Using Micro-robots as a Tool for Insect Behavior Studies

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Abstract— **This paper presents a multidisciplinary experiment setup inspired on the LEURRE project where robots are used for animal behavior studies in a robot/animal mixed-society. Crickets of the species *Gryllus bimaculatus* are let interact with micro-robots equipped with decoys or lures. Unlike previous work, here we use the robots as tools focusing on the insect study itself rather than attempting to design an autonomous robot that mimics cricket behavior. A set of experiments is performed demonstrating the new ways in which even simple open-loop robot movement playback can be successfully used for animal behavior exploration. These experiments focused on the comparative study of the escaping behavior of dominant and subordinate male crickets after a dominance dispute is settled. Results are shown and discussed.**

I. INTRODUCTION

Social behavior studies require the observation of elaborate biological agents interacting among themselves and with the environment resulting in a system of difficult intertwined complexity. Consistent controllability and repeatability is often required in order for one to obtain probabilistically reliable results. In what regards to social insects, Halloy *et all* have recently shown in the LEURRE project how miniature robots can help on the study of the social behavior of cockroaches [4]. Inspired by that work, this paper presents a similar attempt to the use of micro-robots for the behavioral study of field crickets. This work focus on the potential of using such multidisciplinary setup for as a tool for insect behavior research.

Field crickets are among the most widely studied non-social insects. Despite the lack of sophistication of their neural anatomy, crickets show some social behaviors similar to those found in mammals, birds and reptiles, sometimes even reminiscent of behaviors observed in species of much higher cognitive capacity such as dolphins, apes or humans (e.g. agonistic dominancy disputes, female calling rituals). On the other hand, if compared to mammals, crickets are arguably less complex animals given their simplified neural anatomy and body construction, making their study more tractable. Crickets are also excellent subjects for studies on animal behavior because they can be easily found around the globe and because of the ease with which they can be handled both in the lab and in the field.

By unlashing such prototypical behaviors in terms of their causing stimuli and underlying neural circuitry one hopes to better understand fundamental principles that may relate to similar behaviors found more complex animals, including humans.

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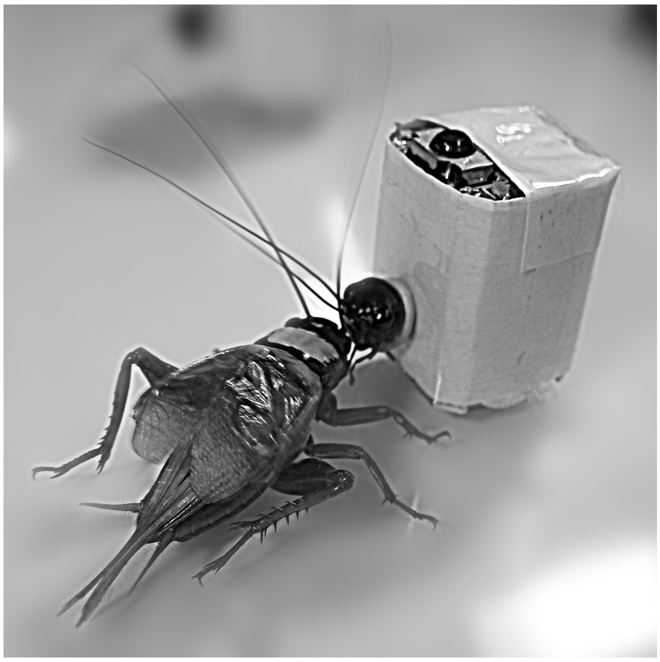


Fig. 1. Cricket and robot interacting.

