

Is the Largely used Analgesic Paracetamol without any Adverse Effects? A Study on Ants as Models

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Abstract

The nowadays most used analgesic is paracetamol. Its mode of action is not yet elucidated and its adverse effects have been only partly studied. In the present work, using ants as models, we examined the potential effect of a large dose (4g per day) on 22 physiological and ethological traits. It appeared that paracetamol increased the ants' sinuosity of movement, decreased their orientation ability, trail-following behavior, tactile (pain) perception, cognition, visual and olfactory conditioning as well as memory. It did not affect the ants' food consumption, general activity, audacity, brood caring, aggressiveness against nestmates and aliens, as well as ability in escaping from an enclosure. Paracetamol impacted thus some traits depending from the central nervous system. On the basis of other researchers' studies, it can be advanced that such an impact depends on the consumed dose. Ants presented slight adaptation, as well as a significant habituation to paracetamol, two traits which are unfavorable to this drug use. Moreover, ants developed some dependence on paracetamol, and the effect of this drug rapidly decreased after weaning, nearly vanishing in about 4 hours. We suggest some control and limitation of usage as for the access to and the use of paracetamol. Note also that pain perception is a vital function and continuously inhibiting it may imperil the health.

Keywords: Addiction; Cognition; Habituation; Memory; Pain Perception

Abbreviations

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

Introduction

Paracetamol, also named acetaminophen, is a chemical compound used as an analgesic and an antipyretic. It is not a non-steroidal anti-inflammatory drug, such as the salicylates, nor an opioid drug, such as morphine. The history of its use, access and packaging is long, with many ups and downs (<https://fr.wikipedia.org/wiki/Paracétamol>). Its success is partly due to the fact that the other analgesics often have severe adverse effects. Its actual chemical structure is hydroxyphenylethanamide (C₈H₉NO₂). It is used for largely decreasing the pain sensation, and, at a lower extent, for decreasing fever. It is nowadays the most sold drug in France, and the active constituent of the three most prescribed drugs all over the world, i.e. Dafalgan®, Efferalgan®, Doliprane®. It is also the active substance of the most used analgesic for children, i.e. Perdolan®. However, even after 100 years of use, its mode of action is still unknown. It does not act as aspirin®, nor as ibuprofen®, nor as opioids. All what is known for sure is that a consumption of more than 4g per day by adult humans is dangerous and causes severe liver damages. Other occasional adverse effects have also been reported [1-3]. Recently, paracetamol has been suspected to act on the central nervous system. It may inhibit the production of prostaglandin, a hormone playing a part in the pain sensation [4]. A serotonergic activity is also suggested [5], and paracetamol might limit the production of endomorphine. However, all these effects are

not yet confirmed and fully understood, and we have to admit that paracetamol is very largely used, by everybody, at high dose, that it is free of access in every drugstore (no document is required), and that until now, nearly nothing is precisely known about its mode of action. Even if accidents have occurred, even if persons died after paracetamol consumption, this drug goes on being the most accessible and used analgesic in the world [6-10]. The internet sites about paracetamol, and the notice joined to the different packages of this drug put forward its advantages, its efficiency in reducing the pain perception, and neglect or slightly mention its potential adverse effects (www.doctissimo.fr/principe-actif-5687-PARACETAMOL.htm; <https://www.antidouleur.be> › Les substances actives; <https://www.creapharma.ch/paracetamol.htm>; pharmacomedicale.org/medicaments/par-specialites/item/paracetamol). Generally, the persons consuming paracetamol affirm perceiving no adverse effect. This is not a proof that paracetamol has no adverse effect; this drug largely reduces the pain perception and thus, at the same time, the perception of painful adverse effects.

Paracetamol is absorbed by the organism rapidly and totally, in 15 - 60 minutes. Its half-life equals about 2 hours. It is nearly completely degraded into glycu- and sulfo- products which are highly toxic (<https://fr.wikipedia.org/wiki/Paracétamol>). On the other hand, paracetamol and its degraded products eliminated by humans are susceptible to be changed into highly toxic substances when waste water is treated with bleach. As a matter of fact, it is not yet known to which extent paracetamol and its toxic residues contaminate natural water, due to the human large consumption of paracetamol.

Given the easy availability of paracetamol, its very large use, its possible effect on the central nervous system, its rapid elimination (what may lead to addiction [11]), the nowadays ignorance of all its effects, and its potential presence in natural water, we estimated useful to examine the potential ethological and physiological effects of this analgesic in the manner we are now accustomed to work, i.e. using ants as biological models.

Here below, we briefly explain why we use ants as models, which species we used, what we know about this species, and what we can and intended to examine using this species as for the potential effects of paracetamol.

Biological functions, systems and processes are primarily studied on animals used as models. Most biological discoveries, in genetics, embryology, physiology and ethology for instance, have been made working on *Escherichia coli*, *Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Apis mellifera*, *Rana esculenta*, *Rattus norvegicus*, *Mus musculus*, *Pan troglodytes* among others. Invertebrates, and among them insects, are advantageously used [12,13] since they have a small size and a short life cycle, and are generally available in large numbers. Ants can also serve as models. We became conscious of this opportunity when studying on them the impact of manmade electromagnetism [14]. As a matter of fact, 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 [15]. They are highly evolved as for the resting position of their mouth parts [16], and their production of several performing pheromones [17]. Generally, they use a nest odor, an entrance odor, an area marking odor, a trail pheromone, a recruiting signal and an alarm signal [18]. They build complex nests, take care of their brood, recruit nestmates, and navigate using memorized cues [18]. They can estimate the distance they walk, expect the presence of food on basis of previous experiences, imitate, learn new cues or methods [18], help callows to learn social signals [19] and even recognize themselves in a mirror [20].

