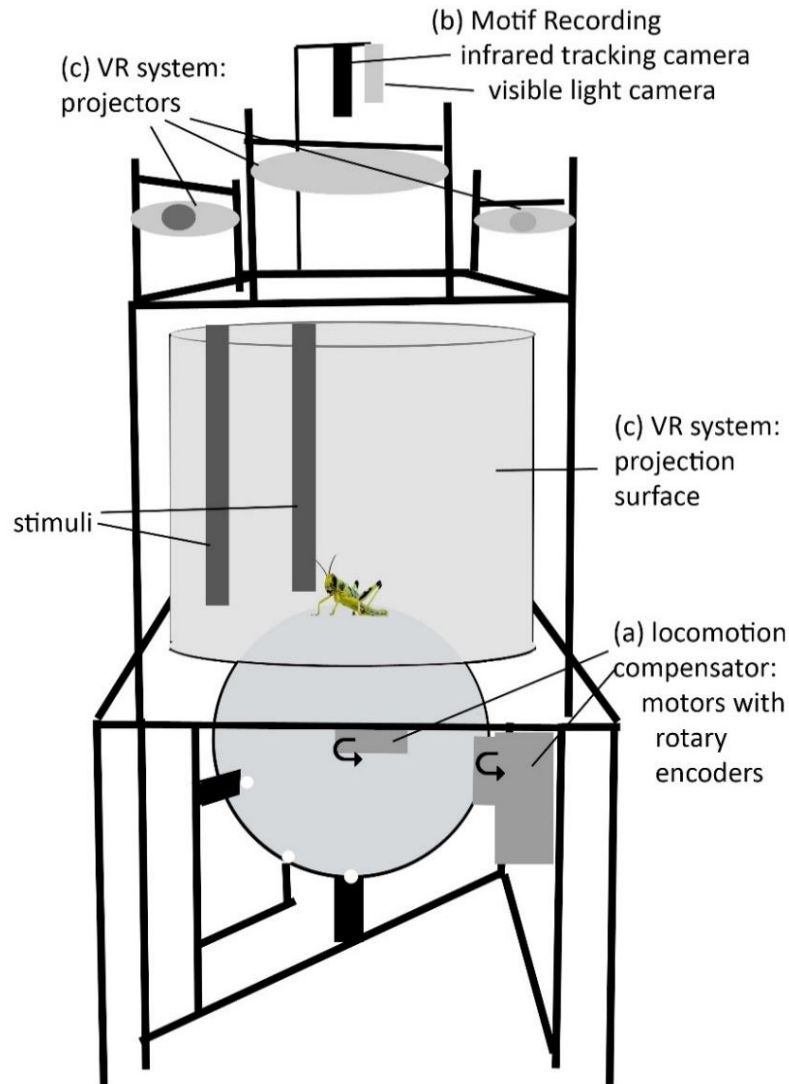
Figure 5. Scheme of major components of the virtual reality system setup. (a) locomotion compensator: motors with rotary encoders, (b) Motif Recording system: infrared tracking camera, (c) the FreemoVR VR system: projectors and projection surface.

feeds animal movement to (c) the VR system. It is composed of three projectors (Optoma GT1070Xe) with overlapping projection on a vertical cylindrical projection surface (70 cm height, 80cm diameter) and a horizontal surface that is the floor of the cylinder (except the central cut-out area for the sphere). A virtual environment is projected in real-time, movement-corrected at 120 fps on the inner projection surface pointing towards the animal. The eye position of the animal is 15 mm above the sphere and for this position the field of view covered for the animal is 360° horizontally and 74.9° vertically.

Concept of coordinates

As the locust was always kept physically centered, it only moved in a virtual coordinate system (CS). Tracking of the locust recorded the locust's position in these `raw coordinates´. Absolute sphere movement was directly converted to the distance the locust had travelled in a certain direction in the raw CS. To simplify positioning of stimuli relative to the locust and later analysis of recorded locust's trajectories, I introduced another CS, the `world coordinate system´. This CS was superimposed with its origin (0,0) on the current raw coordinate of the locust. This allowed starting of each experiment and therefore of each recorded trajectory at (0,0).