Male crickets of the species *Gryllus bimaculatus* DeGeer were used for this study. Typically, when two or more male crickets encounter each other they engage in a kind of agonistic behavior, often involving aggressive stridulation and violent battles, from which a single winner is normally unambiguously defined while losers switch to a fleeing behavior [2]. It is believed that the perception of cuticular pheromones present on the body surface of male crickets is one important cue in the triggering of aggressive behavior. Subordinate crickets tend to flee when they perceive cuticular pheromone of another individual for up to about 60 minutes after loosing a fight. More recently there have been efforts for more rigorously defining the repertoire of isolated stimulus cues in different modalities along with their roles in the ethology of agonistic behavior in individuals of the species *Gryllus bimaculatus* [1]. The study here presented extends that work examining the fleeing behavior of both dominant and subordinate crickets when isolated and exposed to a third unknown agent just after the dominance dispute was over.

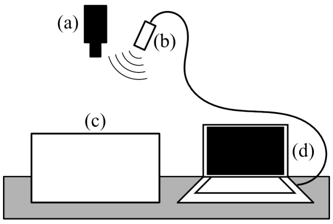


Fig. 2. Sketch of the bench setup used for the experiments. (a) camera, (b) infrared-transmitter, (c) arena, (d) computer. A computer program played a pre-programmed pattern of commands that were sent to the robots using an infrared transmitter. The camera was used for recording all trials for later tracking analysis

II. EXPERIMENT SETUP

The experiment setup used in this work is illustrated in Fig. 2. A laptop equipped with an infrared transmitter was used for controlling a robot's movement while an overhead camera recorded each trial. Fig. 3 shows a top view of the arena – the rectangular area inside which all the experiments were performed. The experiments were designed focusing on how the crickets behaved after a fight when exposed to a new external agent. More specifically we looked for subtle changes in the way subordinate and dominant might differ in their confronting, avoiding, or escaping behaviors and we also looked for changes on how these crickets react to different kinds of new agents.

Usually the biologist can only observe animal's movement, but can't control it. Letting male crickets interact with robots as their opponents rather than using other crickets allowed us to control and rule out the influences of spontaneous opponent movements. Playing back the same sequence of movements at each trial and comparing results from trials that used robots with and without a living upper torso attached we could focus on the influence of the pheromones, and other sensor cues. Attaching the upper-torso then allows to control the movement of the agent while keeping characteristics such as pheromones, antennas fencing, mandible flare, and keeping to some extent also the visual cue of the general silhouette of the insect when observed from certain angles. In each trial an external agent was added, where this agent could be a robot, a cricket, or a robot with a living upper-torso of a cricket attached to it. The robots performed pre-recorded movements played in sequence without controlled feedback – therefore their trajectories were subject to external disturbances due to surface irregularities on the floor, irregular friction, contact with the crickets among other things.

A. Cricket

The animals were reared in plastic cases ($80 \times 45 \times 20$cm) on a 14h:10h light and dark cycle at 28 ± 1°C, $75 \pm 2\%$ humidity. They were fed a diet of insect food pellet (Oriental Yeast Co., Tokyo, Japan), chopped carrot and water ad libitum. Adult sexually mature male crickets that were between 8 and 21 days after they imaginal molt were used in this study. To avoid the effect of copulation on the agonistic behavior, crickets were individually housed in transparent containers for at least 24 hours before the experiment, where they could potentially see, hear and smell conspecifics but could not get involved any kind of tactile interaction.

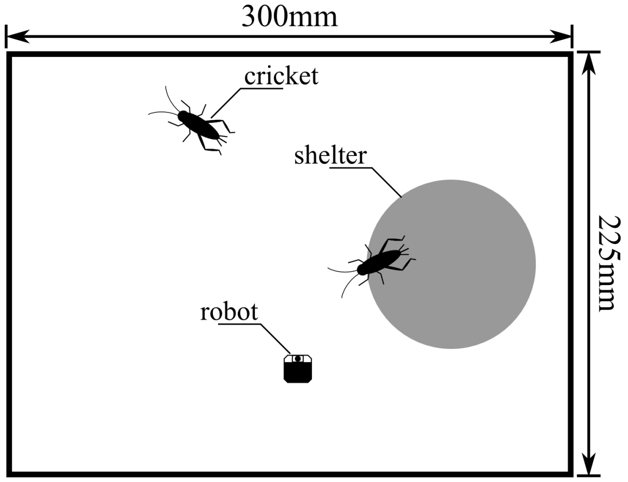


Fig. 3. A top-view illustration of the arena setup for the experiments.