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) [21]. We could do so in optimal conditions thanks to our previous researches on these ants' biological traits [22], ontogenesis of cognitive abilities [19], and extent of cognition [23]. In the present work, we used again the ant *M. sabuleti* Meinert 1861 for examining the potential adverse effects of paracetamol. More precisely, we intended to examine the impact of that drug 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 paracetamol consumption; habituation to paracetamol effect; dependence on paracetamol consumption; decrease of the effect of paracetamol 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 paracetamol (all these experiments lasted four weeks). The four last traits were then examined only on ants consuming or having consumed that drug (this last studies lasted five days).

Material and Methods

Collection and Maintenance of Ants

The experiments were conducted on three colonies of *M. sabuleti* collected in May 2017 from an abandoned quarry located in the Aise valley (Ardenne, Belgium). Two colonies labeled A and B were devoted to the experimental work *sensu stricto*; the other colony, labeled colony C, provided the 'alien ants' and the control ants in the experiments dealing with conditioning, memory and dependence. The colonies nested under stones. They contained 500 - 800 workers, 1 - 2 queens and brood. In the laboratory, the ants nested in 2 - 3 glass tubes half-filled with water and plugged with cotton. These nest tubes were set in trays (34 cm x 23 cm x 4 cm), the sides of which having been slightly covered with talc to prevent ants from escaping. The trays served as foraging areas; food was provided in them. The ants' food consisted of sugared water continuously 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 temperature equaled 18°C - 22°C, and the air relative humidity about 80%. The intensity of the lighting was 330 lux while working on ants. During other time periods, it was provided by natural light and had an intensity of 5 - 120 lux according to the time of the day. The intensity of the electromagnetic field of the laboratory equaled 2 - 3 $\mu\text{W}/\text{m}^2$. The workers are often here named nestmates, as do researchers on social insects.

Solution of paracetamol given to the ants

Two grams of pure paracetamol were provided by the drugstore Wera (Bruxelles). Humans are advised to consume 4 x 1g of paracetamol each day in case of persistent pain. Humans ingest about one liter of water per day (not including the water contained in their food). Humans under paracetamol treatment consume thus 4g of the drug together with 1l of water. Insects and thus ants consume proportionally 10 less water than mammals. For being under a paracetamol diet similar to that of humans treated with that drug, the ants should be provided with a solution of 4g of paracetamol in 100 ml of water. The two grams of paracetamol, duly weighted, were thus dissolved in 50 ml of sugar water, and 5 ml of this solution were poured into the small tubes usually used for providing ants with sugar water. The tubes containing the sugar paracetamol solution were given to the ants, in their tray, after having removed their usual sugar water tubes. It was regularly checked if ants drank the provided solution of paracetamol, and effectively they did.

Food consumption and activity

The ants consuming the sugar water supply and the *T. molitor* larvae as well as those which were active anywhere (on their foraging area, 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). For each of these three counts, the mean was established (Table 1, Daily means). The six means obtained for ants under paracetamol diet were compared to the six means previously obtained for ants under normal diet, 'using the non-parametric test of Wilcoxon' [24]. 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 speed, 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 [11,25]), '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 [26] which calculated the ants' linear speed, angular speed and orientation.' These three variables are defined as previously [11,25]. 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 consuming paracetamol were compared to the previously obtained ones for ants under normal diet, 'using the non-parametric χ^2 test' [24].

Trail-following behavior

The trail pheromone of *Myrmica* ants is produced by the worker's poison gland. Ten poison glands were isolated in 500 μ l hexane and the mixture was set for 15 min at -25°C. The following experimental methodology was that set up about 30 years ago, and very frequently used (for instance in [11]). 'Fifty μ l of the solution was 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 paracetamol 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 [11,25]). 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 minutes. 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

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 1C); if their tactile perception is impacted, they will walk more confidently. The experimental protocol is explained in previous works (for instance, in [25]). '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 paracetamol 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: [25]). '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 1D), and those among these five observed larvae still not replaced in the nest after 30s, 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 paracetamol 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 [11]. It was once more used in the present work. '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 min'. As usual, 'the numbers obtained for the two colonies were added (Table 3, line 2), and the sums obtained for ants consuming paracetamol 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 [25]. 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 paracetamol 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

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 from 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 1E). As previously (for instance: [25]), 'the ants' ability in escaping was quantified by counting, after 30s, 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 paracetamol were compared to those previously obtained for ants under normal diet using the non-parametric Wilcoxon test', as we usually did [25]. Also as usually, 'we calculated the variable "n° of ants escaped after 12 min / 12" for each kind of diet, 12 being the initial number of imprisoned ants (Table 3, line 5)'.

Visual and olfactory conditioning and memory

These traits were quantified on colonies A and B having consumed paracetamol for 10 days (and the quantification lasted then 14 days), as well as on the colony (C) never provided with this drug. The methodology has been set up a long time ago, and has already been used many times (for instance in [11,21,25]). Let us briefly recall it. 'At a time, a green 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 visual conditioning experiment, pieces of rosemary 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 green hollow cube or with pieces of rosemary 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 1F)'. The procedure used for conducting a test on a colony is summarized in [11]. For each test, the numbers of ants under paracetamol 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 paracetamol consumption

For examining if ants adapted themselves to some adverse effects of paracetamol, their linear and angular speeds were again assessed after 15 days of that drug consumption. The values then obtained were compared to the control values and to those obtained after one day of the drug consumption 'using the non-parametric χ^2 test' (Table 5, upper block).

