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Biological Effects of *Curcuma*, a Potential Safe Analgesic; a Study on Ants as Models

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Abstract

We previously showed that the largely used analgesic paracetamol has several adverse effects, impacts the central nervous system, and leads to habituation as well as dependence. An extract of the rhizome of *Curcuma longa* is known for having analgesic properties, and may thus be used instead of paracetamol if it has no or very few adverse effects. Examining this food complement exactly as we examined paracetamol, we discovered that it is entirely safe. Contrary to paracetamol, it did not impact the ants' orientation ability, trail following behavior, cognition, as well as visual and olfactory conditioning and memory. Like paracetamol, it did not affect the ants' food consumption, general activity, audacity, relationship with brood and congeners, escaping ability. Moreover, *curcuma* leaded to no habituation (its effect remained intact over time) and to no dependence. It has an obvious analgesic effect. The latter effect persisted intact for about 5 hours after weaning, then decreased slowly over time, and vanished in a total of 11 - 13 hours after weaning, while the effect of paracetamol rapidly vanished in 4 hours. We thus recommend the use of *curcuma* instead of paracetamol for reducing pain perception, however without abuse since perceiving pain is an essential physiological trait informing the organism of some health problem.

Keywords: *Cognition; Dependence; Habituation; Memory; Paracetamol*

Abbreviations

ang.deg.: Angular Degrees; ang.deg./cm: Angular Degrees Per Cm; °C: Centigrade Degrees; h: Height; l: Liter; mm/s: Millimeter Per Second; χ^2= Chi Square; vs: Versus; n°: Number; cm: Centimeter; mm: Millimeter; ml: Milliliter; µl: Micro Liter; R: Radius, s: Second; min: Minute; h: Hour; t: Time; %: Percentage

Introduction

The most consumed drugs all over the world are antidepressants, anxiolytics, hormones, antibiotics, carbamazepine and analgesics. The latter ones are necessary in some medicinal circumstances and cannot be suppressed without making the patients' live very difficult. The most used analgesic is nowadays paracetamol. Its mode of action is not yet clearly defined, but it presumably acts on the central nervous system [1,2]. Working on ants as models, we showed that the large dose of paracetamol commonly used by humans, i.e. 4g per day, has several adverse effects [3]. Together with reducing the pain perception, this drug impacted the ants' locomotion, orientation ability, trail following, cognition visual and olfactory conditioning ability as well as memory. Other traits, such as relationship with congeners and brood, general activity and food consumption were not affected. Paracetamol, consumed at the efficient analgesic dose of 4g per day, impacts thus the brain functioning, at least some brain functions. Moreover, on basis of our experiments on ants, this drug leaded to habituation (its analgesic effect on ants' pain perception became weaker and weaker over time) and to addiction (the ants preferentially drunk liquid containing paracetamol than liquid free of that drug) [3]. The two latter observations are not in favor of paracetamol use since humans will be induced to consume again that drug and to consume larger amount in the course of time, and such large doses are those which have harmful effects [4].

It should be of high interest to find another, safe, analgesic for medicinal use. An extract of the rhizome of *Curcuma longa* is known since centuries for having some analgesic properties, and this has recently been proved [5]. The active substance of such an extract is curcumin. A few studies have been made on it, though not clearly examining if it has some adverse effects [6-10]. In the same way, links devoted to *curcuma* use, prone beneficial effects on pain perception and inflammatory problems and relate very few about potential adverse effects, stating only that this food complement should not be used for children and pregnant women [a few links: *www.doctissimo.fr/html/sante/phytotherapie/plante-medicinale/curcuma.htm*

www.e-sante.be/curcuma-epice-consommer-tous-jours/actualite/1643

www.leboncomplement.com/curcuma/

www.mr-plantes.com/2016/02/curcuma/

curcumabio.fr/curcuma-posologie/]

Since we have examined in details the effects of paracetamol and understood the usefulness of having at disposal another analgesic, efficient and without adverse effects, we intended to examine the effects of an extract of *curcuma* on 22 physiological and ethological traits in the manner we have studied the effects of paracetamol. The powder of the *curcuma* rhizome contains curcumin but this molecule is not well assimilated by living organisms. An extract of rhizome of *curcuma* is nowadays available in several food complements such as Curcudyn®, Flexicure®, Flexipure®, in which curcumin is present under an easily assimilated form by living organisms. In the notices of these available food complements, nearly nothing is related about potential adverse effects of the product; as in links devoted to *curcuma*, the only advice is to avoid the product for children and pregnant women. In such food complements, other elements are added. For instance, Curcudyn® contains curcumin, ginger, vitamin C and D. Flexipure® contains curcumin and krill oil. When making an aqueous solution of this food complement, its lipid fraction moves above, floats, and can thus easily be eliminated. If a very small amount of oil of krill still remains in the aqueous fraction, it will not perturb the ants since they are accustomed to eat fat of insects, thus of arthropods. We thus finally opted for using the food complement 'flexipure' in order to examine the effects of *curcuma*, a potential efficient and safe analgesic, using ants as models.

Here below, we explain why ants can be used as biological models, which traits we intend to examine on them first while under normal diet, then under *curcuma* diet, before explaining our methods and relating our results, for finally concluding about the potential use of *curcuma* as an analgesic on basis of our results and on what is known or more exactly divulgated about the effects of that food complement.

Biological functions and processes are firstly and essentially studied on living organisms other than humans. Most biological discoveries, in genetics, embryology, physiology and ethology for instance, were made using among others *Escherichia coli, Saccharomyces cerevisiae, Drosophila melanogaster, Apis mellifera, Rana esculenta, Ratus norvegicus, Mus musculus, Pan troglodytes*. Invertebrates, and above all insects, are advantageously used [11,12] because they have a small size and a short life cycle, and are generally available in large numbers. Ants can also be used. We became conscious of this opportunity when studying on them the impact of manmade electromagnetism [13]. 'Several colonies, each containing hundreds of individuals, can be maintained at a very low cost during entire years in a laboratory. Ants are eu-social insects. They live in highly organized colonies presenting some age polyethism, division of labor, social regulation and communication systems between members [14]. They are highly evolved as for the resting position of their mouth parts [15], and their production of several performing pheromones [16]. Generally, they use a nest odor, an entrance odor, an area marking odor, a trail pheromone, a recruiting signal and an alarm signal [17]. They build complex nests, take care of their brood, recruit nestmates, and navigate using memorized cues [17]. They can estimate the distance they walk, expect the presence of food on basis of previous experiences, imitate, learn new cues or methods [17], help callows to learn social signals [18] and even recognize themselves in a mirror [19]'.

