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Defining the critical vertical distance between two visual cues for allowing ants to mentally add them

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ABSTRACT

Having previously defined the critical distance between two horizontally located visual cues allowing workers of the ant *Myrmica sabuleti* to mentally add them, and knowing that their sensitivity to a horizontal and a vertical change of orientation differs, we tried to define the distance between two vertically located cues beyond which limit these ants can no longer add up the cues. Making eight experiments on four colonies, and using different vertical distances between two presented cues, we found that this critical distance equals 4 cm, what is in agreement with what we know about this species' visual perception. Moreover, during every performed experiment, it appeared that these ants better responded to, and thus better perceived, the cues located higher than those located lower. This is to be put in relation with the morphology of their eyes and their position on the head.

KEYWORDS: *Myrmica sabuleti*, numerosity ability, operant conditioning, summation, visual perception.

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The present work was done after their retirement.

INTRODUCTION

The workers of the ant *Myrmica sabuleti* Meinert, 1861 are able to mentally add two visual cues when they are sighted simultaneously, but not when they are seen consecutively [1, 2], and when they are identical in shape, color and size [3]. Horizontally positioned cues are mentally added when they are separated by no more than 5 cm from each other; beyond this critical distance, the ants react as seeing the cues separated from each other (i.e. such as if the cues were seen consecutively) [4]. In the wild, cues (parts of plants, ground characteristics) can be vertically positioned. The workers of *M. sabuleti* are known to be differently sensitive to a horizontal and a vertical change of orientation of a cue [5]. They statistically perceive the rotation of a horizontal segment (i.e., a vertical change) if it amounts at least to 15 angular degrees and the rotation of a vertical segment (i.e., a horizontal change) if it amounts at least to 30 angular degrees. They are thus more sensitive to a vertical change than to a horizontal one, and the critical distance for adding or not adding identical elements may differ between elements horizontally and vertically positioned. The aim of the present work is to define the critical distance between vertically positioned elements below which *M. sabuleti* workers mentally add the elements and beyond which they no longer add them.

M. sabuleti workers can distinguish differently long segments as well as segments located at different height, but can no longer see a 0.5 cm x 3 cm segment located at a height of 15 cm or more. This allowed measuring their subtended angle of vision, which equals 5° 12’ [6]. A subsequent work revealed that *M. sabuleti* workers distinguish shapes, forms, numbers of elements, and differently oriented elements, pointing out their different sensitivity to a horizontal and a vertical change of orientation [5]. Other works on the subject demonstrated that *M. sabuleti* workers distinguish colors [7], perceive perspective [8], and have a very low light threshold, particularly for some colors [9, 10].

Ants make use of visual cues for navigating, e.g., for going to food sites and returning to nest, according to the size and morphology of their eyes. Several theories have been developed for describing how ants make use of their vision to navigate [11, 12]. Nocturnal ants, those foraging in the canopy, and desert ants also use their visual perception to navigate [13-15]. *Myrmica sabuleti* workers use visual cues for finding their way [16], but only when olfactory cues are absent [17]. During the below reported experimental work and laboratory maintenance of the ants, the environment contained no particular odor allowing navigating, with the exception of the unavoidable nest and area marking odors. These ants used thus essentially the presented visual cues to navigate.

The numerosity abilities of *M. sabuleti* workers have largely been studied [18, 19]. They can add identical elements simultaneously seen (see above) [1-3]. They can also expect the subsequent numbers of an arithmetic sequence [20] as well as the subsequent size of a geometric sequence [21]. The largest horizontal distance between two identical cues for allowing *M. sabuleti* workers to mentally add them has been defined [4]. These ants being differently sensitive to horizontal and vertical orientation changes [5], it was thus of interest to define the largest vertical distance between cues beyond which they no longer mentally add them.

The skill of adding has been examined in several vertebrates and invertebrates. A lot of information can be found in some of our previous works [e.g., 1, 2, 3, 18, 19 and references therein]. We here briefly summarize such information. Some birds (e.g., newborn chicks and robins) and monkeys have been proved to be able to add or subtract numbers of identical elements [22-26 and references therein]. As for the invertebrates, experiments on spiders deprived of some of their prey items suggest that these arthropods may be able to add and subtract elements [27]. Bees have been shown to be able to learn adding or subtracting one element to or from 1 to 5 presented according to the respective blue or yellow color of these elements [28]. In these mentioned works, the maximum distance between perceived elements allowing the individuals to add them has not been defined. The present experimental work has the novelty character to define the critical vertical distance between elements allowing their mental adding-up by an animal.

MATERIALS AND METHODS

The present work being a continuation of a previous one [i.e. 4], and their results having to be compared, the experimental designs and methods explained below are quite similar to those of the previous work, and therefore, some self-plagiarism is inevitable.

Collection and maintenance of ants

The experiments were performed on four *M. sabuleti* colonies collected in the Aise valley (Ardenne, Belgium) on the same site from where we previously collected four colonies to define the critical horizontal distance between two cues beyond which the ants no longer add these cues. The date of the collection was also the same (May 2021) [4]. The colonies were labeled A, B, C, D, and maintained in the laboratory in one to two glass tubes half-filled with water with a cotton plug separating the ants from the water. The nest tubes of each colony were set in a tray (34 cm x 23 cm x 4 cm) which served as a foraging area. A cotton-plugged tube containing sugared water was permanently present in these trays, and pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) were deposited in them three times per week. The ambient lighting equaled ca. 330 lux, the temperature ca. 20 °C, the humidity ca. 80%, and the electromagnetic field ca. 2 μWm^2, which were suitable conditions for the used species. We here often name the ants ‘workers’ or ‘nestmates’ as commonly do researchers on social insects.

Experimental planning

A summary of this planning is given in Figure 1. Eight experiments were performed, i.e.