Habituation to paracetamol consumption

For examining if ants became habituated to the analgesic effect of paracetamol (i.e. the beneficial effect of the drug), their linear and angular speeds on a rough substrate were assessed after 16 days of that drug consumption, as it was 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 'using the non-parametric χ^2 test' (Table 5, lower block).

Dependence on paracetamol

This physiological trait was studied after the ants had consumed paracetamol for 17 days, using the same methodology as that previously used for several substances consumed by humans (for example: [11,25]). We recall the method. 'For each colony, 15 ants were transferred into a small tray (15 cm x 7 cm x 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 the drug (the same solution as that used throughout the entire work). Each tube was plugged with cotton (Figure 1G). 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' [24].

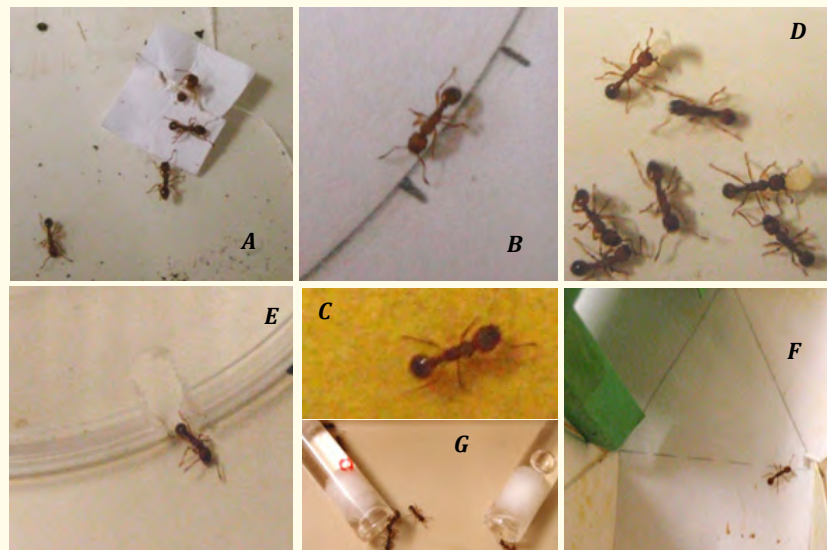


Figure 1: Some views of the experiments. A: ants under paracetamol diet reacting to a tied congener. B: an ant under paracetamol diet following a circular trail along a short distance. C: an ant under normal diet moving with difficulty on a rough substrate. D: ants under paracetamol diet taking care of the brood. E: an ant under normal diet escaping from an enclosure. F: an ant under paracetamol diet, trained to orient to a visual cue (a hollow green cube), and giving the wrong response in a Y apparatus. G: ants under paracetamol diet preferring the sugar solution containing that drug (left tube with a red mark) to the drug-free sugar solution.

Decrease of the effect of paracetamol after its consumption ended

The methodology was similar to that used when examining this trait for several previously studied substances (for instance: [11,25]). The ants were deprived of paracetamol for 12 hours, then provided with a fresh solution of that drug for again 12 hours, and then, the solution of paracetamol was replaced by a usual solution of pure sugar. Since this weaning time, the ants' linear and angular speed on a rough substrate were assessed exactly as previously (before and after consuming the drug for 5 and 17 days), doing so after several time periods. 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 from the probabilities P associated with the Z value of each time period, provided by a Kruskal-Wallis test for multiple comparisons, using Statistica® v.10 software. This analysis was made for some time periods, the values obtained being compared to the control ones and to those obtained at $t = 0$, just before weaning. The total number of periods being k , the error probabilities were calculated as explained in [24]: $P(Z) \cdot (k-1)$.

The results of the present experiment are numerically 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 drug; 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 [27] was used for fitting polynomial regression models.

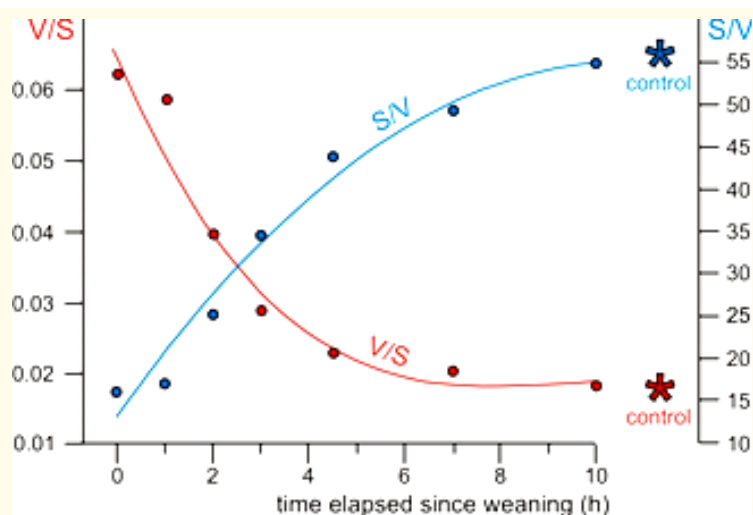


Figure 2: Decrease of the effect of paracetamol on pain perception after its consumption was stopped. The numerical values of V (linear speed) and S (angular speed) are given in table 6. Statistics are summarized in this table and detailed in the text. V/S (red circles and curve) quantified the effect of paracetamol on pain perception; S/V (blue circles and curve) quantified the ants' pain perception. These variables varied over time according to polynomial (quadratic and cubic) functions defined in the text. Briefly, the effect of the drug rapidly vanished in about 4 hours, what incites consuming it again and may lead to addiction.

