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Effect of Monosodium Glutamate on Behavior and Cognition: A Study Using Ants as Biological Models

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Abstract

Monosodium glutamate, a food additive largely consumed since 1909, has been suspected these last twenty years to impact health. Working on ants as biological models, we examined its physiological and ethological effects, without any conflict of interest. We found that glutamate does not impact ants' consumption of sugar, audacity and brood caring behavior, that it slightly affects their locomotion and aggressiveness towards nestmates, that it increases their meat food consumption and largely decreases their precision of reaction, response to pheromones, cognition as well as their learning and memorization abilities. Having the choice between intact meat and meat imbibed with glutamate, the ants prefer the latter. This is not the case when they have the choice between natural sugar water and sugar water containing glutamate. It was thus found that, enhancing the taste of not sugared food, glutamate may lead to over consume such food, and that this substance impacts behavior requiring cognition and memory, thus the brain and nervous system functioning. Indeed, hydrolyzing into glutamic acid, it may act as a neurotransmitter and may be excitotoxic. Glutamate consumption should thus be carefully limited, essentially when it is used together with aspartame, a sweetener giving rise to aspartic acid, a substance which may also act as a neurotransmitter.

INTRODUCTION

Monosodium L-glutamate, the sodium salt of the glutamic amino acid (Figure 1), is a food additive largely used all over the world for enhancing the savor of soups, sauces, eggs, pastas, meats and fishes, for instance. Added to meals, this substance has a pronounced particular taste, the "umami" taste, recalling the stock taste. Its use in cooking allows reducing the amount of added cooking salt. It is employed since 1909, is presently industrially produced and is present in numerous prepared meals. Its name or the label E621 must be written on the package of these prepared meals. It is, for instance, the tastiest constituent of the condiment 'Aromat' produced by Knorr ®. In Asian countries, monosodium glutamate at a purity of 99% is sold in packages of 100 g, 500 g and 1 kg, the most common producer being Aji-no-moto®. In the present work, we used the latter product, at a concentration based on one hand on that recommended by the producer as well as on internet sites, and on the other hand on the ants' water intake compared to that of mammals.

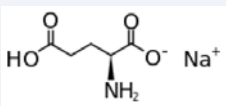

Figure 1 Structure of monosodium glutamate, a food additive used for enhancing savor of meals.

Monosodium L-glutamate is stable, soluble in water, and was considered as having no adverse effects during about 80 years [1-5]. However, during the last 20 years, controversy has arisen about its potential effects on health [6-17]. According to the latter works, potential adverse effects of glutamate may be due to its rapid assimilation and the resulting quick and large increase of glutamic acid in the organism. Glutamate might enhance individuals' allergy and/or hypersensitivity. It might induce headaches, tiredness, heart pain, nausea. Able to be a neurotransmitter, it might act in several physiological systems and be implicated into neurological diseases. For instance, glutamate may perturb the individuals' food demand, this inducing an increase of carbohydrates and proteins consumption, and ultimately, some obesity. It might also impact individuals' learning and memory. It may act as an excitotoxical substance, leading then to nervous cells' death (brain lesions, neurodegenerative diseases). These adverse effects may be more pronounced in young individuals, as well as when glutamate is consumed together with other potential neurotransmitter substances, such as aspartic acid derived for instance from the largely used sweetener aspartame.

Works stating that glutamate has no adverse effects were made in Asian countries. On the contrary, studies revealing the adverse effects of glutamate were performed by non Asian researchers. Glutamate is essentially used in Asian countries; it is present in numerous meals which would have no or few savor without it. One may wonder if researches concluding that glutamate has no adverse effect were really made without any conflict of interest.

Since controversy obviously exists about glutamate, and since this substance goes on being largely used, we aimed to study its potential effect (1) using ants as biological models (2) on several ants' physiological and ethological traits (3) and without any conflict of interest, simply trying to point out the truth.

Why using ants as biological models?

Most biological processes are similar for all animals, including humans (i.e. genetics, metabolism, nervous cells functioning). Consequently, a lot of invertebrates and vertebrates are used as models for studying biological subjects [18]. Invertebrates are more and more used because they offer scientists advantages, such as a short life cycle, a simple anatomy, and being available in large numbers [19]. Some species are largely used as biological models, e.g. the flatworm *Dendrocelium lacteum*, the nematode worm *Caenorhabditis elegans*, the mollusk *Aplysia californica*, the beetle *Tribolium castaneum*, the fruit fly *Drosophila melanogaster*, and the domestic bee *Apis mellifera*. Among the invertebrates, insects, especially social hymenoptera and among them bees, are advantageously used as biological models [20]. Ants too can be used. Indeed, colonies containing thousands of ants can be maintained in laboratories, at low cost and very conveniently, throughout the entire year. Ants are among the most complex and social invertebrate animals as for their morphology, physiology, social organization and behavior. They are among the most morphologically evolved hymenoptera, having a unique resting position of their labium, mandibles and maxilla, as well as a lot of glands emitting numerous efficient pheromones [21,22]. Their societies are highly organized with a strong division of labor, an age-based polyethism and a social regulation. Their behavior is well developed: they care for their brood, build sophisticated nests, chemically mark the inside of their nest, and, differently, their nest entrances, nest surroundings and foraging area. They generally use an alarm signal, a trail pheromone, and a recruitment signal; they are able to navigate using memorized visual and olfactory cues; they efficiently recruit nestmates where, when and as long as it is necessary, and finally, they clean their nest and provide their area with cemeteries. All this is detailed in many handbooks [23]. According to the complexity of their society and behavior, it looks reasonable to use ants as biological models for studying physiological and ethological effects of substances, treatments or situations.

