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Reaction of ants to the simultaneous sight of two identical or different cues

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ABSTRACT

It has previously been shown that workers of the ant *Myrmica sabuleti* can distinguish different numbers of graphic elements sighted as cues even if the shape, color, size, and relative position of these elements differ somewhat. Moreover, these ants were shown to mentally add numbers of identical elements when seeing them simultaneously but not when seeing them consecutively. A further step is needed to examine whether the adding capability of these ants applies only to identical elements or to elements differing by their shape, color, size or location. We first performed an experiment that confirmed that *M. sabuleti* workers mentally added visual elements identical in their appearance when they perceived them simultaneously. We then experimentally established that these ants did not mentally add up elements seen simultaneously when these elements differ in shape, color or size, but added up similar elements that differ only in spatial location. Such behavior in front of encountered visual cues could help *M. sabuleti* workers correctly perform tasks such as foraging and navigating between nest and food sites.

KEYWORDS: cognition, *Myrmica sabuleti*, mental addition, numerosity ability, operant conditioning.

INTRODUCTION

It has previously been shown that the workers of the ant *Myrmica sabuleti* Meinert, 1861, can discriminate different numbers of graphic elements sighted as cues even if the shape, color, size, and relative position of these elements differ somewhat [1]. It has also been shown that these workers mentally add numbers of elements when they see them simultaneously (i.e., at the same time) [2, 3]. Moreover, it has been demonstrated that these ants can make additions using learned symbols (for numerosities corresponding to numbers 1, 2, 3, 4 and 0) and that they do so only when the symbols are set aside from each other [4, 5]. *Myrmica sabuleti* workers were also shown to be able to guess the next number of elements of an increasing or decreasing arithmetic sequence, but only when in the presence of the sequence and not in its absence [6, 7]. Similarly, these ants could guess the size of the next element of an increasing or decreasing geometric sequence, once more doing so only in the presence of the sequence [8]. All these results indicate that *M. sabuleti* workers respond to the sighted result of a numerical operation only if in the presence of its different addends. We can thus presume that these workers would mentally add visual cues only if these cues are similar and not if they differ

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The present work was done after their retirement.

by some of their characteristics (shape, color, size…). The present work aims to check this latter presumption by experimenting on three ant colonies and using cues differing by three of their characteristics as well as identical cues differing only by their relative location. We first performed a preliminary experiment to confirm that *M. sabuleti* workers mentally add simultaneously perceived visual elements when they are identical in appearance and location.

As a preamble to our experimental methods and results, we recall what is currently known about addition and subtraction abilities in several vertebrate and invertebrate species. This recall will not be exhaustive since the subject has been largely presented in previous works [2-5].

The skill of adding a number of elements was acquired during the evolution of the animal kingdom through four successive steps. These steps are: (1) discriminating amounts of elements, (2) obtaining a precise assessment of numbers of elements by counting them, (3) being able to add and subtract several amounts of numbers, and (4) making correspondence between numbers and symbols and using the latter for adding or subtracting. The more evolved the species, the more steps could be reached. Fishes [9, 10], even newborn ones [11], can discriminate different amounts of elements when concretely seeing them. Amphibians [12, 13] are also at this first level. Several bird species, such as robins [14], corvids [15-17], parrots [18], and even newborn chicks [19], can evaluate quantities and count elements. Such true counting is also an ability exhibited by some mammals, e.g., rodents [20, 21] and monkeys [22-24]. Moreover, some birds and some primates can associate symbols with numbers of elements. Indeed, pigeons can associate the number of pecks with numerosity symbols [25], and parrots can acquire a phonetic representation of the numbers 1 to 9 [18]. Monkeys [26] and chimpanzees [27] can acquire a visual representation of the numbers 0 to 9. Regarding invertebrates, golden orb-web spiders can assess the number of prey items in their larder, mastering thus some numerosity concepts [28], and a spider-eating jumping spider [29] can discriminate among sights of 1, 2 or more prey. Mealworm beetles adapt the duration of their mate guarding as a function of the number of competitors [30]. Honeybees can learn adding as well as subtracting one element (blue as well as yellow respectively) to or from 1 to 5 ones [31]. They are also able to associate symbols to numbers of elements [32]. Concerning our experiments on the ant *M. sabuleti*, let us recall that these hymenoptera can discriminate amounts of elements, add them when they see them simultaneously, can acquire symbolisms corresponding to 1, 2, 3, 4 and 0 elements (they have to acquire the notion of zero through experiences) and can use such symbolisms for adding [2-5, 33, 34]. In addition, they can increment the last element of an increasing or decreasing arithmetic or geometric sequence when in the presence of the sequence [6-8]. Let us repeat that we here aim to examine whether *M. sabuleti* workers mentally add only similar elements (i.e., elements of the same kind) or add them regardless of their appearance (different kinds of shape, color, size and position).

MATERIALS AND METHODS

Collection and maintenance of ants

The experiments were conducted on three colonies containing approximately 500 workers, a queen and brood, which were parts of a large natural colony containing more than 2,000 workers collected in September 2019 in an abandoned quarry located at Olloy/Viroin (Ardennes, Belgium). The colonies were maintained in one to two glass tubes half-filled with water, and a cotton plug was used to separate the ants from the water. The nest tubes of each colony were set in a tray (34 cm x 23 cm x 4 cm) that served as the foraging area. In these areas, a cotton-plugged tube containing a sugar water solution was permanently provided, and pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) were delivered three times per week. The laboratory had an illuminance of ca. 330 lux, and the temperature of the room was ca. 20 °C, the humidity ca. 80%, and the electromagnetic field ca. 2 μWm^2, these environmental conditions being suitable to *M. sabuleti*. Here, we often name ants ‘workers’ or ‘nestmates’, as commonly done by researchers on social insects.

