Estimation of elapsed time by ants

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Abstract

Myrmica sabuleti workers submitted to visual operant conditioning 'learned' to estimate time lapses of 5, 10, 15 minutes (training based on food delivering) and of 20 minutes (training based on food retrieving).

Keywords: Behavior; *Myrmica sabuleti;* operant conditioning.

Introduction

Insects can be ethologically conditioned and have been submitted to classical and operant conditioning. Experiments have been performed, among others, on *Drosophila* (ISABEL et al., 2004; CHABAUD et al., 2009), bees (PHAM-DELÈGUE et al., 1990; DECOURTYE et al., 1999; DEISIG et al., 2001, 2002) and ants (JAISSON, 1980; DE CARLO & ABRAMSON, 1989; CAMMAERTS, 2004 a, b; DUPUY et al., 2006; GUERRIERI & D'ETTORRE, 2010). Although this learning ability is recognized and even applied in physiological studies, very little is known about a hypothetical notion of elapsed time in insects. Clearly, such a notion would differ considerably according to the species, its activity and life expectancy (FERRON et al., 1990).

Temporal learning has been demonstrated in *Myrmica sabuleti* MEINERT 1861(CAMMAERTS, 2004 a): the response was very precise, whereby the ants came to the food site precisely at 01:00. A similar, independent study was conducted later on the same species, where the foragers were required to be at the food site from 20:00 until 20:05. Again, the ants responded correctly and precisely (DARRAS, personal communication). Moreover, during that study, the ants 'recognized' that the due time was approaching: they became more numerous at the nest entrance a few minutes before 20:00; they then walked towards the food site at 20:00. They left the site 5 minutes later.

CAMMAERTS & RACHIDI (2009) performed differential olfactory learning experiments on *M. sabuleti*. This involved depositing one kind of cue at a time at one place, then retrieving that cue and depositing another kind of cue at another place 3 to 5 minutes later. The result was that the ants left the first place when the cue was retrieved and moved towards the other place 3 to 5 minutes later, precisely when the second kind of cue was delivered there. The ants were apparently able to estimate a time lapse of a few minutes.

The recruitment behavior of bees indicates that this species is also able to estimate such 'elapsed time' (CHAUVIN, 1954; SCHNEIDER et al., 1986; CNAANI et al., 2003, references therein).

We investigate this issue in greater detail by performing a simple, yet lengthy and meticulous experiment on twelve *M. sabuleti* colonies, maintained in the laboratory, using collective operant visual conditioning.

Material and methods

Ant collection and maintenance

The experiments were performed on twelve colonies, six having been collected at Höhes Martelingen (G D Luxemburg) and six others in two abandoned quarries in the Aise valley and at Linglé (Belgium, Ardenne Region).

The twelve colonies were arranged in three series (1 to 3) of four colonies (named A to L). These colonies were demographically similar, each containing a queen, brood and about 500 workers. They were maintained in the laboratory in artificial nests made of one to three glass tubes half-filled with water, a cotton-plug separating the ants from the water. The glass tubes were deposited in trays (37 cm × 52 cm × 8 cm) whose