Stimulus design

According to previous work (Wallace, 1958) and pilot experiments (Section S1) I designed three-dimensional black posts of 0.2 m radius and 100 m (infinite) height as stimuli, and a white cube as the background in the 3D graphic design program blender. The objects were shadeless so the edges of the cube could not be perceived from within.

Interacting systems

I wrote an interacting python script that had two major functions. It served as an adapter between the VR system and experiments and it contained the actual experimental script. As an adapter, it interacted on one side with the VR system's software to receive coordinates, superimpose the world coordinate system to the raw coordinates, reset the superimposition to a new origin (0,0) and start recording of the cameras. On the other side, it interacted with two databases and an output csv file.

The project database (adapted from Sridhar *et al.*) contained randomized experimental setups. An experiment for one locust always consisted of five stimuli; the first one and the last one were single post controls and stimuli 2-4 were two post or three post experiments. Positions of the first post always occupied one of 8 randomized positions, the other posts were positioned relative to it. Stimuli 2-4 contained three different angular conditions: 30° and 45°

between posts and a symmetric setup of 180° or 120°, according to two- or three-choice experiments. The order of angular conditions in stimuli 2-4 and the randomization position were defined in the project database. The second database was the experiment database. Here, the python script stored the experimental conditions of each completed experiment, that were the chosen randomization from the project database, a unique ID for each experiment and a timestamp. The interaction with the two databases enabled the treatment of large data sets in an ordered fashion and manage randomizations in a way that blinded the experimenter to the positions where the stimuli would appear. The x and y coordinate of the locust's position were saved in the csv output file at 0.04 s timeframes. Here, current stimulus and trial number were recorded, too. I refer to recorded coordinates as trajectories.

Experiments and python script

I gently placed locusts on the center of the VR sphere, started the locomotion compensator and then started tracking and the experimental python script. At the beginning of each experiment, the script started the camera recording and called the white background object only. Locusts could adapt to the VR for 30 seconds. I then presented locusts with a single post as control and with two or three posts for different choice experiments. The script randomly chose experimental conditions from the project database and saved conditional setups in the experiment database. Controls at the beginning and end of experiments lasted 6 minutes each, experimental setups lasted 12 minutes each. All stimuli contained several trials. Within one stimulus, the randomized position for all trials stayed the same. I presented posts at randomized positions at a virtual distance of 2 m from the starting position. As the animal moved towards the post, the visual stimulus was updated in real-time, increasing in visible size for the animal as if the distance to the post was decreasing. At the starting position (0,0), angles between posts were 30°, 45° or were presented symmetrically at 180° or 120° for two- or three-choice experiments, respectively. When the locust reached a distance less than 0.3 m from a post's center or a distance greater than 2.3 m from all posts, a new trial was initialized. With a new trial, all posts disappeared for 3 seconds, the world coordinate system was reset to a new (0,0) position according to the locust's current position and the posts reappeared at

the former starting position. If locusts did not reach any post within 5 minutes of a stimulus, a new trial was started.

Data preprocessing

I rotated the trajectories of experiments with equal angular conditions of the posts to an equal orientation to eliminate randomization of post positions. I applied a rotational matrix that put the posts symmetrically on the x-axis. I excluded trajectories that ended outside a radius of 0.3 m around the center of one of the posts from further analysis to reduce noise. Each trajectory ending at a post I considered as a trial. I visualized bifurcation patterns in trajectories as heat maps by normalizing all trajectory data of one experimental condition bin-wise in columns of x coordinates and creating density maps. To quantify bifurcation points, I fit a piece-wise phase-transition function to the trajectories.

$$y = \begin{cases} 0 & x \leq x_c \\ A|x - x_c|^\alpha & x > x_c \end{cases}$$

where x_c is the critical bifurcation point, α is the critical exponent, and A is the proportionality constant.

