Active Antennal Searching Suggesting Anticipatory Capability in Pill Bugs (*Armadillidium Vulgare*)

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Abstract
This research shows that pill bugs can actively search for stimuli anticipating elicitation of adaptive behavior in critical conditions. The bugs were placed respectively onto an annulus-shaped substrate surrounded by water. Small obstacles were introduced at regular intervals in the middle of the annulus. They moved along the edge of the water, touching the obstacles with their antennae repeatedly. Moving along the water is dangerous for them as they risk drowning. Under such a critical condition, they demonstrated an adaptive behavior: mounting the obstacles. We further investigated the time series of antennal touching time on the obstacles, which can be assumed to be related to searching of stimuli. The log-log plots of the cumulative frequency distribution of the touching times showed a power law distribution.
Keywords: Active antennal searching, Anticipatory capability, Pill bug, Power law

1 Introduction

Most behavioral patterns in pill bugs have been interpreted as reactions to stimuli received passively. However, if all types of behavior were solely caused by this mechanism, this animal would not survive in novel critical situations where active searching of stimuli becomes necessary to enable adaptive behaviors.

It has been experimentally demonstrated that pill bugs can display tentative adaptive behavior in critical situations [1-4] and that the antennae may play a significant role in obtaining external spatial information [5, 6]. However, active antennal searching has not been identified quantitatively because of the lack of equipment that can precisely observe every antennal movement of an ambulatory animal of millimeter size.

In this study, we report findings obtained by using new equipment: a 'micro-locomotion compensator' [7]. The experimental settings were the same as those in the previous research [3]. In the experiments, each bug was placed onto an annulus-shaped substrate surrounded by water (test condition) or acrylic wall (control condition). Small obstacles were introduced at regular intervals in the middle of the annulus.

In the test condition, the bugs moved along the edge of the water, touching the obstacles with their antennae repeatedly. Moving along the water is dangerous for the bugs as they risk drowning. Under such critical conditions, the bugs demonstrate adaptive behavior: mounting the obstacles. In the control group, mounting behavior happened rarely.

We try to analyze the time series of antennal touching time on the obstacles, which can be assumed to be related to searching of stimuli.

2 Materials and Methods

2.1 Subjects

About two hundred individuals were caught for main stock and kept in a plastic container with soil to a depth of 2 cm and an opaque lid. They were fed slices of carrot once a week. A moist atmosphere was maintained by wetting the soil every day. The lid was slightly opened, and the illumination -a fluorescent light having an intensity of 250 lx at a distance of 100 cm from the soil- was on from 10:00 hr to 17:00 hr. The temperature of the laboratory was kept at 23-25 °C; the humidity was at 40-50 %.

After two months, six individuals, each of which was 9-10 mm in length, were selected and placed one by one into petri dishes (6 cm in diameter, with a thin layer of wet soil).

Each individual was fed on a small piece of carrot for three days and on nothing for consecutive three days in order to equalize their digestive condition. The condition of atmosphere and illumination were the same as those for the main stock.

2.2 Apparatus

The experimental apparatus for test condition consisted of a black acrylic annulus (20 mm in width, 120 mm in internal diameter) surrounded by water (Fig. 1). That for control condition consisted of a black acrylic annulus surrounded by black acrylic wall.

Thirty two small black columnar acrylic obstacles (2 mm in height, 4 mm in diameter) were introduced at regular intervals in the middle of the annulus.

2.3 Micro Locomotion Compensator (MLC)

The MLC comprised a pair of motorized slides, a video camera with a microscopic lens, a video tracker and a PC (Fig. 1). The motorized slides were connected orthogonally each other, and the top table of the slides could be positioned with a precision of just micrometer.

A pill bug walking on the apparatus on the top table was observed by the video camera, and after image-processing at the video tracker, the position of the bug was reported to the PC at 60Hz.

The PC controlled the pair of slides to return the bug to the previous position, so the bug was maintained at a position just under the video camera.

2.4 Position Measuring System

A pill bug walking on the apparatus on the top table was also observed by the micro video camera (Fig. 1) at 30 Hz and stored in the PC as image files.

