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Food collection and response to pheromones in an ant species exposed to electromagnetic radiation

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We used the ant species *Myrmica sabuleti* as a model to study the impact of electromagnetic waves on social insects' response to their pheromones and their food collection. We quantified *M. sabuleti* workers' response to their trail, area marking and alarm pheromone under normal conditions. Then, we quantified the same responses while under the influence of electromagnetic waves. Under such an influence, ants followed trails for only short distances, no longer arrived at marked areas and no longer orientated themselves to a source of alarm pheromone. Also when exposed to electromagnetic waves, ants became unable to return to their nest and recruit congeners; therefore, the number of ants collecting food increases only slightly and slowly. After 180 h of exposure, their colonies deteriorated. Electromagnetic radiation obviously affects social insects' behavior and physiology.

Keywords: alarm pheromone, area marking, food collection, *Myrmica sabuleti*, trail pheromone

Introduction

Our long-term goal is to examine the impact of electromagnetic waves on living organisms. Reversed magnetic fields were shown to affect ant navigation and orientation (Banks & Srygley, 2003; Riveros & Srygley, 2008). Other observations reveal adverse effects of electromagnetic waves on animals (e.g. flies, Panagopoulos, Karabarbounis, & Margaritis, 2004; bats, Nicholls & Racey, 2009; rats, Benlaidi & El Kharroussi, 2011; birds, Everaert & Bauwens, 2007). Several of these studies on waves have controversial reports by researchers and some works have reported that electromagnetic field has no impact on living animals (Leonard, Berteaud, & Bruyére, 1983; Pinholster, 1993; Verschaeve, 1995). The latter works deal with biological phenomena (DNA, cancer and so on) that do not change easily and/or rapidly (i.e. in a few days or weeks). Examining whether electromagnetic waves have an effect on living animals should be done on sensitive and easily perturbed living systems. Social insects and their precise responses to their pheromones, their learning abilities and sophisticated behavior are adequate biological models for such an examination.

The members of an ant colony accomplish several tasks (foraging, collecting food, recruiting congeners) based on what they perceive and memorize. They must recognize individual, colony and species-specific pheromones and correctly respond to them for ensuring the functioning of the colony. Individual deficiencies in such discriminations and/or responses will cause perturbation in the colony. In other words, deficiencies in social behavior (nestmates recruitment, trail laying and following behavior, trophallaxis) and in activities (food collection, nest relocation, brood caring) may imperil the survival of the colony.

Here we used the ant *Myrmica sabuleti* Meinert 1861 as a model to examine whether electromagnetic waves have an impact on living organisms. *M. sabuleti* can be easily maintained in a laboratory. Its biology is presently well known. Congeners communicate using several pheromones, among others the trail pheromone is produced by the poison gland (3-ethyl-2,5-dimethyl pyrazine; Evershed, Morgan, & Cammaerts, 1981), a complex area-marking pheromone is secreted by the Dufour's gland (alkanes, alkenes, farnesene; Morgan, Tyler, & Cammaerts, 1977) and an alarm pheromone is produced by mandibular glands (essentially composed of 3-octanone and 3-octanol, Morgan, Inwood, & Cammaerts, 1978). The trail pheromone is identical in many species of *Myrmica* (Evershed, Morgan, & Cammaerts, 1982), whereas the contents of the Dufour's gland (Attygalle, Evershed, Morgan, & Cammaerts, 1983) and the mandibular glands (Cammaerts, Evershed, & Morgan, 1982, 1983) are species specific. Food collection in *Myrmica* is achieved by group recruitment that requires the use of the species' pheromones (Cammaerts, 1978; Cammaerts & Cammaerts, 1980). The orientation system of *M. sabuleti* has been elucidated (Cammaerts & Lambert, 2009; Cammaerts & Rachidi, 2009); its visual perception has been largely studied (i.e. Cammaerts, 2006, 2007) and its olfactory and visual conditioning have been analyzed (Cammaerts, Rachidi, & Cammaerts, 2011b). On the other hand, the assessment of such ant species responses to their pheromones is established since a long time. It is well known how to obtain pheromones from workers, present them to the ants, quantify the ants' reactions and statistically analyze such reactions. *M. sabuleti* is thus an excellent model for performing our planned experimentation.

We have shown that olfactory and visual learning are affected by electromagnetic waves (Cammaerts et al., 2012). Several physiological effects were then observed: foragers moved slowly, many workers died outside and inside the nest, dead ants were not transported to cemeteries, one queen died, and at a later stage some workers developed ovaries. The health of the exposed colonies was clearly deteriorated which was similar to the colony collapse disorder (CCD) observed in honeybees, becoming a universal problem http://bembem.skynetblogs.be/archive/2010/11/11/gsm-et-disparition-des-abeilles.html; http://www.sante-de-labeille.com. So, after having shown that electromagnetic waves affect ants' learning (Cammaerts et al., 2012), here we aimed to examine whether these waves have an impact on the ants' responses to pheromones and food collection. This would at least partly explain the colony deterioration observed in our previous study and may contribute solving the mystery of CCD in honeybees.

We analyzed *M. sabuleti* workers' response to their trail, their area marking and their alarm pheromones. These responses were previously studied in *Myrmica rubra* (Cammaerts-Tricot, 1973, 1974a) and adequate methods have then been set for each of the three pheromones. *M. sabuleti* workers have somewhat different sensorial organs and pheromonal glands; their responses to their pheromones may slightly differ from those of *M. rubra* workers. So, we first quantified *M. sabuleti* workers' responses to their pheromones in the absence of electromagnetic waves and achieved this in two steps. In a first step, we used colonies never exposed to

radiation; in a second step, we experimented on the colonies exposed to electromagnetic waves 6 months before the present tests, aiming to evaluate whether these colonies had recovered and could be used for future experimentation. The latter colonies showed signs of good health: they reacted correctly to their pheromones, normally cared for brood and queen, collected food, recruited nestmates and oriented themselves around their nest. They were deemed adequate for experimentation. So, we repeated the tests on these previously exposed colonies, subjecting them again to electromagnetic waves. Therefore we tested the same ants without exposure and then under the influence of electromagnetic waves, we could detect the effect of radiation on the ants' response to their pheromones. Lastly, we examined the ants' food collection with and without the influence of electromagnetic waves. After the experimentation, we observed the same deterioration of the exposed colonies as the one seen at the end of our previous work (Cammaerts et al., 2012).