Movement classification

I calculated speed at every timeframe. To classify frames either as stop or movement, I applied the python-implemented pomegranate general mixture model with two distribution components (exponential and normal distribution) on noise reduced speed data distribution. Supplementary Figure S3a shows the distribution of speed. The function performed an expectation maximization (EM) to iteratively estimate parameters for functions to fit on the data. The learned model of the EM described the locust's speed distribution well (cmp. Fig. S3b). The function then classified speeds to belong to one of the two distributions. I assumed these distributions to be a stop or a movement frame. Distribution of stops are robust to different speed thresholds of stop classification (Fig. S6).

Correcting direction by stopping

To investigate how the precision of directedness of a locust towards the post was influenced by stops, I evaluated its angle of walking direction to the post before and after stops in the one post control condition. I only accounted for stops of at least 25 frames (= 1 second) length. I averaged the walking direction of 25 frames before and after stops, respectively. To validate the degree of angular improvement after a stop towards the post, I assumed the observed distribution of angular improvement to represent a sample of the true distribution of angular improvement around stops. I bootstrapped the data and fit a kernel density function to means of the samples. As a control, I investigated the angular improvement during walking periods. The mean length of stops was 150 frames, so I investigated the angular change during 150 frames in walking periods. I proceeded with walking periods as mentioned above with stops.

Stopping at the bifurcation: dependency on angle between posts

I investigated how locusts' stopping behavior was dependent on the angle they perceived between two posts as they were walking towards them. I assumed the observed distribution of stops to represent a sample of the true distribution of stops. I removed datapoints with an x coordinate < 0.01 m from noise reduced data. I bootstrapped the entire data and calculated the probability of stops to occur anywhere in the trajectory per resampled set. Subsequently I bootstrapped data in bins of 3° steps dependent on the angle between targets perceived by the locust. I calculated the probability of stop frames per bin by the proportion of stop to all frames that occurred per bin. Comparing the occurrence per bin to the general occurrence of stops at any position allows the evaluation if stopping is overrepresented at certain angles, e.g. if locusts are more likely to stop at the bifurcation angle.

Acknowledgements

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Supplementary Material

Section S1: Pilot experiments on setup and stimulus design

For stimulus design and experimental setup conditions, I ran pilot experiments. Kennedy (1937) described locusts to be very active at high humidity levels, but did not describe them as particularly undirected. I found locusts to show a rather undirected, circling behavior at high humidity, and to be calm but still attracted to posts at dry conditions, relative humidity below 23 %. Based on Wallace' (1958) findings on attractive posts, I designed three-dimensional, straight, vertical black posts with a high perimeter-area ratio as stimuli. I tested different sizes of stimuli and different distances. I started with posts of 1 m radius at 5 m distance and found locusts to lose interest in rather far stimuli. Sobel (1990) described similar reactions of locusts losing interest in objects that seemed to be very far away. I found locusts to be attracted to targets presented at 2 m distance with a radius of 0.2 m and 100 m height. I tested if locusts were attracted to posts differently when fed ad libitum or starved for 1, 2 or 3 days and could not find differences. This is coherent with former investigations (Dkhili *et al.*, 2019; Ellis, 1951).

Figure S1: Locust trajectories of two-choice experiments
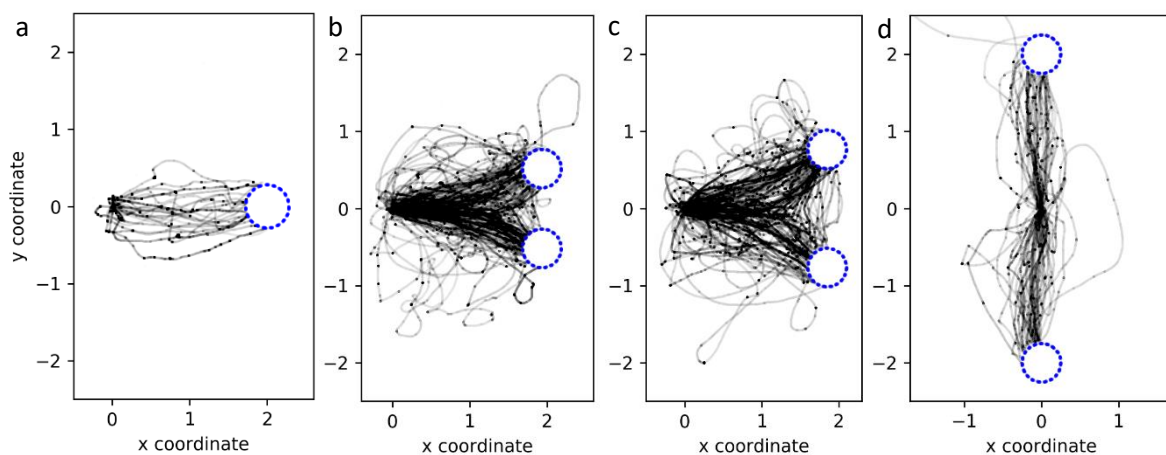