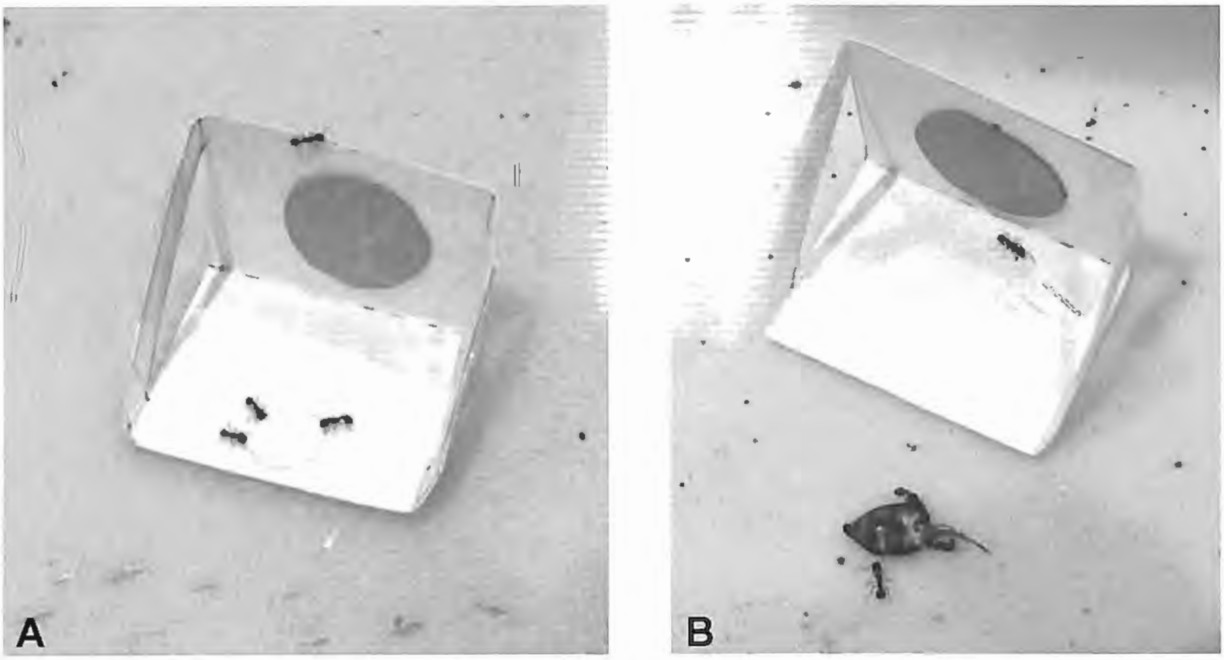

Figure 1. Experimental design and apparatus. **A**: training of ants using an experimental apparatus provided with sugared water as a reward. **B**: ants pulling away a delivered piece of cockroach from an experimental apparatus, a few minutes before the retrieving of that food, in the course a fourth training experiment.

borders were covered with talc. The trays served as foraging areas, food being delivered to them. Conditioning and testing the ants took place on the floor of these trays.

Temperature was maintained ca. 20 ± 2 °C and humidity at about 80%, both optimal values for the studied species. Lighting intensity was usually ca. 600 lux, but 10,000 lux while feeding and testing the ants.

A saturated solution of brown sugar was permanently offered in a glass tube plugged with cotton, except during training when sugared water was used as a reward. Pieces of dead cockroaches were provided twice a week, except during training when meat was used as a reward.

Experimental apparatus

Ants were conditioned and tested using an experimental apparatus built of white extra-strong paper (Fig. 1, A, B). Identical but separate apparatus were used for conditioning and testing. Each colony had its own training and test apparatus.

These apparatus consisted of a piece of paper (4 cm × 6 cm) folded at 90° in order to present a horizontal and a vertical part (4 cm × 3 cm) (Fig. 1). Attached glued paper supports helped maintain the configuration of the apparatus (Fig. 1, A, B). A circle (diam. 2 cm) of green strong paper (Canson®) was glued in the middle of the vertical part of the apparatus. A cover glass slide (1.8 cm × 1.8 cm) was deposited on the horizontal part, the reward being delivered on that glass slide during training (Fig. 1, A). No reward was given during the test.

The reward consisted either of 0.2 ml sugared water (saturated solution) (Fig. 1, A) or a piece (about 0.5 cm^3) of dead cockroach (Fig. 1, B), depending on the kind of experiment performed.

Experimental protocol

Two types of experiments were conducted, one using sugared water as a reward, the other using pieces of cockroach. For each type, three experiments were performed, each one on a series of 4 colonies. These three experiments differed in the elapsed time the ants had to learn. Since ants may memorize learned elapsed times, these periods changed between the first and the second types of experiments. Receiving sugared water as a reward, ants of the first series (colonies A, B, C, D) were required to learn an elapsed time of 5 min, ants of the second series (colonies E, F, G, H) a 10 min period, and ants of the third series (colonies I, J, K, L) 15 min. Then, with the cockroach reward, the first ant series (A-D) were required to learn an elapsed time of 10 min, the second ant series (E-H) a time of 15 min, and the third ant series (I-L) 5 min.