B. Robot

Originally designed for an educational robotic competition [3], the micro-robots borrowed for this setup are of compatible size of that of a typical cricket of the species *Gryllus bimaculatus*. In Fig. 1 a cricket interacts with a robot wrapped with a paper shell to which the head of a dead male was attached. The robot has dimensions of $18 \times 18 \times 22mm$, is driven by two differential wheels and has no sensors except for an infrared receiver used for receiving commands encoded into pulses of infrared light. Commands are pre-recorded in a computer and played in a loop causing the robot to move without the use of any feedback. A small series of small random movements are performed before each repetition in order to disturb the trajectory thus avoiding systematic preference towards specific paths.

The main robot components are (numbers in accordance to Fig. 4):

1) Motor – Customized from wristwatch motor unit for higher torque, this micro-stepper motor was originally designed for adjusting auto-focus in miniature camera/lens mechanisms such as those included in mobile phones.
2) Battery – Miniature one-cell rechargeable $3.7V$ lithium ion polymer battery with capacity of $65mAh$.
3) Control board – Currently based on the Microchip $8bit$ PIC18 family of microcontrollers, each robot comes equipped with a PIC18LF1220 which features 4kb of re-programmable flash memory.
4) IR sensor – An IR sensor is used in order to listen for commands from the PC. The sensor operates at the $40kHz$ bandwidth modulation (same of most home-appliance remote controls).

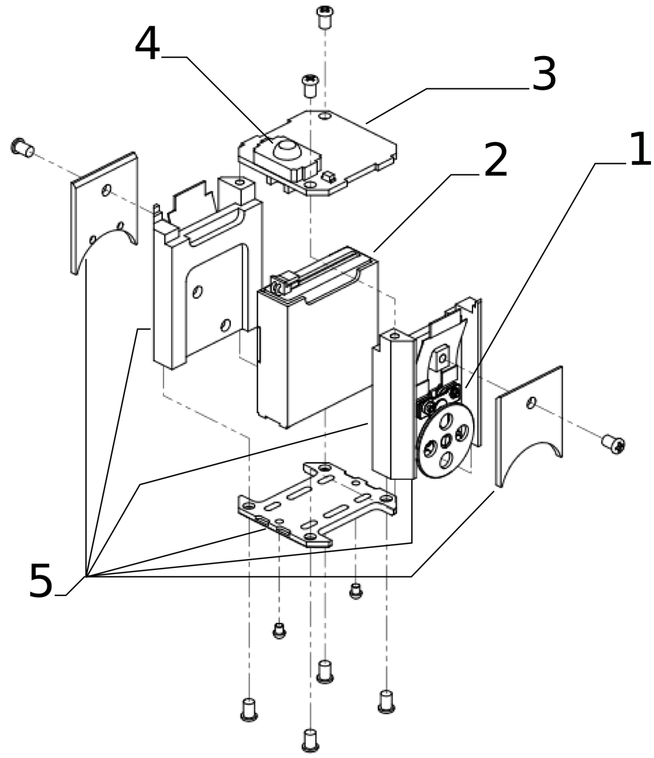


Fig. 4. Exploded view of the robot revealing its components

5) Body – The resistant durable body of the robot is micro-machined in aluminum using high precision CNC machines.

C. Arena

The arena was a rectangular area of dimensions 300×225mm delimited by transparent acrylic walls of 150mm of hight, separating it from the external environment of the lab.

D. Shelter

In the field crickets protect themselves from predators and other threads by hiding in burrows (often excavated by themselves) [2]. We hypothesized seeking darker environments to be an important factor in their strategy for determining an appropriate shelter. In our preliminary studies we designed a single shelter as a circular area delimited by a semi-transparent red disk of 120mm in diameter hanged 30mm above the floor so as to cast a shadow just bellow it but still allowing a top camera to track of the insects and robots underneath (depicted in Fig. 3). The dimensions of this shelter were defined so that the area was not large enough to accommodate more than one cricket without interaction with each other.