During these last years, we used ants of the genus *Myrmica* for examining the harmful effects of products consumed by humans (alkaloids, antidepressants, anxiolytics, drugs, sweeteners, statins and so on) [20]. We did so in optimal conditions because we had made previous researches on these ants' biological traits [21], ontogenesis of cognitive abilities [18], and extent of cognition [22]. In the present work, we used again the ant *M. sabuleti* Meinert 1861 for examining the potential adverse effects of *curcuma*. More precisely, we intended to examine the impact of that food complement on the 22 following traits: meat consumption; sugar consumption; general activity; linear speed; angular speed; orientation ability; trail-following behavior; audacity; tactile (pain) perception; brood caring behavior; cognition; aggressiveness against nestmates; aggressiveness against aliens; ability in escaping from an enclosure; visual conditioning; visual memory; olfactory conditioning; olfactory memory; as well as: adaptation to *curcuma* consumption; habituation to *curcuma* effect; dependence on *curcuma* consumption; decrease of the effect of *curcuma* after its consumption ended.

The 18 first traits were examined on ants living under normal diet (this lasted three weeks), then on the same ants consuming *curcuma* (all these experiments lasted four weeks). The four last traits were then examined only on ants consuming or having consumed that food complement (this last studies lasted four days).

Material and Methods

Collection and maintenance of ants

The experiments were made on three colonies of *M. sabuleti* collected in May 2017 from an abandoned quarry located in the Aise valley (Ardenne, Belgium). Two colonies (A and B) were used for the experimental work *sensu stricto* while the third colony (C) provided the control and the alien ants used in a few experiments. The colonies nested under stones, and contained 500 - 800 workers, 1 - 2 queens and brood. We maintained them in 2 - 3 glass tubes half-filled with water and plugged with cotton. These nest tubes were deposited in trays (34 cm x 23 cm x 4 cm), the sides of which being covered with talc to prevent ants from escaping. The trays served as foraging areas; food was delivered there. The food consisted of sugar water permanently given in tubes (diameter: 1.5 cm, length: 7 cm) plugged with cotton, and of cut *Tenebrio molitor* larvae (Linnaeus, 1758) given three times a week. The air temperature of the laboratory equaled 18°C - 22°C, and the air relative humidity about 80%. The intensity of the lighting was 330 lux when working on ants, and 5 - 120 lux during other time periods, being then provided by natural light and varying with the time of the day. The electromagnetic field had the weak intensity of 2 - 3 $\mu W/m^2$. The workers are here named nestmates, as often done by researchers on social insects.

Solution of *curcum*a given to the ants

Among all the available extracts of *curcuma,* we opted for 'Flexipure'®, a food complement anybody can buy in any drugstore without special recommendation. This product contains an assimilable extract of *curcuma* and krill oil. The latter element causes no problem for ants since these insects are accustomed to consume insects' fat. A package of Flexipure® was provided by the drugstore Wera (Bruxelles). Humans are advised to consume 2 tablets of Flexipure® per day, one in the morning, one in the evening. Humans consume about one liter of water per day (not including the water contained in their food). Humans ingesting Flexipure® consume thus 2 tablets of the food complement together with 1l of water. Insects, including ants, consume proportionally 10 less water than mammals. For being under a Flexipure treatment similar to that of humans, the ants should receive a solution of 2 tablets of that food complement in 100 ml of water. One tablet of Flexipure® was thus opened and its content dissolved in 50 ml of sugar water. Five ml of the obtained solution were poured into the small tubes usually used for providing ants with sugar water. Most of the supernatant oil of krill was eliminated from the tubes, and the latter were then plugged with cotton as usual. The tubes containing the extract of *curcuma* were then given to the ants, in their tray, instead of their usual sugar water tubes. The ants came soon drinking the provided solution, and went on drinking it during the whole experimental work. The cotton plug of the tubes was refreshed each two days, and the entire solution was renewed each seven days.

Preliminary remark

Since our aim was to compare the beneficial and the adverse effects of *curcuma* on one hand and of paracetamol on the other hand, the methodology (experimental apparatus, protocols, methods, and analysis) here employed was exactly the same as that used for examining the effects of paracetamol [3].

Food consumption and activity

The ants of colonies A and B drinking the sugar water supply and eating the *T. molitor* larvae, as well as those being active anywhere (in their tray, near the food sources, in their nest) were counted, during six days, three times between 12:00 and 15:00 o'clock, and three times between 21:00 and 24:00 o'clock (west European winter time = UT + 1) (Table 1, Daily counts). The mean was then established for each of these twelve counts (2 x (3 + 3)) (Table 1, Daily means). The six means obtained for ants under *curcuma* diet were compared to the six means previously obtained for ants under normal diet, 'using the non-parametric test of Wilcoxon' [23]. The mean of the daily means was also calculated for each kind of diet and of count (Table 1, Average of daily means).

Linear and angular speeds, orientation

The linear and the angular speeds of foragers walking in their tray were quantified without presenting any stimulus to the ants; the orientation ability was assessed by presenting them with a nestmate tied to a piece of white strong paper (Figure 1A). This tied worker emitted its alarm pheromone. As done in previous studies (among others [24]), 'the movement of 20 ants of each colony (n = 20 ants x 2 colonies = 40 trajectories) was recorded on an adequate support until the ants walked along 6 cm or reached the tied worker. The running time was appreciated listening to a metronome set at 1s'. 'The trajectories were then copied on a substrate which could remain affixed to a PC monitor screen. They were analyzed using specifically designed software [25] which calculated the ants' linear speed, angular speed and orientation.' These three variables are defined as previously, for instance in [24]. Concerning the orientation, let us recall that when it was lower than 90°, the ants had a tendency to orient themselves towards the tied worker, and when it was larger than 90°, the ants had a tendency to avoid the tied worker. Each distribution of 40 values was characterized by its median and quartiles (Table 2, lines 1, 2, 3) and those corresponding to ants under *curcuma* diet were compared to the previously obtained ones for ants under normal diet, 'using the non-parametric χ^2 test' [23].

Trail-following behavior

The trail pheromone of *Myrmica* ants is produced by the workers's poison gland. Ten poison glands were isolated in 500 µl hexane and the mixture was kept for 15 minutes at -25°C. The following experimental methodology was that set up about 30 years ago, and very frequently used (for instance in [24]). 'Fifty µl of the solution were deposited with a metallic normograph pen, on a circle (R = 5 cm) pencil-drawn on a piece of white paper and divided into 10 angular degrees arcs (Figure 1B). One minute later, this piece of paper was deposited in the ants' tray, and the response of 20 ants of each colony to the trail was assessed. More precisely, the number of arcs of 10 angular degrees each ant walked along the trail without departing from it was counted.' The distribution of the obtained values was characterized by its median and quartiles (Table 2, line 4), and the distribution corresponding to ants consuming *curcuma* was compared to that corresponding to ants under normal diet 'using the non-parametric χ^2 test'.