Results and Discussion

Food consumption and activity

These traits were not impacted by paracetamol consumption (Table 1). The mean number of ants consuming meat food equaled 1.11 when under paracetamol diet and 0.92 while under normal diet. The slight increase of meat food consumption was not significant: $N = 6$, $T = -5, +14$, $P = 0.281$. The mean number of ants drinking sugar water equaled 4.88 for paracetamol diet and 4.11 for normal diet. The difference was not significant: $N = 6$, $T = -6, +15$, $P = 0.219$. As for the ants' general activity, the slight increase observed after ants consumed

paracetamol (the mean number of active ants equaled 9.42 instead of 8.90) was also not significant: N = 6, T = -8, +13, P = 0.344. Thus, paracetamol did not affect food (meat as well as sugar food) consumption, nor the activity of the ants. This is a positive result for a drug, at least for an analgesic.

Normal diet with paracetamol						
Days	Meat	Sugar Water	Activity	Meat	Sugar Water	Activity
Daily counts						
IA	0 1 1 2 2 2	1 1 1 2 1 2	6 7 6 5 7 7	0 1 0 1 1 1	3 3 3 1 1 0	5 7 7 7 8 8
B	3 3 2 2 2 1	6 5 7 6 6 8	14 13 15 10 15 13	0 0 1 0 1 0	4 5 5 5 5 4	5 6 6 5 4 5
IIA	0 1 0 1 0 0	5 4 5 1 2 2	9 8 9 7 8 9	1 1 1 1 2 2	1 1 1 2 2 1	5 6 5 8 8 7
B	2 2 2 0 1 0	7 8 7 6 7 8	13 11 12 8 9 9	0 1 0 1 0 0	10 11 11 4 5 7	9 9 8 12 13 15
IIA	0 1 0 1 1 1	1 1 1 2 2 3	8 7 8 6 8 7	0 1 0 2 2 2	2 2 1 1 1 1	9 9 8 5 6 7
B	1 1 0 3 3 3	5 5 6 3 5 5	7 8 7 12 14 15	2 2 2 1 2 1	5 5 4 9 10 10	9 12 14 12 13 13
IVA	1 1 1 3 2 3	1 0 0 0 1 1	7 8 8 6 7 8	0 0 1 3 3 4	2 0 1 2 2 1	9 9 10 9 9 11
B	0 0 1 0 1 0	6 6 7 7 8 9	9 8 8 9 10 11	1 0 0 1 1 1	6 6 7 3 4 3	12 13 12 7 8 9
V A	1 0 1 1 1 2	1 1 1 2 2 3	6 7 7 7 6 8	1 1 1 1 1 1	2 2 1 1 0 1	11 10 9 9 10 11
B	0 1 0 2 2 2	6 5 6 8 9 10	10 8 8 9 9 10	1 1 1 1 2 2	14 15 13 9 15 9	14 16 16 9 16 9
VIA	1 0 0 0 1 1	1 1 0 2 2 2	7 8 8 9 8 10	2 2 1 3 3 4	2 2 1 3 3 2	7 6 6 8 8 9
B	0 1 0 1 1 1	6 7 6 7 8 7	7 12 9 11 11 12	1 0 1 1 0 0	9 14 13 9 13 15	9 14 11 9 14 13
Daily means						
I	1.75	3.83	9.75	0.50	3.25	7.58
II	0.75	5.17	9.33	0.83	4.67	8.75
III	1.25	3.25	9.00	1.42	4.25	9.75
IV	1.08	3.83	8.25	1.25	3.08	9.83
V	1.08	4.50	7.75	1.17	6.83	11.67
VI	0.58	4.08	9.33	1.50	7.17	8.92
Average of daily means						
	0.92	4.11	8.90	1.11	4.88	9.42

Table 1: Impact of paracetamol on food consumption and general activity.

Assessments were made during 6 days: the ants, of colonies A and B, eating meat, drinking sugar water, and being active were counted six times per day. Daily means and average of them were then established. Paracetamol did not statistically impact the three examined traits.

Linear and angular speed

After having consumed paracetamol during one day, the ants appeared to walk in an unusual manner. They presented jerks and jolts, they made very short steps. They often briefly stopped, then moved again, turned repeatedly and sometimes appeared having difficulties in walking. The obtained numerical results confirmed this observation (Table 2, lines 1, 2). The ants' linear speed was somewhat lower than before consuming paracetamol, although the difference was not significant ($\chi^2 = 10.77$, $df = 3$, $0.01 < P < 0.02$). The angular speed (the sinuosity) was much higher than when under normal diet, the difference being significant ($\chi^2 = 25.64$, $df = 2$, $P < 0.001$). However, after several days under paracetamol diet, the ants' locomotion seemed to be less affected, a potential adaptation to the drug consumption we checked after the ants had consumed paracetamol for 15 days (see below).

Orientation ability

This ability appeared to be somewhat affected by paracetamol consumption (Figure 1A; Table 2, line 3). Indeed, even if the ants consuming this drug obviously perceived the alarm pheromone, they oriented themselves towards it less well than when being under normal diet. The difference was significant ($\chi^2 = 11.12$, $df = 3$, $0.01 < P < 0.02$). This experimental result may indicate some impact of the drug on the individuals’ movement control and/or central nervous system, a hypothesis considered in the forthcoming experiments.

Trail following behavior

This trait was affected by paracetamol consumption (Table 2, line 4). Indeed, ants under normal diet followed a circular trail meanly along 8.5 arcs of 10 ang. deg., while ants consuming paracetamol followed the trail along only 3.5 arcs of 10 ang. deg (Figure 1B). The difference of trail-following ability between ants under the two kinds of diet was significant: $\chi^2 = 23.97$, $df = 2$, $P < 0.001$. The presumption of an impact on the nervous system seemed to be corroborated.