Material and methods
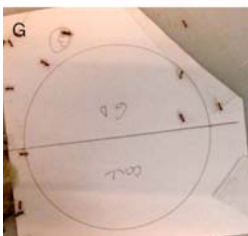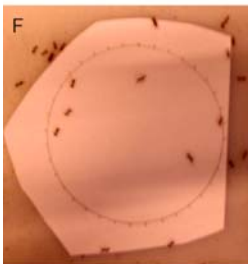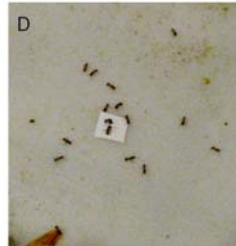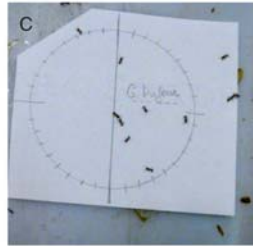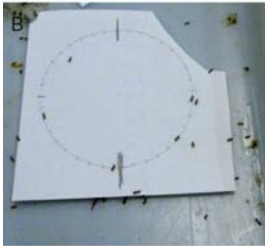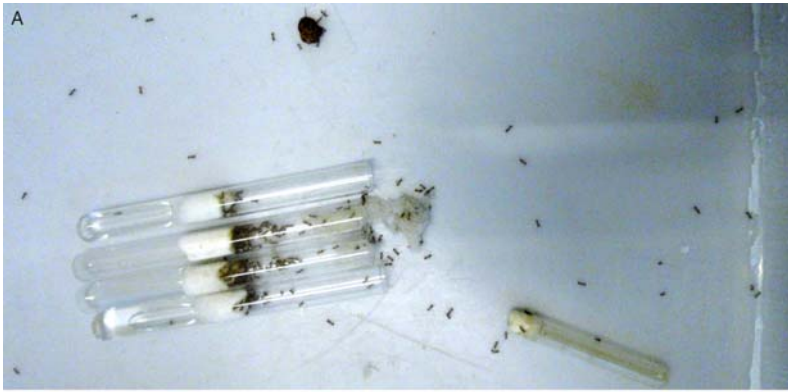
Collection and maintenance of ants

Two series of six colonies were used: one never exposed to electromagnetic waves and one exposed to electromagnetic waves four times, for 2–3 days, 6 months before the beginning of the present experiments (details in Cammaerts et al., 2012). The first colonies were collected at Höhes Martelingen (G D Luxembourg) and in Lorraine (France), the second colonies in the Aise valley (Ardenne, Belgium). These original localities were geographically not far from each other and their environmental characteristics (soil, plants, humidity and luminosity) were similar. The responses of the ants of both the series were cautiously analyzed and compared; the second series of ant colonies was so proved to be adequate for conducting the present experimentation. Therefore, the effect of electromagnetic waves on ants' responses to their trail, area marking and alarm pheromones was examined using only the second series of colonies which came from one geographical place, the Aise valley.

The 12 colonies, labeled 1–6 for each series, were demographically similar, each containing a queen, brood and about 500 workers. They were identical in terms of health and were cared for in the same way. They nested in three glass tubes half-filled with water and a cotton plug separated the ants from the water (Figure 1A). The glass tubes were set in trays (37 cm × 52 cm × 8 cm) and talcum powder was dusted over the edges of the trays. These trays served as foraging areas on which food was offered and testing took place (Figures 1 and 3).

Temperature was maintained at $20 \pm 2\,^{\circ}$C and humidity at about 80%, these values were optimal for the species. Lighting intensity was ca. 600 lux but 10 000 lux when feeding and testing a small ants. Sugar water was permanently offered in the glass tube plugged with cotton and pieces of dead cockroaches were provided twice a week, deposited on a glass slide (Figure 1).

Electromagnetic wave delivery

The radio frequency signal was produced by a Rohde & Schwarz (Munich, Germany) dual-channel SMATE200A vector signal generator. The frequency was set to 900 MHz, which is typical for Global System for Mobile communication (GSM). The signal was continuous and Gaussian Minimum Shift Keying (GMSK) was modulated in accordance to the GSM standard. The signal was produced via two channels, each of which delivered electromagnetic waves to three nests (Figure 1E). Each set of three nests was exposed to waves by means of an omni-patch antenna connected to one channel of the source, with horizontal polarization and gain about 1.6. The manufacturer of the antennae was Fractus® (Barcelone, Spain) and the model of the

patch antenna was Fractus EZConnect 868 MHz Chip antenna FROS-S1-R-O-IOS. The manufacturer stipulated that, for such an antenna, the radiation pattern was unidirectional; the antenna was placed 1 m above the ants; this gave an uniform electrical field pattern over the ants. The source power was set to 10 dBm [= dBmW = power ratio in decibels (dBs) of the measured power referenced to 1 mW, a convenient measure of absolute power] for each channel, theoretically giving an electric field value of about 1 V/m over each nest. The latter value is approximate, the only precise one being 10 dBm. Theoretically, such a power density gives an electromagnetic field of 0.77 V/m which corresponds to 1.6×10^{-3} W/m^2 = 1.6 mW/m^2 = 1.6×10^{-4} mW/cm^2.