Table 1. Mean numbers of ants present on the experimental apparatus at times *ta* (food deliverance), *tb* (food retrieval), *tb'* (1 min after meat retrieval), *tc* (5 min before meat retrieval), *to* (times other than *ta*, *tb* and *tc*). For *ta, tb, tb'*: mean of 5 values; for *tc*: mean of 10 values; for to: mean of 41, 51, 61 or 71 values depending on experiment. *: significantly larger or smaller than mean number at *to*. (*): significantly larger (instead of smaller as expected) than mean number at *to*. NS: not significantly different from mean number at *to*. Level of probability = 0.05.

reward	tested colonies	test	to	ta	tc	tb	tb'
sugared water	A, B, C, D	1	12.6	19.6 *		10.4 *	
		2	18.3	22.8 *		13.4 *	
	E, F, G, H	1	11.7	18.2 *		4.2 *	
		2	14.5	26.2 *		7.0 *	
	I, J, K, L	1	8.1	16.0 *		5.2 *	
		2	14.5	25.0 *		14.2 NS	
meat	A, B, C, D	1	1.0	2.8 *	1.2 *	0.0*	0.2 *
		2	2.2	4.4 *	6.4 *	2.0 NS	1.6 *
	E, F, G, H	1	4.5	10.0 *	7.1 *	3.6 *	1.8 *
		2	2.2	9.4 *	8.0 *	2.8 NS	2.4 NS
	I, J, K, L	1	4.0	7.4 *	8.8 *	6.4 (*)	5.8 (*)
		2	4.7	9.2 *	6.7 *	6.0 (*)	4.6 NS

An entire experiment consisted successively of a control, six first training (= conditioning) experiments, a first test, six second training (= conditioning) experiments, and finally a second test.

Ant conditioning

The six first and second training experiments were made one per day, at different times to avoid temporal learning (possible in this species, Cammaerts, 2004 a).

A training experiment (simultaneously performed on four colonies) consisted of:

- firstly placing the experimental (training) apparatus onto the foraging area of the four used colonies at a given time of day;
- then waiting the elapsed time that the ants of these four colonies were required to learn;
- immediately thereafter, delivering, onto the experimental apparatus, on the cover glass slide, the adequate reward (sugared water/piece of cockroach);
- from that moment, waiting for exactly 20 min;
- thereafter promptly removing the reward and the experimental apparatus from the foraging area of the four used colonies, gently putting back on their tray those ants present on the removed objects.

Ant testing

Each control, first and second test were performed as follows:

- the experimental (test) apparatus were deposited on the foraging area of the four used colonies (no reward given here);
- then, immediately, for each of the four used colonies, the ants present on the experimental apparatus were counted each 30 s. This was done without interruption, successively for: the elapsed time the ants had to learn (5, 10 or 15 min), the 20 min during which the reward had previously been given, and the 5 min following the time at which the reward and the apparatus were removed at the training sessions;
- thereafter, the test apparatus were removed from the foraging area of the four used colonies, and those ants present on the apparatus gently returned onto their tray.

Statistical analysis

The mean numbers of ants (of the four colonies used to perform one experiment) present on the experimental apparatus at the time at which food was delivered during training (ta = mean of numbers at ta – 30'' to ta + 1'30''; mean of 5 values), at the time at which food was retrieved during training (tb = mean of numbers at tb to tb + 2'; mean of 5 values) and, only when ants were trained with meat as a reward, during the five minutes preceding the time tb (tc = mean of numbers at tb – 5'to tb – 30''; mean of 10 values) as well as during the elapsed time 1 minute after tb (tb' = mean of numbers at tb + 1'30'' to tb + 3'30''; mean of 5 values) were compared to the mean numbers of ants present on the apparatus at times other than ta, tb and tc (i.e. mean of 41, 51, 61 or 71 values depending on the experiment), using the non-parametric 2 × 2 contingency table χ^2 test (SIEGEL & CASTELLAN, 1989).