Figure S1. Locust trajectories walking towards one post control (a) and two posts at different angular conditions (b - d). Locusts showed a bifurcation pattern when approaching two posts. Trajectories of locusts walking to posts presented at 2 m distance at 30° (b), 45° (c) and 180° (d). Blue dotted lines are representing post positions.

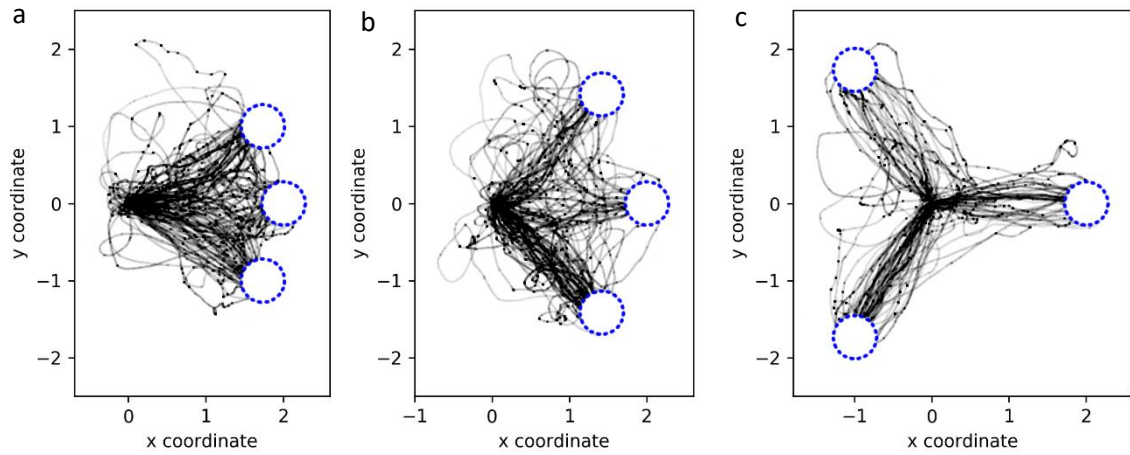## Figure S2: Locust trajectories of three-choice experiments

Figure S2. Locust trajectories walking towards three posts at different angular conditions (a-c). Locusts showed a multi-bifurcation pattern when approaching three posts. Trajectories of locusts walking to posts presented at 2 m distance at 30° (b), 45° (c) and 120° (d). Blue dotted lines are representing post positions.

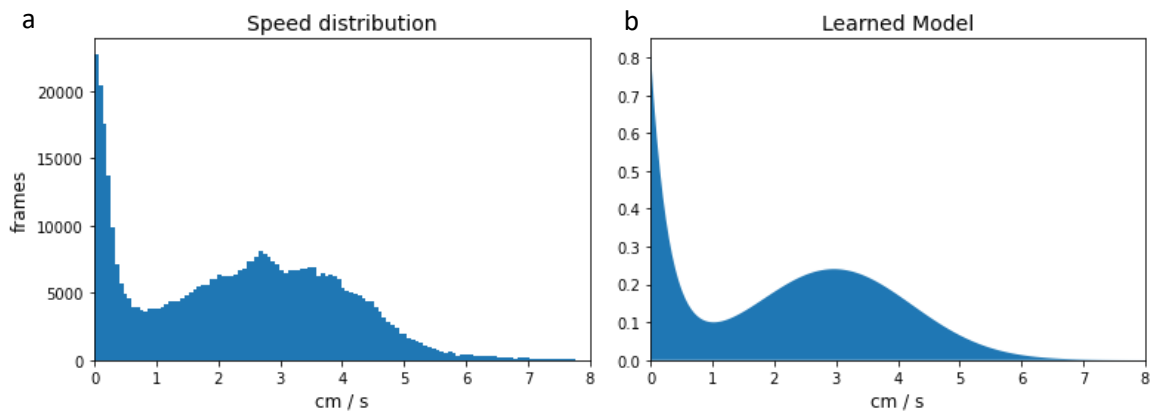## Figure S3: Locust speed distribution and speed classification

