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Research Article

Ethological and Physiological Effects of Ibuprofen, the Recently Most Used Analgesic; a Study on Ants as Models

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Abstract

The previously most used analgesic, paracetamol, was found to have adverse effects, and nowadays, ibuprofen is often preferentially used. Here we examined the potential adverse effects of ibuprofen exactly as we examined those of paracetamol. We found that ibuprofen decreased the ants' food consumption, speed of locomotion (and increased their sinuosity), orientation and trail following ability, audacity and escaping behavior, as well as their tactile perception (what resulted from the analgesic effect of the drug). Ibuprofen also affected the ants' brood caring and social relationship, inducing some aggressiveness between nestmates. It did not impact the ants' cognition and conditioning ability, nor their visual and olfactory memory. Ants did not adapt themselves to the impact of ibuprofen on their locomotion, became habituated to the analgesic effect of the drug, and developed no dependence on it. The effect of ibuprofen slowly decreased after weaning and vanished in about 10 hours. Ibuprofen presented thus several adverse effects which differed from those induced by paracetamol. Paracetamol impacted cognition, conditioning and memory while ibuprofen affected social relationship. For safely obtaining an efficient analgesic effect, these two analgesics could be used successively, each one for three days, at a rather low dose, with the use of acetylsalicylic acid during the following seventh day. For moderate pain, curcuma can be used as a substitute. We should also remember that pain perception is a vital physiological function allowing the individuals taking care of themselves.

Keywords: *Aggressiveness; Audacity; Locomotion; Habituation; Straightforwardness*

Abbreviations

ang.deg.: Angular Degrees; ang.deg./cm: Angular Degrees Per cm; mm/s: Millimeter Per Second; χ^2: Chi Square; vs: Versus; n°: Number; cm: Centimeter; mm: Millimeter; ml: Milliliter; µl: Microliter; mg: Milligram; s: Second; min: Minute; h: Hours; t: Time; %: Percentage

Introduction

Analgesics are among the most consumed drugs by humans. These last years, the most used of them was paracetamol, available in any drugstores, for instance under the labels Dafalgan® and Perdolan®. Its use has recently been debated [1], and using ants as models, we effectively found that paracetamol has several adverse effects [2]. It increased the ants' sinuosity of movement, decreased their orientation and trail following ability and affected their cognition, conditioning ability and memory, though not impacting their activity, food consumption and social relationship. Paracetamol impacted thus the ants' nervous system. Ants developed habituation to paracetamol as well as some dependence, two drawbacks not wished for a drug. Such dependence is caused by the quick loss (in 4 hours) of the effect of paracetamol just after weaning. According to the adverse effects of paracetamol, it was not surprising to learn that a novel analgesic, the alpha-methyl-[4-(2-methylpropyl) phenyl] propanoic acid (Figure 1) or ibuprofen, has been produced, available in any drugstore, for instance under the labels Brufen®, Nurofen® and Advil®. Ibuprofen inhibits, among others, the enzyme cyclo-oxygenase (COX) which allows producing prostaglandins, decreasing so inflammatory processes and, consequently, pain perception. Is this new analgesic entirely safe? Little information is given on this topic on internet or in the directions for use joined to the drugs. However, digestive problems, headaches and some allergic reactions (at the skin and the respiratory system levels) are mentioned. Several works have been done on ibuprofen by practitioners and researchers. Rainsford [3] explained that this analgesic is the product of a long research program, that it is a 'super aspirin', and that it has no adverse effect. On the basis of results during oral surgery, Dionne [4] also showed that ibuprofen is an efficient analgesic producing exactly the effects wanted by the patients, i.e. decrease of local pain, stress and inflammation. However, Dionne and Cooper [5] observed adverse effects, essentially on the intestine, but concluded that, finally, this analgesic is no more toxic than other similarly efficient ones, and that the solution could be limiting the consumption of ibuprofen at 1,200 mg per day. Schou., *et al*. [6] carefully examined the impact of different ingested doses of ibuprofen: they showed that the analgesic effect efficiency increased with the dose, that some adverse effects occurred, but that these effects did not statistically differ with those presented by control patients treated with a placebo. Also, Henry., *et al*. [7] stated that the digestive problems observed after ingestion of ibuprofen are very probably due to the simultaneous consumption of aspirin®. A critical bibliographical review on all the known harmful effects of ibuprofen has been written by Rainford, with the participation of many other practitioners and researchers [8]. These authors proved that ibuprofen has adverse effects on the intestine (and on the digestive tract in general), the kidneys, and the respiratory system, but is effectively a very efficient analgesic. They stated that ibuprofen should be used in case of pain, inflammation, and so on, but should not be consumed after a time period of 72 hours. A comparison between the effects of paracetamol, ibuprofen and aspirin has been made in 2002, with the conclusion that ibuprofen is as well tolerated as paracetamol and better tolerated than aspirin [9]. More information on this last point is given in the 'Conclusion' section.