Audacity

The methodology is identical to that previously used (for instance in [24]). Let us summarize it. 'A cylindrical tower made of strong white paper (Steinbach®, height = 4 cm; diameter = 1.5 cm) was deposited in the ants' tray, and the ants present at any place of this apparatus were counted 12 times in 12 min (Figure 1C). The mean and extremes of the obtained values were established' (Table 2, line 5). The values obtained for ants under one and the other kinds of diet were compared using the non-parametric Wilcoxon test, pulling the values obtained for the two colonies as well as those obtained during each successive time period of two minutes.

Tactile (pain) perception

As for paracetamol [3], 'the examined product is an analgesic. It should affect the sensitive nervous system. It is why we assessed the ants' locomotion on a rough substrate: if they perceived correctly the uncomfortable character of the substrate, they will walk slowly, sinuously (Figure 1D); if their tactile perception is impacted, they will walk more confidently.' The experimental protocol is explained in previous works (for instance, in [24]). 'A duly folded piece (3 cm x 11 (i.e. 2 + 7 + 2) cm) of rough emery n° 280 paper was tied to the bottom and the borders of a tray (15 cm x 7 cm x 4.5 cm) dividing so the tray into a 3 cm long zone, a 3 cm long zone where the ants' moving was made difficult, and a 9 cm long smooth zone. Each colony had its own apparatus. For each colony, 12 ants were deposited in the small zone. They tried to move away from that small zone and walked for a time on the rough paper. The linear and the angular speeds of ants walking on that rough substrate were quantified' (n = 12 trajectories x 2 colonies = 24; Table 2, line 6). 'The distribution of the obtained values was characterized by their median and quartiles, and the distributions corresponding to ants consuming *curcuma* were compared to those corresponding to ants under normal diet thanks to the non-parametric χ^2 test'.

Brood caring behavior

We proceeded as previously (for instance: [24]). 'For each colony, larvae were removed from their nest and set in front of the entrance. The ants' behavior in front of five of these larvae was observed (Figure 1F), and those among these five observed larvae still not replaced in the nest after 30 s, 2, 4, 6, 8, and 10 min were counted. The results obtained for each colony were pulled (Table 3, line 1), and the sums obtained for ants consuming *curcuma* were compared to the sums obtained for these ants under normal diet using the non-parametric Wilcoxon test'.

Cognition

The experimental protocol has been set up for examining the effect of nicotine on this trait [24]. 'Two duly folded pieces of white extra strong paper (Steinbach®, 12 cm x 4.5 cm) were inserted in a tray (15 cm x 7 cm x 4.5 cm) in order to create a path with twists and turns between an initial small loggia and a large loggia'. Each colony had its own apparatus. 'For each colony, 15 ants were set all together in the initial loggia, and thereafter, the ants present in this initial loggia and in the large one were counted after 30s, 2, 4, 6, 8, 10 and 12 minutes'. As usual, 'the numbers obtained for the two colonies were added (Table 3, line 2), and the sums obtained for ants consuming *curcuma* were compared to those obtained for these ants under normal diet using the non-parametric Wilcoxon test'.

Aggressiveness against nestmates and aliens

The same method as that previously used was again employed [3,24]. 'Five dyadic encounters were realized for each colony. Each encountering was conducted in a small cylindrical cup (diameter = 2 cm, height = 1.6 cm), the borders of which being slightly covered with talc. Each time (5 x 2 = 10 encounters with nestmates, 5 x 2 = 10 encounters with aliens), one ant of colony A or B was observed during 5 min and its encounter with the opponent was defined by the number of times it did nothing (level 0 of aggressiveness), touched the other ant with its antennae (level 1), opened its mandibles (level 2), gripped and/or pulled the other ant (level 3), tried to sting or stung the other ant (level 4)'. For each level, the numbers obtained for the two colonies were added (Table 3, line 3 and 4), and the totals corresponding to ants consuming *curcuma* were compared to those corresponding to ants under normal diet using the non-parametric χ^2 test. As previously, 'the ants' aggressiveness was also assessed by a variable 'a', which equaled the number of recorded aggressiveness levels 2 + 3 + 4 divided by the number of recorded levels 0 + 1'.

Ability in escaping from an enclosure

As for the study of the effects of paracetamol [3], 'the enclosure was a reversed polyacetate glass (h = 8 cm, bottom diameter = 7 cm, ceiling diameter = 5 cm) deposited in the ants' tray. For each colony, six ants were introduced into it through a hole (diameter = 3 mm) in the center of its ceiling. The lower part of its inner surface had been slightly covered with talc to prevent the ants of climbing. The rim of the bottom had been provided with a small notch (3 mm height, 2 mm broad) for giving to the ants the opportunity to escape (Figure 1H)'. As previously (for instance: [24]), 'the ants' ability in escaping was quantified by counting, after 30 s, 2, 4, 6, 8, 10 and 12 min, the ants still under the glass and those escaped. The results obtained for each colony were pulled' (Table 3, line 5), and 'the sums obtained for ants consuming *curcuma* were compared to those previously obtained for ants under normal diet using the non-parametric Wilcoxon test'. Also as usually, 'we calculated the variable "n° of ants escaped after 12 minutes / 12" for each kind of diet, 12 being the initial number of imprisoned ants (Table 3, line 5)'.

Visual and olfactory conditioning and memory

For comparative purpose, we proceeded as for studying the effects of paracetamol. We worked on colonies A and B having consumed *curcuma* for 10 days (and the quantification lasted then 14 days), as well as on colony C never provided with this food complement. The methodology has been set up a long time ago, and has already been used many times (for instance in [24]). Briefly, 'at a time, a yellow hollow cube under which ants could walk was set above the sugar water supply, and the ants underwent so visual operant conditioning. The cubes were made of strong paper (Canson®). One week later, after the end of the visual conditioning experiment, pieces of basilica were set near the sugar water supply, and the ants underwent then olfactory operant conditioning. Tests were performed while ants were expected to acquire conditioning, and after removal of the cue, while they were expected to lose their conditioning. As usually, 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). Each colony had its own Y-apparatus. The Y-apparatus was provided with a yellow hollow cube or with pieces of basilica in one branch; half of the tests were conducted with the cue in the left branch and the other half with the cue in the right branch. Choosing the way with the cue was considered as giving the correct response (Figure 1I)'. The procedure used for conducting a test on a colony is summarized in [24]. For each test, the numbers of ants under *curcuma* diet (10 ants x 2 colonies = 20 choices), and of ants under normal diet (n = 10), which gave the correct response were recorded, and the percentage of correct responses established (Table 4). Of course, 'the numerical results obtained for the ants under the two kinds of diet were statistically analyzed thanks to the non-parametric Wilcoxon test'.

Adaptation to *curcuma* consumption

Having found, until now, no adverse effect due to *curcuma* consumption, the ants had not to adapt themselves to that food complement diet, and consequently no assessment was made concerning this non-existing trait. This was of course in favor of *curcuma* use.

Habituation to *curcuma* consumption

For examining if ants became habituated to the analgesic effect of *curcuma*, their linear and angular speeds on a rough substrate were assessed after 16 days of that food complement consumption, as it had been done after five days of consumption. The results then obtained were statistically compared to the control ones and to those obtained after five days of consumption by 'using the non-parametric χ^2 test' (Table 5).