Figure S3. (a) Speed distribution of locusts presented with two posts and (b) respective learned general mixture model. To classify locust speed as stop or movement, a general mixture model with exponential and normal distribution was applied on the experimental speed data. The learned parameters then classified all frames to belong to one of the distributions, I considered these stop and movement frames.

Figure S4: Locusts' stops are associated to angular shifts in walking direction
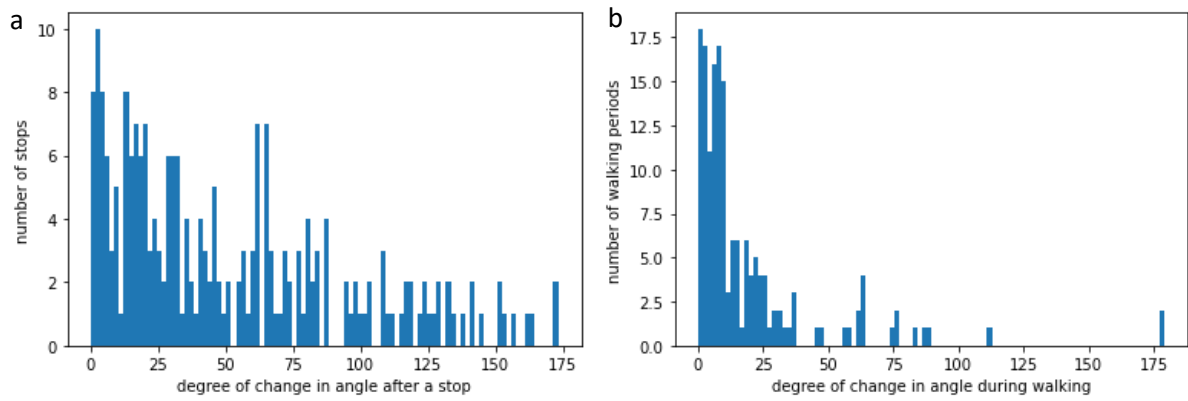

Figure S4. Locusts' stops are associated to angular shifts in walking direction. (a) Stops were associated with a median angular shift of 38.2° in the one post control setup. (b) In 150 frame-long walking periods, locusts showed a median angular shift of 9.2°.