Which traits can we effectively correctly examine?

During many years, we worked on ant's species belonging to the genus *Myrmica*, and among others, on *Myrmica ruginodis* Nylander, 1846. We know about its ecological traits, eye morphology, subtended angle of vision, visual perception, navigation system, visual and olfactory conditioning capabilities, and recruitment strategy [24-29]. The ontogenesis of cognitive abilities of *Myrmica* species has also been approached [30]. Studies on the impact of age, activity and diet on the conditioning capability of *M. ruginodis* led to presume that ants could be good biological models [31]. This was confirmed by the study of the effect, among others, of caffeine, theophylline, cocaine, and atropine, of nicotine, of morphine and quinine, of fluoxetine (an 'ISRS' antidepressant), of anafranil (an 'ACT' antidepressant) and of efexor (an 'IRSNa' antidepressant), of carbamazepine, of buprenorphine and methadone and of aspartame for instance [32-35 among others]. Each time, we observed effects related to those observed on humans, and brought information and precision on them. Here, we aimed to use the ant *M. ruginodis* as a biological model for examining physiological and ethological effects of the monosodium L-glutamate.

The ants' food being given on their foraging area, at a clearly visible place, it is easy to assess food consumption. We can precisely assess the ants' locomotion (linear and angular speeds), precision of reaction (orientation towards an alarm signal), response to a pheromone (trail following behavior), audacity, brood caring, cognition and aggressiveness. The ants' acquisition of a visual conditioning and their visual memory can be quantified using an already set up protocol. The ants' preference between two kinds of food can be quantified using a well-tried technique. These experimental methods are here again shortly explained.

Why have we no conflict of interest?

Making fundamental research on ants' ethology without external funding, being not glutamate consumers and not attempting to prevent anybody to use it, we are outside any conflict of interest.

Experimental Planning

We first assessed eight ants' traits while ants were under a diet without glutamate. Then, we assessed the same traits while these ants consumed glutamate. After that, we assessed two other traits, the ants being still under glutamate consumption. Finally, the ants received again a diet without glutamate.

The ten assessed traits were:

- Food consumption, on basis of ants coming on their sugar water and meat food,

- Locomotion (linear and angular speeds),

- Precision of reaction through orientation towards an isolated worker's head, a source of alarm pheromone,

- Response to pheromones, through the trail following behavior,

- Audacity through the number of ants coming on an unknown and unusual apparatus,

- Brood caring,

- Cognition through the ants' behavior in front of a twists and turns way,

- Aggressiveness against nestmates, assessed in the course of dyadic encountering,

- Visual conditioning and memory through the ants' ability in acquiring conditioning to a visual cue and in retaining that cue,

- Preference between food with and without glutamate, the food being meat or sugar water.

MATERIAL AND METHODS

Collection and maintenance of ants

The experiments were performed on four colonies of *Myrmica ruginodis* Nylander, 1846 collected in an old quarry of the Aise Valley (Ardenne, Belgium), on the borders of a forest, the ants nesting under stones or in wood. The colonies were demographically similar, each containing a queen, brood and about 500 workers. They were maintained in the laboratory in artificial nests made of one to three glass tubes half-filled with water, a cotton-plug separating the ants from the water. The glass tubes were deposited in trays (34 cm x 23 cm x 4 cm), which internal sides were slightly covered with talc to prevent the ants from escaping. These trays served as foraging areas, food being delivered in them. The ordinary feeding of the ants were a 30% saccharose aqueous solution provided *ad libitum* in a small glass tube plugged with cotton, along with two cut *Tenebrio molitor* Linnaeus 1758 larvae (mealworms) provided twice a week on a glass slide. Temperature was 18° - 22° C and relative humidity circa 80%. Lighting had a constant intensity of 330 lux while caring for the ants, training and testing them; during other time periods, it was dimmed to 110 lux. The ambient electromagnetic field had an intensity of 2-3 $\mu W/m^2$. All the members of a colony are here named nestmates, as commonly done by researchers on social hymenoptera.