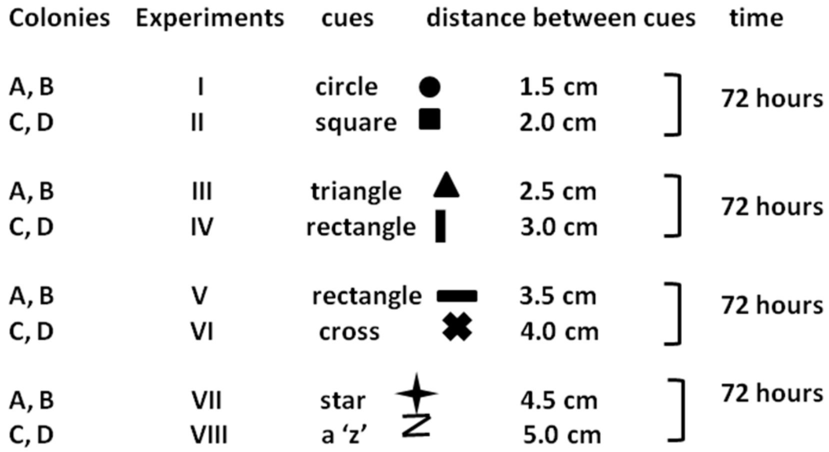

Figure 1. Experimental planning.

experiments I, III, V and VII on colonies A and B, and experiments II, IV, VI, and VIII on colonies C and D. Two experiments were performed at the same time, i.e. I and II, III and IV, V and VI, VII and VIII. A resting time period of 24 hours was managed between experiments I, II and III, IV, between experiments III, IV and V, VI and between experiments V, VI and VII, VIII. During each experiment, the ants were trained for 72 hours in their foraging area to two visual cues set aside one another either on the left or on the right of the nest entrance, the cues making an angle of 45° with the axis of the nest tube(s). The ants saw thus the two cues while entering and going out of their nest. One cue was located in a low position (at 2-3 mm from the surface of the ants’ tray), while the other cue was located higher, at a vertical distance of 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 cm according to experiments I to VIII respectively. It was checked if the ants could perceive the highest presented cues (see the following subsection). Over their 72 training hours, the ants were tested six times in front of three stands, one stand bearing the cue located at a lower level, another stand bearing the cue located at a higher level, and a stand bearing the two superimposed cues, keeping the height at which they were presented separately during training (see the three following subsections).

Experimental design

Figures 2 (experimental design and maintaining of the cues in place during 72 hours) and 3 (concerning the perception of cues presented at a height of 5 cm) help to understand the description given below. During training, two stands bearing identical cues set aside each other were deposited close to the nest entrance either on its left (colonies A, C) or on its right (colonies B, D). One of the cues was positioned at a lower level; the other was positioned at a higher level (see below). Due to their position at the nest entrance, the cues could often be sighted by the ants. The kind of cues and their exact relative position are detailed below. Over their training, the ants were tested, after 7, 24, 31, 48, 55, and 72 hours, in a separate tray (21 cm x 15 cm x 7 cm), each colony having its own tray devoted to testing. In this tray, three cues were deposited: the two cues used for training the ants (i.e. with their elements located at the lower and higher level) separately set, and a cue made of these two elements vertically superimposed. The cues used for testing the ants were identical to those used during training but were novel and never used. The assessment of the ants’ response is detailed below. The cues presented at the highest level were here located at a height of 5 cm, and their width and height equaled 7.5 mm. Therefore, it had to be checked if the ants could see such large cues presented at that height. According to the location of their eyes, the ants see well above them, and the workers of the ant *M. sabuleti* have a subtended angle of vision of 5° 12’ [6]. Being near a cue presented at a height of 5 cm, these workers can thus see a cue having a dimension of

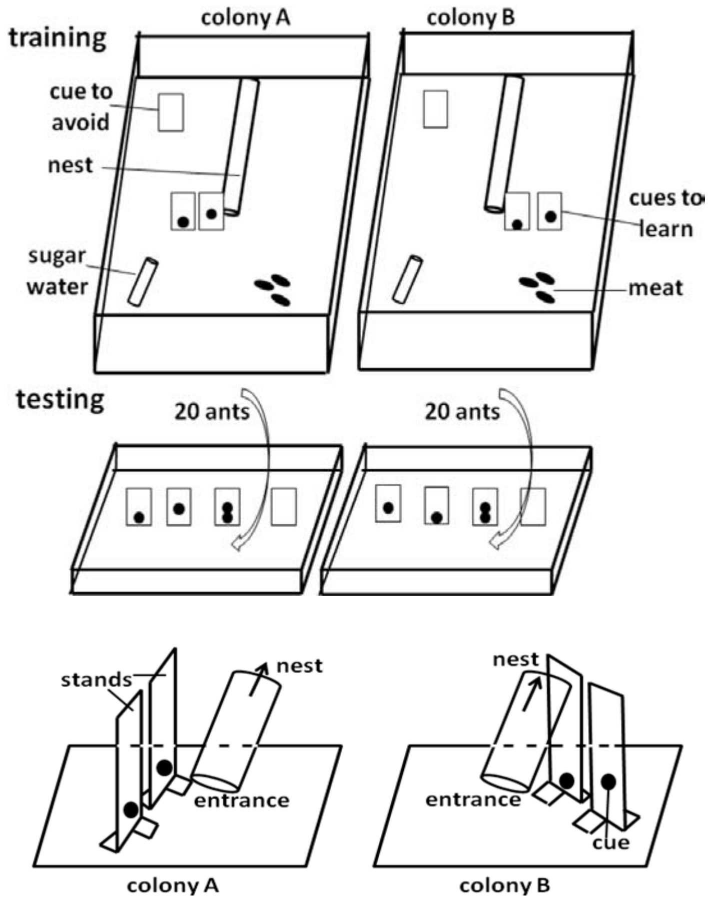

Figure 2. The experimental design used to define the maximum vertical distance between two cues beyond which the ants no longer mentally add them, and the device allowing to maintain the cues in place (at nest entrance) for 72 hours.

0.5124 cm (value calculated using trigonometry; Figure 3, left part). They thus saw cues 0.75 cm wide presented (located) at a height of 5 cm. At a distance of 2, 4, 5, and 6 cm from the foot of a presented cue, the ants can see a cue located at a height of 5 cm as small as 0.5513 cm, 0.6558 cm, 0.7240 cm and 0.8000 cm, respectively (values calculated using Pythagoras's theorem; Figure 3, right part). Consequently, the here trained and tested ants could see a cue (7.5 mm high and wide) located at a height of 5 cm while being at a maximum distance of about 5 cm from the foot of its stand. Of course, the ants could *a fortiori* see the cues presented at a smaller height than 5 cm.