An activated GSM device (e.g. Nokia brand), located at a normal distance from a common communication mast, transmits a short message every 2 h. According to experts in electromagnetic waves delivery, it has a radiated power of about 2 W during one time slot and, in the mean time, has a radiated power of 250 mW. The power density at a distance r (in m) from such a GSM can be calculated using the formula: p (W)/[4 × Pius × (r (m))^2] with p = 2 W. If r = 1 cm = 0.01 m, p = 2/4 × 22/7 × 0.01^2 = 1590.9 W/m^2 = 159.1 mW/cm^2. However, for short distances (less than the wave length), the formula is not relevant since the GSM reflects electromagnetic waves, retains energy, and suddenly transmits it. Consequently, the electromagnetic field generated at 1 cm from a mobile phone largely varies. Moreover, it may sometimes increase i.e. (i) when the nearest antenna mast power is weak and (ii) when the phone connects to another located farther mast (study done by the Association Française des Opérateurs Mobiles (AFOM), http://www.afom.fr; Wiedemann, Thalmann, Grutsch, & Schütz, 2006). In anyway, the electromagnetic field experimentally generated in the present work did not have a higher power density than the field surrounding a common activated GSM device.

As for the electromagnetic field surrounding the GSM communication masts, it largely varies with the distance from the masts, the kind of antennae and the countries. In several countries, limits have been given for the power densities of such electromagnetic fields (i.e. 2 V/m is a recommendation applicable in Paris I.C.N.I.R.P, 1988). Generally, such a field does not exceed 2 or 3 V/m where public has an access. However, insects, birds and bats can approach masts nearer than human. A precise analysis has been made near a common mast (http://www.issep. be/open.asp?f = /files/…/CEM%20a%20proximite%20antennes). One common antennae of that mast was set to 37 dBm. The power density of the electromagnetic field surrounding it equaled 5 W and the electric field existing at a few 100 m from that antennae had a value of 0.250 V/m. Living organisms flying or moving near such antennae are thus exposed to radiations of the electromagnetic field similar to the one produced here over six ant colonies. The exposure consisted of three periods of 60 h and one of 18 h with a delay of 3 days between each exposure. Since they nest in ground, ants may not be exposed to such electromagnetic fields. They are used here as a model to investigate the effect of natural magnetic exposure on animals, in general.

Figure 1. Ant response to their pheromones without (B, C, D) and under the influence (F, G, H) of electromagnetic waves. A: One of the six colonies exposed to radiation 6 months ago: the ants had regained health and are suitable for the present experiment. E: Experimental design of electromagnetic waves delivery on six colonies. B, F: Response to trail pheromone. The response was assessed by the numbers of arcs the ants walked on the trail (Table 1). C, G: Response to area-marking pheromone. The right half of the circle was marked; the response was assessed by the number of ants coming to the two half circles and those present on these two areas (Table 2). D, H: Response to alarm pheromone. The pheromone was emitted by a congener's head. The response was assessed by the ants' orientation toward the head and their linear and angular speed near the head (Figure 2, Table 3).

Ant response to trail pheromone

The trail pheromone of *Myrmica* ants is produced by the workers' poison gland. Ten glands were isolated in 1 ml hexane and stored for 15 min at $-25\,°C$. For each experiment on a colony, 0.1 ml of the solution was deposited using a normograph pen on a circle ($R = 5$ cm) drawn on a piece of white paper with a pencil and divided into $10\,°$ arcs. After 1 min, the piece of paper with the artificial trail was placed in the foraging area of the colony and 20 foragers coming into contact with the trail were observed. Their responses were assessed by the number of $10\,°$. arcs they walked without departing from the trail, even if they turned back on the trail (Figure 1B,F). If an ant turned back on its way when being in front of the trail, its response was assessed as "zero arcs walked". When an ant crossed the trail without following it, its response equaled "one arc walked". This procedure allows discriminating between species: in front of a trail traced with an alien poison gland, an ant often turns back; in front of a trail made with an extract of own species glands, a worker crosses the trail and generally follows it along variable distances. The "zero" and the "1" values are thus essential. The six colonies of each series were accordingly tested, leading to a total of 120 ants (Table 1). In one set of experiment, congeners' poison glands were used; in another set, the poison glands of ants from the other series were used. So, four kinds of experiments were conducted in the absence of electromagnetic waves. A control experiment was also conducted using the colonies never exposed and pure hexane (0.1 ml). Thereafter, the previously exposed colonies were subjected to the same tests but in the presence of electromagnetic waves (total ants observed: 120). These six colonies were exposed to radiation for 12 h before testing and again for the 2 days when the tests were performed. These experiments were conducted only once since they severely impacted the health of the colony.

For each series of experiments, the median and quartiles of the distribution of the obtained numbers of walked arcs were documented (Table 1). Such an assessment was used because the numbers of arcs the ants walked along a trail are not normally distributed (many ants walk along a few arcs, only a few ones walk along more than 20 arcs; e.g. Cammaerts-Tricot, 1974a). The obtained distributions cannot be characterized by their mean value; no parametric statistics can be applied. Being interested in differences in the ant population response, so in the distributions of the obtained values, we compared these distributions to one another using the nonparametric χ^2-test and checked that the median test corroborated the obtained statistical results (Siegel & Castellan, 1989).

Ant response to their area-marking pheromone

The area-marking pheromone is a mixture of nonvolatile and volatile compounds produced by the workers' Dufour's gland. It attracts nestmates to the area and gives a "known" character to the marked area. Ants became so inclined to approach and move about to marked areas (Cammaerts-Tricot, Morgan, Tyler, 1977). To determine the ethological activity of the area-marking pheromone, it is proper to count the ants entering and those remaining in the area scented with that pheromone.

To find out, 10 Dufour's glands were isolated in 1 ml hexane and the solution was kept for 15 min at $-25\,°C$. To conduct an experiment on a colony, 0.1 ml of the extract was randomly deposited using a normograph pen on half of a circle ($R = 5$ cm) drawn on a piece of white paper with a pencil, the other not marked half circle serving as a control area. After 1 min, the circle was placed in the colony's foraging area (Figure 1C,G) and the foragers' reaction was assessed for 5 min as follows. For each two halves of the circle, were simultaneously assessed (by two persons; Table 2A–C):

Table 1. Ants' response to their trail pheromone.