Cues presented to the ants

The cues are schematized in Figure 1. Those used to train the ants were either two stands (see below) bearing elements identical in their appearance and relative location (preliminary experiment) or two stands bearing elements differing from each other either by their shape (Experiment I), color (Experiment II), size (at least by one of their dimensions, Experiment III) or only by their location (Experiment IV). The elements of each cue were drawn inside a 2 cm x 2 cm white square using Microsoft Word® software, cut, and tied with extra transparent sticky paper on the front face of a stand three to six days before starting the experiments to avoid the remaining odor. Each stand was made of Steinbach® (Malmedy, Belgium) strong white paper (250 g/m^2), had a vertical part (2 cm x 2 cm) and was maintained vertically thanks to a horizontal part [2 x (1 cm x 0.5 cm)] duly folded. The cues used to test the ants were similar to the cues used to train them, but novel, never used before.

Experimental planning

A preliminary experiment and then four successive experiments were performed each with a delay of two days between each of them. The preliminary experiment aimed to check whether ants effectively add identical cues simultaneously seen, i.e. cues of either the same shape (colony A), same color (colony B) or same size (colony C). These cues were different from those presented to the same colonies during Experiments I, II, III, and moreover were only horizontally juxtaposed. The experiments I to IV were performed to examine whether ants respond to the simultaneous sight of cues with a different shape (Experiment I), color (Experiment II) or size (Experiment III) or to the simultaneous sight of similar cues, but differently located (Experiment IV). Each of these experiments was conducted according to the same protocol, time periods, and mathematical and statistical analyses.

Experimental design and protocol

A schema of the design and protocol is given in Figure 2. Photos of the experiments are shown in Figures 3 and 4.

For each experiment, the ants were trained in their foraging area. One cue was set on the left, and another cue was set on the right of the nest entrance, which has been shown to be a valuable reward for *M. sabuleti* workers [33]. The ants were expected to memorize these two cues. A blank stand, i.e., a cue to avoid, not bearing any element, was set far from the nest entrance and any food, thus far from any reward.

Over this 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), with each of the three colonies having its own tray devoted to testing. For the preliminary experiment, three stands were set in the testing tray, one bearing the cue presented twice during training, a second bearing the two juxtaposed cues, and a third bearing nothing. For Experiments I, II, III, and IV, four stands were deposited, one bearing the cue set on the left of the nest entrance during training, a second bearing the cue set on the right of the nest entrance during training, a third stand bearing the two cues drawn beside one another (i.e., the two cues added), and a fourth stand bearing nothing (= a blank stand).

To perform a test on one colony, 15 ants were transferred in the middle of their tray devoted to testing. The ants freely moved in the tray, saw the cues, went toward them, and stayed 2 to 20 seconds near those of their choice. Half a minute after the ants were in their tray devoted to testing, every 30 seconds over 10 experimental minutes (thus 20 times), the ants present at less than 2 cm of each cue were punctually counted, and the sum of these counts was established for each kind of cue. The sums for each colony and each training period are given in Tables 1 and 2, respectively. After each test, the ants were placed back in their foraging area, near the entrance of their nest.

Statistical analysis

As reported above, Tables 1 and 2 give the sum of the 20 counts made during the testing at the end of each of the six training time periods for each of the three colonies (A, B, C) and for each cue. For each training time period, the results obtained for the three colonies were correspondingly added, and the percentage of ants' visits to each cue was calculated and graphically presented in Figures 3 (preliminary experiment) and 5 (Experiments I to IV).