Dependence on *curcuma* consumption

This trait was studied after the ants had consumed *curcuma* for 18 days, using the same methodology as that previously used while studying the effects of substances consumed by humans (for example: [3,24]). 'For each colony, 15 ants were transferred into a small tray (15 cm × 7 cm × 5 cm), the borders of which had been covered with talc, and in which two tubes (h = 2.5 cm, diam. = 0.5 cm) had been laid, one of them containing sugar water, the other containing a sugar solution of *curcuma* (the same solution as that used throughout the entire work). Each tube was plugged with cotton. In one tray, the tube containing the drug was located on the right; in the other tray, it was located on the left. The ants drinking each provided liquid were counted 12 times over 15 min, and the mean value was calculated for each kind of liquid. The sums of the values obtained for each colony were compared to the numbers expected with a random choice by the ants, using the non-parametric goodness of fit χ^2 test' [23].

Decrease of the effect of *curcuma* after its consumption ended

The methodology was similar to that used when examining this trait for several previously studied substances (for instance: [24]). 'The ants were deprived of *curcuma* for 12 hours, then provided with a fresh solution of that food complement for again 12 hours, and then, the solution of *curcuma* was replaced by a solution of pure sugar. Since this weaning time, the ants' linear and angular speeds on a rough substrate were assessed exactly as previously (before and after consuming the food complement for 4 and 16 days), doing so after several time periods. The only difference was that, for avoiding removing ants from their colonies too often, we experimented on 16 ants (8 from each two colonies) each time instead of 24. The experiment ended when the ants' locomotion on a rough substrate was again similar to that presented under normal diet.'

The inequalities between the values obtained for given time periods were assessed by a Kruskal-Wallis test for multiple comparisons, using Statistica® v.10 software.

'The numerical results are given in Table 6' and two variables (V/S and S/V, V = linear speed, S = sinuosity) are graphically presented in figure 2. V/S assessed the effect of the food complement; S/V quantified the ants' tactile (pain) perception. The mathematical functions describing the variation of these two variables over time were established using Statistica® v. 10 software, and the procedure explained in [26] was used for fitting polynomial regression models.

Results and Discussion

Food consumption and activity

Briefly, food consumption was not affected by *curcuma* consumption, while general activity was somewhat increased under that food complement diet (Table 1).

Normal diet with curcuma extract						
Days	**Meat**	**Sugar Water**	**Activity**	**Meat**	**Sugar Water**	**Activity**
Daily counts						
I A B	1 1 0 2 2 2 1 1 0 2 2 2	2 2 2 4 3 2 1 1 2 1 2 1	12 13 12 6 8 9 6 7 8 6 6 5	1 1 0 0 0 1 1 1 1 2 1 0	1 1 2 2 2 3 1 2 1 1 1 3	11 12 11 10 10 10 8 9 9 10 11 12
II A B	1 1 2 1 1 1 2 2 2 1 1 1	3 3 2 1 1 1 2 2 1 3 3 3	10 11 12 8 9 9 6 7 6 7 8 8	0 0 1 1 1 0 1 0 0 0 0 1	2 2 3 2 2 2 1 1 2 2 2 3	13 12 11 9 10 12 12 11 13 9 8 10
II A B	4 5 4 3 3 2 2 3 2 2 2 1	2 3 2 5 4 5 1 0 1 2 1 2	8 15 10 9 10 11 7 8 8 9 9 10	2 2 1 2 2 2 0 1 0 2 2 3	2 2 2 2 2 1 1 1 2 3 2 2	10 11 12 9 10 12 7 8 9 12 13 12
IVA B	2 2 1 1 1 1 1 1 0 1 0 1	1 1 1 2 3 2 1 1 1 1 1 1	9 9 8 8 8 9 9 8 10 7 8 8	1 0 1 1 1 1 1 1 1 2 2 2	1 1 1 2 2 3 1 2 2 3 3 2	13 12 13 9 10 11 9 8 8 9 10 8
V A B	1 1 2 3 3 2 1 1 0 1 1 0	2 2 1 1 2 1 1 0 1 2 1 1	10 9 10 12 13 12 8 9 10 6 7 6	2 2 1 2 2 3 1 1 1 4 4 5	1 1 1 1 1 2 2 1 3 1 2 2	12 13 12 10 12 12 9 8 9 8 7 6 7
VIA B	1 1 1 4 4 3 1 1 0 2 2 1	1 0 1 1 1 2 2 1 1 1 1 1	10 11 10 12 11 13 6 7 8 7 6 7	2 2 2 3 2 2 1 1 2 1 1 1	2 1 2 3 3 2 1 1 2 1 1 1	10 11 10 11 12 13 13 10 11 9 8 10
Daily means						
I	1.33	2.08	8.17	0.75	1.67	10.42
II	1.33	2.08	8.42	0.42	2.00	10.80
III	2.75	2.33	9.50	1.58	1.83	10.42
IV	1.00	1.33	8.42	1.17	1.92	9.25
V	1.33	1.25	9.33	2.33	1.50	9.92
VI	1.75	1.16	9.00	1.67	1.67	10.67
Average of daily means						
	1.58	1.71	8.81	1.12	1.83	10.24

***Table 1:** Impact of a curcuma extract on ants' food consumption and general activity. During six days, the ants of colonies A and B eating meat, drinking sugar water, and being active were counted six times per day. Then, daily means and average of these daily means were established. The used food complement did not affect the three examined traits.*

Though ants consumed a little less meat while under *curcuma* diet (average: 1.12 *vs* 1.58 ants counted on the meat), there was finally no significant difference as for meat consumption between the ants under normal diet and the same ants under *curcuma* diet (N = 6, T = -15, P = 0.219). Even if ants under the food complement diet were slightly more numerous on the sugar water supply than when under normal diet (1.83 *vs* 1.71 in average), the difference of that food consumption between the ants under normal and *curcuma* diet was not significant (N = 6, T = +13, P = 0.344).

Compared with what occurred under normal diet, under *curcuma* diet ants became a little more active. This was obvious to the observer and was statistically significant (N = 6, T = +21, P = 0.016). Even after three weeks, the ants consuming the food complement appeared to be a little more active than usually, though being not at all nervous. It can thus be concluded that, even if having an analgesic effect (see below), *curcuma* induces not sleepiness.

These first results are in favor of *curcuma* use.

Linear and angular speeds, orientation

The ants' locomotion was not affected by *curcuma* consumption (Table 2, lines 1, 2). Even if being somewhat more active (see above), the ants went on walking at the same linear and angular speeds as when under normal diet. The small difference concerning these two traits between ants under normal and *curcuma* diets was not significant (linear speed: χ^2 = 1.59, df = 3, 0.50 < P < 0.70; angular speed: χ^2 = 3.38, df = 2, 0.10 < P < 0.20).