Walked arcs	Control	Intact glands, intact ants	Previously exposed glands, intact ants	Intact glands, previously exposed ants	Previously exposed glands and ants
(A) In the absence of electromagnetic waves					
0	20	0	0	0	0
1	62	2	2	1	0
2–3	27	2	2	1	3
4–5	8	4	10	3	4
6–8	2	6	11	12	14
9–12	1	14	16	10	27
13–20		45	55	31	36
21–30		30	17	30	20
31–50		15	5	22	15
51–80		2	2	10	1
N	20	120	120	120	120
Median	1.0	19.0	15.5	20.0	14.0
Quartiles	0–2	13–26	11–19	14–31.3	10–23

Walked arcs	In the absence of electromagnetic waves	In the presence of electromagnetic waves						
		Colonies						
		1	2	3	4	5	6	Total
(B) In the absence and the presence of electromagnetic waves (previously exposed ants tested with their own glands)								
0	0	0	0	1	0	0	1	2
1	0	7	5	2	4	4	4	26
2–3	3	6	9	6	7	8	5	41
4–5	4	3	5	3	5	2	3	21
6–8	14	2	1	3	4	3	5	18
9–12	27	1	0	3	0	2	1	7
13–20	36	1	0	2	0	1	0	4
21–30	20	0	0	0	0	0	1	1
31–50	15							
51–80	1							
N	120	20	20	20	20	20	20	120
Median	14.0							3.00
Quartiles	10–23							2–5.3

Notes: Pure hexane (control) or extracts of poison glands were deposited on a circle presented to the ants (Figure 1B,F) which response was assessed by the number of $10°$ arcs walked along the trail. (A) In the absence of electromagnetic waves, 20 ants of each 6 never exposed colonies and of each 6 previously exposed ones were confronted with trails made with glands of either intact or previously exposed ants. (B) In the absence (results already given in (A)) and in the presence of electromagnetic waves, 20 ants of each 6 colonies previously exposed were confronted with trails made with their own poison glands. Each distribution of the number of walked arcs was characterized by its median and quartiles; results of nonparametric χ^2 tests comparing these distributions to one another are given in the "Results" section.

- E = the numbers of ants entering the area during 30 successive periods of 10 s.
- P = the numbers of ants staying on the area at the end of 30 successive periods of 10 s.

For the two variables, the counts were totaled for the marked and the unmarked area.

The experiment was performed, initially in the absence of electromagnetic waves, on each 6 colonies of each two series. In a first set of experiments, the Dufour's glands of unexposed workers and in a second set, Dufour's glands of exposed foragers were used. Experiments conducted without electromagnetic waves were under four settings: (i) ants never exposed were tested with an extract of their own glands (detailed in Table 2A); (ii) ants never exposed were tested with an extract of glands from ants previously exposed; (iii) previously exposed ants were tested with an extract from never exposed ants; (iv) previously exposed ants were tested with an extract of their own glands (Table 2B). In each case, the six totaled values of E and P obtained for the marked areas were

Table 2. Ants' response to their area-marking pheromone.

(A)

(A) Variables		1	2	3	4	5	6	Sum	Mean	N	T	P
Control		11	41	63	66	77	45	303	1.68	6	21	0.016
E	Test	20	70	107	166	187	132	682	3.78			
	Control	12	28	35	34	59	29	197	1.09			
P	Test	10	24	37	95	107	50	325	1.79	6	13.5	NS

Colonies: 1–6

(B)

(B) Ants' \ Glands	Previously unexposed	Previously exposed
Previously unexposed	E control: 1.68 Test: 3.78 $P = 0.016$ P control: 1.09 Test: 1.79 NS	E control: 1.57 test: 2.76 $P = 0.016$ P control: 0.84 test: 1.10 NS
Previously exposed	E control: 1.79 Test: 3.97 $P = 0.016$ P control: 0.91 Test: 2.40 $P = 0.016$	E control: 1.46 Test: 3.18 $P = 0.016$ P control: 0.84 Test: 1.43 $P = 0.016$

(C)

(C) Variables		1	2	3	4	5	6	Sum	Mean	N	T	P
Control		13	78	85	32	44	27	279	1.55	6	21	0.016
E	Test	20	93	103	55	53	44	368	2.04			
	Control	4	59	58	7	33	10	171	0.89			
P	Test	5	66	78	13	48	16	226	1.25	6	21	0.016

Colonies: 1–6

Notes: Ants were presented with circles half marked with the pheromone (produced by the workers' Dufour's gland, Figure 1C,G). The ants coming to the unmarked and the marked areas in 10 s (E) and those present on these areas at the end of time laps of 10 s (P) were counted during 5 min for each 6 colonies in both series using the unexposed and previously exposed ants' glands. N, T, P: results of nonparametric Wilcoxon tests made between the six test sums of E and P and the 6 corresponding control sums (Siegel & Castellan, 1989). (A) Details of the experiment with unexposed ants and their own glands. (B) Mean control and test values of E and P, as well as statistical results for the four kinds of experiments made without the influence of electromagnetic waves. (C) Numerical and statistical results of experiments conducted under the influence of electromagnetic waves on previously exposed colonies using their own Dufour's glands. The results are to be compared with those given in (B), last line and column.

compared with the six totaled values relative to the unmarked areas using the nonparametric Wilcoxon test (Siegel & Castellan, 1989). This test was the more powerful one; the strongest significance occurs with $N = 6$, for $T = 21$, the probability of wrongly rejecting the null hypothesis then equaling 0.016. The mean value of the E and P was also determined for each kind of experiment and both areas (Table 2).

Thereafter, the same tests and calculations were performed again on the second series of colonies but under the influence of electromagnetic waves (Table 2C). The ants were exposed for 12 h preceding the tests and for the 2 testing days. This was conducted only once, due to the negative impact of exposure. The values obtained in the course of the latter experiment were compared, in the same way as explained earlier, with those obtained using the same ants and glands but without the influence of electromagnetic waves.

Ant response to their alarm pheromone

The alarm pheromone is elaborated by two mandibular glands located in the worker's head. Ants secrete it by widely opening their mandibles. To avoid secretion of chemical materials other than these glands content, entire heads were isolated and used for the ants, being presented in the foraging area on a piece ($1 \, cm^2$) of white paper. They so naturally emitted the required pheromone (Figure 1D,H).