Audacity

This behavioral trait was not affected by paracetamol consumption (Table 2, line 5). Being under normal diet, or consuming paracetamol, the ants always cautiously came onto the presented apparatus, and did not stay long time periods on it. The difference between the numbers of ants on the apparatus while under either kind of diet was not significant ($N = 5$, $T = +7, -8$, $P = 0.50$).

Tactile (pain) perception

This trait was largely impacted by the drug. This was logical since the wanted effect of paracetamol is effectively a reduction of pain perception. This effect of the drug was obvious while looking to the ants moving on a rough substrate. Under normal diet, the ants walked very slowly and sinuously on such a substrate (Figure 1C). While consuming paracetamol, they appeared to walk more easily on the rough substrate, as if they less perceived its uncomfortable character. The obtained numerical results confirmed these observations (Table 2, line 6). Under paracetamol diet, the ants’ linear speed was higher and their angular speed was lower than when under normal diet. These two differences were significant (linear speed: $\chi^2 = 28.45$, $df = 2$, $P < 0.001$; angular speed: $\chi^2 = 40.33$, $df = 1$, $P < 0.001$).

Traits	Normal diet	Diet with paracetamol
Linear speed (mm/s)	13.0 (11.5 - 14.9)	12.2 (10.8 - 13.3)
Angular speed (ang.deg./cm)	141 (120 - 154)	183 (164 - 214)
Orientation (ang. deg.)	40.9 (29.2 - 57.2)	65.2 (46.1 - 85.3)
Trail-following (n° arcs)	8.5 (5.0 - 12.0)	3.5 (2.0 - 6.0)
Audacity (n° ants)	1.05 [0 - 2]	1.05 [0 - 2]
Tactile (pain) perception:		
linear speed (mm/s)	5.4 (4.8 - 6.7)	9.1 (8.2 - 10.4)
angular speed (ang.deg./s)	304 (259 - 335)	177 (166 - 195)

Table 2: Impact of paracetamol on six physiological and/or ethological traits.

The linear speed, the angular speed, the orientation to an alarm signal, the trail-following, the audacity, and the linear and angular speed on a rough substrate of ants of two colonies were measured before and while these ants consumed paracetamol. Statistically, the drug did not change the ants’ linear speed and audacity, but increased their sinuosity of movement, decreased their orientation and trail-following ability, and reduced their tactile perception.

Brood caring behavior

This behavioral trait was very slightly and not statistically affected by paracetamol consumption (Table 3, line 1). While consuming this drug, the ants went on taking care of their brood (Figure 1D). Within the ten experimental minutes, ants under normal diet replaced in the nest the ten larvae experimentally removed from it. While consuming paracetamol, the ants also did so, but they took a little more time for accomplishing this task. However, the difference of behavior between ants under each kind of diet was not significant ($N = 5$, $T = -2.5$, $+12.5$, $P = 0.125$). Thus, paracetamol did not affect the ants' brood care, and so presumably not their social interactions, a hypothesis checked here below.

Cognition

This trait was affected by paracetamol consumption (Table 3, line 2). Under normal diet, 3 ants among 30 could cross the twists and turns path and reach the large loggia located beyond, and only 16 ants were still in the small loggia after the 12 experimental minutes. While consuming paracetamol, only 1 ant among 30 reached the large loggia, and 19 ones were still in front of the twists and turns path after the 12 experimental minutes. The difference in ability of crossing the twists and turns path between ants consuming or not consuming paracetamol was significant: small loggia: $N = 7$, $T = +28$, $P = 0.008$; large loggia: $N = 5$, $T = -15$, $P = 0.031$. Paracetamol may thus impact the central nervous system, a presumption emerged from two previous experiments, and checked in two following ones.

Working on humans and using a small dose of paracetamol, Pickering and co-authors [28] showed that this drug does not affect and even sharpens reflection and memory. This important at first sight contradiction with our results is discussed here below at the end of the section devoted to conditioning and memory.

Aggressiveness against nestmates and aliens

This trait was not at all affected by paracetamol consumption (Table 3, line 3). In presence of a nestmate, ants under normal diet as well as under paracetamol diet either did nothing, or touched the opponent with their antennae, and seldom opened their mandibles. The variable assessing the ants' 'aggressiveness' against a nestmate equaled 0.07 while under normal diet, and 0.10 while consuming paracetamol. The difference of behavior between ants under each kind of diet was not significant: $\chi^2 = 0.91$, $df = 2$, $0.50 < P < 0.70$. In presence of an alien, ants under paracetamol diet reacted as they did when under normal diet. They contacted the opponent with their antennae, very often opened their mandibles, and sometimes gripped and even tried to sting the opponent. The variable assessing the ants' aggressiveness against an alien equaled 3.16 for those under normal diet, and 2.25 for those consuming paracetamol. The difference of ants' aggressiveness between the two kinds of diet was not significant: $\chi^2 = 0.20$, $df = 2$, $P = 0.90$.

We could thus conclude that paracetamol did not affect the ants' social relations with nestmates (adults and larvae) and individuals being not nestmates.

Ability in escaping from an enclosure

This ability was very slightly impacted by paracetamol consumption (Table 3, line 4). Under normal diet, 6 ants among 12 could escape from the enclosure within the 12 experimental minutes (Figure 1E). The variable assessing this ability equaled 0.50. While consuming paracetamol, only 5 ants could escape; the variable equaled thus 0.41. The difference in escaping ability between ants under one or the other kind of diet was at the limit of significance: ants still captive as well as ants which could escape: $N = 4$, $T = +15$ as well as -15 , $P = 0.031$. In fact, the ants under paracetamol diet were no more excited than while under normal diet. They simply took some more time for finding the exit. Such slowness might reveal a weak impact of the drug on the central nervous system, a hypothesis already emitted on basis of previous observations (see above). The following experiment allowed looking again to such an impact.