Aqueous solution of glutamate 0.4% (= 4/96 w/w)

The monosodium glutamate used was that provided by Aji-no-moto®, at a purity of 99%, that easily found in any shop in Asia, and also available in Europe in Asian groceries. The concentration of glutamate recommended on the package, as well as that recommended on internet sites is about 0.4% what is equivalent to 0.4/99.6 w/w. However, the glutamate concentration of meals cooked for humans is not uniform, being at the estimation of their manufacturer and often higher than 0.4%. Ants, as any insect, drink proportionally about ten less than mammals. For giving to ants a quantity of glutamate proportionally similar to that ingested by humans, we used an aqueous solution of glutamate at the concentration 4%, i.e. 4 gr of glutamate into 100 ml of tap water. We used this solution throughout the whole experimental work. For giving glutamate to the ants, we immersed pieces (2-3 mm length) of *T. molitor* in 1-2 ml of that solution, and after at least two hours, gave 6 such pieces to the ants. On the other hand, the 30% sugar solution intended for the ants was made using the 4% glutamate solution and this sugar glutamate solution was provided to the ants in the usual small tubes plugged with cotton. The meat and sugar food containing glutamate were renewed each two days and we checked each day if ants effectively consumed the two kinds of food. Ants consuming glutamate-added food are here said as being 'under glutamate diet'.

Food consumption

This trait was assessed first while ants were under a diet without glutamate and, when all these controls were finished, while the ants were under a glutamate diet. Each time, during six days, the ants of the four colonies coming on the sugar water as well as on the meat food (Figure 2A), were counted 12 times between 13:00 and 16:00 o'clock. The daily sums of these counts were established (Table 1, Daily counts). These daily counts obtained for the four colonies were added (Table 1, Daily sums = sums of 12 x 4 = 48 counts) for each kind of food. Finally, the total mean (of 12 x 4 x 6 = 288 counts) for each kind of food and each kind of diet, as well as that for each kind of diet (n = 576) were established (Table 1, Total means).

Linear and angular speeds, orientation

The movement of ten ants of each colony (n = 4 colonies x 10 ants = 40 trajectories) moving on their foraging area was analyzed. Their linear and angular speeds were assessed without presenting them any stimulus. Their orientation towards an alarm signal (allowing examining the ants' precision of reaction) was assessed by presenting them an isolated worker's head. Such a head is a source of alarm pheromone identical to that of an alarmed worker, in terms of the dimensions of the emitting source (the mandibular glands' opening) and of the quantity of pheromone emitted [36].

Trajectories were manually recorded using a water-proof marker pen, on a glass slide horizontally placed 3 cm above the foraging area. A metronome set at 1 second allowed assessing the total time of each trajectory. Each trajectory was recorded until the ant reached the stimulus or walked for about 6 cm. All the trajectories were copied with a water-proof marker pen onto transparent polyvinyl sheets which could be affixed to a PC monitor screen, remaining in place due to their own static electricity charge. The trajectories were analyzed using specifically designed software [37]. Each trajectory was entered in the software by clicking as many points as wanted with the mouse. The location of the presented worker's head was then entered. After that, the total time of the trajectory was entered, and the software was asked to calculate the three following variables. The linear speed (V, here measured in mm/s) is the length of the trajectory divided by the time spent moving along this trajectory. The angular speed (S, i.e. the sinuosity, here measured in angular degrees/cm) is the sum of the angles made by each segment 'point i to point i – 1' and the following one 'point i to point i + 1', divided by the length of the trajectory. The orientation (O, here measured in angular degrees) towards a source (here an ant's head) is the sum of the angles, measured at each successive point of the trajectory, made by each segment 'point i of the trajectory - given source' and each segment 'point i - point i + 1', divided by the number of measured angles. When O is lower than 90°, the animal has a tendency to orient itself towards the source and when it is larger than 90°, the animal has a tendency to avoid the source.

Each distribution of 40 values of each variable was characterized by its median and quartiles (Table 2).