III. METHODS

A. Preliminary study

Initially a preliminary batch of tests was performed in order to determine feasibility of the robot/cricket mixed-society setup. The goal of this initial study was to explore the alternative setups and methodologies to be refined later into a more focused experiment setup. For this purpose an arena was equipped with a shelter as illustrated in Fig. 3. In each trial two new male crickets were let to freely interact with each other inside the arena for five minutes or longer until a dominant/subordinate relationship could be clearly observed, and then a third agent was added, where this third agent was one of the following:

1) Clean robot;
2) Another cricket;
3) Robot equipped with 8 artificial odorless antennas made of acrylic thread;
4) Robot equipped with a real amputated male cricket head (as depicted in Fig. 1);
5) Robot equipped with four real male cricket wings attached to each side;
6) Robot equipped with the living upper half of a male cricket body;

A tracking camera recorded all trials and the resulting footage was later processed for the tracking of both robots and crickets using SwisTrack [5].

B. Fleeing Study

After qualitatively analyzing results of the preliminary study the authors decided to abolish the shelter and focus only on the fleeing behavior of either dominant or subordinate in isolation. For this study again two male crickets were let to freely interact just as they did in the preliminary study, except that this time the arena had no shelter and the crickets just interacted long enough so that a clear dominant/subordinate relationship could observed. After that one individual (either dominant or subordinate) was removed and one of the following added in its place:

1) Clean robot;
2) Another cricket;
3) Robot equipped with the living upper half of a male cricket body;

Again a tracking camera recorded all trials and the resulting footage was later processed using SwisTrack [5]. This time each cricket as well as the robots were marked with a distinct color dye in order to facilitate the automatic tracking of the coordinates of their location.

IV. RESULTS

A. Preliminary Study

The preliminary study was performed as described in subsection III-A. From the preliminary study no preference for staying in the area shaded under the shelter could be noticed. The preliminary study also made clear how the overwhelming complexity of the interaction between the two original male crickets make it difficult to analyze the influence of robot behavior in isolation. Robot movements purposely included some randomness to it so as to allow the robot to stochastically cover a wide portion of the territory of the arena but crickets, on the other hand, showed a clear preference to staying near walls. Fig. 5 shows the

TABLE I

SIX TESTED GROUPS

	dominant cricket	subordinate cricket
robot with upper cricket torso attached	a	b
clean robot	c	d
new male cricket	e	f

TABLE II

MEAN OF MEANS ABSOLUTE ESCAPE DIST., MWW U-TEST $p(z(U))$

	b	c	d	e	f
a	43.2111%	22.0431%	4.8815%	57.5511%	14.9800%
b		61.7887%	51.2691%	48.4991%	38.2660%
c			50.1594%	28.5851%	77.1056%
d				13.0039%	82.7259%
e					11.6143%

TABLE III

MEAN OF MEANS RELATIVE ESCAPE DISTANCE, MWW U-TEST $p(z(U))$

	b	c	d	e	f
a	54.1193%	34.2782%	4.0057%	88.4574%	23.8646%
b		93.3739%	38.2733%	63.1171%	52.0051%
c			34.2782%	57.9991%	58.8909%
d				19.1362%	79.3428%
e					23.8646%

typical trajectories in a robot/cricket trial. In every batch of trials crickets spent in average more than 80% of their time wandering along the edge of the walls of the arena (less than 2.5cm from the wall edge).

B. Fleeing Study

The fleeing study was performed as described in subsection III-B. For data analysis, firstly all trials were separated in three different kinds depending on the type of agent lastly added to the arena: (1) robot with the upper torso of a cricket attached to it, (2) clean robot, (3) a new male cricket. Then each of these three kinds was further divided in two groups according to whether the subordinate or the dominant was the cricket left inside the arena after the dominance dispute. For easy reference the six groups were labeled with the alphabet letters from *a* to *f* as shown in Table I.