Paracetamol was found to increase the ants' sinuosity of movement [3]. Consequently, as for this physiological trait, *curcuma* is safer than paracetamol.

Orientation to an alarm signal

This trait was not at all impacted by *curcuma* consumption (Table 2, line 3). Indeed, ants under that food complement consumption oriented themselves towards an alarm signal as well as ants under normal diet (Figure 1A). The difference between the two diets was not significant (χ^2 = 0.68, df = 2, 0.70 < P < 0.80). *Curcuma* may thus not negatively affect the brain functioning, a presumption again examined in following experiments.

Paracetamol decreased the ants' orientation ability [3]; *curcuma* was here shown to have not that adverse effect.

Trail-following behavior

This trait was not impacted by *curcuma* consumption (Table 2, line 4). Under normal diet as well as under *curcuma* diet, the ants followed a circular trail meanly along 9 angular arcs of 10 degrees (Figure 1B). The difference of ants' behavior in front of a trail between ants under normal and *curcuma* diet was not significant (χ^2 = 0.38, df = 2, 0.80 < P < 0.90). The here above presumption seemed thus to be corroborated: *curcuma* may not affect the brain functioning.

Once more, paracetamol negatively affected the here examined trait [3]; *curcuma* seemed thus until this step safer than that drug.

Audacity

Curcuma consumption did not affect this ethological trait (Table 2, line 5). Indeed, under normal or this food complement diet, the ants were not inclined in walking on an unknown and risky apparatus (Figure 1C). The difference of ants' behavior in front of such an apparatus between ants under one or the other kind of diet was not significant (N = 4, T = +6, -4, P = 0.437).

Paracetamol also did not impact the ants' audacity [3].

Tactile (pain) perception

This physiological trait was largely impacted by *curcuma* consumption (Table 2, line 6). Under normal diet, the ants moved on a rough substrate very cautiously, at a low linear speed and a high angular speed, even stopping from time to time (Figure 1D). Under *curcuma* diet, they moved far more frankly, at a higher linear speed and a lower angular speed, even running (Figure 1E). The difference of ants' locomotion on a rough substrate according to their diet (normal or with *curcuma*) was significant (linear speed: $\chi^2 = 24.05$, df = 2, P < 0.001; angular speed: $\chi^2 = 40.62$, df = 1, P < 0.001). This result revealed the efficient analgesic effect of an extract of *curcuma*. A following experiment examined if such an effect persists as it is initially in the course of the food complement consumption or if some individual's habituation occurs (see below 'Habituation to *curcuma* consumption').

Of course, paracetamol also presented an analgesic effect on ants, though being somewhat weaker than that of *curcuma* [3].

Traits	Normal Diet	Diet with *Curcuma* Extract
Linear speed (mm/s)	14.0 (12.7 - 15.8)	13.7 (11.6 - 15.5)
Angular speed (ang.deg./cm)	128 (116 - 139)	118 (108 - 136)
Orientation (ang. deg.)	44.4 (35.7 - 59.1)	41.2 (29.0 - 53.7)
Trail-following (n° arcs)	9.0 (6.0 - 15.0)	9.0 (5.0 - 12.3)
Audacity (n° ants)	1.20 (0 - 3)	1.15 (0 - 3)
Tactile (pain) perception:		
linear speed (mm/s)	5.7 (4.9 - 6.6)	9.1 (7.8 - 9.9)
angular speed (ang.deg./cm)	254 (215 - 279)	137 (115 - 147)

***Table 2:** Impact of a curcuma extract on six ants' physiological and ethological traits. The six traits listed in column 1 were assessed on ants of two colonies, first while they were under normal diet, secondly while they consumed an extract of curcuma. This food complement did not affect the first five examined traits, and largely reduced the sixth one, i.e. the pain perception. It might thus be used as a safe analgesic, if it did not impact other traits, what following experiments tested.*

Brood caring behavior

This trait was neutrally affected by *curcuma* consumption (Table 3, line 1). Ants under that food complement diet took care of their brood just like the same ants under normal diet. They soon replaced in their nest the larvae artificially removed from it (Figure 1F), but seemed to do so somewhat more quickly since the difference of ants' score for re-entering larvae between ants under *curcuma* or normal diet was slightly significant (larvae no re-entered: N = 5, T = -15, P = 0.031). This result was in agreement with that concerning the orientation of ants towards an alarm signal. In the present experiment, the ants must perceive the presence of larvae, orient themselves towards them, then orient themselves towards the nest entrance. So, like in in the experiment on ants' orientation, the ants must perceived the alarm signal and orient themselves towards it. Each time, such abilities were not impacted and were even somewhat enhanced by *curcuma* consumption. The presumption of a non-negative impact of *curcuma* on the brain functioning became more plausible, and was again examined in following experiments.

Paracetamol also did not impact the ants' brood caring behavior [3].

Cognition

This physiological and ethological trait was enhanced by *curcuma* consumption (Table 3, line 2). Under normal diet, after 12 experimental minutes, 3 ants could cross the twists and turns path and reach the large zone beyond it while 15 ants were still in the small zone located in front of the difficult path. Under the food complement diet, 5 ants could reach the large zone beyond the twists and turns path while 13 ants were still in front of that path. The difference of ants' ability in crossing the twists and turns between ants under the two kinds of diet was significant (small zone: N = 7, T = -28, P = 0.0078; large zone: N = 4, T = 10, P = 0.062). This result corroborated the previous presumption of a favorable impact of *curcuma* on some brain activities, what was still checked by following experiments.

Paracetamol impacted the ants' cognition [3], what was not in favor of that drug use, while *curcuma* did not induce such an adverse effect

Aggressiveness against nestmates and aliens

These two traits were not or very slightly affected by *curcuma* consumption (Table 3, lines 3, 4). In front of a nestmate, an ant under normal or *curcuma* diet either did nothing or touched the opponent with its antennae, or seldom opened their mandibles (Figure 1G). The difference of ants' behavior according to their diet was not significant, even if ants under *curcuma* diet a little more often opened their mandibles ($\chi^2 = 7.51$, df = 2, $0.05 < P < 0.10$). In front of an alien, an ant under normal or *curcuma* diet seldom did anything, contacted the opponent with their antennae, often opened their mandibles, and sometimes gripped or even tried to sting the opponent. The difference of behavior between ants under the two kinds of diet was not significant ($\chi^2 = 0.32$, df = 2, $0.80 < P < 0.90$). The ants' relationships with their nestmates and not nestmates were thus not changed by the food complement consumption, a result in agreement with the previously observed similarity of ants' behavior in front of their brood (see above).

In the same manner, paracetamol did not affect the ants' relationship with their nestmates and aliens [3].