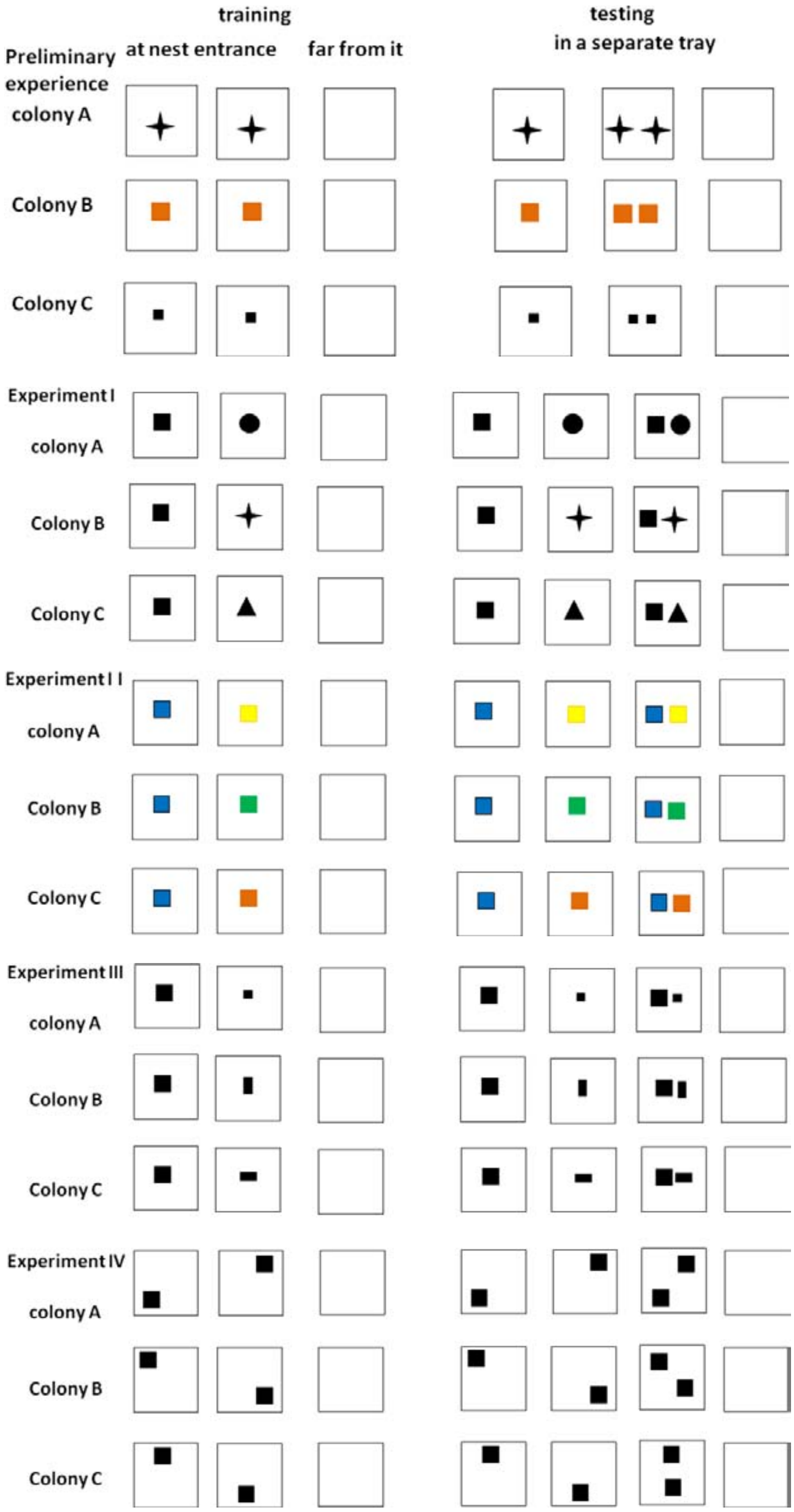

Figure 1. Visual cues used during the four experiments performed, each one on three ant colonies. Each cue was presented tied to a stand (see Figures 2 and 3). The ants were trained in their foraging area to two identical cues (preliminary experiment) or to two cues differing by their shape, color, size, or location. They were tested in a separate tray in front of one or two cues (if different), their sum and a blank stand.

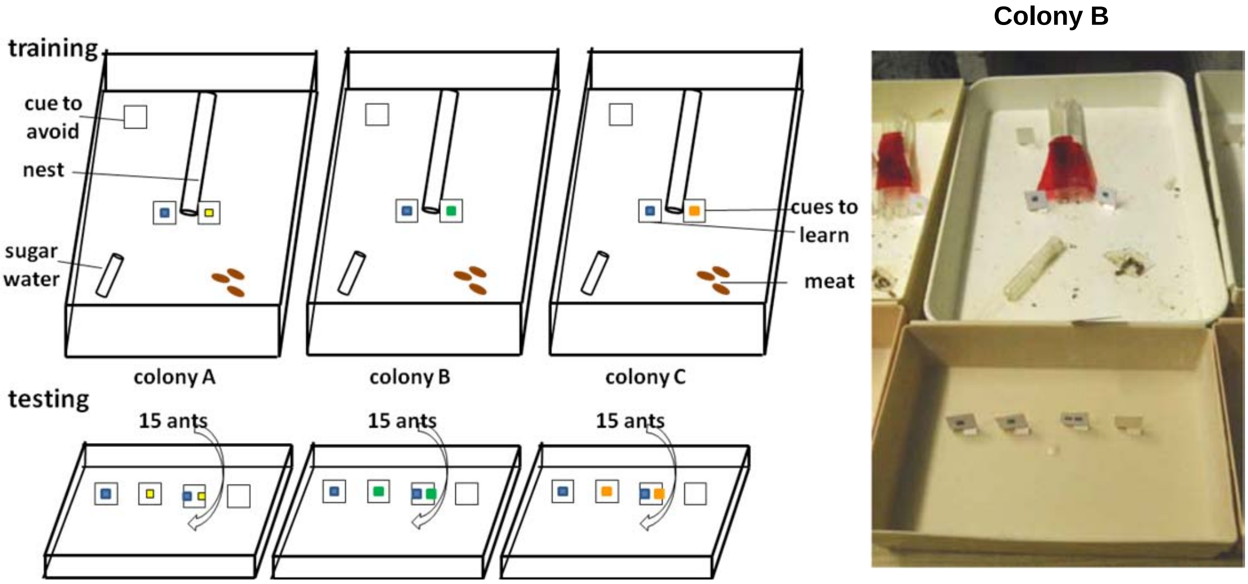

Figure 2. Experimental design used to examine whether ants add visual cues of different kinds. Only the experiment using differently colored cues is schematized here; three other experiments were performed, with cues of different shapes, sizes or locations on the stand. The ants were trained in their foraging area, the cues being set at the nest entrance and a blank stand being set far away. The ants were tested over time in a separate tray in front of each of the two cues used during training, their sum and a blank stand.