Results

Control

Before any conditioning, only 1 to 4 ants were present, at a given time, on the experimental apparatus deposited, without reward, on the foraging area of the twelve ant colonies used. The mean number randomly varied over the course of time (Fig. 2, graph 1).

Learning with sugared water as a reward

Ants of the first series (colonies A-D) were more numerous (P < 0.01) on the experimental apparatus 5-6 min after the apparatus were deposited on the colonies' foraging area. After that, the ants were somewhat less numerous but still climbed onto the apparatus. Twenty-five minutes after the beginning of the test, i.e. at the time of food retrieval during training, they became less numerous (P < 0.01) (Fig. 2, graph 2; Tab. 1 upper part, A, B, C, D).

The numbers of ants of the second series (E-H) present on the experimental apparatus increased 10 min after depositing the apparatus on the foraging area (P < 0.01). Immediately thereafter it decreased, finally dropping down (P < 0.01) 30 min after this deposit, i.e. exactly when the reward was retrieved during training (Fig. 2, graph 3; Tab. 1 upper part, E, F, G, H).

In the third series (I-L), significantly more ants climbed onto the experimental apparatus 15 min after its deposit (P < 0.01). Immediately thereafter, fewer ants climbed on the apparatus. Then, suddenly, 35 min after the beginning of the test, significantly fewer (P < 0.01, first test) – or as many ants as at times other than the 'food delivering' and the 'food retrieving' times (NS, second test) – still remained on the apparatus (Fig. 2, graph 4; Tab. 1 upper part, I, J, K, L).

Learning with meat as a reward

The ants of the first series (A-D) present on the experimental apparatus became more numerous 10 min after the apparatus was deposited (P < 0.01). Their numbers declined until about 28 min after the beginning of the test. At that time, they again became very numerous (P < 0.01). After that, about 30 to 32 min after the beginning of the test (i.e. just after food retrieval during training), the ant numbers dropped drastically (P < 0.01; Fig. 2, graph 5; Tab. 1 lower part, A, B, C, D).

The ants of the second series (E-H) climbing on the apparatus became more numerous 15 min after apparatus deposition (P < 0.01). Numbers dropped again until about 30 min after test begin. At that time, numbers again increased considerably (P < 0.01). About 35 to 37 min after apparatus deposition (i.e. just after food was retrieval during training), they stopped climbing onto and even left the experimental apparatus. In fact, their numbers dropped below (P < 0.01, first test) or were similar to (NS, second test) those at times other than 'food deliverance', 'before food retrieval' and 'food retrieval' (Fig. 2, graph 6; Tab. 1 lower part, E, F, G, H).

In the third series (I-L), ant numbers on the experimental apparatus increased 5 min after apparatus deposition (P < 0.01), then decreased and again increased (P < 0.01) about 22 min after deposition. The numbers again decreased about 25 to 27 min after the initial presentation of the apparatus (i.e. after the time of food retrieval during training), but were not statistically smaller than the values at times other than 'food deliverance', 'before food retrieval' and 'food retrieval' (NS or statistically larger; Fig. 2, graph 7; Tab. 1 lower part, I, J, K, L)

Qualitative observations

During the training phase of those experiments using meat as a reward, after two to three training (conditioning) experiments, the ants pulled the delivered piece of cockroach away from the apparatus (about 5 min before the time that the experimenter removed that piece of cockroach) (Fig. 1, B). Note that while the present work was performed, 32 other ant colonies were maintained in the laboratory without being tested: none of them pulled the delivered meat away as quickly as that observed for the twelve trained colonies (e.g. 10 to 24 hours later in untested ants).