Positions of top of the antennae in each file were measured by the position measuring software.

2.5 Experiment

A small piece of reflective sheet was pasted on the back of the individual to be detected by the video camera. Each individual was placed onto the annulus of the apparatus. The annulus was surrounded by water for the water group (n=3) and by acrylic wall for the wall group (n=3). They were left for two hours.

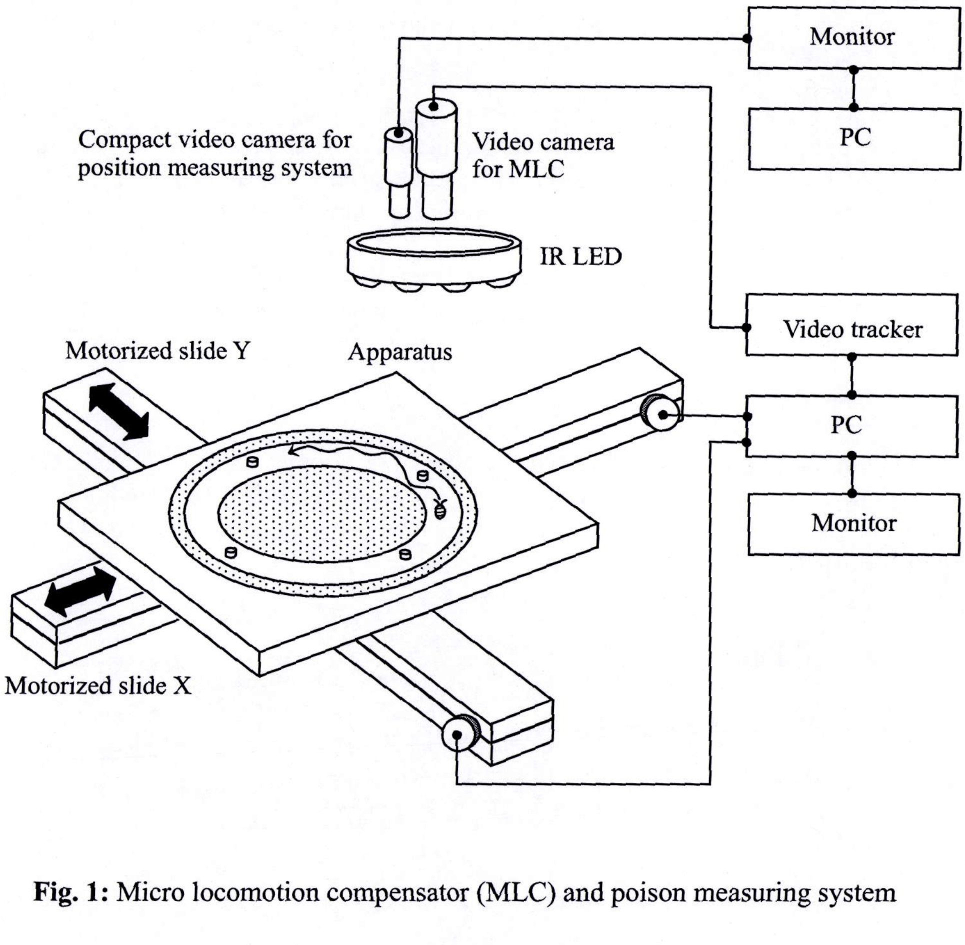


Fig. 1: Micro locomotion compensator (MLC) and poison measuring system

On the annulus, the temperature was 24-25 °C; the humidity was 50-60 % in the test condition and 40-50 % in the control one. Infrared light was illuminated from the IR LED (Fig. 1).

3 Results

3.1 Categorization of Behavioral Patterns

In each individual, behavioral patterns to the obstacles in for five minutes after 0, 20, 40, 60, 80 and 100 min from the beginning of the experiment were investigated.

In both groups, individuals moved most of the time along the outer wall or water with their one antenna touching the wall or water and another one touching the obstacles. We call this touching obstacles by one antenna as 'one-antennal touching' (Fig. 2 (a)).