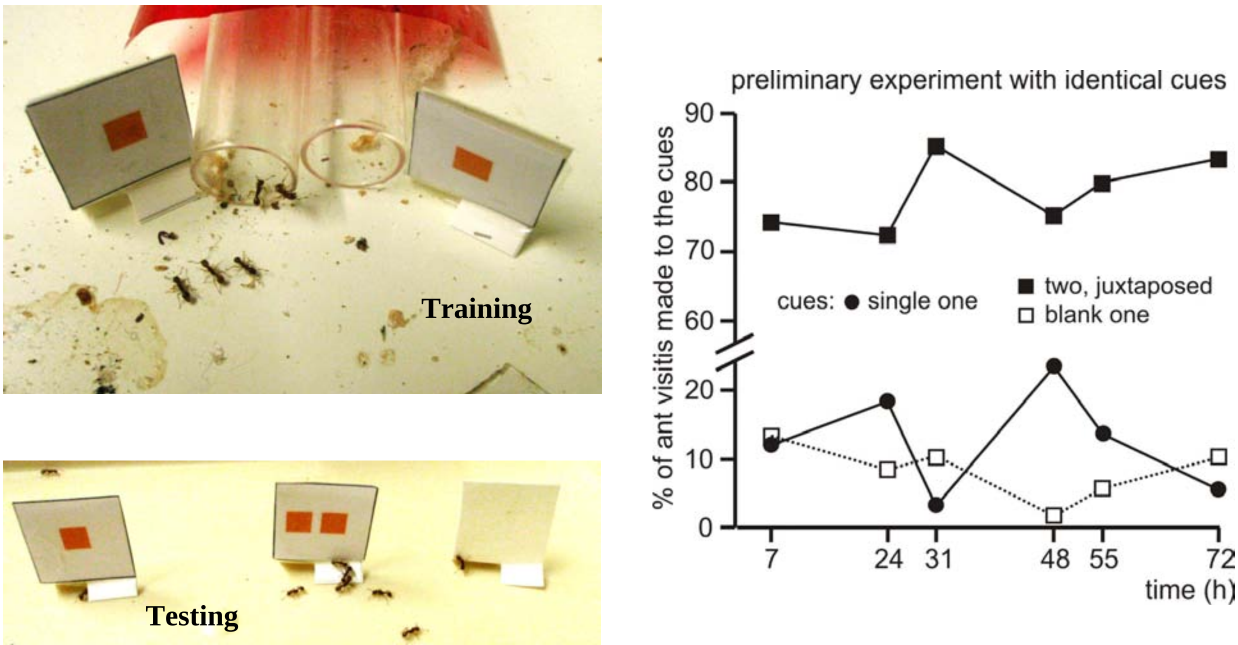

Figure 3. Two photos and a graphical summary of the results of the preliminary experiment. The ants of the three colonies were presented with two identical cues at their nest entrance, and these cues differed from those received during Experiments I to IV. They were tested in front of a single cue (the kind of cue they saw during training), of the two juxtaposed cues (horizontally presented on a stand) and of a blank stand. They essentially responded to juxtaposed identical cues.

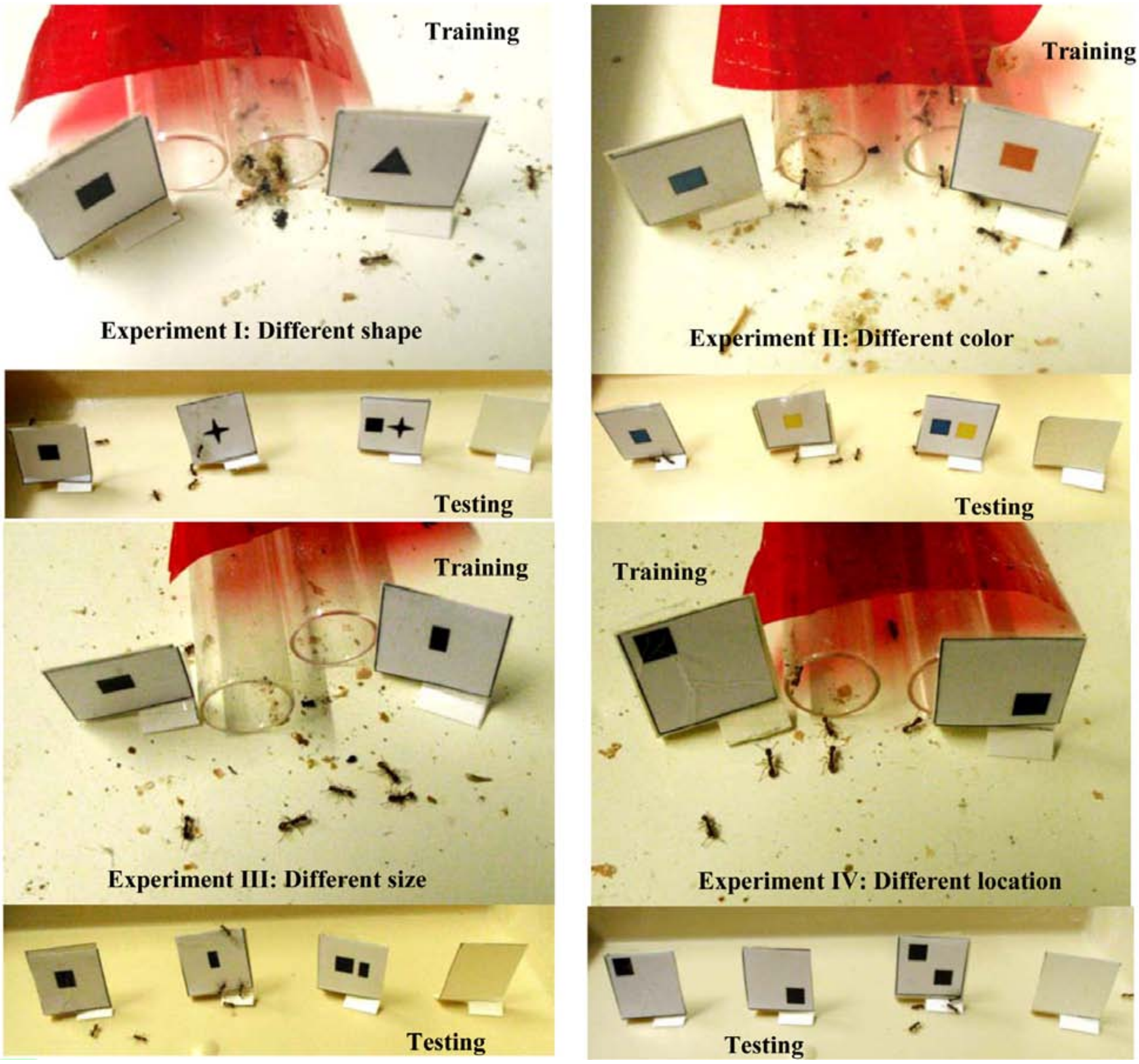

Figure 4. Some views of Experiments I to IV. The ants were trained to two cues set near each other at the nest entrance. The two cues differed by their shape, color, size or location on the stand. The tested ants responded to each of the two presented cues and not to their juxtaposition when the two cues differed by their shape, color, or size (Experiments I, II, III) and responded to the two juxtaposed cues when the latter were identical but differently located (Experiment IV).