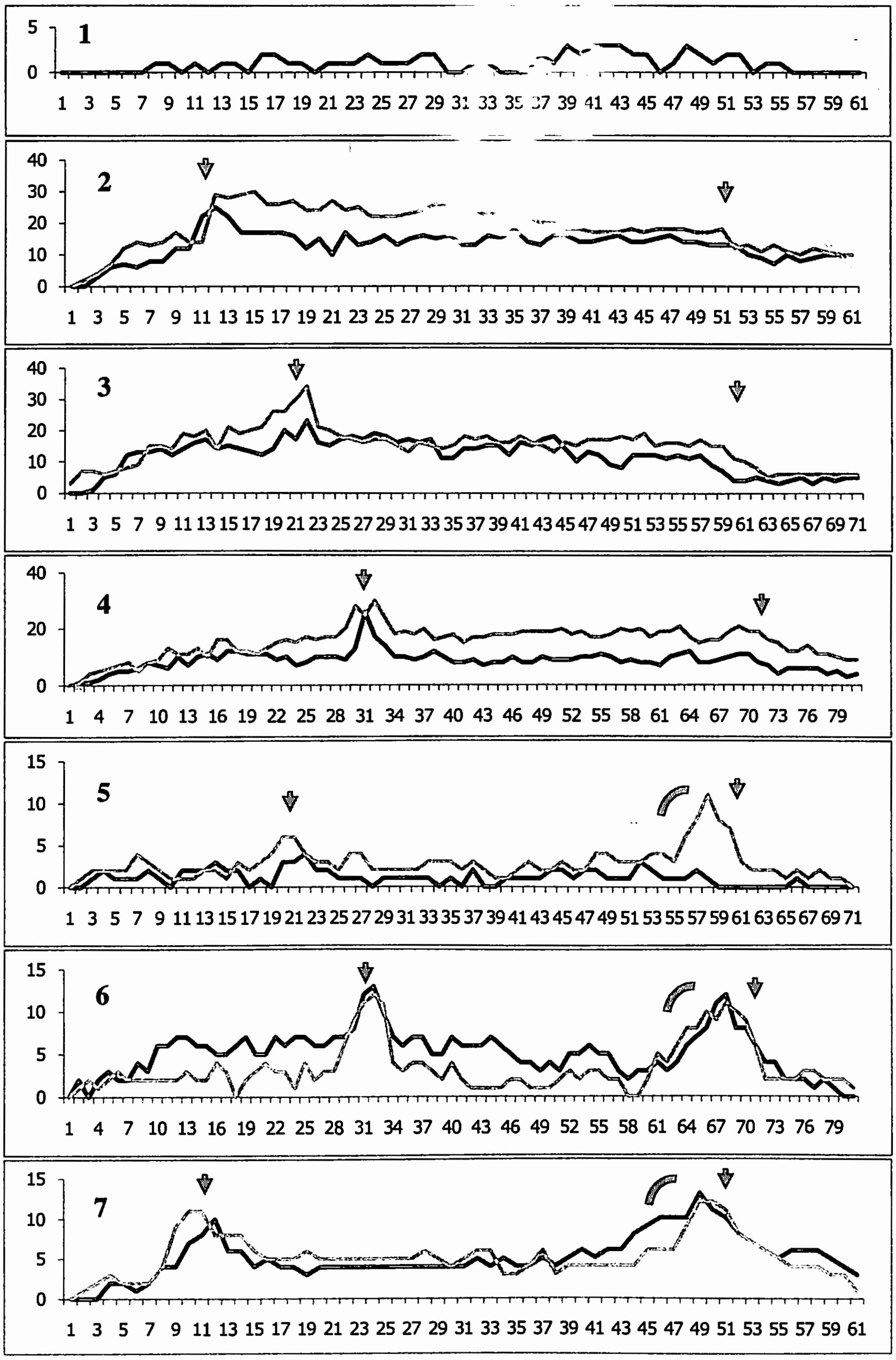

Figure 2. Mean number of ants (y-axis) present, in the course of time (x-axis: numbers of counts, one count each 30 sec, t = 0: deposit of the experimental apparatus) on the experimental apparatus during the experiments. Graph 1 = control experiment. Graphs 2 to 7 = test experiments. With sugared water as a reward, colonies A, B, C, D had to learn a 5 min elapsed time (graph 2), colonies E, F, G, H, a 10 min (graph 3) and colonies I, J, K, L, a 15 min (graph 4). With meat as a reward, colonies A, B, C, D had to learn a 10 min elapsed time (graph 5), colonies E, F, G, H, a 15 min (graph 6) and colonies I, J, K, L, a 5 min (graph 7). Left arrows: times at which food was delivered during training; right arrows: times at which food was retrieved during training. Arcs of circle: time periods preceding meat retrieving during training. Black curves: first test; grey curves: second test.

During training and testing experiments, foraging ants were often seen changing their direction of movement, moving towards the deposited apparatus and walking around it until the precise time at which food was (training experiments) or should have been (test experiments) delivered. At that precise time, they climbed onto the apparatus.

Conclusion – Discussion

Our results allow the conclusion that

- during the tests, ants were always more numerous on the experimental apparatus at the time corresponding to that at which, during training, they received food on these apparatus, than at the other times;
- during the tests, ants were also always more numerous on the apparatus at a time preceding that at which, during training, meat food was retrieved from the apparatus, than at the other times;
- during the tests, ant numbers on the apparatus dropped at the time corresponding to that at which, during training, the delivered sugared water was retrieved;
- during the tests, ants became progressively, and not immediately, less numerous on the apparatus a little later than at the time at which, during training, meat was retrieved. This delay is because many ants climbed onto the apparatus a few minutes before the time at which meat was retrieved during training;
- after a few training experiments using meat as a reward and during the following training experiments, ants pulled the meat away from the experimental apparatus a few minutes before its retrieval and transported it towards their nest. We consider such behaviour, never observed in not trained colonies, as being anticipation of an expected event.