Ability in escaping from an enclosure

This behavioral trait was improved by *curcuma* consumption (Table 3, lines 5, 6). Under normal diet, 9 ants among 12 could escape from an enclosure; under *curcuma* diet, 10 ants could do so (Figure 1H). The difference of ability in escaping between ants under the two kinds of diet was significant (ants still enclosed or escaped: N = 6, T = - or + 21, P = 0.016). This result corroborated the previous presumption of a positive, or at least a non-negative impact, of the food complement on some brain functioning (see above), a fact again examined the following experiment.

Paracetamol also did not statistically impact the ants' ability in escaping from an enclosure [3].

Traits	Normal Diet	Diet with *Curcuma* Extract
Brood caring: n° of larvae among 10 not replaced in the nest in the course of 10 min	t: 30s 2 4 6 8 10 min n° 10 8 6 4 2 0	t: 30s 2 4 6 8 10 min n° 10 8 5 3 2 0
Cognition: ants in front of and beyond twists and turns in the course of 12 min	t n° in front n° beyond 30s 27 0 2 24 1 4 21 1 6 22 1 8 18 3 10 16 3 12 15 3	t n° in front n° beyond 30s 26 0 2 22 1 4 19 1 6 17 2 8 14 4 10 13 5 12 13 5
Aggressiveness against nestmates	levels 0 1 2 3 4 var 'a' n° 84 26 10 0 0 0.09	levels 0 1 2 3 4 var 'a' n° 64 44 12 0 0 0.11
Aggressiveness against aliens	levels 0 1 2 3 4 var 'a' n° 3 27 72 22 0 2.68	levels 0 1 2 3 4 var 'a' n° 8 28 71 16 2 2.47
Escaping from an enclosure: ants in and out of the enclosure in the course of 12 min	t: 30s 2 4 6 8 10 12 n° in 12 11 9 8 6 4 3 n° out: 0 1 3 4 6 8 9 variable = 9/12 = 0.75	t: 30s 2 4 6 8 10 12 n° in : 12 10 8 7 5 3 2 n° out: 0 2 4 5 7 9 10 variable = 10/12 = 0.83

Table 3: *Effects of a curcuma extract on four ants' ethological and physiological traits. The five traits, listed in column I, were assessed on ants of two colonies before then after they consumed an extract of curcuma. This food complement did not affect (on the contrary) the examined traits, and may thus be safely used if not impacting other studied traits.*

Visual and olfactory conditioning and memory

These ethological traits were not affected by *curcuma* consumption (Table 4, Figure 1I).

Traits	Under normal diet		Under *curcuma* diet		
Time (hrs)	colony C	%	colony A	colony B	%
Visual conditioning					
7	7	70	6	6	60
24	8	80	7	6	65
31	8	80	7	7	70
48	8	80	8	8	80
55	8	80	8	9	85
72	8	80	8	9	85
Visual memory					
7	8	80	9	7	80
24	7	70	8	7	75
31	8	80	7	8	75
48	7	70	8	8	80
55	7	70	8	7	75
72	7	70	7	7	70
Olfactory conditioning					
7	7	70	6	7	65
24	8	80	8	7	75
31	8	80	9	7	80
48	9	90	9	9	90
55	9	90	9	10	95
72	9	90	9	8	85
Olfactory memory					
7	9	90	9	9	90
24	8	80	8	8	80
31	8	80	8	7	75
48	7	70	8	9	85
55	8	80	8	7	75
72	8	80	8	8	80

Table 4: *Impact of curcuma on conditioning ability and memory. Ants were trained to a visual or an olfactory cue, and tested in a Y apparatus provided with the cue in one of its branch in the course of their conditioning acquisition then of their loss. The food additive did not at all affect the visual and the olfactory conditioning ability (so the short time memory), nor the middle term memory, what is in favor of that food complement use.*