Cues

The cues are schematically presented in Figure 4 and can be seen in Figures 5 & 6. They were black graphic elements, and according to the experiment, their shape was a circle, a square, a triangle, a vertically oriented rectangle, a horizontally oriented rectangle, a cross, a star, or a 'Z', the dimensions of which are given in Figure 4. Moreover, according to the experiment, they were either located at a lower level, at a height of ca. 3-5 mm, or located at a higher level, at a vertical distance of 1.5 cm, 2.0 cm, 2.5 cm, 3.0 cm, 3.5 cm, 4.0 cm, 4.5 cm or 5.0 cm above the cue located at the lower level. During the tests, the cues that were presented to the ants were single cues such as those located at the lower and at the higher level of the stands during training, and a cue superimposing these single signals that kept the position they had during training, including the vertical distance between them. Using the Word® software, the single cues (presented during

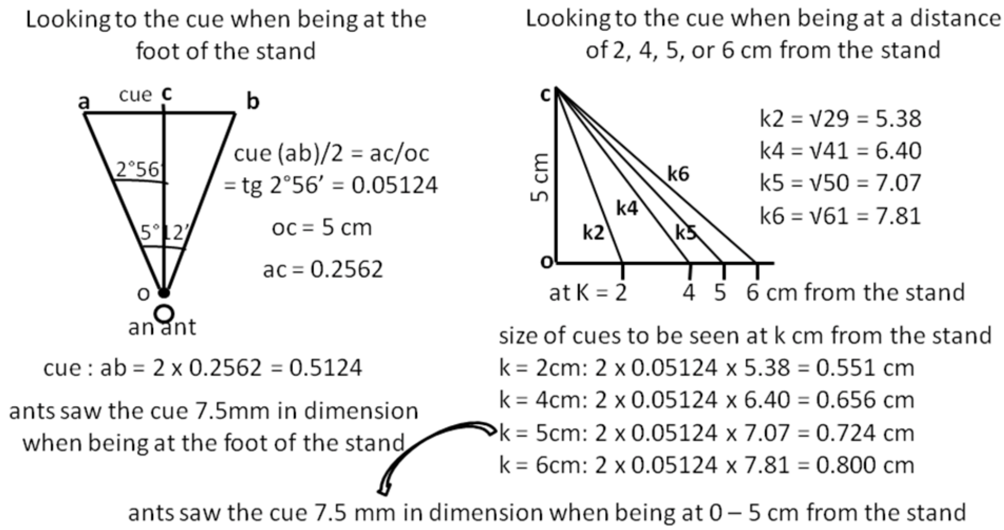

Figure 3. Calculating the minimum dimension required for a cue located at a height of 5 cm in order to be perceived by *M. sabuleti* workers staying at the foot of this cue (left part of the figure) or at several distances from it (right part of the figure).

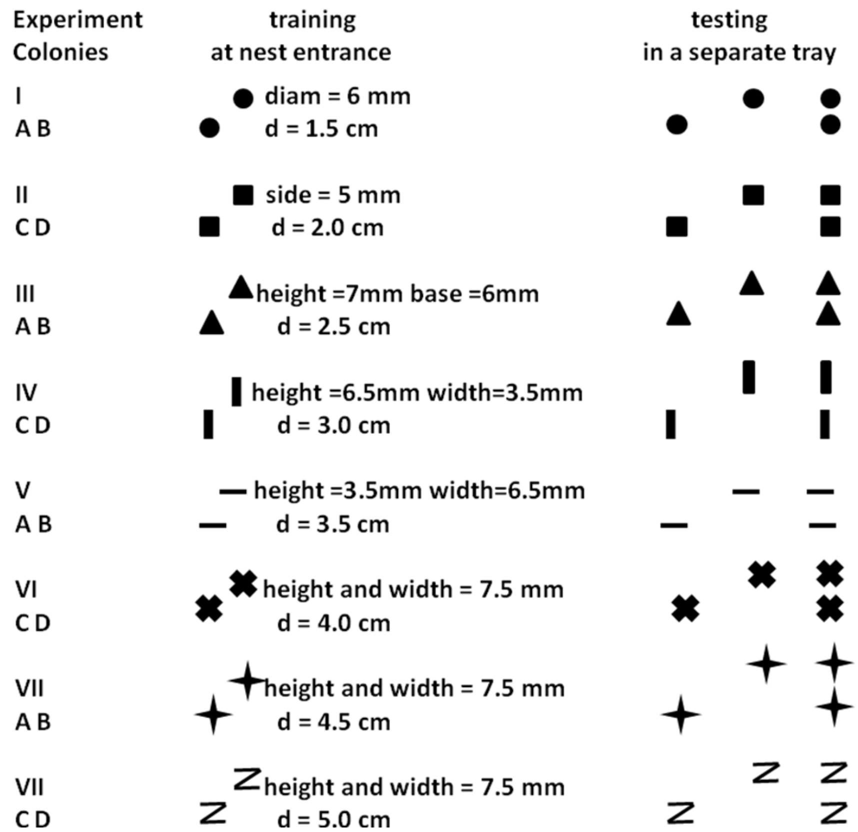

Figure 4. Cues presented to ants for defining the largest vertical distance between two cues beyond which the ants no longer mentally add them. d = the distance between the cues located at the lower and higher levels. The distance between the cues is the one indicated in column two, not the one that is figured in column three.