For every trial the raw trajectories from the recorded videos were tracked and a smooth operator with a window of 30 frames (roughly 1 secound) was applied. The resulting smoothed trajectories were then used for finding all disjoint intervals in which the distance between the two studied agents was less than 4cm. For each of those intervals the minimum distance between the two agents was then computed and that corresponding frame marked as a *touch frame*. Later for each touch frame a corresponding *touch interval* was computed, defined to start at the touch frame and finish at the frame in which the distance between both agents was more than 4.5cm and the velocity of the dominant or subordinate cricket crossed below the lower threshold of 2mm/s.

Finally for each escape interval two metrics were computed: (1) the final distance between the two agents at the last frame of the escape interval, and (2) the distance between the position the dominant or subordinate cricket was at the touch frame and the position it was at the last frame of the corresponding touch interval. For simplicity we will refer to each of these metrics respectively as *relative escape distance* and *absolute escape distance* during the rest of this work.

Statistics of the results were calculated in two different ways: (1) *average analysis* and (2) *mixed analysis*. The average analysis was performed by first calculating mean values for relative and absolute escape distances within each trial and then later comparing these mean trial values across the groups. This allows every trial to account to a same weight disregard of the number of touches computed. The drawback of allowing only a single sample value per trial is that statistical evidence becomes more difficult to be determined for such small samples. To cope with that problem the mixed analysis was performed by mixing all values from different trials of same group into a single bucket and later comparing this data across the groups. This allows a larger number of samples thus giving chance for more robust statistics analysis. By doing so data from all trials within a group ends up mixed together so that trials with larger number of touches offer stronger influence in the final results. Both average and mixed analysis results are plotted in the graphs shown in figure 6. As it can be seen from these graphs, there was a consistent tendency for subordinate crickets to escape further than dominants for all three kinds of trials, both in absolute and relative distances, with the exception of the results from the mixed analysis of groups *a* and *b* (right-hand side graphs of Fig.6).

In order to look for some habituation evidence the same mixed analysis was performed for the absolute distances but this time mixing only data from touches that happened within the first, second and third minutes of every trial. These results are plotted in the graphs of figure IV-B. These graphs seem to show some tendency for crickets to escape smaller distances over time when confronted with robots, while they increasing the escaping distances over time when confronted with other crickets.

For all 15 two-group permutations the Mann-Whitney U-test (MWW U-test) was performed in order to access the statistical significance of the changed features on the measured data for absolute and relative mean escaping distances in both average and mixed analysis. The null-hypothesis is that the data of absolute or relative mean escaping distances of two given groups are samples from a same population, i.e. the effects of the differing feature between the two compared groups was negligible. The approximate p-value associated with the found U-values was then computed assuming a normal distribution. The MWW U-test results of the average analysis are shown in Table II and Table III for mean absolute and mean relative escaping distance respectively. Similarly the mixed analysis MWW U-test results are shown in Table IV and Table V. Finally the habituation MWW U-test results are shown in Table VII.