Trail following behavior

This trait was assessed on ten ants of each colony (n = 4

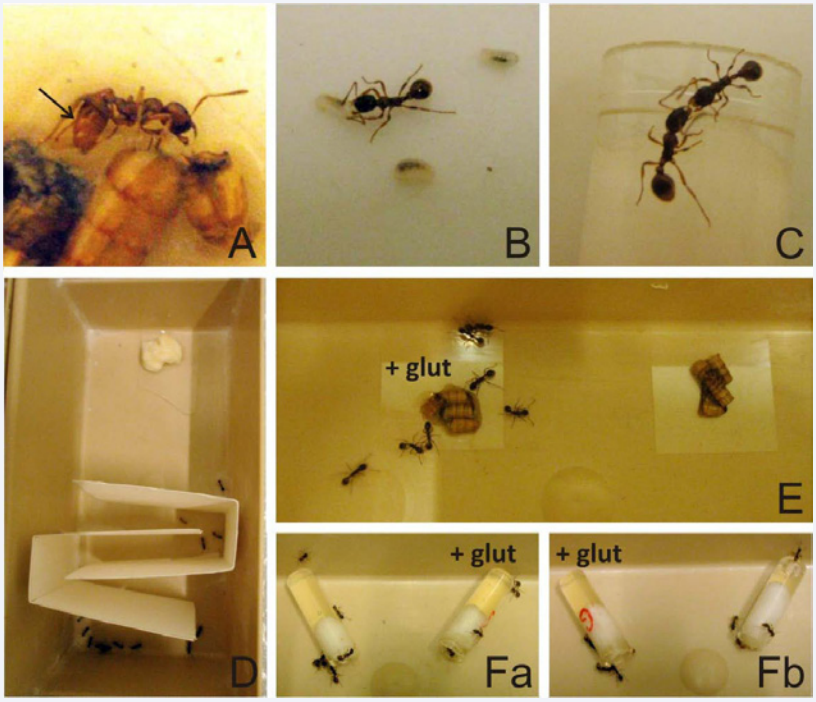

Figure 2 Some views of the experiments. A: an ant having eaten meat food imbibed with glutamate. Its gaster is enlarged; the ant died. B: an ant under glutamate diet taking care of a larva. C: two nestmates, under glutamate diet, presenting some slight aggressive behavior. D: ants under normal diet finding their way through twists and turns. E: ants preferring meat imbibed with glutamate. F: ants of two colonies (Fa, Fb) drinking equally sugar water with and without glutamate.

Table 1: Food consumption under a diet without or with glutamate.

Diet:	**without glutamate**		**with glutamate**	
Food:	sugar water	meat	sugar water	meat
Colonies:	A B C D	A B C D	A B C D	A B C D
	Daily counts			
Day 1	2 15 12 7	2 9 3 6	6 0 12 0	4 18 2 18
Day 2	2 6 12 8	2 1 4 11	2 0 12 24	12 0 0 12
Day 3	0 2 23 12	4 4 2 14	0 12 0 12	12 0 12 6
Day 4	2 2 0 9	7 6 6 7	12 12 4 0	4 6 12 12
Day 5	0 9 10 10	6 1 2 2	4 2 6 12	0 18 12 6
Day 6	4 2 0 0	5 3 6 6	6 12 8 10	12 8 12 0
	Daily sums			
Day 1	36	20	18	42
Day 2	28	18	38	24
Day 3	37	24	24	30
Day 4	13	26	28	40
Day 5	29	11	24	36
Day 6	6	20	36	32
	Total means			
Days 1-6	149/288 = 0.52	119/288 = 0.41	168/288 = 0.58	204/288 = 0.71
	0.47		0.65	

The ants of four colonies (A – D) coming on sugar water as well as on meat were counted 12 times, each day for 6 consecutive days (total number of counts = 4 x 12 x 6 = 288). The table gives the sums of the counts made each day for each colony (Daily counts) as well as the sums of these counts (Daily sums). These sums obtained for each kind of diet were statistically compared (see results section). The means of all the counts made for each kind of food and diet, as well as for each kind of diet were also established. Briefly, the ants did not eat more sugar water containing glutamate than sugar water free of this food additive, but they obviously eat more meat imbibed with glutamate than intact meat.

colonies x 10 ants = 40 trail followings) to examine the ants' general response to their pheromones. The trail pheromone of *Myrmica* ants is produced by the workers' poison gland. Ten of these glands were isolated in 500 µl hexane and stored for 15 min at -25 °C. To perform one experiment, 50 µl of the solution was deposited, using a metallic normograph pen, on a circle (R = 5 cm) pencil drawn on white paper and divided into 10 angular degrees arcs. One minute later, the paper with the artificial trail was placed in the ants' foraging area. When an ant came into contact with the trail, its movement was assessed by the number of arcs of 10 angular degrees it walked without departing from the trail, even if it turned back. If an ant turned back when coming in front of the trail, its response was assessed as "zero arc walked"; when an ant crossed the trail without following it, its response equaled "one walked arc". Before testing the ants on a trail, they were observed on a "blank" circumference imbibed with 50 µl of pure hexane, control numbers being so obtained (Table 2, C = control, T = test). On experimental trails, *Myrmica* workers do not deposit their trail pheromone because they do so only after having found food or a new nest site. Each distribution of values was characterized by its median and quartiles (Table 2).

Audacity

This trait was assessed on the four colonies. A cylindrical tower built in strong white paper (Steinbach®, height = 4 cm; diam = 1.5 cm) was set on the ants' foraging area, and the ants present on it, at any place, were counted 10 times, in the course of 10 min. The mean and extremes of the obtained values were established (Table 2, audacity).

Brood caring behaviour

This trait was assessed on colonies A and C which contained numerous larvae. A few larvae were removed from the inside of the nest and deposited in front of the nest entrance. For each colony, five of these larvae were observed, as well as the ants' behavior in front of a larva (Figure 2 B). The larvae among the five observed ones still remaining out of the nest after 5 seconds, 2, 4, 6, 8, and 10 minutes were counted, the numbers recorded for each colony being added (Table 3, brood caring).