Traits	Normal diet	Diet with paracetamol
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° 9 7 5 3 0 0	t: 30s 2 4 6 8 10 min n° 8 8 6 4 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 24 0 2 22 0 4 21 1 6 19 2 8 17 2 10 16 3 12 16 3	t n° in front n° beyond 30s 28 0 2 26 0 4 27 0 6 25 1 8 22 0 10 19 0 12 19 1
Aggressiveness against nestmates	levels 0 1 2 3 4 var 'a' n° 81 56 9 0 0 0.07	levels 0 1 2 3 4 var 'a' n° 75 48 12 0 0 0.10
Aggressiveness against aliens	levels 0 1 2 3 4 var 'a' n° 4 28 74 27 0 3.16	levels 0 1 2 3 4 var 'a' n° 0 36 75 25 1 2.25
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 10 9 7 7 6 6 n° out: 0 2 3 5 5 6 6 variable = 6/12 = 0.50	t: 30s 2 4 6 8 10 12 n° in : 12 10 10 8 8 7 7 n° out: 0 2 2 4 4 5 5 variable = 5/12 = 0.41

Table 3: Impact of paracetamol on four ethological or physiological traits.

Brood caring behavior, cognition, aggressiveness against nestmates and aliens, and ability in escaping from an enclosure were assessed on ants of two colonies before then when they were provided with paracetamol. On basis of observations and statistical analysis, the drug appeared to have nearly no impact on ants' relation with their brood, their nestmates and aliens, and on their ability in escaping from an enclosure, but to impact their cognition.

Visual and olfactory conditioning and memory

These physiological traits depending on the central nervous system were largely impacted by paracetamol consumption (Table 4, Figure 1F). Let us recall that these observations began after the ants had consumed paracetamol for 10 days, and lasted 14 days.

Under normal diet, ants reached a visual conditioning score of 70% after 7 hours, and a final score of 80%. Under paracetamol consumption, they reached a score of only 55% after 7 hours of training, and a low final score of 60%. The difference of conditioning ability between ants under the two kinds of diet was significant: N = 6, T = -21, P = 0.016. The ants' short term memory was thus impacted by paracetamol. After removal of the visual cue, ants under normal diet kept a conditioning score of 80% after 7 hours, and still of 70% after 72 hours. Under paracetamol diet, ants immediately lost their conditioning, presenting scores of 45% - 55% continuously over time. The difference of visual memory between ants consuming or not paracetamol was significant: N = 6, T = -21, P = 0.016. The drug impacted thus the ants' middle term visual memory.

Under normal diet, ants reached an olfactory conditioning score of 70% after 7 hours, and a final score of 90%. Under paracetamol consumption, they reached a score of only 55% after 7 hours of training, and a low final score of 60%. The difference of conditioning ability between ants under the two kinds of diet was significant: N = 6, T = -21, P = 0.016. The ants' short term olfactory memory was thus

impacted by paracetamol. After removal of the olfactory cue, ants under normal diet kept a conditioning score of 90% after 7 hours, and still of 80% after 72 hours. Under paracetamol diet, ants immediately lost their conditioning, presenting scores of 45% - 55% continuously over time. The difference of olfactory memory between ants consuming or not paracetamol was significant: $N = 6$, $T = -21$, $P = 0.016$. The drug impacted thus the ants' middle term olfactory memory.

Traits Time (hrs)	Under normal diet		under paracetamol diet		
	colony C	%	colony A	colony B	%
Visual conditioning					
7	7	70	6	5	55
24	8	80	6	5	55
31	8	80	7	5	60
48	8	80	7	6	65
55	8	80	6	6	60
72	8	80	7	5	60
Visual memory					
7	8	80	6	4	50
24	7	70	5	5	50
31	8	80	6	5	55
48	7	70	4	5	45
55	7	70	5	5	50
72	7	70	5	5	50
Olfactory conditioning					
7	7	70	6	5	55
24	8	80	5	5	50
31	8	80	6	6	60
48	9	90	6	6	60
55	9	90	6	5	55
72	9	90	6	6	60
Olfactory memory					
7	9	90	5	4	45
24	8	80	4	6	50
31	8	80	7	4	55
48	7	70	4	7	55
55	8	80	5	5	50
72	8	80	5	5	50

Table 4: Effect of paracetamol on visual and olfactory conditioning ability and memory.

Ants were trained to a visual, than an olfactory cue, and were tested over time while expected to acquire then retain some conditioning. The drug (a high dose) statistically significantly reduced these abilities, affecting thus the ants' memory (short term as well as middle term one).

The ants still remembered what they knew before consuming paracetamol: the location of their meat food, sugar supply and nest entrance, the odor of their nestmates, nest inside, nest entrance and foraging area. The ants' long term memory was thus not impacted by paracetamol.