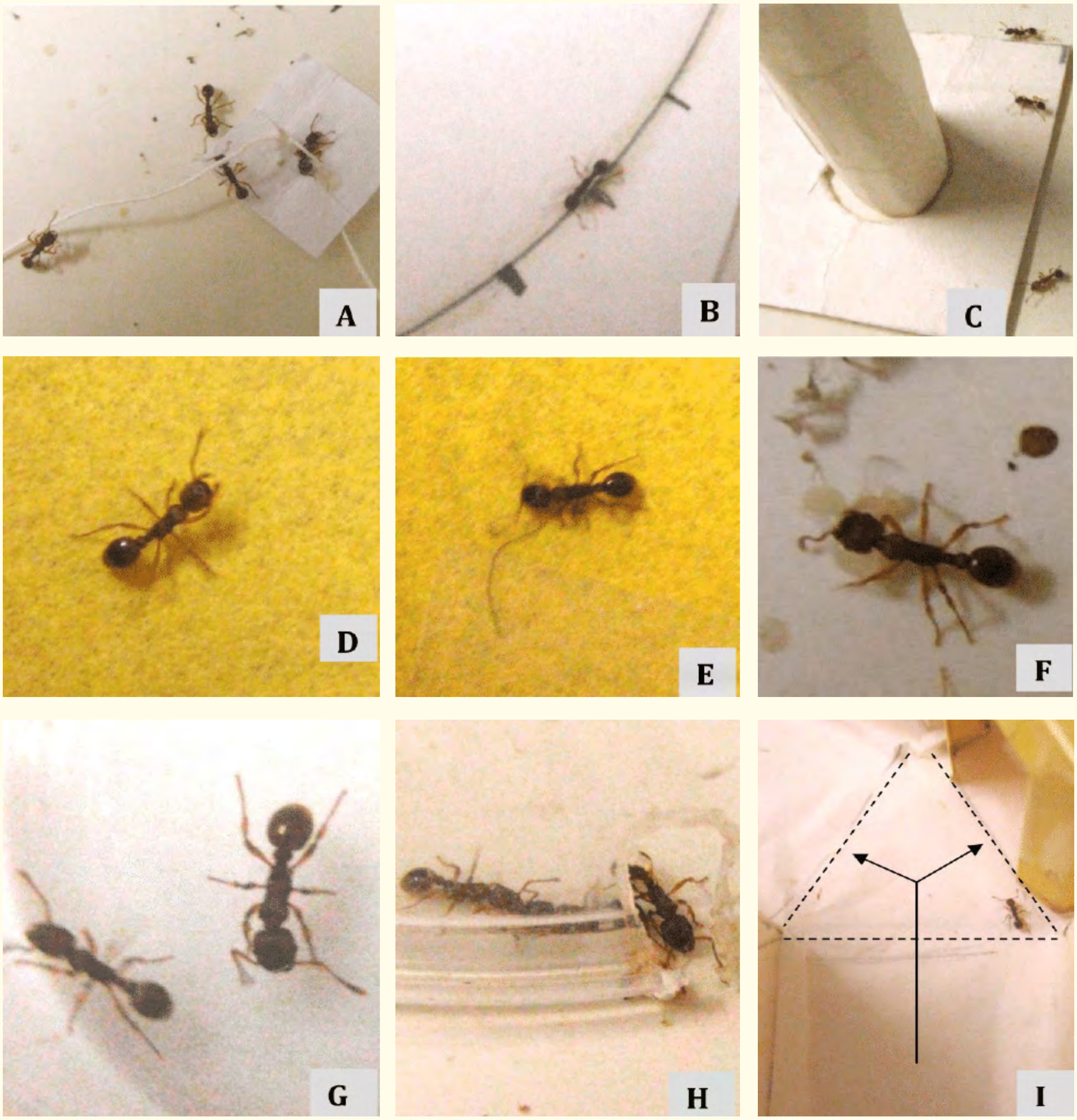

Figure 1: *Some views of the experiments. A: ants under curcuma diet coming to a tied worker which emitted its attractive alarm pheromone. B: an ant under curcuma diet following a circular trail. C: ants under curcuma diet not inclined in coming onto an unknown and risky apparatus. D: an ant under normal diet moving with difficulty on a rough substrate; it is stopping for a short time. E: an ant under curcuma diet moving frankly on a rough substrate; it is running. F: an ant under curcuma diet transporting a larva to the nest. G: two ants under curcuma diet being not aggressive to one another in the course of a dyadic encounter. H: an ant under curcuma diet escaping from an enclosure, and two other ones in the nick of following it. I: an ant under curcuma diet, trained to a hollow yellow cube, giving the correct response when tested in a Y apparatus provided with that cue.*

Ants trained to a visual cue reached a conditioning score of 70% and 60% after 7 hours under normal and *curcuma* diet respectively, as well as a score of 80% and 85% after 72 hours under normal and *curcuma* diet respectively. The difference of conditioning ability between ants under the two kinds of diet was not significant: N = 5, T = +12, P = 0.156. After removal of the visual cue, 7 hours later, ants under each of the two kinds of diet kept all their conditioning, and retained 20% (70% minus 50%) of it after a total of 72 hours. The difference of visual memory between ants under one and the other kind of diet was not significant: N = 4, T = +8, P = 0.187.

Trained to an olfactory cue, ants under normal and *curcuma* diet reached after 7 hours a conditioning score of 70% and 65% respectively, and after a total of 72 hours, a score of 90% and 85% respectively. The difference of olfactory conditioning acquisition between ants under one and the other kinds of diet was not significant: N = 4, T = -7.5, P = 0.250. After having been no longer trained, ants under normal diet and those under *curcuma* diet presented an unchanged conditioning score after 7 hours, and lost each ones *ca* 10% of it after 72 hours. The difference of olfactory memory between ants under the two kinds of diet was not significant: N = 3, T = +3, -3, P = 0.625.

Thus, *curcuma* consumption did not impact the conditioning ability as well as the short, middle and long term memory of the experienced individuals.

On the contrary, paracetamol largely affected the ants' conditioning ability, as well as their short and middle term memory [3].

We could thus definitively conclude that *curcuma*, contrary to paracetamol, does not impact the central nervous system. Consequently, it does not act in the same manner as paracetamol, on the cerebral pain perception. Studies of other authors [5] showed that this food complement does not act as acetylsalicylic acid, nor as opiacae (morphine, for example). As suggested by other authors, it may act otherwise, for instance on the cellular membrane and other cytological function [27-30]. This is detailed in the link www.wikiphyto.org/wiki/Curcuma. It may thus finally have an action on the nervous transmission of the pain perception to the brain. This could lead to impact several nervous transmissions to the brain, what would be an important adverse effect of the food complement. At our mind, this does not occur. Indeed, all along our work, ants went on perceiving odors (they moved towards a chemical alarm signal, they follow their trail, they perceived their brood, their perceived the provided basilica), visual cues (they saw their nest entrance, the notch allowing them to escape, the provided yellow hollow cube) and tactile contacts (they often touched themselves with their antennae). It may thus be presumed that *curcuma* decreases only nervous transmissions occurring in the case of pain perception. We can advance a hypothesis. On basis of our observations and results (related above and below) and of what has found until now about the mode of action of *curcuma* (see references here above and in the introduction section), it is not nonsense to presume that this food complement might enhance the natural adaption, habituation and reaction of the organism in case of pain perception. In some physical and psychological circumstances, organisms are able to support high level of pain thanks to physiological mechanisms. *Curcuma* might act, together with acting as an anti-inflammatory product, on such mechanisms. Note that pain perception is a complex physiological function still under investigation (gepi.org/save/Lise/mecanismes_evitement_douleur.pdf).

Adaptation to *curcuma* consumption

Let us recall (see the 'material and methods' section) that having revealed no adverse effect for the studied food complement, this physiological trait could not be taken into account, what is favorable to the use of *curcuma.*

Habituation to *curcuma* consumption

After 16 days of the food complement consumption, the ants moved on a rough substrate as frankly as after 4 days of such a consumption (Table 5). The difference of locomotion between ants having consumed *curcuma* for 16 days and those having consumed it for 4 days was not significant: linear speed: $\chi^2 = 0.40$, df = 2, $0.80 < P < 0.90$; angular speed: $\chi^2 = 4.76$, df = 2, $0.05 < P \sim 0.10$. Consequently, the ants presented no habituation to the analgesic effect of *curcuma*, what is in favor of that food complement for reducing pain perception.

Variables assessed	Normal diet	Diet with *curcuma* for 4 days	Diet with *curcuma* for 16 days
Linear speed (mm/s)	5.7 (4.9 - 6.6)	9.1 (7.8 - 9.9)	9.8 (7.8 - 10.8)
Angular speed (ang.deg./cm) on a rough substrate	254 (215 - 279)	137 (115 - 147)	145 (120 - 175)

Table 5: *Habituation to the analgesic effect of curcuma. The ants' locomotion on a rough substrate was quantified while living under normal diet, then when having consumed curcuma for 4 days (what revealed the analgesic effect of the product), and when having consumed it for 16 days (what revealed a similar analgesic effect). The ants did not habituate themselves to the effect of curcuma, what is excellent for an analgesic.*

On the contrary, paracetamol induced obviously some habituation over consumption time [3].

Dependence on *curcuma* consumption

In front of a sugar solution free of *curcuma* and a similar solution containing this food complement, 20 ants of colony A chose the former liquid and 14 ants the latter one, while 20 ants of colony B also chose the former liquid and 18 ants went drinking the latter one. In total, 40 ants preferred the pure sugar water and 32 ants went drinking the sugar water containing *curcuma.* These numbers did not differ from those expected (36 and 36) if ants went randomly drinking each kind of sugar water presented: $\chi^2 = 0.002$, df = 1, $0.95 < P < 0.98$. Consequently, after having consumed *curcuma* for 14 days, the ants developed no dependence at all on that food complement consumption, a fact which is in favor of its use.

As for paracetamol, an ants' dependence was revealed. Since not only addiction, but also some habituation occurred, humans may be progressively incited to consume again this drug, and to consume larger amount of it over time, what is not in favor of paracetamol use.