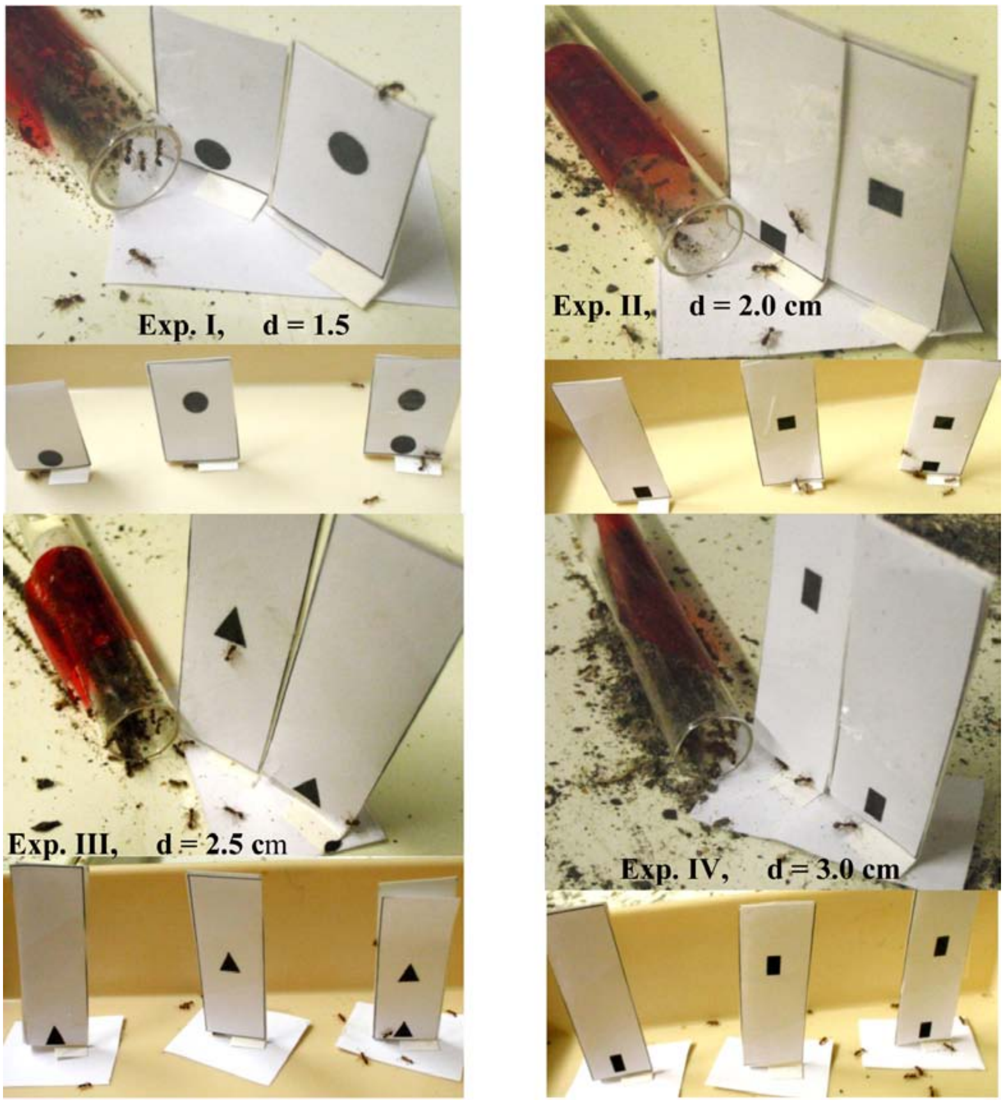

Figure 5. Some views of the experiments I to IV. In each of the experiments, the upper photo concerns the ants' training, and the lower photo, their testing. The ants trained to two cues set at a vertical distance of 1.5 cm to 3.0 cm from each other mostly reacted to the two superimposed cues.

training and testing) and superimposed cues (presented only during testing) were drawn inside a rectangle whose dimensions (2 cm wide x 3 to 8 cm high) allowed maintaining the position that the single cues had during training and thus their vertical distance. All these rectangles containing a single or two superimposed cues were printed on white paper, and each one was tied on the front face of a stand made of extra strong white paper (Steinbach®) whose dimensions were those of the rectangle to be tied (2 cm x 3 to 8 cm). Each stand had also a duly folded foot (twice 1 cm x 0.5 cm) allowing its vertical maintenance. Both stands were tied by their foot to a piece of white paper (3 cm x 5 cm) placed at the nest entrance on the floor of the tray as schematized in the lower part of Figure 2, for ensuring their perfect maintenance in place for 72 hours. The base of the 6 to 8 cm high stands devoted to testing was tied to a piece of paper (4 cm x 3 cm) to ensure their vertical maintenance throughout the test which lasted about 12 minutes (10 experimental minutes with two more ones for making photos) (Figures 5 & 6). The cues were tied on the stands, and the foot