Cognition

The assessment was made on colonies B and D using an experimental apparatus previously presented by us [32]. This apparatus consisted in a small tray (15 cm x 7 cm x 4.5 cm) inside of which pieces of white extra strong paper (Steinbach ®, 12 cm x 4.5 cm) were inserted in order to create a way with twists and turns between a loggia too narrow for 15 ants at a time (the initial loggia) and a larger one (the free loggia) (Figure 2 D). Each colony had its own apparatus. To conduct an experiment, 15 ants were set all together, at the same time, in the initial loggia of the apparatus, and those located in this loggia as well as in the free loggia were counted after 5 seconds, 2, 4, 6, 8 and 10 minutes. The numbers obtained for the two colonies were added (Table 3, cognition).

Aggressiveness against nestmates

This trait, as the previous ones, was quantified before the ants consumed glutamate, and later on while they continuously consumed that food additive. Ants' potential aggressiveness against nestmates was assessed in the course of dyadic encounters of five pairs of ants of each four colonies, the encountering being conducted in a small cylindrical cup (diameter = 2 cm, , height = 1.6 cm), the borders of which had been slightly covered with talc. Each time (in total 5 x 4 = 20 encounters), one ant of the tested pair was observed for 5 minutes and its encounter with the other ant was characterized by the numbers of times it did nothing (level 1 of aggressiveness), touched the other ant with its antennae (level 2), opened its mandibles in front of the other ant (level 3), gripped and/or pulled the other ant (level 4), or tried to sting or stung the other ant (level 5) (Figure 2 C). The numbers recorded for each four colonies were added (Table 3, aggressiveness against nestmates).

Visual conditioning ability and memory

This was examined on the four colonies. At a given time, a yellow hollow cube was set above the pieces of *T. molitor* larvae, the ants undergoing so visual operant conditioning. These cubes were made of strong paper (Canson®) according to previously published instructions [28]. The wavelengths reflections of the yellow paper have been determined [38]. The ants could see the cube and enter it. Tests were performed in the course of time, while the ants were expected to acquire conditioning then, after having removed the yellow hollow cube, while the ants were expected to partly lose their conditioning. During these tests, choosing the way with the yellow cube was considered as giving the 'correct' choice. Control experiments had previously

Table 2: Effect of glutamate on five traits.

Traits ↓ Diet →	without glutamate	with glutamate
linear speed (mm/s)	13.1 (11.4 – 14.1)	14.5 (13.5 – 15.9)
angular speed (angular degrees/cm)	134 (113 – 157)	149 (124 – 162)
orientation (angular degrees)	38.5 (25.5 – 62.1)	69.2 (45.2 – 89.8)
trail following (n° of walked arcs)	C: 1.0 (1.0-1.0) T: 12.5 (8.0 – 19.3)	C: 1.0 (1.0 – 1.0) T: 5.0 (3.0 – 8.0)
'audacity'(n° of ants)	0.63 [0 – 1]	0.80 [0 – 1]

For each trait, 40 values were collected. The table gives their median (and quartiles) or their mean [and extremes]. Experimental details and statistical results are given in the text. Briefly, glutamate slightly increased the ants' speed of locomotion and audacity, and reduced their ability in orienting themselves and in following a trail. C = control (a blank circumference); T = test (a circumference drawn with trail pheromone).

Table 3: Effect of glutamate on brood caring, cognition and aggressivness.

Traits ↓ Diet →		without glutamate	with glutamate
Brood caring:			
n° of not re-entered larvae after:	5 sec	10	10
	2 min	8	8
	4 min	6	6
	6 min	5	6
	8 min	4	4
	10 min	2	1
Cognition:			
n° of ants in front and beyond the twists and turns		in front beyond	in front beyond
after:	5 sec	30 0	30 0
	2 min	24 0	26 0
(in front = in the small initial loggia	4 min	22 0	27 0
	6 min	19 2	25 0
beyond = in the large free loggia)	8 min	16 3	22 0
	10 min	13 4	21 1
Aggressiveness against nestmates:			
levels:	1	148	66
	2	113	104
	3	53	61
	4	0	0
	5	0	0

Ants of two (for brood caring and cognition) or four (for aggressiveness) colonies were tested while being firstly under a diet without glutamate, then under a diet with glutamate. Experimental details and statistical results are given in the text. Briefly, glutamate did not impact the ants' brood caring behavior, largely affected their cognition and slightly changed their behavior in front of nestmates.

been made on never conditioned ants as well as on trained ants consuming no glutamate [28]. This had to be done because, once an animal is conditioned to a given stimulus, it becomes no longer naïve for such an experiment. Ants were individually tested in a Y apparatus constructed of strong white paper, and set in a small tray (30 cm x 15 cm x 4 cm) apart from their tray as previously explained [28]. Each colony had its own Y apparatus. The sides of the apparatus were slightly covered with talc, and the floor was changed between tests. The Y-apparatus was provided with a yellow hollow cube in one or the other branch, half of the tests being conducted with the cube in the left branch and the other half with the cube in the right branch of the Y maze. To conduct a test on a colony, 10 workers were transferred one by one onto the area at the entrance of the Y-apparatus. Each ant was observed until it turned either to the left or to the right in the Y-tube, and its first choice was recorded when the ant was beyond a pencil drawn line indicating the entrance of a branch. Afterwards, the ant was transferred into a polyacetate cup, until 10 ants were so tested, this avoiding testing twice the same ant. All the tested ants were then placed back on their foraging area. For each test, the numbers of ants (n = 10 x 4 = 40) which chose the "correct" way with the yellow cube, or went to the "wrong" empty branch of the Y were recorded, and the percentage of correct responses was established (Table 4).