For the preliminary experiment and for each of the four experiments (I, II, III, IV), the sum of the counts obtained for each colony after 7, 24, 31, 48, 55 and 72 training hours were added (Tables 1 and 2: ∑ visits), and the numbers totalized for the three colonies were used for statistical analysis.

The goodness-of-fit χ^2 test [35] was used to compare: (1) the total numbers of ants' visits to the three (preliminary experiment) or four (Experiments I, II, III, IV) presented cues, (2) for the preliminary experiment, the numbers of visits to the cue set either on the left or on the right of the nest entrance during training and to the cue representing the two juxtaposed identical cues, and for Experiments I, II, III, IV, the numbers of visits to the cue set on the left of the nest entrance

Table 1. Number of ants' visits over time to the simultaneous presentation of a stand bearing only one cue similar to the two identical ones seen by the ants at the left and at the right of the nest entrance during training, of a stand bearing these two cues juxtaposed, and of a blank stand.

Time (hours)	Colony A: same shape			Colony B: same color			Colony C: same size		
	one cue	added	blank	one cue	added	blank	one cue	added	blank
7 h	5	40	14	3	52	5	15	49	7
24 h	5	35	9	12	38	4	15	51	2
31 h	1	37	4	2	36	11	2	46	0
48 h	12	35	2	10	40	1	12	38	0
55 h	4	29	3	4	32	2	8	31	2
72 h	4	37	14	4	29	2	1	60	0
∑ visits:	31	213	46	35	227	25	53	275	11
Statistics:	goodness-of-fit test: 119, 715, 82 *vs.* equally distributed: $\chi^2 = 756.81$, df = 3, P < 0.001 119, 715 *vs.* equally distributed: $\chi^2 = 425.92$, df = 1, P < 0.001 Wilcoxon test (one cue *vs.* added cues): 23, 32, 5, 34, 16, 9 *vs.* 141, 124, 119, 113, 92, 126: N = 6, T = 0, P = 0.028								

during training and to the cue representing the juxtaposed two cues, (3) for Experiments I, II, III, IV, the numbers of visits to the cue set on the right of the nest entrance during training and to the one representing the juxtaposed two cues, and (4) for Experiments I, II, III, IV, the numbers of visits to the cue set on the right and to the cue set on the left of the nest entrance during training. The P values obtained using goodness-of-fit tests were adjusted for multiple comparisons by using the Benjamini-Hochberg procedure with a false discovery rate of 0.05 [36].

By using the Wilcoxon nonparametric test running under Statistica® v10 software (StatSoft, Maisons-Alfort, France), the six successive numbers of visits for each cue totalized for the three colonies and obtained after the 7 to 72 hours of training enabled us to compare the ants' responses to: (1) the 'single' cue and the two juxtaposed cues, for the preliminary experiment, and (2) the cue set on the left of the nest entrance during training and the cue set on the right of the nest entrance, for Experiments I, II, III and IV. The level of probability was set at P = 0.05.

RESULTS

Numerical and statistical results are given in Table 1 (preliminary experiment) and Table 2 (Experiments I to IV). Some illustrations and a graphical summary of the results are shown in Figure 3 (preliminary experiment) and in Figures 4 and 5 (Experiments I to IV).

Preliminary experiment: Reaction to the juxtaposition of two cues of the same shape, color and size and relative location

The ants of colony A were trained to two identical black stars, those of colony B to two identical orange squares, and those of colony C to two identical small black squares. These cues with the same design were set one at the left and one at the right of the nest entrance. At the same time, a blank stand was set far from any reward. In this experiment, each colony was presented with a cue whose shape, color or size differed from those of the cues presented in Experiments I, II or III.

The ants were tested over time simultaneously in front of one of the visual cues presented during training, of these two cues horizontally juxtaposed, and of a blank stand. The numerical and statistical results are given in Table 1. During testing, the ants of the three colonies more often visited the two identical cues juxtaposed on a stand than a single cue and the blank cue. The percentage of visits obtained over the ants' training to the single cue, to the two added cues, and to the blank cue can be seen in Figure 5. The numbers of ants' visits to each of the three

Table 2. Number of ants' visits over time to the simultaneous presentation of two cues differing either by their shape, color, size or relative location on their stand, these cues being seen near each other by the ants during training, as well as to these cues presented in juxtaposition (i.e., 'added' on the same stand) and to a blank stand (a stand bearing no cue).