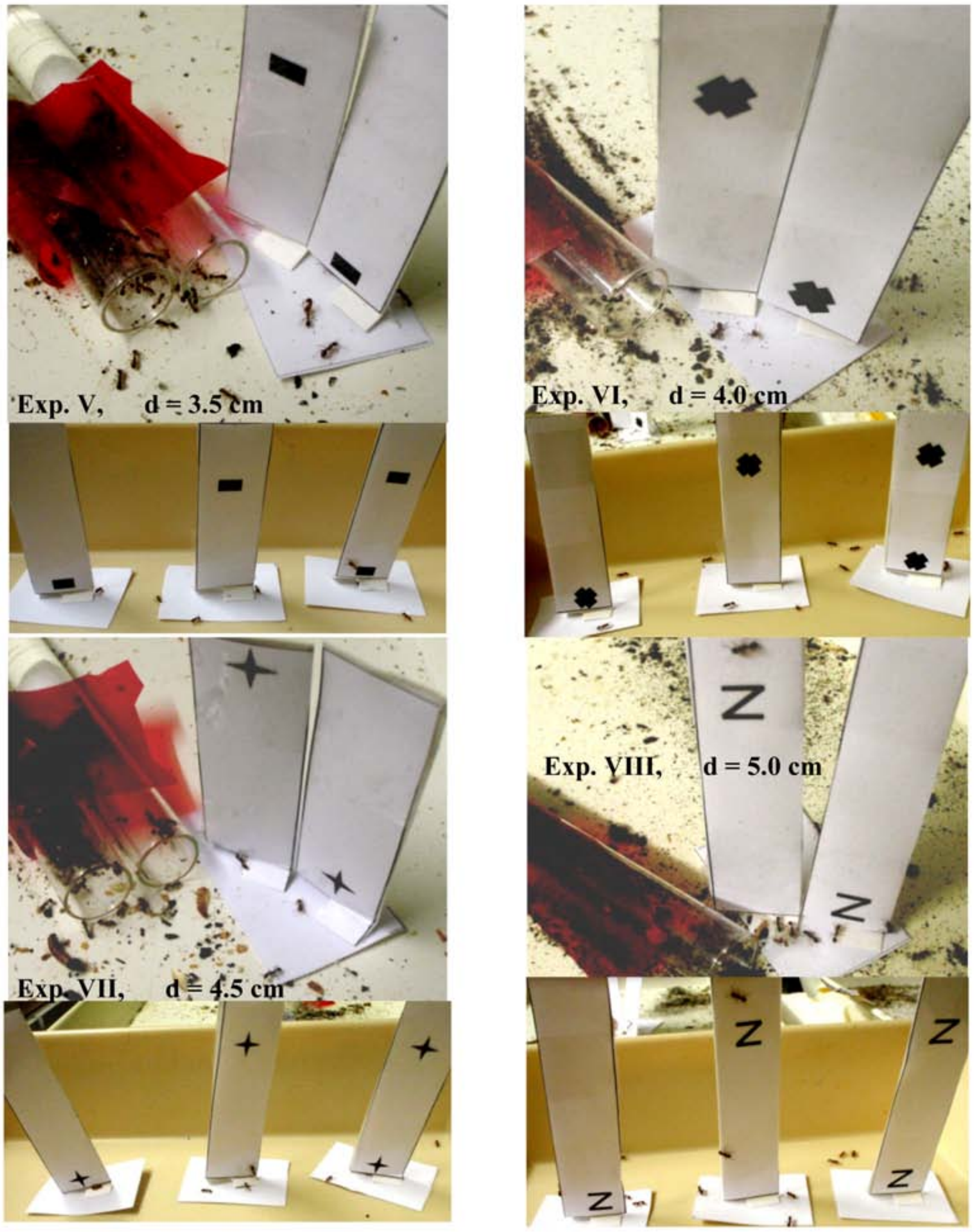

Figure 6. Some views of the experiments V to VIII. In each of the experiments, the upper photo concerns the ants' training, and the lower photo, their testing. The ants trained to cues set at a vertical distance of 3.5 cm from each other reacted to the two superimposed cues, though less than during the experiments I to IV (Figure 5). The ants trained to two cues set at a vertical distance of 4.0 to 5 cm from each other reacted essentially to each separated single cue, mostly to the one located at the higher level, and no longer mentally added two such distant cues.

of the stands was tied on the paper shelf, in order to ensure their vertical maintenance, by using an extra transparent sticky paper and this was made 1 to 4 days before their use to ensure that no odor remained.

Experimental protocol, statistical analysis

For training the ants, the cues used for each experiment were deposited close to the nest entrance which acted as a valuable reward [e.g., 3, 4] and the ants then started to acquire operant

(= operative) conditioning. To make a test on a colony, 20 ants of that colony were transported to their tray devoted to testing. The ants freely moved in the tray, perceived the three presented stands, and went towards those of their choice. They stayed near a stand for about 2 to 60 seconds. Half a minute after the ants were in their tray, those present at less than 2 cm from each cue were counted 20 times, i.e., every 30 seconds over 10 minutes. The number of counts made for each cue and during each of the six tests equaled 20 per colony, thus 40 for the two colonies. For each experiment, this gave a total of 120 counts (20 x 6) on one colony and 240 on two colonies. For each presented stand, the sums of the 20 counts made for each colony and during each test as well as the total of the obtained six sums (six tests were performed for each kind of cue) are given in Tables 1a and 1b. For each experiment, these six sums obtained for each of the two colonies were correspondingly added, and the obtained totals allowed establishing the proportion of ants counted in front of each of the three presented stands. These proportions are reported in the section ‘Results’, and graphically presented in Figure 7. The totals of the counts made for each experiment on the two colonies during the six tests were used for statistical analysis.

The goodness-of-fit χ^2 test was used to compare the numbers expected if the ants randomly went to the cues presented during testing with (1) the number of ants counted in front of the three presented cues, (2) the number of ants counted in front of the cue located at the lower level and of the two superimposed ones, and (3) the number of ants counted in front of the cue located at the higher level and of the two superimposed ones [29]. By using Statistica® software (StatSoft, Maisons-Alfort, France), the non-parametric test of Wilcoxon allowed comparing (1) the six successively recorded numbers of ants in front of the cue located at the lower level with the six successively recorded numbers in front of the two superimposed cues, (2) the six successively recorded numbers of ants in front of the cue located at the higher level with the corresponding numbers recorded in front of the superimposed cues, as well as, though this was not the aim of the present work, (3) the successively recorded numbers of ants in front of the cues located at the lower and the higher levels. The results of these analyses are given in Tables 1a and 1b, and are summarized and commented in the text.

RESULTS

Photos of each experiment can be seen in Figures 5 & 6. Numerical and statistical results are detailed in Table 1 and Figure 7 summarizes the conclusions developed hereunder. When two P values are given, the first one concerns the χ^2 test and the second one the Wilcoxon test.

Experiment I, colonies A and B, circles, vertical distance between cues = 1.5 cm

Measured over the 72 training hours, the average proportion of tested ants counted in front of the cue located at the lower level equaled 7.30%, that in front of the cue located at the higher level equaled 20.38%, and in front of the two superimposed cues, the proportion was 72.31%. Statistically, the ants did not randomly go to the three presented cues ($P < 0.001$). They preferred the cue with the superimposed elements to those with a single element ($P < 0.001$ and $P = 0.028$). It could thus be concluded that the ants mentally added the two cues presented with a vertical distance of 1.5 cm between them. Moreover, and unexpectedly, they appeared to react more to the cue located at the higher level than to the cue located at the lower level ($P = 0.028$).

Experiment II, colonies C and D, squares, vertical distance between cues = 2.0 cm

Taking account of the six testing sessions, the mean proportion of ants moving near the cue located at the lower level equaled 5.79%, that near the cue located at the higher level equaled 23.43%, and that near the two superimposed cues equaled 70.78%. Statistically, the ants did not randomly go to the three kinds of cues ($P < 0.001$). They more often visited the cue with the superimposed elements than the single elements located at the lower and the higher levels separately presented ($P < 0.001$ and $P = 0.028$). They thus mentally added the two cues which were located at a distance of 2.0 cm from each other. As in the previous experiment, between the two single cues they had some preference for the cue located at the higher level ($P = 0.028$).

Table 1a. Ants' responses to the two cues presented during training and to their superimposition. The vertical distance (d) between the two cues differed according to the experiment (I to IV).