The behaviour after the deposition of the experimental apparatus and before reward delivery underlines an anticipatory behaviour in some ants.

Ants thus apparently perceived and evaluated elapsed time and, unexpectedly, anticipated following events. Such abilities are clearly advantageous in situ. Certain events may occur at precise elapsed times after other events and may persist during rather precise time periods (f.i. the presence of dew). If they are able to evaluate such elapsed times, ants can come to the correct place at the correct time and leave when no further advantages can be expected. They can even exhibit appropriate behaviour before the occurrence of an event. Taking food away from the food site towards the nest avoids the loss of that food, which might otherwise be picked up by other animals, destroyed, relocated or covered by material if not transported very near the nest. In general, for any behaviour, elapsed time evaluation may improve animals' survival.

On one hand, the exhibition of such behaviour requires a previous training (or learning) phase. Does such a learned notion persist? On the other hand, we show here time elapsed estimation at a collective level. One next step would be to determine whether ants also have a notion of 'elapsed time' at an individual level.

Physiological mechanisms exist which allow an animal to evaluate elapsed time. This is proved by Pavlov's experiments on dogs and Ruch's experiments on rats (RUCH, 1931 and references therein). Such a phenomenon, however, has rarely been demonstrated in invertebrates, though circadian rhythms have been pointed out (PASSERA & ARON 2005). Bees probably have that notion (CHAUVIN, 1954; SCHNEIDER et al., 1986; CNAANI et al., 2003, references therein). SRINIVASAN et al. (2004) demonstrated the phenomenon in bees based on certain visual motor computations. However, this possible evaluation system does not explain how bees continue to correctly assess elapsed time when their movement is delayed or stopped (e.g. by wind, obstacles). The present experiments argue for a rather precise notion of elapsed time in ants, even without movement.

Acknowledgements

We sincerely thank Dr R. CAMMAERTS for improving the text and the figures. We feel once again indebted to Dr M. STACHOWITSCH for copyediting our English.

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