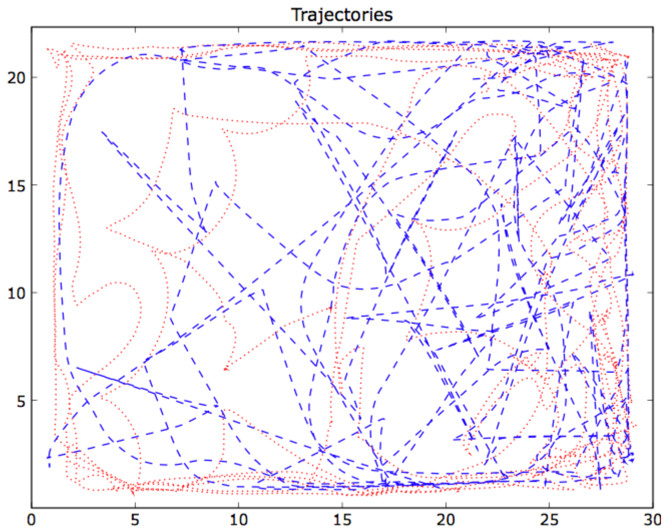
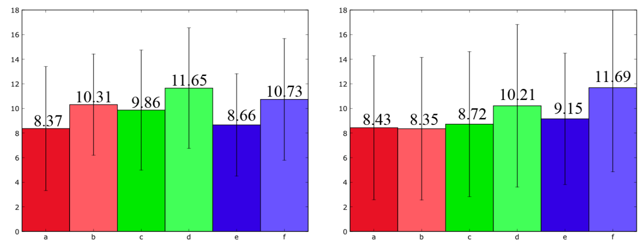
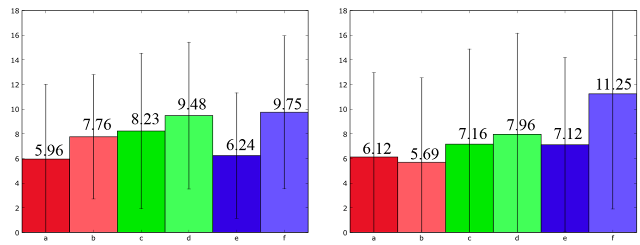


Fig. 5. Sample of trajectories showing a typical interaction of a cricket (dotted track) with a robot (dashed track). Notice how the cricket tends to follow the edges along the walls of the arena.



(a) Mean of means of the distance between agents after escape.

(b) Mixed mean of the distance between agents after escape.



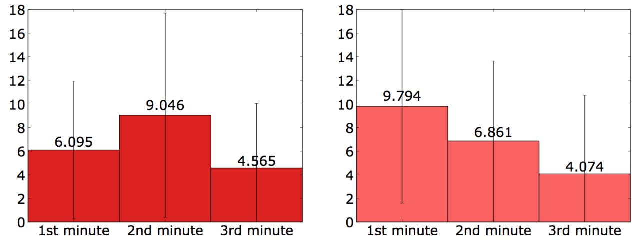
(c) Mean of means of the escaping distance.

(d) Mixed mean of the escaping distance.

Fig. 6. The two top graphs show respectively the average and mixed analysis of the escaping distance obtained by subtracting the position the subordinate or dominant cricket was at the moment of touch from the position it was after it first stopped moving after touch (velocity below threshold of 3mm/s). The two bottom graphs show respectively the average and mixed analysis of the distance between two agents after they touch each other, measured at the moment after the touch when the subordinate or dominant cricket first stopped moving (velocity below threshold of 3mm/s). The two agents are *a* dominant cricket and robot with upper torso attached (15 trials), *b* subordinate cricket and robot with upper torso attached (14 trials), *c* dominant cricket and robot (17 trials), *d* subordinate cricket and robot (15 trials), *e* dominant cricket and another cricket (15 trials), *f* subordinate cricket and another cricket (14 trials).

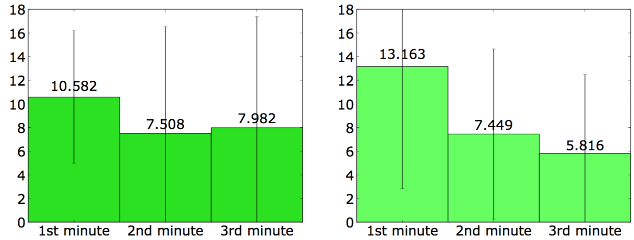
V. Discussion

The authors presented here an effective behavioral research framework based on the multidisciplinary mixing of micro-robots and insects. Although qualitative observation seems to confirm the hypothesis that subordinate crickets are more sensitive to external stimulus, the statistical significance was data was not conclusive. More trials are needed in order to determine the statistical significance based on mean trial results instead of mixing data from different trials within a same group. Further analysis of other metrics regarding the escaping behavior may also show interesting information. For future work the authors plan to combine the use of the