Experiment, cue, distance (d), training time (h)	**Ant visits to the lower cue, the higher cue and the superimposed cues for colony A or C; colony B or D**						**Summing the two colonies: statistics (χ^2) on the three cues, on the lower cue and the superimposed, on the higher cue and the superimposed, as well as (Wilcoxon) on the lower cue and the superimposed, on the higher cue and the superimposed, and on the lower and the higher cues**
I, circles,							goodness-of-fit χ^2 test: 53, 148, 525 *vs.* 242, 242, 242
d = 1.5 cm							χ^2 = 515.05, df = 2, P < 0.001
7 h	4	8	38	13	13	35	goodness-of-fit χ^2 test: 53, 525 *vs.* 289, 289
24 h	4	13	40	6	20	47	χ^2 = 385.44, df = 1, P < 0.001
31 h	0	4	25	0	15	56	goodness-of-fit χ^2 test: 148, 525 *vs.* 336.5, 336.5
48 h	5	11	33	3	10	51	χ^2 = 211.19, df = 1, P < 0.001
55 h	4	10	33	2	12	49	Wilcoxon tests:
72 h	8	19	35	4	25	83	17,10,0,8,6,12 *vs.* 73,87,81,84,82,118; P = 0.028
							21,33,19,21,22,44 *vs.* 73,87,81,84,82,118; P = 0.028
Σ of visits	25	65	204	28	83	321	17,10,0,8,6,12 *vs.* 21,33,19,21,22,44; P = 0.028
II, squares,							goodness-of-fit χ^2 test: 47, 190, 574 *vs.* 270, 270, 270
d = 2.0 cm							χ^2 = 549.55, df = 2, P < 0.001
7 h	0	5	42	6	14	65	goodness-of-fit χ^2 test: 47, 574 *vs.* 310.5, 310.5
24 h	9	20	52	4	17	37	χ^2 = 447.23, df = 1, P < 0.001
31 h	5	15	45	1	15	61	goodness-of-fit χ^2 test: 190, 574 *vs.* 382, 382
48 h	0	8	36	3	15	42	χ^2 = 193.01, df = 1, P < 0.001
55 h	1	16	52	3	14	36	Wilcoxon tests:
72 h	7	23	57	8	28	49	6,13,6,3,4,15 *vs.* 107,89,106,78,88,106; P = 0.028
							19,37,30,23,30,51 *vs.* 107,89,106,78,88,106; P = 0.028
Σ of visits	22	87	284	25	103	290	6,13,6,3,4,15 *vs.* 19,37,30,23,30,51; P = 0.028
III, triangles,							goodness-of-fit χ^2 test: 25, 157, 539 *vs.* 240, 240, 240
d = 2.5 cm							χ^2 = 593.06, df = 2, P < 0.001
7 h	1	14	36	1	18	44	goodness-of-fit χ^2 test: 25, 539 *vs.* 282, 282
24 h	4	10	47	4	19	43	χ^2 = 468.43, df = 1, P < 0.001
31 h	1	11	36	2	12	60	goodness-of-fit χ^2 test: 157, 539 *vs.* 348, 348
48 h	1	10	42	2	14	66	χ^2 = 209.66, df = 1, P < 0.001
55 h	4	9	35	2	14	48	Wilcoxon tests:
72 h	1	14	39	2	12	43	2,8,3,3,6,3 *vs.* 80,90,96,108,83,82; P = 0.028
							32,29,23,24,23,26 *vs.* 80,90,96,108,83,82; P = 0.028
Σ of visits	12	68	235	13	89	304	2,8,3,3,6,3 *vs.* 32,29,23,24,23,26; P = 0.028
IV, rectangles ▮ ,							goodness-of fit χ^2 test: 34, 155, 548 *vs.* 369, 369, 369
d = 3.0 cm							χ^2 = 514.76, df = 2, P < 0.001
7 h	2	8	33	4	13	30	goodness-of-fit χ^2 test: 34, 548 *vs.* 291, 291
24 h	1	11	38	3	12	38	χ^2 = 453.94, df = 1, P < 0.001
31 h	2	15	38	3	12	44	goodness-of-fit χ^2 test: 155, 548 *vs.* 351.5, 351.5
48 h	1	11	65	6	10	55	χ^2 = 219.70, df = 1, P < 0.001
55 h	1	9	32	6	16	55	Wilcoxon tests:
72 h	0	10	59	5	28	61	6,4,5,7,7,5 *vs.* 63,76,82,120,87,120; P = 0.028
							21,23,27,21,25,38 *vs.* 63,76,82,120,87,120; P = 0.028
Σ of visits	7	64	265	27	91	283	6,4,5,7,7,5 *vs.* 21,23,27,21,25,38; P = 0.028

Table 1b. Ants' responses to the two cues presented during training and to their superimposition. The vertical distance (d) between the two cues differed according to the experiment (V to VIII).

Experiment, cue, distance (d), training time (h)	**Ant visits to the lower cue, the higher cue and the superimposed cues for colony A or C; colony B or D**		**Summing the two colonies: statistics (χ^2) on the three cues, on the lower cue and the superimposed, on the higher cue and the superimposed, as well as (Wilcoxon) on the lower cue and the superimposed, on the higher cue and the superimposed, and on the lower and the higher cues**
V, rectangles ▬ ,			goodness-of-fit χ^2 test: 134, 325, 513 *vs*. 324, 324, 324
d = 3.5 cm			$\chi^2 = 221.67$, df = 2, P < 0.001
7 h	8 22 25	16 38 37	goodness-of-fit χ^2 test: 134, 513 *vs*. 323.5, 323.5
24 h	10 24 42	17 27 37	$\chi^2 = 222.00$, df = 1, P < 0.001
31 h	8 23 27	17 42 53	goodness-of-fit χ^2 test: 325, 513 *vs*. 419, 419
48 h	5 18 44	21 35 49	$\chi^2 = 42.18$, df = 1, P < 0.001
55 h	5 20 53	12 33 65	Wilcoxon tests:
72 h	5 20 37	10 23 44	24,27,25,26,17,15 *vs*. 62,79,80,93,118,81; P = 0.028
			60,51,65,53,53,43 *vs*. 62,79,80,93,118,81; P = 0.028
sums	41 127 228	93 198 285	14,27,25,26,17,15 *vs*. 60,51,65,53,53,43; P = 0.028
VI, crosses,			goodness-of-fit χ^2 test: 174, 406, 282 *vs*. 287, 287, 287
d = 4.0 cm			$\chi^2 = 93.82$, df = 2, P < 0.001
7 h	15 31 27	22 34 33	goodness-of-fit χ^2 test: 174, 282 *vs*. 228, 228
24 h	7 18 18	23 30 24	$\chi^2 = 25.58$, df = 1, P < 0.001
31 h	5 35 30	9 55 31	goodness-of-fit χ^2 test: 406, 282 *vs*. 344, 344
48 h	12 29 15	22 37 36	$\chi^2 = 22.35$, df = 1, P < 0.001
55 h	6 32 30	17 42 15	Wilcoxon tests:
72 h	17 26 10	19 37 13	37,30,14,34,23,36 *vs*. 60,42,61,51,45,23; P = 0.075
			65,48,90,66,74,63 *vs*. 60,42,61,51,45,23; P = 0.028
sums	62 171 130	112 235 152	37,30,14,34,23,36 *vs*. 65,48,90,66,74,63; P = 0.028
VII, stars,			goodness-of- fit χ^2 test: 216, 424, 306 *vs*. 315, 315, 315
d = 4.5 cm			$\chi^2 = 69.01$, df = 2, P < 0.001
7 h	23 32 14	24 33 9	goodness-of-fit χ^2 test: 216, 306 *vs*. 261, 261
24 h	19 30 14	12 41 30	$\chi^2 = 15.52$, df = 1, P < 0.001
31 h	15 32 19	19 31 17	goodness-of-fit χ^2 test: 424, 306 *vs*. 365, 365
48 h	15 35 30	20 42 31	$\chi^2 = 19.07$, df = 1, P < 0.001
55 h	18 27 41	23 53 36	Wilcoxon tests:
72 h	13 25 30	15 43 35	47,31,34,35,41,28 *vs*. 23,44,36,61,77,65; P = 0.116
			65,71,63,77,80,68 *vs*. 23,44,36,61,77,65; P = 0.028
sums	103 181 148	113 243 158	47,31,34,35,41,28 *vs*. 65,71,63,77,80,68; P = 0.028
VIII, Z,			goodness-of-fit χ^2 test: 226,471,124 *vs*.274, 274, 274
d = 5.0 cm			$\chi^2 = 232.07$, df = 2, P < 0.001
7 h	6 28 7	19 45 20	goodness-of-fit χ^2 test: 226, 124 *vs*. 175, 175
24 h	30 58 15	17 52 18	$\chi^2 = 297.26$, df = 1, P < 0.001
31 h	6 23 2	30 44 10	goodness-of-fit χ^2 test: 471, 124 *vs*. 297.5, 297.5
48 h	9 31 6	19 41 10	$\chi^2 = 101.35$, df = 1, P < 0.001
55 h	5 30 2	25 46 14	Wilcoxon tests:
72 h	32 53 6	28 50 14	25,47,36,25,30,60 *vs*. 27,33,12,16,16,20; P = 0.046
			73,110,67,72,76,103 *vs*. 27,33,12,16,16,20; P = 0.028
sums	88 193 38	138 278 86	25,47,36,25,30,60 *vs*. 73,110,67,72,76,103; P = 0.028