We sometimes observed a sequential behavioral process that the individual oriented its head to an obstacle (Fig. 2 (b)) after one-antennal touching, mounted the obstacle and stayed for a while with its antennae circling (Fig. 2 (c)). We call this sequential pattern consisting of mounting and staying on an obstacle as 'mounting'.

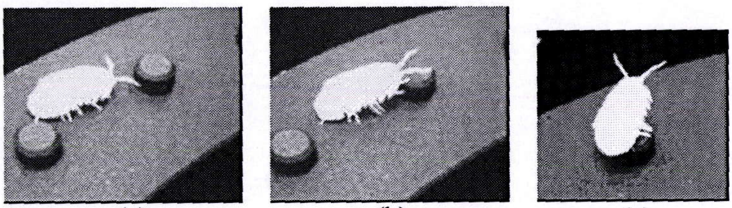
3.1.1 One-Antennal Touching

One-antennal touching was a popular behavior in both groups. Time sequential change of total number of the behavior in each group was shown in Table 1. Total number for the wall group was almost significantly larger than that for the water group (U=0, p=.05).

In both groups, it decreased gradually from 0 to 100 min (Wall group, R^2=0.861, $p<.01$; Water group, R^2=0.945, $p<.005$). This tendency is the same as that in the previous study [4].

3.1.2 Mounting

Mounting was a specific behavior for the water group. Time sequential change of total number of mounting in each group was shown in Table 1. It appeared only once in the wall group and total number of the behavior for the water group was almost significantly larger than that for the wall group (U=0, p=.05).



(a) (b) (c)

Fig. 2: (a) One-antennal touching, (b) Orientation, (c) Mounting to an obstacle

In the water group, it increased rapidly from 0 to 20 min and decreased gradually after that (R^2=0.797, $p< .05$). In the previous study, it was shown that the frequency of the mounting rapidly increased and reached a peak at 33 min after the beginning of the experiment [4]. From these results, we assumed that there is a peak between 20 and 40 min in the present experiment.

Mounting is considered as tentative adaptive behavior in this experimental condition because it decreases time to encounter water which is dangerous for the bugs as they risk drowning. In this paper, we define the first period (0 min) as 'initial phase', the second and third ones (20 and 40 min) as 'adaptation phase' and the rest (60, 80 and 100 min) as 'de-adaptation phase', and call them as the first, second and third phase respectively.

3.2 Power Law Distribution of the One-Antennal Touching Time

As individuals in the water group were motivated strongly to escape from the annulus, we assumed that they tried to search stimuli to express novel adaptive behaviors in the first phase.

As a result, they learned to choose the obstacles as a stimulus to express mounting as an adaptive behavior in the second phase. In this phase, one-antennal touching became a releaser to express mounting. However, frequency of mounting gradually decreased in the third phase.

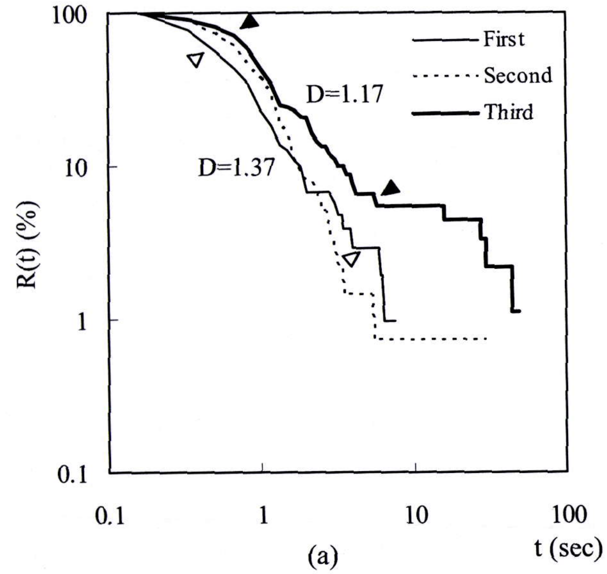
From these results, we assumed that the function of the one-antennal touching was different among three phases and distributions of touching times to the obstacles by one-antennal touching were analyzed.