To assess the ants' response to the alarm pheromone, we recorded for one colony:

- The trajectory of 10 foragers moving in the surroundings of the presented head in order to quantify the orientation towards such a head.
- The trajectory of 10 foragers having perceived the alarm pheromone in order to assess the linear and angular speed in the presence of the alarm pheromone.

The orientation (O) is the mean of the angles (°) made at successive points of the trajectory between the direction of the trajectory (= the segment joining two adjacent points of the trajectory) and the direction "point of the trajectory – deposited isolated head" (Figure 2).

The linear speed (V) is the length of the trajectory (mm) divided by the time (s) spent to travel it (Figure 2). Time was evaluated by listening to a metronome beating each second.

The angular speed is the sum of the angles made by the segments joining the successive points of the trajectory (°) divided by the length of the trajectory (cm; Figure 2).
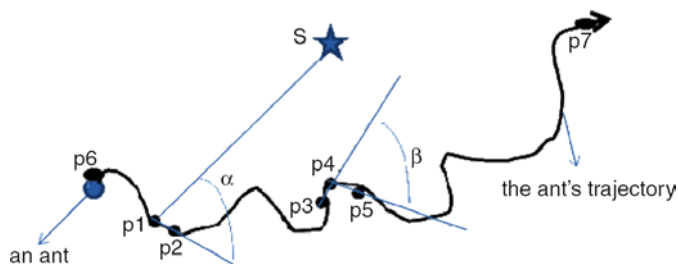

Figure 2. Ant locomotion analysis. A trajectory was characterized by: the orientation toward the source (S), which is the mean of the angles (as α, in °) made at each point of the trajectory between the direction of the trajectory and the segment 'point of the trajectory – S'; the linear speed which is the length of the trajectory (p6–p7, in mm) divided by the time (in s) required to travel it; the angular speed which is the sum of the angles (as β, in °) made along the trajectory divided by the length of the trajectory (p6–p7, in cm).

These variables have previously been defined differently (Cammaerts-Tricot, 1973) and used (e.g. Cammaerts & Mori, 1987; Cammaerts-Tricot, 1974b). Software was set (Cammaerts, Verhaeghe, Cammaerts, & Lesseux, 1991) to assess the variables more simply (e.g. Cammaerts & Cammaerts, 2000, 2001). Recently, updated software (Cammaerts et al., in press) was created using the above definitions; this software was applied in the present study.

In the absence of electromagnetic waves, each variable was assessed for both series of ant colonies, under two experimental conditions (Table 3A): a control using blank pieces of paper (1 cm^2) and a test using heads of congeners. After that, control and test experiments were again performed but under the influence of electromagnetic waves and exclusively on ants and congeners' glands previously exposed to waves (Table 3B). The ants were exposed to electromagnetic waves for 12 h preceding the tests and for the 2 days during which the tests were performed.

For each (three in total) kind of experiments, 10×6 values of O, V, S were obtained using first a blank paper and later a congener's head. The distributions of the 60 O, V, S values were not normal; they were thus characterized by their median and quartiles and were compared with one another by the nonparametric χ^2- test (Table 3). Median tests were also made for checking the statistical results.

Social food collection

Two colonies (labeled 4 and 5) of the first series and two (labeled 3 and 6) of the second series were starved for 4 days. Then to colonies 3 and 6, electromagnetic waves were delivered for 12 h and thereafter for 6 h while the following tests were performed on the four colonies. A piece of dead cockroach was placed in the ants'

Table 3. Ants' response to their alarm pheromone.

Colonies	Experiments	O (°)	V (mm/s)	S (°/cm)
(A) Without the influence of electromagnetic waves				
Unexposed	Control	91.7 (72.7–109.9)	10.9 (9.8–13.1)	178 (160–204)
		$P < 0.001$	$P < 0.001$	$P < 0.001$
	Test	40.7 (32.0–53.2)	20.8 (18.2–25.3)	128 (116–146)
Previously exposed	Control	89.3 (66.6–105.3)	12.8 (11.8–14.7)	184 (148–211)
		$P < 0.001$	$P < 0.001$	$P < 0.001$
	Test	44.4 (35.1–57.3)	22.8 (19.3–24.1)	126 (106–143)
(B) Under the influence of electromagnetic waves				
Previously exposed	Control	81.8 (65.7–96.4)	13.4 (11.8–15.8)	172 (149–204)
		NS	$P < 0.001$	NS
	Test	64.7 (44.5–88.9)	19.8 (16.2–21.6)	141 (125–160)
(C) Comparison of responses without and under the influence of electromagnetic waves				
Previously exposed	Test without	44.4 (35.1–57.3)	22.8 (19.3–24.1)	126 (106–143)
	waves	$P < 0.01$	$P < 0.05$	$P < 0.05$
	Test with	64.7 (44.5–88.9)	19.8 (16.2–21.6)	141 (125–160)

Notes: This pheromone being produced by the workers' mandibular glands, workers' heads were presented to ants which response was analyzed (Figure 1D,H). Control experiments were made using blank paper. Three variables were assessed: O: the ants' orientation toward the stimulus (°); V: the ants' linear speed (mm/s) near the stimulus; S: the ants' angular speed (°/cm) near the stimulus (Figure 2). Each variable was quantified for 10 ants' control and test trajectories for each six colonies of each series. This led, each time, to a total of 60 control and test values of O, V and S. The distributions were characterized by their median and quartiles, and were compared with one another using the nonparametric χ^2-test (Siegel & Castellan, 1989). P = level of probability; NS: not significant difference for $P = 0.05$. (A) Unexposed and previously exposed colonies, tested without the influence of electromagnetic waves. (B) Previously exposed colonies tested under the influence of electromagnetic waves. (C) comparison of values obtained without and under the influence of electromagnetic waves using the same six ant colonies (values reported in (A), last line and in (B), last line).

foraging area. The ants leaving the food site were closely observed to describe their behavior (see "Results" section) and photographs were taken at 7 and 12 min after the beginning of the experiment (Figure 3). The ants present at the food site were also counted every half a minute for 12 min (Figure 4).