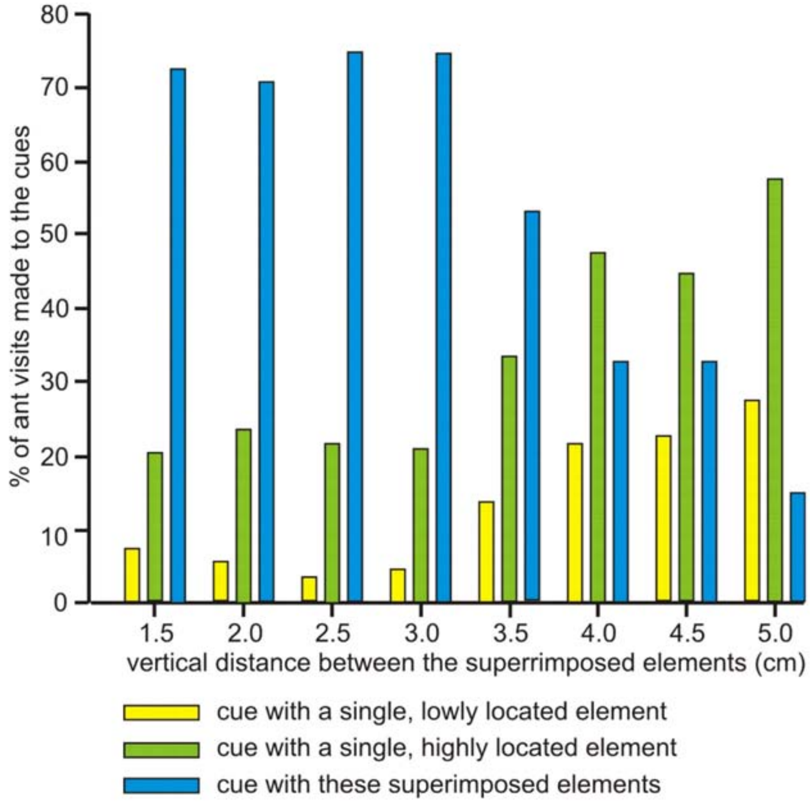

Figure 7. Graphical summary of the results. Trained to two separate cues which were at a vertical distance of 1.5 cm to 5.0 cm from each other, the ants were tested at the same time in front of these two cues and in front of their superimposition. The ants reacted mostly to the superimposed cues when these elements were at a distance of less than 4 cm from each other. When the cues were at a distance of 4 cm from one another and onwards, the ants began to react mostly to the single cues, and they did so totally when the cues were at a distance of 5 cm from each other. The critical vertical distance between cues enabling ants to mentally add them equals thus *ca.* 4 cm. In addition, the ants appeared to react more to the cue located at the higher level than to the one located at the lower level.

Experiment III, colonies A and B, triangles, vertical distance between cues = 2.5 cm

Taking account of the six tests, the proportion of ants which visited the cue located at the lower level equaled 3.47%, that near the cue located at the higher level equaled 21.77%, and that near the two superimposed cues equaled 74.76%. Statistically, the ants did not randomly go to the three presented cues ($P < 0.001$), but they went more often to the superimposed cues ($P < 0.001$ and $P = 0.028$). The ants thus mentally added the two cues presented at a vertical distance of 2.5 cm between them. As during the two previous experiments, the ants more often visited the cue located at the higher level than the lowly located one ($P = 0.028$).

Experiment IV, colonies C and D, vertically oriented rectangles, vertical distance between cues = 3.0 cm

Overall, the proportion of ants that visited the cue located at the lower level equaled 4.61%, while that visited the cue located at the higher level equaled 21.03%, and that visited the two superimposed cues equaled 74.35%. The ants did not statistically randomly choose each stand ($P < 0.001$), but preferred that with the two superimposed cues ($P < 0.001$ and $P = 0.028$). Thus, the ants mentally added the two cues presented with a vertical distance of 3.0 cm between them. As during the three previous experiments, they went more often to the cue located at the higher level than to the one located at the lower level ($P = 0.028$).

Experiment V, colonies A and B, horizontally oriented rectangles, vertical distance between cues = 3.5 cm

Taking into account the six testing sessions, the average proportion of ants sighted in front of the cue located at the lower level equaled 13.89%, that sighted in front of the cue located at the higher level equaled 33.43%, and that sighted in front of the two superimposed cues equaled 52.79%. Statistically, the ants did not randomly go towards each presented stand ($P < 0.001$), but went more often to that bearing the two superimposed cues ($P < 0.001$ and $P = 0.028$). They thus mentally added the two cues presented during training with a vertical distance of 3.5 cm between them. However, the ants chose the superimposed elements less often than during the four previous experiments. As during the four previous experiments, more ants were sighted in front of the cue located at the higher level than in front of the one located at the lower level ($P = 0.028$).