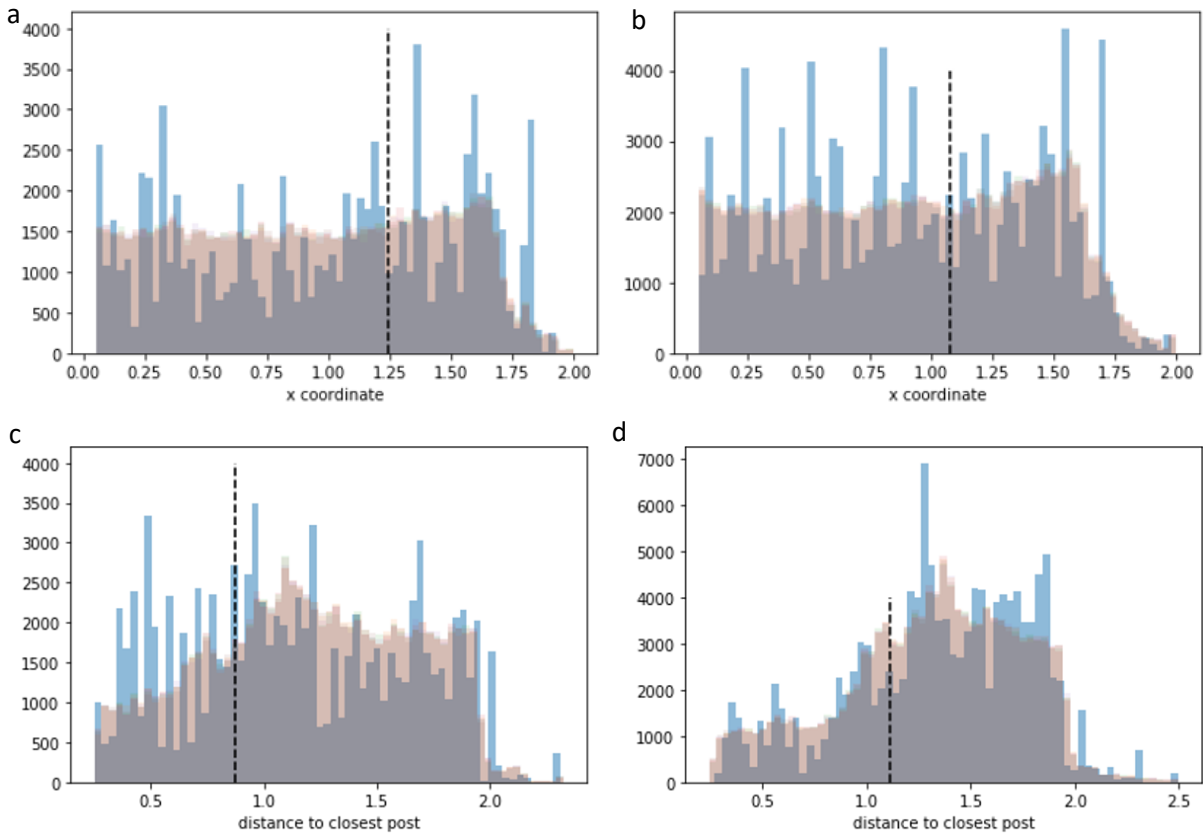Figure S5: Stop frame distribution relative to the x coordinate in trajectories and closest post

Figure S5. Stop frame distribution in relation to (a, b) the x coordinate in trajectories and to (c, d) the closest post from the locust's position. Stop frames are depicted in blue, brown shows bootstrapped data of all frames, sample size equal to respective stop frame size. Dashed lines indicate the bifurcation. (a, c) 30° between posts. (b, d) 45° between posts.

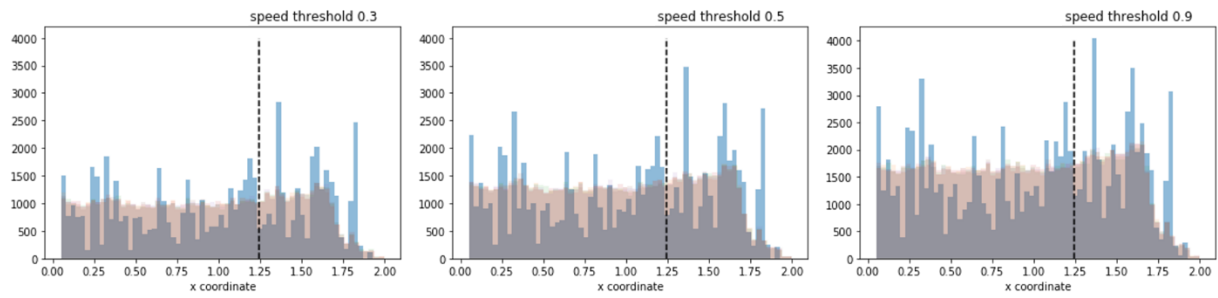Figure S6: Stop frame distribution is robust to different thresholds of stop frame classification.

Figure S6. Stop frame distribution is robust to different thresholds of stop frame classification. Arbitrary thresholds (0.3, 0.5, 0.9 cm/s) do not change stop distribution on the x-coordinate of trajectories. Stop frames are depicted in blue, brown shows bootstrapped data of all frames, sample size equal to respective stop frame size. Dashed lines indicate the bifurcation.