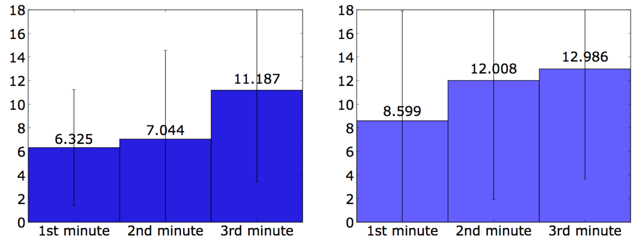
(a) Habituation in group *a*

(b) Habituation in group *b*



(c) Habituation in group *c*

(d) Habituation in group *d*



(e) Habituation in group *e*

(f) Habituation in group *f*

Fig. 7. Study of the habituation within each of the six groups. Here the mixed mean escaping distances are plotted for the touches that happened during the first, second and third minutes of every trial.

TABLE IV

Mixed absolute escape distance, MWW U-test $p(z(U))$

	b	c	d	e	f
a	33.0147%	65.8411%	38.5299%	25.8007%	0.0002%
b		36.4023%	16.7971%	3.3365%	0.0000%
c			61.1237%	50.9663%	0.0303%
d				96.8543%	0.5867%
e					0.0401%

TABLE V

Mixed relative escape distance, MWW U-test $p(z(U))$

	b	c	d	e	f
a	99.6309%	74.8794%	4.4171%	2.3561%	0.0003%
b		74.1647%	5.0258%	2.5362%	0.0012%
c			8.9959%	10.9708%	0.0123%
d				77.7851%	6.5190%
e					0.4777%

TABLE VI

ABSOLUTE ESCAPE DISTANCE HABITUATION, MWW U-TEST $p(z(U))$

Group A	2nd minute	3rd minute
1st minute	33.3265%	29.6169%
2nd minute		7.5076%
Group B	2nd minute	3rd minute
1st minute	25.7820%	0.8679%
2nd minute		19.8959%
Group C	2nd minute	3rd minute
1st minute	3.6479%	5.9375%
2nd minute		95.4829%
Group D	2nd minute	3rd minute
1st minute	15.8464%	2.8333%
2nd minute		53.9255%
Group E	2nd minute	3rd minute
1st minute	83.3184%	4.8341%
2nd minute		10.9745%
Group F	2nd minute	3rd minute
1st minute	18.4039%	14.6145%
2nd minute		68.3325%

TABLE VII

ABSOLUTE ESCAPE DISTANCE HABITUATION, MWW U-TEST $p(z(U))$

Group A	2nd touch	3rd touch	4th touch
1st touch	91.7411%	39.5158%	79.9991%
2nd touch		44.2877%	83.5780%
3rd touch			39.4098%
Group B	2nd touch	3rd touch	4th touch
1st touch	74.2557%	19.4702%	78.4846%
2nd touch		36.3722%	74.1182%
3rd touch			14.1482%
Group C	2nd touch	3rd touch	4th touch
1st touch	63.5256%	22.7425%	42.8014%
2nd touch		69.6270%	65.4721%
3rd touch			72.2339%
Group D	2nd touch	3rd touch	4th touch
1st touch	62.8609%	32.9114%	19.6706%
2nd touch		54.9626%	0.9130%
3rd touch			2.0638%
Group E	2nd touch	3rd touch	4th touch
1st touch	88.4574%	82.7259%	22.1700%
2nd touch		96.5189%	23.8646%
3rd touch			21.4758%
Group F	2nd touch	3rd touch	4th touch
1st touch	96.3352%	85.4178%	81.8295%
2nd touch		92.6781%	67.9219%
3rd touch			52.0051%

mixed-society setup here presented with online sampling of neural activity by using electrodes directly connected to the nervous system of the animals during the experiment. By doing so, the controlled repeatability allowed by the use of the robots will help on the behavioral study allowing the mapping of the corresponding neural activity captured from the insects in action.

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