Decrease of the effect of *curcuma* after its consumption ended

Numerical values of ants' linear and angular speeds on a rough substrate are given in table 6, and two variables (defined in the section 'Material and Methods') are graphically presented in figure 2. At t = 0 (just before weaning), the ants' pain perception was weak, and consequently, their linear speed on a rough substrate was high, and their angular speed rather small. After weaning, the analgesic effect of *curcuma* did not decrease for about 5 hours. After a total of 6 hours following weaning, this effect was lower though still efficient since the ants' locomotion on a rough substrate went on differing from the control one (linear speed: P = 0.010; angular speed: P = 0.015), and was statistically similar to that before weaning (linear speed: P = 1.000; angular speed: P = 0.489). Eight and an half hours after weaning, the analgesic effect of *curcuma* became less efficient. At that time, the ants' locomotion on a rough substrate was at the limit of differing from the control one (linear speed: 0.143; angular speed: P = 0.065), though being still not different from that before weaning (linear speed: 0.541; angular speed: 0.167). Eleven hours after weaning, the analgesic effect of *curcuma* became statistically similar to the control one (linear speed: P = 1.000; angular speed: P = 1.000), and different from that before weaning (linear speed: P = 0.001; angular speed: P = 0.002). After that, the analgesic effect of *curcuma* went on somewhat decreasing. The efficient analgesic effect of *curcuma* vanished thus in a total of about 9 - 10 hours, staying intact during the first 6 hours, what is in favor of a safe use of that food complement.

Experiment Time	V = Linear speed (mm/sec)	P (*vs* control)	P (*vs* t=0)	S = Angular speed (ang.deg./cm)	P (*vs* control)	P (*vs* t=0)
Control	5.7 (4.9 - 6.6)			254 (215 - 279)		
4 days	9.1 (7.8 - 9.9)			137 (115 - 147)		
12 days	9.8 (7.8 - 10.8)			145 (120 - 175)		
Weaning						
t = 0 h	9.4 (9.2 - 9.9)			139 (110 - 154)		
2	9.0 (8.6 - 10.1			135 (110 - 146)		
4	9.5 (8.9 - 10.1)			148 (127 - 175)		
6	8.1 (7.2 - 8.8)	P = 0.010	P= 1.000	183 (147 - 204)	P = 0.015	P = 0.489
8 ½	7.5 (6.6 - 8.8)	P = 0.143	P = 0.541	192 (167 - 213)	P = 0.065	P = 0.167
11	6.3 (5.4 - 7.2)	P = 1.000	P = 0.001	215 (176 - 234)	P = 1.000	P = 0.002
14	5.4 (4.9 - 5.9)			237 (211 - 250)		
16 ½	4.9 (4.1 - 5.5)			235 (202 - 292)		

Table 6: *Decrease of the analgesic effect of curcuma after its consumption was stopped. The ants' linear and angular speeds on a rough substrate (what revealed pain perception) were assessed at different times after weaning. After 6 hours, the analgesic effect was still efficient; after 8 ½ hours, it was weak; after 11 hours, it nearly vanished. The analgesic effect of curcuma persisted thus for about 5 hours after weaning, then decreased slowly and fully vanished in about 12 hours. Such a slow decrease allows avoiding addiction. The variables V/S (assessing the effect of the drug) and S/V (assessing the ants' pain perception) are graphically presented in figure 2.*

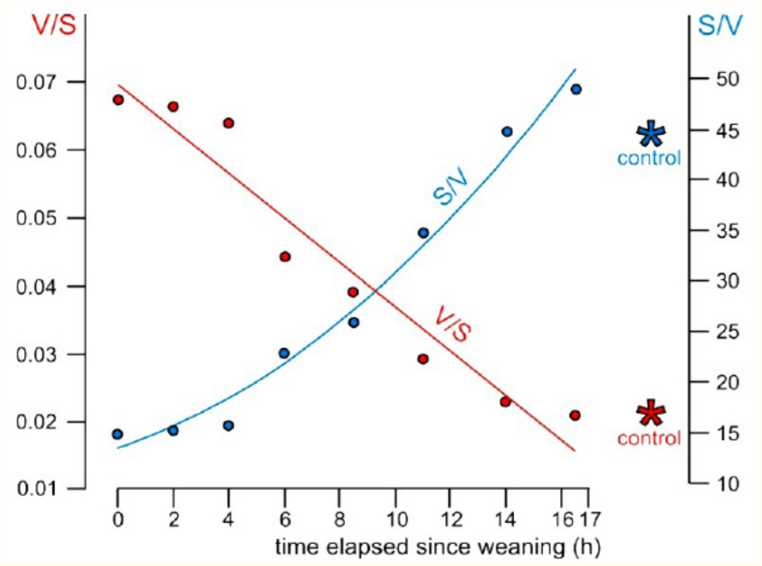

Figure 2: *Decrease of the analgesic effect of an extract of curcuma after its consumption was stopped. The numerical values of V (linear speed) and of S (angular speed) are given in table 6; statistics are summarized in this table and detailed in the text. V/S (red circles and line) quantified the analgesic effect of the food complement; S/V (blue circles and curve) quantified the ants' pain perception. V/S varied over time according to a linear function, S/V according to a quadratic one (details in the text). Briefly, the analgesic effect of curcuma slowly vanished in about 12 hours, what allows avoiding addiction and is thus in favor of the use of that food complement as a safe analgesic.*

This result was far better than that obtained for paracetamol, the analgesic effect of which vanished in only 4 hours [3]. A slow decrease of the effects of a drug allows avoiding addiction [20,24]. This deduction was once more corroborated: paracetamol leaded to dependence, an extract of *curcuma* did not induce such an addiction.

Assessed by V/S, the decrease over time of the analgesic effect of *curcuma* was graphically best fitted by the linear function: V/S = 0.0697 + 0.0033t with $R^2 = 0.94$. The variation over time of S/V, accounting for the ants' tactile (pain) perception, was best fitted by the quadratic function: $S/V = 13.2988 + 0.9081t + 0.0793t^2$ with $R^2 = 0.98$. It has been checked that such functions account better than other functions for the observed changes over time of the two variables.

Conclusion

In conclusion, we recommend the use of an extract of *curcuma* in order to reduce pain perception, instead of paracetamol, the nowadays largely used analgesic which was proved to have many adverse effects, and to lead to habituation and dependence at the commonly dose used by humans. We simply add that *curcuma* should be used without abuse since perceiving pain is an essential physiological trait which can inform the organism about a health problem. An extract of *Curcuma longa* detains also several other appreciable properties [31]. Practitioners and researchers should examine if this food complement could act on, and enhance, some natural physiological mechanisms allowing organisms to support pain perception.

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Conflict of Interest

We affirm having no conflict of interest as for the use of *curcuma* as an analgesic. We are ethologist, working on ants, and receive no money for performing our research. We also affirm having maintained the ants in the best possible conditions.

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