Results

Response to trail pheromone

The concentration of the extracts was sufficient to obtain obvious but not excessive responses from the workers (Table 1, Figure 1B,F).

Without the influence of electromagnetic waves, the ants in both series responded perfectly to their trail pheromone, regardless whether the pheromone was obtained from one or the other of the two series of colonies (Figure 1B). Slight differences (commented in the "Discussion" section) appeared which leaded to the fact that the results pertinent to intact glands and workers were not statistically different from those relative to previously exposed glands and workers [Table 1A third column vs. sixth column: quartiles = 13–26 vs. 10–23, not significant (NS)]. The impact of electromagnetic waves could be studied using the previously exposed colonies and their own poison glands.

Under the influence of electromagnetic waves, the ants of the six colonies behaved similarly (Table 1B): they perceived the trail pheromone, generally crossed the trail and retreated, often followed the trail for short distances (Figure 1F), and sometimes stopped moving. The median of the numbers of arcs walked along the trail was 3.00 (Table 1B) which was higher than the control (1.00, $P < 0.001$) but lower than the usual ants' trail following abilities (14.00, $P < 0.001$). The median test corroborated the significant difference between the two results. Electromagnetic waves so affect the ants' trail following behavior.
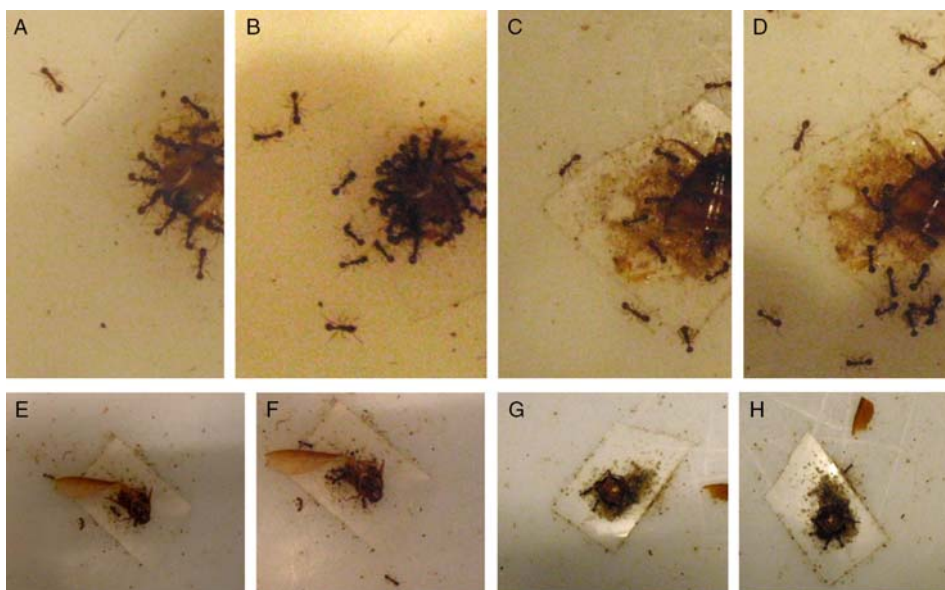

Figure 3. Food collection without (A, B; C, D) and under the influence (E, F; G, H) of electromagnetic waves. After a period of starvation, two nonexposed colonies (one: A, B, the other: C, D) and the two exposed ones (one: E, F, the other: G, H) received a piece of dead cockroach. The food site was photographed at $t = 7$ min (A, C, E, G) and $t = 12$ min (B, D, F, H). Exposed foragers were unable to return to their nest and recruit congeners (E, G, F, H).
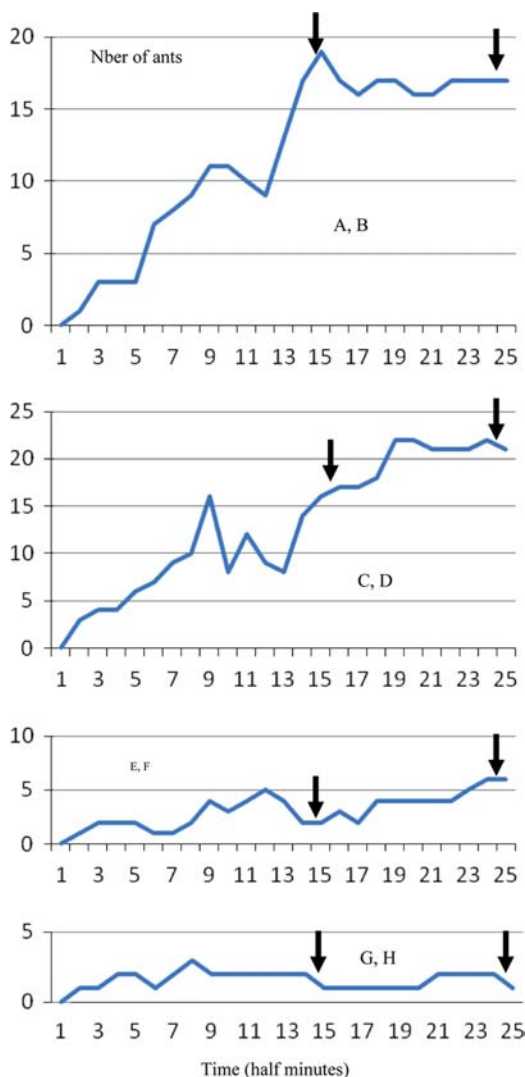
Figure 4. Same legend as in Figure 2. The numbers of foragers present on the food site are given on the y-axis, over time, given on the x axis, for 24 periods of 30 s. A, B, C, D, E, F, G, H refer to the photographs presented in Figure 3. The numbers obtained at $t = 7$ and 12 min (vertical arrows) were statistically analyzed (see the "Results" section).