Pickering and co-authors worked on humans and showed that paracetamol ameliorates reflection and memory [28]. How can we explain the contraction between these authors' results and our ones? First, the assessments made on humans concerned other cognitive

abilities than those acting during conditioning. They are rather similar to those prevailing in our experiments on cognition and escaping ability. In the latter experiments, we observed a slight impact of paracetamol on the ants' behavior, lower than that observed on conditioning ability. Paracetamol may thus more impact conditioning, association between a cue and a reward, than true reflection. However, in our conditioning experiments as well as in the experiments of Pickering and co-authors, memory played an important part. There exists thus, effectively, at first sight, some contradiction. The explanation is given by the work of Ishida and co-authors [29]. Working on mice, the latter authors clearly showed that low dose of paracetamol increased these mammals' memory, middle dose had no effect, and high dose impaired the memory. Here, we used a high dose of paracetamol (corresponding to 4 g/day by humans); Pickering, *et al.* [28] experimented with a low dose of paracetamol (corresponding to 1 g/day). Other authors [30] examined the effect of subcutaneous paracetamol administration on rats' spatial learning and memory, and neurotransmission. The doses used were 10 mg/kg and 50 mg/kg, what corresponded to 700 mg (= 0.7g) and 3,500 mg (= 3.5g) for a human of 70 kg, and was lower than the dose (4 g/day) we used. They observed some improvement of the tested rats' response, what corroborates the fact that low doses of paracetamol favorably impact memory. In fine, all this shows that there is no contraction between our works and those of other researches. Paracetamol very probably acts on neurotransmission [30]. If so, it is logical that low doses do not impact and even ameliorate the nervous system functioning, while high doses disturb it.

The association of the works of the three here above cited groups of researchers [28-30] and of our present work is very instructive and may help elucidating the modality of action of paracetamol. This analgesic might first act on the pain perception faculty, then on basic, instinctive reactions such as operant conditioning, and later on or at higher doses, on more complex cognitive abilities, on refectation, on memorization. As stated in the introduction section, the mechanism of action of paracetamol is not yet fully elucidated, even if some pieces of the puzzle are yet in place [31,32]. Our work on ants adds some new information on the topic.

Adaptation to paracetamol consumption

The ants only slightly adapted themselves to adverse effects of paracetamol (Table 5, upper block). After having consumed the drug for 15 days, the ants' sinuosity was somewhat smaller than, but not statistically different from that presented after 3 days of paracetamol consumption ($\chi^2 = 4.58$, $df = 2$, $P \sim 0.10$), but it was still larger than that presented under normal diet ($\chi^2 = 16.47$, $df = 3$, $P < 0.001$). Such a weak adaptation to paracetamol is not in favor of its use.

The experiments dealing with the ants' conditioning ability were conducted after the ants continuously consumed the drugs for 10 days and lasted 14 more days. A strong impact of the drug on the examined trait was noted. There was thus no adaptation to the effect of paracetamol on the learning ability and the memory. This was of course not in favor of the drug use.

Habituation to paracetamol consumption

Ants developed some slight habituation to paracetamol consumption (Table 5, lower block). After having consumed the drug for 16 days, the ants moved on a rough substrate still a little more quickly than when under normal diet ($\chi^2 = 7.45$, $df = 2$, $0.02 < P < 0.05$), but statistically more slowly than after 3 days of paracetamol consumption ($\chi^2 = 16.82$, $df = 2$, $P < 0.001$). As for the ants' sinuosity on a rough substrate after 16 days of paracetamol consumption, it was still lower than the control one ($206 < 304$ ang. deg./cm; $\chi^2 = 20.27$, $df = 2$, $P < 0.001$) although somewhat higher than that presented after 3 days of consumption (177 ang. deg./cm), from which it statistically differed ($\chi^2 = 12.22$, $df = 2$, $0.001 < P < 0.01$).

A habituation to the drug effect on pain perception is not in favor of the drug use since one may become inclined to increase over time the amount of drug consumed for obtaining a similar effect. Moreover, when some sites of the unit membrane are made inefficient (due to a particular product for instance), the cell progressively extends its membrane and set in place additional sites, in order to stay efficient in spite of the circumstances [33]. If the number of cellular sites becomes larger in the course of time, the individual's sensitivity enlarges; it may even become sensitive to elements previously causing it no pain. Consequently, a larger amount of the product (for instance an

analgesic) will be necessary for decreasing the efficiency of the cells, and thus the sensitivity and the pain perception of the individual. In other words, this physiological reaction of the concerned cells also leads to an increase of the amount of consumed product. As discussed at the end of the section devoted to conditioning and memory, harmful effects of paracetamol appear at high dose. Increasing the consumed amount of that drug is thus unfavorable.

Traits, variables	Normal diet	2 - 3 days of drug diet	15 - 16 days of drug diet
Adaptation			
Linear speed (mm:sec)	13.0 (11.5 - 14.9)	12.2 (10.8 - 13.3)	12.6 (10.9 - 13.8)
Angular speed (ang.deg./cm)	141 (120 - 154)	183 (164 - 214)	169(147 - 192)
Habituation			
Linear speed (mm/sec)	5.4 (4.8 - 6.7)	9.1 (8.2 - 10.4)	7.5 (6.2 - 7.9)
Angular speed (ang. deg./cm) on a rough substrate	304 (259 - 335)	177 (166 - 195)	206 (193 - 253)

Table 5: Adaptation and habituation to paracetamol consumption.

The ants' linear and angular speeds were again assessed after 15 days of paracetamol consumption, and a slight adaptation to the drug effect was statistically observed (upper part of the table). The ants' locomotion on a rough substrate was again assessed after 16 days of paracetamol consumption, and some habituation to this drug decrease of pain perception was so revealed (lower part of the table). This is not in favor of paracetamol use.