	Colony A				**Colony B**				**Colony C**			
Exp, time	**left**	**right**	**added**	**blank**	**left**	**right**	**added**	**blank**	**left**	**right**	**added**	**blank**
Exp I, 7 h	5	25	19	0	20	14	4	3	28	61	4	0
24 h	7	26	8	2	35	16	5	2	17	56	11	0
31 h	3	41	7	1	9	21	2	1	47	20	0	0
48 h	28	29	9	0	54	20	2	1	34	24	1	3
55 h	17	49	5	5	20	44	4	1	36	26	0	0
72 h	39	27	7	1	28	16	3	0	35	17	1	3
∑ visits:	99	197	55	9	166	131	20	8	197	204	17	6
Statitstics:	goodness-of-fit: 462, 532, 92, 23 *vs.* equally distributed: χ^2 = 714.12, df = 3, P < 0.001											
# shape	462, 92 *vs.* equally distributed: χ^2 = 247.11, df = 1, P < 0.001											
	532, 92 *vs.* equally distributed: χ^2 = 310.26, df = 1, P < 0.001											
	462, 532 *vs.* equally distributed: χ^2 = 4.93, df = 1, 0.025 < P < 0.050											
	Wilcoxon: 53, 59, 59, 116, 73, 102 *vs.*100, 98, 82, 73, 119, 60: N = 6, T = 7, P = 0.46											
Exp II, 7 h	16	18	6	2	16	23	15	6	22	16	1	1
24 h	31	34	4	1	13	21	3	1	21	28	4	0
31 h	15	35	7	4	24	16	2	0	21	19	1	0
48 h	32	35	3	2	27	14	4	0	27	24	5	1
55 h	20	30	6	1	19	18	1	3	44	31	0	0
72 h	37	21	1	0	22	20	5	1	33	26	2	1
∑ visits:	151	173	27	10	121	112	30	11	168	144	13	3
Statistics:	goodness-of-fit: 440, 429, 70, 24 *vs.* equally distributed: χ^2 = 628.67, df = 3, P < 0.001											
# color	440, 70 *vs.* equally distributed: χ^2 = 268.43, df = 1, P < 0.001											
	429, 70 *vs.* equally distributed: χ^2 = 258.28, df = 1, P < 0.001											
	440, 429 *vs.* equally distributed: χ^2 = 0.139, df = 1, 0.50 < P < 0.75											
	Wilcoxon: 54, 65, 60, 86, 83, 92 *vs.* 57, 83, 70, 73, 79, 67: N = 6, T = 9, P = 0.75											
Exp III, 7 h	35	22	11	0	19	33	3	1	26	28	0	0
24 h	23	27	5	2	21	33	4	2	52	21	1	0
31 h	30	36	9	1	48	30	3	1	33	39	3	8
48 h	26	19	4	5	38	46	8	1	47	32	1	0
55 h	24	52	5	0	18	20	3	0	22	21	7	3
72 h	27	23	3	0	14	24	8	0	15	27	0	0
∑ visits:	165	179	37	8	158	186	29	5	195	168	12	11
Statistics:	goodness-of-fit: 513, 533, 78, 24 *vs.* equally distributed: χ^2 = 782.00, df = 3, P < 0.001											
	513, 78 *vs.* equally distributed: χ^2 = 320.18, df = 1, P < 0.001											
# size	533, 78 *vs.* equally distributed: χ^2 = 338.83, df = 1, P < 0.001											
	513, 533 *vs.* equally distributed: χ^2 = 0.38, df = 1, 0.50 < P < 0.70											
	Wilcoxon: 80, 91, 111, 111, 64, 56 *vs.*83, 81, 105, 97, 93, 74: N = 6, T = 9, P = 0.75											

Table 2 continued..

	Colony A				Colony B				Colony C			
Exp, time	**left**	**right**	**added**	**blank**	**left**	**right**	**added**	**blank**	**left**	**right**	**added**	**blank**
Exp IV, 7 h	3	7	36	2	0	6	39	5	17	7	33	1
24 h	10	3	32	5	5	5	36	1	3	2	42	1
31 h	1	3	28	2	0	10	31	5	1	6	37	0
48 h	2	8	28	3	0	12	52	4	1	4	45	0
55 h	0	2	48	4	0	2	29	7	6	11	33	0
72 h	3	2	41	2	1	4	30	1	8	3	43	0
∑ visits:	19	25	213	18	6	39	217	23	36	33	233	2
Statistics: # location	goodness-of-fit : 61, 97, 663, 43 *vs.* equally distributed: χ^2 = 940.39, df = 3, P < 0.002 663, 158 *vs.* equally distributed: χ^2 = 310.63, df = 1, P < 0.002 61, 97 *vs.* equally distributed: χ^2 = 8.20, df = 1, P ~ 0.004 Wilcoxon: 20, 18, 2, 3, 6, 12 *vs.*20, 10, 19, 24, 15, 9: N = 5, T = 3 P = 0.23											

presented cues significantly differed from the numbers randomly expected (Table 1, P < 0.001), and the numbers of visits between stands bearing the single and the juxtaposed cues also differed from those expected with random visits (P < 0.001). The latter result was confirmed by the use of the Wilcoxon test (P = 0.028; Table 1). Consequently, the ants responded essentially to the two identical cues simultaneously seen and less to one of the two cues; they mentally added the vision of the two cues, as confirmed by the result of Experiment IV: the ants mentally add identical cues set near each other (thus simultaneously seen), even if they are somewhat differently located on their stand.

Experiment I: Reaction to the juxtaposition of two cues of different shapes

The number of ants' visits to each of the two cues that were set beside the nest entrance during training, to these two cues presented side by side on a same single stand and to the blank stand after 7, 24, 31, 48, 55, and 72 training hours is given in Table 2, and the percentages corresponding to the sum of these numbers for the three colonies are illustrated in Figure 5. The statistical analysis of the sums of the ants' visits observed over their testing (Table 2) showed that the ants did not randomly go toward each presented cue (P < 0.001) but went more to the cues they saw on the left or on the right of the nest entrance during training than to these two cues presented side by side on a single stand (P < 0.001). As an ancillary result, the ants went slightly more to the cue they saw on the left than to the one they saw on the right of their nest entrance during training (0.025 < P < 0.050), possibly due to more ants walking on the right side of the nest entrance because the meat food was located there, and they had to furnish meat to the brood. Nevertheless, the ants' conditioning scores fluctuated over their learning, which commonly occurs [37]. In this situation, using the more appropriate Wilcoxon test revealed that the ants responded statistically similarly to the two cues set near their nest entrance (Table 2). We can thus conclude that, trained to two cues that differed in shape and that were seen near each other, the ants responded more to each of these single cues than to their presentation on a same support, side by side. The ants thus memorized each of the two cues differing by their shape but did not mentally add them.