Response to area-marking pheromone

The extracts were of adequate concentration to assess ants' response. Without the influence of electromagnetic waves, more "intact" as well as previously exposed ants came to marked areas than to unmarked ones ($P = 0.016$, Figure 1C,G, Table 2). Previously exposed workers were also more numerous on marked areas (Table 2B, $P = 0.016$), while intact ones were only so slightly (Table 2A,B; NS). So, previously exposed ants responded somewhat better than never exposed ants. But Dufour's gland of previously exposed workers may contain less pheromone than the gland of intact ones (2.76 vs. 3.78, 1.10 vs. 1.79, 3.18 vs. 3.97, 1.43 vs. 2.40). This led to clear differences between the results relative to intact workers and previously exposed glands (2.76, 1.10) and those concerning previously exposed workers and intact glands (3.97, 2.40). Only slightly perceptible differences occurred between the

experiments using intact glands and workers (3.78, 1.79) and those using previously exposed glands and workers (3.18, 1.43, Table 2B). The latter glands and workers are thus suitable to detect the effect of electromagnetic waves on the ants' response to their marking pheromone.

Under the influence of electromagnetic waves, the ants' response was qualitatively similar (Table 2C compared with Table 2B last line and column). More ants entered the marked area (variable E, $P = 0.016$) and ants were also more numerous in such an area (variable P, $P = 0.016$, Figure 1G). But, this response was quantitatively lower than that exhibited under normal conditions. Wilcoxon tests carried out between the values of E and P obtained, on the same ants, with and without the radiation revealed that (i) ant numbers entering the marked area statistically differed (2.04 instead of 3.18, $P = 0.016$) and (ii) ant numbers on the area did not significantly differ (1.25 instead of 1.43, NS). Thus, under the influence of electromagnetic waves only fewer ants reached the marked areas.

Response to alarm pheromone

Without electromagnetic waves, unexposed and previously exposed ants were attracted (positive taxis) to a congener's head ($P < 0.001$, Figure 1D) then walking rapidly (positive orthokinesis, $P < 0.001$) and somewhat less sinuously (negative klinokinesis, $P < 0.001$; Figure 1D, Table 3). No differences existed between the results of the two kinds of experiments: $O = 40.7°$ vs. $44.4°$, $V = 20.8$ mm/s vs. 22.8 mm/s, sinuosity = $128°$/cm vs. $126°$/cm. However, small differences may exist between the content of unexposed and previously exposed glands, as well as between the behaviors of unexposed and previously exposed workers, a point which could not be elucidated. Indeed, a worker's head contains the alarm pheromone as well as colony- and individual-specific elements. So, only congeners' heads, and not alien ones, could be used. Nevertheless, previously exposed ants and glands are suitable to observe the ants' response to their alarm pheromone under the influence of electromagnetic waves.

Under radiation (Table 3B), ants slightly reacted to a blank paper (regarding orientation, linear speed and sinuosity). Near an isolated head, movements were quick and erratic, but this alarm reaction was less efficient than under normal conditions. Instead of exhibiting clear positive taxis ($O = 44.4°$., Table 3A), the ants showed an orientation which was not statistically different from the control ($64.7°$., NS, Table 3B). The difference between 44.4 and $64.7°$. was significant ($P < 0.01$, Table 3C). Linear speed increased (19.8 mm/s vs. 13.4 mm/s, $P < 001$, Table 3B) but not as much as under normal conditions (22.8 mm/s vs. 12.8 mm/s, $P < 0.001$, Table 3A). The difference between 19.8 and 22.8 mm/s was slightly significant ($P < 0.05$, Table 3C). Angular speed decreased but not statistically ($141°$/cm vs. $172°$/cm, NS, Table 3B) as it did under normal conditions ($126°$/cm vs. $184°$/cm, $P < 0.01$, Table 3A). The difference between 141 and $126°$. was slightly significant ($P < 0.05$, Table 3C). The ants' large sinuosity and slight increase of linear speed explain the strong alarm reaction observed under radiation (Figure 1H). But this alarm resembled a "general alarm", as if ants perceived some danger, and differed from that induced by the alarm pheromone.

Social food collection

In the absence of electromagnetic waves, a few ants quickly found the food, fed for 1–2 min, and then returned to the nest without hesitating (Figures 3 and 4). Shortly thereafter, several ants reached the food and the number of ants present there increased rapidly (Figures 3A–D and 4A–D). Under the influence of electromagnetic waves, only one to two ants reached the food site, fed for 1 or 2 min and then left the

food, walked randomly around it, moved to the nest, went back to the food, and returned again toward the nest but failed in reaching it and recruiting congeners. Consequently, the number of ants present on the food only slightly and slowly increased due to a random arrival of foragers (Figures 3E–H and 4E–H). At $t = 7$ and 12 min, a total of 35 and 38 ants, respectively, collected food in the absence of electromagnetic waves while only 3 ants and 8 ants, respectively, did so under radiation. These ant numbers under the two conditions statistically differed ($P < 0.001$; χ^2 2×2 table contingency).

Last observation
At the end of the present experimentation, the six exposed colonies presented signs of deterioration: foragers moved slowly, food collection decreased, larvae no more developed, and many workers died. This also occurred at the end of our previous work dealing on effects of electromagnetic waves on ants' conditioning and memory (Cammaerts et al., 2012).

Discussion and conclusion

The life of many insects is controlled by pheromones emitted, perceived and reacted to by each individual. Food collection is a key task, especially for social insects such as wasps, bumblebees and honeybees. Deficiencies in any species' system of communication and food collection imperil its survival. The present work examines the influence of electromagnetic waves on such social functions in an ant, *M. sabuleti*, used as a biological model. The experimenters were blinded to the exposure situation and the same ants, proved to be in good health, were examined first without exposure then under the influence of electromagnetic waves.