Preference between meat and sugar water, with or without glutamate

Fifteen ants of colony A, as well as of colony B, were transferred into a small tray (15 cm × 7 cm × 5 cm), the borders of which had been covered with talc to prevent escape, and in which two *T. molitor* larvae cut in four pieces were set, one unchanged, the other imbibed with 100 µl of the glutamate 4% solution. In one of the trays, the pieces of *T. molitor* imbibed with glutamate were located on the right; in the other tray, they were located on the left. The ants coming onto and eating each kind of larva (Figure 2 E) were counted 12 times in 12 min, the mean values being then established for each kind of larva. An identical experiment was made on fifteen ants of colony C, as well as of colony D, using sugar water instead of *T. molitor* larvae. In each tray, two tubes (h = 2.5 cm, diam. = 0.5 cm) were laid, one containing a solution of sugar in tap water, the other a solution of 30% sugar in the 4% solution of glutamate, each tube being plugged with cotton. In one of the trays, the tube containing glutamate was located on the right; in the other tray, it was located on the left (Figure 2 F). The ants drinking each kind of liquid were counted 12 times in 12 min, and the mean values were established for each kind of liquid.

Statistical analysis

All the results obtained for ants consuming glutamate were statistically compared to those previously obtained for ants under normal diet using non-parametric tests [39].

The numerical results of the experiments about the ants' food consumption, brood caring, cognition, visual and memory were analyzed using the non-parametric Wilcoxon test, the values of N, T and P being given in the 'Results' section. Those of the experiments dealing with the ants' linear and angular speeds, orientation, trail following, aggressiveness against nestmates were analyzed using the non-parametric χ^2 test. The results of the assessment of the ants' audacity were analyzed using the non-parametric Mann-Whitney U test, the values of U, Z and P being given in the 'Results' section. The results of the experiment examining the ants' preference between food with and without

Table 4: Effect of glutamate on ants' visual conditioning ability, and memory.

Traits↓ Diet →	with glutamate nest 1 2 4 5 %	without glutamate %
Visual conditioning		
after: 7 hrs	5 5 6 6 55.0	47.0
24hrs	5 5 4 6 50.0	60.0
31hrs	7 5 4 4 50.0	63.3
48hrs	6 4 5 5 50.0	65.0
55hrs	4 3 5 5 42.5	75.0
72hrs	4 5 4 5 45.0	81.7
Visual memory		
after: 7 hrs	no memory	70.0
24hrs		65.0
31hrs		62.0
48hrs		50.0
55hrs		62.0
72hrs		60.0

The ants were trained to a yellow cube and tested in a Y apparatus provided with such a cube in one of its branch. The table gives the numbers of ants giving the 'correct' response and the proportion of correct responses for the tested population. Under glutamate diet, the ants were unable to acquire visual conditioning, though the studied species, M. ruginodis, has a good vision, relies essentially on it for navigating and detains a long lasting visual memory [26, 27, 28].

glutamate were statistically compared to the values expected if ants randomly went eating each kind of food, using the non-parametric goodness of fit χ^2 test. Non significant results are noted 'NS'.

RESULTS

Food consumption

It did not matter if the ants were or not under glutamate diet, their consumption of sugar water was similar (Table 1): the daily sums of the counts of ants on the sugar water were statistically similar whatever the ants' diet (N = 6, T = +12, -9, P = 0.42) even if they were slightly more numerous while under glutamate diet. Concerning the consumption of meat, statistically significant differences were observed between ants under a normal diet and the same ants under a glutamate diet (Table 1). When under glutamate diet, more ants were present on the meat (complete) food. The daily sums of the counts statistically differed (N = 6, T = 21, P = 0.016). Within the six experimental days, we saw three times an ant never stopping eating, especially the juice (haemolymph + glutamate) located around the cut *T. molitor* larvae (Figure 2 A), and this until it could no longer walk and finally died. We never noticed so with ants normally fed, either for meat or for sugar food.

The total means of the different counts summarized the situation (Table 1). On sugar water, meanly 0.52 and 0.58 ants were counted while under normal diet and glutamate diet respectively, and on meat food, 0.41 and 0.71 ants were counted while under normal diet and glutamate diet respectively. The total mean for all kinds of food, meat and sugar water, was larger when ants were under glutamate diet (0.65) than when they were under a normal diet (0.47). Consequently, glutamate increased ants' food consumption, particularly meat (complete food) consumption, which food has probably a nicer taste when imbibed with glutamate, while sugar water already has a pleasant taste without glutamate.

We also observed that ants having consumed a rather large amount of sugar water containing glutamate did not fail in walking, did not die, but walked abnormally, hesitating, stopping, turning and walking again with difficulties. However, about an hour later, they walked normally and rather quickly.