Dependence on paracetamol

This trait was examined after the ants consumed paracetamol for 17 days (Figure 1G). In front of a drug-free sugar solution and a sugar solution containing it, 10 ants of colony A choose the former solution and 12 ants the latter one, while 17 ants of colony B went drinking the former solution and 31 ones the latter solution. In total, 43 ants chose the solution containing paracetamol and 27 ants chose the solution free of that drug. Such numbers did not statistically differ from those expected if ants randomly drank each kind of solution ($\chi^2 = 1.42$, $df = 1$, $0.20 < P < 0.30$). However, in presence of a sugar solution with paracetamol and drug-free sugar water, ants that have never consumed paracetamol (belonging to colony C) made a completely different choice. Indeed, 15 ones preferred the drug-free solution, and only 3 choose the solution containing paracetamol. The numbers (27 vs 43) obtained for ants consuming paracetamol statistically differed from those (15 vs 3) obtained for ants having never consumed that drug ($\chi^2 = 9.77$, $df = 1$, $0.001 < P < 0.01$). On the basis of this last statistical result, it could be concluded that ants developed some dependence to (addiction on) paracetamol consumption.

The weak preference of the ants of colonies A and B for the sugar paracetamol solution may be due to the unpleasant taste of the drug (personal experience). The ants may have perceived a difference of taste between the sugar solution containing the drug and the drug-free solution, and tended to prefer the latter one. However, their dependence on the drug led them to somewhat prefer the solution containing it.

This effect – the development of some addiction - is not beneficial to persons treated with the drug; problems may occur at weaning.

Decrease of the effect of paracetamol after its consumption ended

Briefly, the effect of paracetamol on pain perception very rapidly decreased in the course of a few hours, nearly vanishing in 4 - 4 ½ hours (Table 6, Figure 2).

As soon as 3 hours after weaning, the effect of the drug was already weak. The ants' linear speed on a rough substrate was not yet similar to the control one, but near the limit of significance ($P = 0.06$). Moreover, it was then already largely different from that presented just before weaning (at $t = 0$) ($P < 0.001$). However, paracetamol had still some effect 3 hours after weaning since the ants' sinuosity on a

rough substrate was still different from the control one ($P < 0.001$), but this effect was weaker than the initial one ($P < 0.001$). Thereafter, 4 ½ hours after weaning, the effect of paracetamol was weakly perceptible. The ants' linear speed on a rough substrate was, at this time, identical to the control one ($P = 0.90$) and very different from that presented before weaning ($P < 0.001$). The ants' sinuosity then approached the control one ($P \sim 0.05$), and was very different from the initial one ($P < 0.001$). Seven hours after weaning, the ants' sinuosity on a rough substrate was similar to the control one ($P = 0.30$), and of course, very different from the initial one ($P < 0.001$). Thus, after weaning, the effect of paracetamol on the pain perception rapidly decreased, became weak after 3 hours, nearly no longer perceptible after 4 ½ hours, and null after 7 hours. In fact, the effect of the drug nearly fully vanished in 4 hours.

Assessed by V/S , the decrease over time of the analgesic effect of paracetamol was graphically best fitted by the cubic function: $V/S = 0.0654 - 0.0159t + 0.0018t^2 - 0.0000645t^3$ with $R^2 = 0.96$, although we could accept the quadratic function $V/S = 0.0635 - 0.0125t + 0.0008t^2$ as the optimum fit since the cubic term does not significantly improve the model. The variation over time of S/V , accounting for the ants' tactile (pain) perception, was best fitted by the quadratic function: $S/V = 12.9771 + 7.9718t - 0.3792t^2$ with $R^2 = 0.98$. It has been checked that such polynomial functions account better than other functions for the observed rapid changes over time of the two variables.

Such a rapid loss of the drug effect after weaning can be perceived by persons having consumed paracetamol, what leads to addiction, a relation between quick loss of the effect and addiction we have often observed in the course of our studies of the effects of substances (for instance, drugs) consumed by humans (among others [11,21,34] and unpublished data). In the case of paracetamol, since some habituation occurred (see above), consumers not only may consume again the drug each 4 - 5 hours, but also might want larger amount of it days after days, what may cause risk (end of section devoted to conditioning and memory).

Additional comment

The perception of pain by an organism is a very useful physiological trait; it informs the organism about some unusual functioning, aggression, lack or excess of some elements, etc., the organism having then the possibility to react to the situation. The 'nociceptive' pain is thus a normal alarm signal, beneficial by inducing an appropriate and sometimes essential reaction of the organism. Reducing the pain perception must be done only when necessary, and always together with researching the cause of the pain [<https://www.pure-sante.info/danger-antidouleurs/#KEwfe85LRABJYxOp.99>].

Though being somewhat out of the scope of the present work, it should be noted that 2g of *curcuma* is more efficient in reducing pain perception than 2g of paracetamol, and has no adverse effect [35]. We could not but think that, at least partly, the marketing of paracetamol must persist because, due to habituation and dependence, it is very lucrative.

Conclusion

Working on ants as models, and using a high dose of paracetamol (corresponding for humans to the more used dose: 4 g / day), we observed obvious impact of that most used analgesic on these insects' locomotion, orientation ability, trail-following, tactile (pain) perception, cognition, conditioning ability and memory, and thus on their nervous system. Paracetamol did not affect the other examined traits (such as food consumption, audacity, brood caring, relation with nestmates and aliens, escaping behavior), though very slightly inducing some slowness. Ants developed only slight adaptation, but clear habituation and addiction to paracetamol. Other researchers' works prove that the toxicity of this analgesic increases with the consumed dose. Accordingly, we recommend a parsimonious use of it, the more so since pain perception is a vital function for an organism, and because natural safe solutions exist for momentarily reducing pain perception. Let us add that, nowadays, paracetamol and its degraded products may contaminate some natural water, impacting then the biology of aquatic vertebrates and invertebrates.

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Conflict of Interest and Ethical Responsibilities

We affirm having no conflict of interest at all as for the use of paracetamol. We are independent researchers in ethology and receive no money for conducting our research. We also affirm having maintained the ants in the best conditions possible (food, temperature, humidity, lighting, electromagnetic field).

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