Under normal conditions, *M. sabuleti* workers transverse 17.3 arcs (median value) of $10°$. along a circular trail ($R = 5$ cm). When exposed to electromagnetic waves, they only walked 3.0 arcs. Normally, 3.43 ants came in 10 s to a marked area (a half circle, $R = 5$ cm), but under the influence of electromagnetic waves, only 2.04 ants arrived to the area. *M. sabuleti* workers are attracted by their alarm pheromone (median orientation equals $42.5°$.) but exposed to electromagnetic waves, foragers poorly oriented themselves toward a source of such a pheromone ($64.7°$.). Under normal conditions, foragers easily found food, quickly returned to the nest and recruited congeners, and the number of ants increased so rapidly. Under the influence of electromagnetic waves, foragers moved slowly and randomly, never returned to the nest and did not recruit nestmates. Thus, very few ants located the food.

Eventual effects on ants, noise and ventilation emitted by the generator (i) have been reduced by placing the generator on an adequate support and (ii) examined before experimenting and seen to be absent, the ants went on normally responding to their pheromones and collecting food. Other factors may also affect ants' response to their pheromones: e.g. electromagnetic field produced by electric apparatus, temperature increase or decrease, and presence of volatiles in the air. During experimentation, such factors were absent or identical in the absence and the presence of electromagnetic waves.

As explained in the text, we collected the ant colonies from three places identical as for their environmental characteristics. The effect of electromagnetic field was studied by using exclusively the ants from one place, first testing them without, secondly under the influence of electromagnetic field and the experimental values obtained in these two conditions were then compared. The other colonies were used only to show

that the ants used for the purpose of the present work were in good health, behaved like any *M. sabuleti* ants and correctly responded to their pheromones.

As reported, the latter ants had been previously exposed to electromagnetic waves. Many of them died, those which survived and showed signs of good health were selected for the present study. Slight differences were detected between the never exposed and the previously exposed ants. For instance, the poison gland of previously exposed ants contained less trail pheromone than that of intact ants, while previously exposed ants responded better to the trail pheromone than the unexposed ants. The inoculated colonies might be in better health, stronger – or less sensitive – than the unexposed colonies; they might have been submitted to drastic selection when exposed to radiation. If this assumption is correct, the observed effects of electromagnetic waves would be still stronger on more sensitive animals.

The intensity of the electromagnetic field used here was relatively weak (10 dBm) and similar to that emitted by common commercial GSM devices at a common distance from a communication mast. Information, about the electromagnetic field surrounding an activated GSM, is given in Cammaerts, Debeir, and Cammaerts (2011a). Briefly, at a few centimeters from a GSM, that field usually equals 250 mW and intermittently, about 2 W. In the vicinity of communication masts, the strength of the electromagnetic field varies with the kind of antennae, the country and the distance from the masts. Limits (3 and 2 V/m) are now more and more often applied for such fields, but this procedure does not actually reduces exposure (see the "Material and methods" section in which a scientific analysis made near a common mast is also reported). All this shows that the strength of the electromagnetic field we applied in the present work is realistic and has an intensity range similar to those existing in the human environment (communication masts, GSM, Wifi, and electrical and electronic apparatus). The revealed impact of such electromagnetic fields may apply to all insects whose life depend on pheromonal perception, orientation capability and communication. Among these insects are important pollinators such as bumblebees and bees.

The colonies submitted to electromagnetic waves were exposed only 3 times for 60 h and once for 18 h with a 3 day-recovery period between each exposure. They showed deficiencies in movement, food consumption and larval development, just like in our former work on the subject (Cammaerts et al., 2012). Insect larvae development implicates nervous cells (pars intercerebralis) and cellular membranes. The observed effects can be anticipated in other insects. Electromagnetic waves might so be used to prevent pest insect development.

When exposed to radiation for a few hours, ants became excitable, as if in the presence of a danger. Our results relative to the alarm pheromone corroborated such an observation. After 2–3 days of exposure, the ants seemed in poor health condition. Some researchers and beekeepers are inclined to believe that the large quantities of dead bees found near hives are due to insecticides, while the actual slow decline of bee populations is due to other causes. Among others, bees might no more behave normally when entering electromagnetic fields generated by GSM masts. Biologists reported that bees are disoriented and no longer returned to their hives when exposed to electromagnetic waves (http://agbi.uni-landau.de; http://www.izgmf.deAktionen/Meldungen/Archiov_05/bienen.htlm; http://bembem.skynetblogs.be/archive/2010/11/11/gsm-et-disparition-des-abeilles.html). Sharma and Kumar (2010) placed activated GSM inside bee hives and showed that the bees' life was negatively influenced by electromagnetic waves (e.g. there was neither honey nor pollen in the hives after the experiment). Favre (2011, and references therein) revealed that activated mobile phones induce "piping" in honeybees staying in the hive, a behavior exhibited in disturbed colonies or

to announce a swarming process. At the same time, more and more studies show the impact of electromagnetic waves on organisms and humans (e.g. Balmori, 2006; Benlaidi & El Kharroussi, 2010; Everaert & Bauwens, 2007; Nicholls & Racey, 2009; Panagopoulos et al., 2004; Stever, Kuhn, Otten, Wunder, & Harst, 2005). Our previous work gave rise to the premise that electromagnetic waves act on the nerves cells and the neurotransmitters (Cammaerts et al., 2012). Other researchers also advance this hypothesis (Mausset-Bonnefont et al., 2004). Likewise, working on nerve cells of rats, Orendaeova et al. (2009) found changes in the number of proliferating nerve cells depending on age and dosage used as a response to electromagnetic exposure. The study we conducted on *Paramecium caudatum* revealed the impact of electromagnetic waves on the cellular membrane (Cammaerts et al., 2011a). Working on Wistar rats, Wang et al. (2009) demonstrated the impact of electromagnetic waves on the proteins associated with synaptic vesicles.

The present work demonstrates the incapacity of social insects (ants) to correctly respond to their pheromones and to recruit congeners, and confirmed deficiencies in larvae development when exposed to electromagnetic waves. It provides convincing evidence of a negative impact of electromagnetic waves on insects, at least on those whose life depends on communication and memory. As other researchers, we speculate that the waves affect nerve cells, impacting the cellular membrane (Cammaerts et al., 2011a). Despite its negative consequences, electromagnetic waves may prove beneficial to control pest insects.

Acknowledgements

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