Experiment VI, colonies C and D, crosses, vertical distance between cues = 4.0 cm

According to the six performed tests, the proportion of ants that visited the cue located at the lower level equaled 20.18%, that visited the cue located at the higher level, 47.10%, and that visited the two superimposed cues, 32.71%. The ants did not randomly visit each presented cues ($P < 0.001$), but contrary to the previous experiments, they essentially visited the cue located at the higher level and less often the superimposed cues ($P < 0.001$ and $P = 0.028$). The ants did not mentally add the two cues presented during training with a vertical distance of 4.0 cm between them, but reacted essentially to the cue located at the higher level ($P = 0.028$). The cue with the superimposed elements was not significantly preferred to the cue located at the lower level ($P = 0.075$). Again, the ants significantly more frequently visited the cue located at the higher level than the one located at the lower level ($P = 0.028$).

Experiment VII, colonies A and B, stars, vertical distance between cues = 4.5 cm

On the basis of the six testing sessions, the proportion of ants counted near the cue located at the lower level equaled 22.83%, that counted near the cue located at the higher level equaled 44.82%, and that counted near the two superimposed cues equaled 32.74%. The ants did not randomly visit each presented cues ($P < 0.001$). As in Experiment VI, the ants more often visited the cue located at the higher level than the two superimposed ones ($P = 0.028$) while the visits to the stand with the two superimposed cues were not significantly more frequent than those to the stand bearing the cue located at the lower level ($P = 0.116$). Thus, as during the previous experiment, the ants did not mentally add the two elements presented with a vertically distance of 4.5 cm between them. Also, as during the previous experiments, the ants more often visited the cue located at the higher level than the cue located at the lower level ($P = 0.028$).

Experiment VIII, colonies C and D, 'Z', vertical distance between cues = 5.0 cm

Taking into account the six testing sessions, the proportion of ants that visited the cue located at the lower level equaled 27.53%, that visited the cue located at the higher level equaled 57.37%, and that visited the two superimposed cues equaled 15.10%. The ants did not randomly visit each presented cue ($P < 0.001$). They significantly more often visited the cue located at the higher level than the superimposed cues ($P < 0.001$ and $P = 0.028$), and the cue located at the lower level than the superimposed cues ($P < 0.001$ and $P = 0.046$). They also, again, went more often to the cue located at the higher level than to the one located at the lower level ($P = 0.028$). Consequently, the two single cues located at a vertical distance of 5 cm from each other were not mentally added by the ants, and the ants more often reacted to a cue located at a height of 5 cm than to a cue located near the ground (i.e. close to the floor of their tray).

DISCUSSION

The aim of the present work was to define the maximum vertical distance between visual cues below which *M. sabuleti* ants mentally add these cues and beyond which they no longer do so. This critical distance was found to be 4 cm. In a previous work [4], we assessed the horizontal critical distance between cues enabling the ants to mentally add or not add them, and found that it equals 5 cm. Consequently, similar to the case with horizontally positioned elements, a critical vertical distance between the sighted elements does exist for allowing ants to mentally add them, and this vertical interval is smaller than the horizontal one. This finding is in agreement with the fact that the workers of the ant *M. sabuleti* are more sensitive to a vertical change in orientation of a horizontal segment than to a horizontal change of orientation of a vertical segment [5].

It is interesting to compare our calculation of the maximum vertical distance up to which *M. sabuleti* ants can see cues of different dimensions with that which has been calculated in a previous work [6]. In the present work this distance was calculated (Figure 3) as being equal to about 6 cm for a cue measuring 7.5 mm in dimension. The area of such a cue equals 0.5624 cm^2. In the previous work [6], it was estimated that this distance was larger than 10 cm and lower than 15 cm for a cue measuring 0.5 cm x 3 cm, having thus an area of 1.5 cm^2. One way to compare these vertical maximum distances beyond which *M. sabuleti* ants can no longer see cues of different dimensions is to overlay the geometric representations of the triangular spaces delimited by the ant, the cue and the height at which the cues were located. This leads to two 'similar' triangles whose ratio of the height (15/6 = 2.5) and ratio of the area of the cues (1.5/0.5624 = 2.667) are of the same order of magnitude. The previous and the present work are technically in agreement with each other, demonstrating thus their exactness.

In addition to defining the critical vertical distance between two cues allowing *M. sabuleti* ants to mentally add them, it was found that they better saw the cues located at the higher level than the ones located at the lower level. This peculiarity is in accordance with the ability of the workers of a related species, *Myrmica ruginodis*, Nylander 1846, to distinguish different patterns of luminous spots presented above them [30]. Such a kind of 'celestial' vision is rather common in ants and allows them using celestial cues as well as cues located in the canopy [11]. Seeing well what is above the head may result from the position of the eyes as well as from their morphology, a detailed study of which was previously conducted on three *Myrmica* species [31]. In this last work, the morphology of the *M. sabuleti* workers' eyes has been precisely analyzed and a schema clearly shows their oval configuration which, together with their location on the head, allows a good dorsal visual perception and a different sensitivity to horizontal and vertical views. Photos of *M. sabuleti* workers and of their head can be seen on the internet site 'https://www.galerie-insecte.org/galerie/Myrmica_sabuleti'.

Similar to the previous work defining the maximum horizontal distance between cues allowing ants to add them [4], it here also appeared that the ants somewhat better memorized single cues (Experiment VI: choice of the single cues: 27.53% + 57.37% = 84.9%) than superimposed ones (Experiments I, II, III, IV: choice of the superimposed cues: 72.31%, 70.78%, 74.76%, 74.35% respectively). It thus again seems that it is easier for ants to memorize one cue than to add two cues and memorize the addition.

Mentally adding cues which are identical and located near each other, and not adding cues which differ and/or are located far from one another, is what should be expected for an ant to correctly, easily and rapidly forage, return to food sites and come back to the nest.

CONCLUSION

The present work follows a series of studies in agreement with one another which define when, i.e. under which circumstances and under which conditions, encountered visual cues are mentally added by moving *M sabuleti* ants. These ants add visual cues only when these cues are identical, simultaneously seen, and located at a horizontal distance of less than 5 cm from one another as well as at a vertical distance of less than 4 cm from one another. If not, the cues are not mentally added, but separately memorized.

CONFLICT OF INTEREST STATEMENT

We affirm having no conflict of interest with respect to the subject examined in the present work.

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