Experiment II: Reaction to the juxtaposition of two cues of different colors

The number of ants' visits to the simultaneous presentation of the four cues, this time with the ones presented during training differing by their color, is given in Table 2, and the percentages corresponding to the sum for the

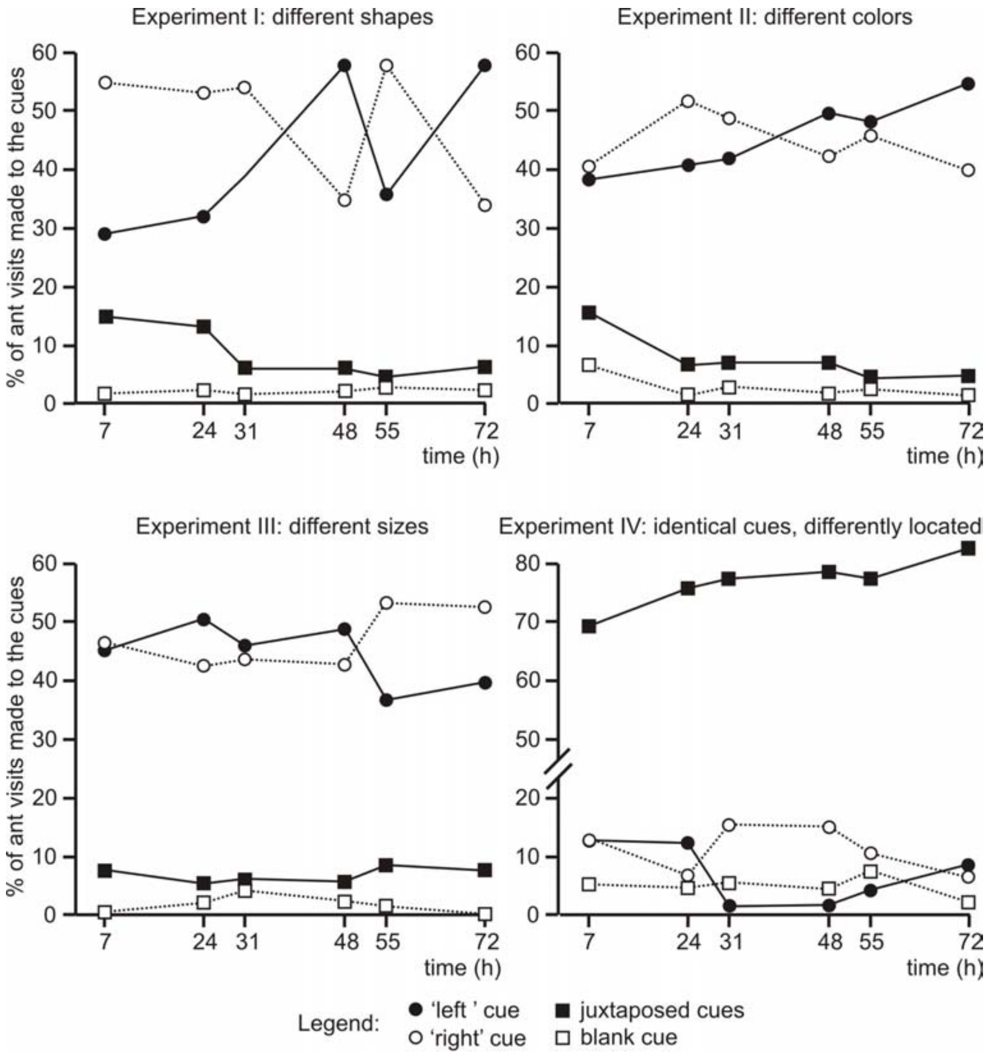

Figure 5. Graphical representation of the results of Experiments I to IV. Percentage of visits made by ants to different cues after having been trained to a cue present on the left and another on the right of their nest entrance versus a blank cue situated far away (the cue to avoid). The stands bearing these single cues plus a novel stand bearing, side by side, the two single cues, which the ants saw at their nest entrance during training, were simultaneously presented 6 times over time at times ranging from 7 to 72 hours in a tray devoted to testing. The two cues set near the nest entrance differed by their shape (Experiment I), by their color (Experiment II), by their size (Experiment III) or only by their position on the stand (Experiment IV). Only when the cues used for training were identical, although differently located on their stand (Experiment IV), did the ants respond significantly more to the 'sum' of these cues than to either of these cues.

three colonies are shown in Figure 5. During testing, the ants responded more to each of the two differently colored cues than to these two cues presented on the same stand as well as to the blank stand. The statistical analysis of the sums of the ants' visits over their testing allowed us to state that the ants did not randomly visit the four presented cues ($P < 0.001$) but more often visited the cues that were set on the left and on the right of the nest entrance during training than these two cues juxtaposed on the same stand ($P < 0.001$) and the blank stand ($P < 0.001$). Statistically, they equally visited each of the two cues that were situated at the left and the right of the nest entrance ($P > 0.50$), which was confirmed by the result of the Wilcoxon test (Table 2). During this experiment, the larvae started to become nymphs and thus no longer required meat food. During their training, contrary to what occurred during Experiment I, the ants saw the cue set on the left of the nest entrance as often as they saw the cue set on the right, and they thus reacted equally to each of them. Consequently, trained to two cues of different color presented side by side, the ants did not react to their juxtaposition on a same stand and equally responded to each of them without mentally adding them.

Experiment III: Reaction to the juxtaposition of two cues of different sizes

The number of ants' visits to the simultaneous presentation of the four cues, the two presented during training differing by their size, is given in Table 2, and the percentages of the sum for the three colonies are shown in Figure 5. The statistical analysis of the numbers of counted ants (Table 2) showed that the ants did not randomly visit the four presented cues ($P < 0.001$) and more often visited the cues they saw on the left and on the right of the nest entrance during training than these two cues juxtaposed on the same stand and a blank stand. They equally visited the cues set on the left and right sides of the nest entrance during training (Table 2: result of two distinct statistical analyses: NS). Consequently, trained to two cues of different sizes simultaneously seen, the ants did not 'add' them but equally responded to each of them.