Linear and angular speed

Ants walked somewhat more rapidly as soon as they consumed glutamate (Table 2, line 1), but the difference between the two kinds of diet was at the limit of significance: $\chi^2 = 7.63$, df = 3, $P \sim 0.05$. Under glutamate diet, the ants seemed also to walk slightly more sinuously (Table 2, line 2), but the difference between the two diets was not significant: $\chi^2 = 2.49$, df = 3, NS. As a matter of fact, glutamate consumption was not found to drastically impact the ants' locomotion.

Orientation towards an isolated worker's head (= precision of reaction)

This trait was affected by glutamate consumption. While being under normal diet, ants oriented themselves correctly towards a source of alarm pheromone (O = 38.5 angular degrees; Table 2, line 3). As soon as they consumed glutamate, their orientation was of lower quality, the ants going aside or beyond the source. Their orientation values were so statistically higher: O = 69.2 angular degrees; Table 2, line 3; $\chi^2 = 10.13$, df = 2, $0.001 < P < 0.01$). Thus, glutamate affected the ants' precision of reaction.

Trail following behavior

This trait was impacted by glutamate consumption (Table 2, line 4). Under normal diet, ants duly followed a trail, moving on it along meanly 12.5 arcs of 10°. While they consumed glutamate, the ants still detected the trail but soon departed from it and meanly followed it along only 5 arcs of 10°. The difference of ants' behavior in front on a trail according to their diet was statistically significant: $\chi^2 = 24.70$, df = 2, $P < 0.001$.

Audacity

Although the ants were somewhat more numerous in coming onto the provided experimental apparatus while under glutamate consumption (Table 2, line 5), this difference of behavior between the two diets was not significant: U = 680, Z = -1.4867, P = 0.1371.

Brood caring behavior

This trait was not affected by glutamate consumption (Table 3, brood caring). While under that diet, the ants went on taking care of their larvae (Figure 2 B). They rapidly perceived the larvae removed from the nest, took them between their mandibles and re-entered them inside the nest. No statistical difference could be seen between the numbers of larvae not yet re-entered in the course of the time, either the ants consumed glutamate or not (N = 2, NS).

Cognition

This trait was affected by glutamate consumption (Table 3, cognition). Under normal diet, the ants of colonies B and D progressively left the small loggia lying in front of twists and turns, moved in these twists and turns, and four ones among 30 could reach the free loggia beyond the twists and turns within the 10 experimental minutes (Figure 2 D). Under glutamate diet, the same ants presented difficulties in doing so. They entered the twists and turns but came back on their way several times, so that, at the end of the experiment, 21 were still in the small loggia, and only one was in the free loggia. The difference of ants' behavior, during this test assessing the ants' cognitive abilities, according to their diet was statistically significant: for the small loggia: N = 5, T = 15, P = 0.031. The significance for the free loggia could not be assessed, due to the smallness of the sample.

Aggressiveness against nestmates

This trait was slightly affected by glutamate consumption (Table 3, aggressiveness against nestmates). An ant under normal diet encountering a nestmate did not interfere or touched the opponent with its antennae, and seldom opened its mandibles. Under glutamate diet, an ant more often touched the nestmate with its antennae and opened its mandibles (Figure 2 C). If the five levels of aggressiveness are taken into account separately, the difference in ants' aggressive behavior according to their diet was significant: $\chi^2 = 20.74$, df = 4, P < 0.001. If the levels 1 and 2 on one hand, and the levels 3, 4 and 5 on the other hand are pooled, the difference was slightly significant: $\chi^2 = 7.55$, df = 1, P < 0.01. Glutamate impacted thus the ant behavior in the course of encounters with nestmates, though these encounters never became as aggressive as for ants consuming fluoxetine [33].

Visual conditioning ability and memory

Glutamate largely impacted these abilities. Under glutamate diet, ants of the four colonies never acquired visual conditioning in the course of the 72 training hours (Table 4). This result was statistically significant: N = 6, T = 20, P = 0.03, all the more because the studied species has a visual perception of good quality, uses essentially its visual perception for navigating and memorizes visual cues during months [26-28].

Preference between meat and sugar water, with or without glutamate

In front of natural pieces of a *T. molitor* larva and of such pieces imbibed with an aqueous solution of glutamate, the ants showed an obvious preference for the latter food (Figure 2 E). In the course of the 12 counts, finally, 17 ants of colonies A and B were counted on the natural cut larva while 49 were counted on the cut larva imbibed with glutamate. These numerical results statistically differed from those expected if ants randomly went on the two kinds of pieces of larvae i.e. 17 and 49 *vs* 33 and 33; $\chi^2 = 15.52$, df = 1, P < 0.001. The ants were thus more inclined to eat meat imbibed with glutamate than to eat glutamate-free meat.