Table 1: Total numbers of one-antennal touching and mounting for 5 min-periods after 0, 20, 40, 60, 80 and 100 min after the start of the experiment

Behavioral pattern	Group[a]	Phases						Total
		First	Second		Third			
		0min	20min	40min	60min	80min	100min	
One-antennal touching	Water	105	76	64	38	28	24	335
	Wall	224	212	184	202	161	140	1123*
Mounting	Water	4	26	12	16	4	4	66*
	Wall	0	0	1	0	0	0	1

[a]$n = 3$ for each group

*$P = .05$



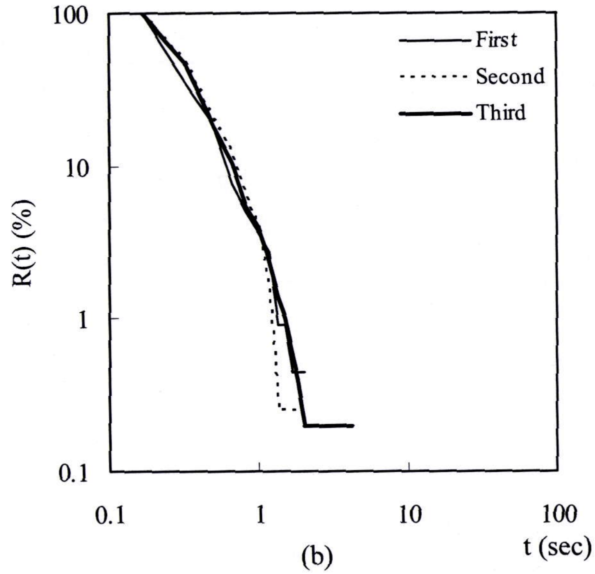


Fig. 3: Cumulative percentage (R(t)) of one-antennal touching time (t) in log-log plot for the first, second and third phases in the water (a) and wall groups (b). Pairs of white and black triangles indicate linear sections. D is the absolute value of the slope for the linear section.

Time series data of touching times to the obstacles by one-antennal touching were obtained in each phase. The total number of the behavior was 105, 140 and 90 in each phase. The touching time frequency histogram was re-plotted into a cumulative percentage of number of the behavior longer than the abscissa in a log-log plot (Fig. 3a).

The distribution, characterized by a linear section between 0.500 and 4.83 sec, shows power law with a slope of -1.37 (R^2= 0.984) on the plots for the first phase. On those for the third phase, the distribution, characterized by a linear section between 0.667 and 6.01 sec, shows power law with a slope of -1.17 (R^2= 0.988). For the second phase, such a linear section was not observed.

In the wall group, there was no linear section in the plots for all phases (Fig. 3b).

4 Discussion

The cumulative frequency distribution of the one-antennal touching times in the first, second and third phase was plotted in a log-log chart to investigate time dependent change in the distribution.

In the wall group, distributions in all phases were exponential. In the water group, the distribution in each phase was power law, exponential, and power law respectively.

The same kind of aspect has been shown in the distribution of food dwelling time in the feeding behavior of flies [8]. This study showed power law distribution immediately after the subjects were placed onto the test plate, exerting all their efforts for searching and sampling to adapt to the new situation. As time elapsed, they began to behave in a characteristic manner described by an exponential distribution, which may indicate an adaptation; power law distribution behavior wasn't observed afterward.

For pill bugs, power law distribution in the first phase implies that they search stimuli to express novel adaptive behavior. Exponential distribution in the second phase implies that they learned to choose the obstacles as a characteristic stimulus to express mounting.

Power law distributions in the third phase, of which the value of slope is different from that in the first phase, implies that they autonomously identified the situation as novel again and began searching for stimuli anticipating elicitation of new adaptive behavior.

5 Conclusion

We investigated the time series of antennal touching time on the obstacles, which can be assumed to be related to searching of stimuli. The log-log plots of the cumulative frequency distribution of the touching times showed a power law distribution. This result shows that pill bugs can actively search for stimuli anticipating elicitation of novel adaptive behavior, mounting, in critical conditions.

From the perspective of phylogeny, our research enhances the knowledge of computing anticipatory systems.

Acknowledgements

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