Experiment IV: Reaction to the juxtaposition of two identical cues differing by their location

As for Experiments I, II, and III, the number of ants' visits to the simultaneous presentation of the two cues they saw during training, both identical in appearance but differing by their relative location on their stand, and to their juxtaposition on a stand as well as to a blank stand, is given in Table 2, and the percentages of the sum for the three colonies are shown in Figure 5. During this experiment, the ants behaved otherwise than during the three previous experiments. The statistical analysis made on the numbers of counted ants (Table 2) showed that the ants visited the stand bearing the two juxtaposed cues much more than the stands separately bearing these cues that were seen on the left and the right of the nest entrance ($P < 0.002$) during training. Consequently, the ants reacted to the addition of cues when they were identical in shape, color or size, even if their relative location differed. In other words, the *M. sabuleti* workers' criterion for mentally adding visual cues is their identical appearance. During this experiment, the tested ants more often visited the cue corresponding to the one that was located on the right of the nest entrance during training than the one located on the left ($P \sim 0.004$). This could be due to the need of sugar water for the newly emerged workers (= the callows) and to the position of the sugar water tube on the right side of the nest entrance. However, using the more appropriate Wilcoxon test, this preference for the cue seen on the right of the nest entrance during training was found to be not significant ($P = 0.23$; Table 2).

Comparison of the operant conditioning scores of learned simple cues and of their mental addition

Concerning the ants' conditioning scores, they were of a high level with only a slight difference in the course of the five experiments conducted over time. For Experiments I, II, and III, let us take the scores relative to the cues located both on the left and the right of the nest entrance during training. These scores for Experiment I (different shape) are, 83.61%, 84.56%, 92.77%, 92.08%, 92.75% and 91.53% (weighted mean = 84.31%), for Experiment II (different color), 78.19%, 91.92%, 90.28%, 91.38%, 93.64% and 94.08% (weighted mean = 90.24%) and for Experiment III (different size), 91.57%, 92.67%, 89.63%, 91.63%, 89.71% and 92.20% (weighted mean = 91.11%) The ants thus acquired excellent conditioning, the nest entrance being a valuable reward and the cues located there being very often seen by the ants. For Experiment IV (same shape, color and size, different location), let us take the scores concerning the two added cues. They equaled 69.23%, 75.86%, 77.42%, 78.60%, 77.46% and 82.61% (weighted mean = 76.74%). For the preliminary experiment (same shape, color, size and location), the juxtaposed cues scored 74.21%, 72.51%, 85.61%, 75.33%, 80.00%, 83.44% (weighted mean = 78.06%). The ants thus made a mental addition and remembered its result somewhat less easily than they learned a single visual cue.

DISCUSSION

Workers of the ant *M. sabuleti* trained to two identical and similarly positioned elements each presented on a separate stand and then tested in front of one of these cues and of their addend on a horizontal line reacted preferentially to the juxtaposed elements. Trained to two stands also presented on both sides of the nest entrance but bearing elements differing by their shape, color or size, the workers did not react to the juxtaposition of these elements on a single stand but essentially responded to each element separately presented. In contrast, when the two stands were bearing elements identical in shape, color and size though differing by their location on the stand, the ants responded more to the elements presented in juxtaposition on the same stand than to each element separately presented. The ants thus mentally added two separated elements seen at the same time only when these elements were identical, although they could be differently positioned. This requires some comments.

When *M. sabuleti* workers count only a number of elements on a cue, their counting is hardly affected by the graphical characteristics of the components of the cue (i.e., the shape, color, size of its elements), the most observed impact, although slight, being due to a change in the relative location of the elements of the cue [1]. These ants have been shown to mentally add (juxtapose) graphic elements when they see them simultaneously (i.e., perceiving thus the result of the operation) and not when they see them consecutively [2, 3]. In the present work, we show that these ants mentally add elements seen near each other during training only when these elements are identical and not when they differ by their structural or color characteristics. In comparison, the honeybee can add or subtract one element to or from a sample of elements even when these elements differ by their size or shape and without seeing the second component or the result of the operation, all this after having learned to make an addition or a subtraction according to the color of the elements [31].

As an ancillary result, we should note that *M. sabuleti* ants somewhat more easily learned a single visual cue than mentally made the addition of two sighted cues and remembered this sum.

In the wild, mentally adding identical cues located next to each other and separately memorizing cues differing by their characteristics may help an ant to navigate or perform several common tasks, such as collecting food and returning to nest.

We can propose that prior to mentally adding, *M. sabuleti* ants evaluate the appearance of visual cues (shape, color, size) and their spatial location. If the cues have a different appearance, the ants do not add them. If the visual cues are not seen simultaneously (i.e., are not present in the same place), they also do not add them. Indeed, non-numerical information such as the sense of magnitude resulting from the sight of particularities of the cues was also found to be important and to precede the sense of number (e.g., in bees [38]) or to accompany it (size and number of cues in bees [39]) or to help it (size and shape of a same kind of cue in dogs and wolves [40]).

Odors must be treated otherwise. Cues with different odors perceived at the same time in the same place should be added since their addition may correspond to a meaningful situation. Animals tested for this ability indeed add the differently odorous cues when they are simultaneously perceived (e.g., bees [41] and ants [42]). In the wild, mixtures of odors abound. Being able to memorize encountered mixtures of odors may also help in performing common tasks such as recognizing nestmates' odor and territorial marking odors as well as navigating from nest to food sites and cemeteries.

CONCLUSION

We showed that *M. sabuleti* workers mentally add visual cues seen simultaneously (e.g., set near each other) when these cues are identical (of the same shape, color or size), even if they differ in their relative position (i.e., being positioned somewhat higher, lower, more to the left or to the right). When cues differ by their shape, color, or size, the ants do not add them. In contrast, ants mentally (sensorily) pool (mix) different odors present at the same place [42]. All of these behaviors may confer an advantage in various social tasks, such as navigating, foraging and recognizing of marked areas.

CONFLICT OF INTEREST STATEMENT

We affirm having no conflict of interest concerning the subject examined here.

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