On the contrary, during a similar experiment made using sugared water, ants showed no preference between the two kinds of sugared liquid (Figure 2 F). More precisely, 59 ants of colonies C and D were counted during the 12 counts on the sugar water free of glutamate, while 53 were counted on the sugared glutamate solution, these numbers not differing from those expected if ants randomly drank each kind of liquid (i.e. 56 and 56; $\chi^2 = 0.32$, df = 1, NS). Thus, mixed to sugar water, glutamate did not enhance the ants' preference for that sugar water with glutamate. The results of this double experiment were in agreement with those of the first experiment i.e. that concerning the ants' meat and sugar water consumption with and without glutamate (Results section, Food consumption paragraph).

DISCUSSION

Monosodium glutamate is a food additive largely used, since it powerfully enhances the savor of meals. It is nowadays added to many culinary preparations. Used without suspicion for about 80 years, it was thereafter suspected to have some adverse effects impacting health; its safety is nowadays under controversy [40-44].

Ants are good biological models because they are among the most physiologically, biologically and behaviorally evolved invertebrates [20-23]. We can assess plenty of their physiological and behavioral traits such as their locomotion, orientation, response to pheromones, audacity, brood caring, cognition, aggressiveness, tactile perception, conditioning ability and memory, and preference between two kinds of food [32-35]. Moreover, hundreds of them can be maintained in a laboratory during the entire year. This is the reason why we used ants as biological models for studying the impact of substances or treatments [32-35]. Furthermore, being without any funding, we have no conflict of interest at all concerning the use of the substances we examine, e.g. glutamate in the present case. Using ants, we here examined physiological and ethological effects of this food additive. We observed that, in comparison with a glutamate-free diet, this substance:

-) enhanced consumption of meat (complete) food,

-) slightly increased speed of locomotion,

-) decreased the precision of reaction,

-) decreased the response to pheromones,

-) impacted cognitive ability,

-) largely reduced learning and memory,

-) slightly increased aggressiveness against nestmates.

On the contrary, glutamate did not affect the ants' audacity and brood caring behavior.

In front of meat imbibed or not with glutamate, the ants preferred the imbibed meat. They did not do so in front of sugar water, with or without glutamate. Such observations were in agreement with those concerning the ants' food consumption: the ants eat more meat imbibed with glutamate, but not more sugar water containing glutamate. We observed ants eating so much meat juice imbibed with glutamate that they could no longer walk and finally died. Glutamate enhances thus efficiently the savor of food and may rather dangerously increase food (meat or complete food) intake. This is in agreement with other researchers' observations [9].

On the other hand, glutamate had adverse effects on physiological and ethological traits depending on brain and nervous system functioning. For example, glutamate consumption affected the ants' orientation towards a punctual source of pheromone, thus their ability in making a positive taxis and their precision of reaction. Following a pheromone trace requires continuously perceiving identical concentrations of trail pheromone by each antenna: this ability was impacted by glutamate consumption. Cognition, learning and memory were also negatively affected by glutamate consumption. On the contrary, for an ant, taking care of the colony's larvae is rather innate or soon learned, and no longer requires cognition: this trait was not impacted by glutamate.

Our results and observations on ants agree with those obtained on mammals [13-16] as well as on humans [6-12,17]. These studies show that glutamate acts negatively on several physiological systems, perturbs individuals' food demand, impacts learning and memory (affecting the hypothalamus) and has adverse effects on nervous cells. This latter effect may be due to the fact that glutamate gives rise to glutamic acid, a neurotransmitter, which can also act as an excitotoxic substance [10,11,17]. These results on vertebrates and those of the present study on ants show all that glutamate has adverse biological effects. We thus conclude that this food additive is not entirely safe, and disagree with the claims of Asian researchers about its safety [1-5]. Glutamate salt is very largely used in Asian countries, and the works of Asian researchers may thus have not been done without some conflict of interest. Our findings on ants result from a financially and deliberately independent research on the etho-physiological effects of a food additive, using an evolved invertebrate as a model. They contribute to solve the controversy concerning the safety of glutamate.

The use of glutamate may even be more toxic in some circumstances. Another substance largely used all over the world is aspartame. This sweetener leads to the presence of aspartic acid in the body, a substance which may also act as a neurotransmitter [13]. Attention should thus be paid when glutamate and aspartame are consumed during the same food intake, e.g. glutamate in a meal and aspartame in a drink. Anyway, on the basis of our observations and results, we recommend that further experimental work should be made, outside any conflict of interest, by practitioners, biologists, pharmacists and nutritionists, on other meat-consuming biological models as well as on humans. In the meantime, precautions should be adopted about glutamate consumption.

CONCLUSION

Using ants as a biological model, we show that monosodium glutamate, a food additive largely used essentially in Asia, decreases precision of reaction, cognition, learning and memorization. Meat food containing glutamate is preferred to natural meat food, and can thus be over consumed. Other researchers have shown that glutamate impacts the nervous system very likely *via* its hydrolysation into glutamic acid. Its use must be revised, or at least limited, especially when occurring together with that of aspartame, a food additive producing aspartic acid, another potential neurotransmitter impacting the nervous system.

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Conflict of interest

We affirm having no conflict of interest concerning the use of monosodium glutamate as a food additive.

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