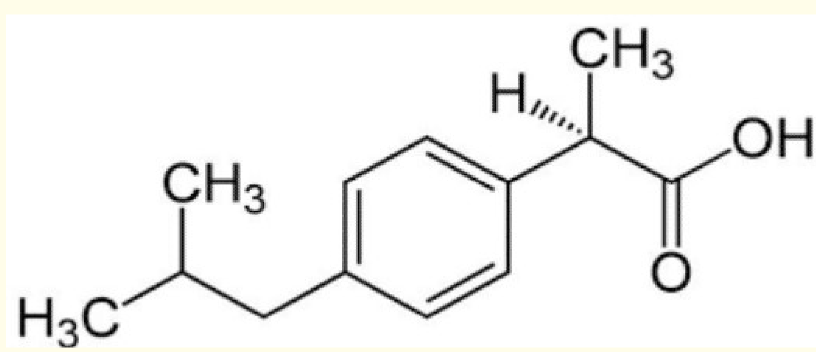

Figure 1: *Chemical structure of the analgesic ibuprofen. Its chemical name is alpha-methyl-[4-(2-methylpropyl) phenyl] propanoic acid. It inhibits among others, an enzyme allowing the synthesis of prostaglandins, decreasing so inflammatory processes and consequently pain perception.*

In fine, all these studies never examined the impact of ibuprofen on the individuals' locomotion, movement coordination, social relationship, cognitive abilities, memory, as well as potential adaptation to, habituation to and dependence on the drug. They also never defined if the effects of ibuprofen are similar to or differ from those of paracetamol or other analgesics. For filing these gaps, we examined the physiological and ethological effects of ibuprofen exactly as we examined those of paracetamol using ants as models, with the same experimental protocols and assessments. Here under, we detail why we used ants as biological models, define the species we used, and enumerate the examined traits. We then briefly relate our methods, report our results and discuss them. Finally, we compare the effects of ibuprofen with those of paracetamol on ants and conclude.

Why ants can be used as models

Most physiological functions are similar for animals and humans [10,11]. This is why these processes are generally examined on animals judiciously chosen as models (fruit flies, cockroaches, bees, mice, monkeys for instance) [12]. Of course, insects are employed, particularly because of their rapid development and easy maintenance in a laboratory [13], and among them, hymenoptera [14]. Ants are morphologically evolved hymenoptera with a particular shutting of their mouth parts [15]. Their colonies present social regulation, labor division, and exchange of information thanks to tactile and, above all, to chemical signals (pheromones) produced by several glands [16-18]. They build sophisticated nests, take care of their queens, larvae and nymphs and chemically mark the different parts of their territory [17]. They navigate, recruit congeners, clean the inside of their nest and create cemeteries [17,18]. These traits are in favor of using ants for examining the effects of substances or events [19].

Which species we used

We have largely worked on ants of the genus *Myrmica*, making research on their ecology, eyes, angle of vision, vision, recruitment, navigation, learning [20], as well as on the ontogenesis of some of their abilities [21]. The study of the effects of electromagnetic fields on ants' conditioning and responses to their pheromones proved that these insects can be used as models [22,23]. This was confirmed in the course of numerous studies on the impact of products used by humans [24-28], which revealed the effects observed in humans, gave precision on them, and pointed out other impacts from which humans may suffer. The present work was performed, once more, on the ant *M. sabuleti* Meinert 1861.

Which traits we examined

We examined the impact of ibuprofen on 18 biological traits, in a first time on ants having never consumed that drug, then on these ants consuming ibuprofen. After that, we looked to the effects of the drug on 4 other traits only on ants consuming ibuprofen or having consumed it. The 18 traits were: the ants' meat and sugar consumption, activity, speed of locomotion, sinuosity, orientation ability, trail following, audacity, pain perception, caring of larvae, cognition, aggressiveness towards nestmates and aliens, escaping ability, visual and olfactory conditioning, visual and olfactory memory. The four last traits were: the ants' adaptation to ibuprofen, habituation to it, dependence on it, and the loss of the effects after weaning.

Adaptation during consumption of a product occurs if its harmful impact decreases over time. Habituation exists if the sought-after effect of a product decreases in the course of its consumption. Dependence appears when an individual having consumed a product prefers going on consuming it than avoiding it.

Material and Methods

The material and methods here used are identical to those used in previous works. Consequently, for avoiding plagiarism and too long text, they are here below only somewhat briefly related, and readers are advised to look for details in previous works, among others [25,26,28].

Ants' maintenance

The experiments were conducted on two colonies of *M. sabuleti* collected in the Aise Valley (Belgium) in June 2016, as well as on another one found on the same site, which allowed having aliens and some control values. These colonies were maintained in glass tubes half filled with water. The nest tubes of each colony were placed in a tray (34 cm x 23 cm x 4 cm) as done in, among others [25,26]. The ants received *ad libidum* sugar water in cotton plugged tubes and, three times per week, pieces of *Tenebrio molitor* larvae (Linnaeus, 1758). Temperature, humidity, lighting and electromagnetism were in agreement with the species' needs [26,28]. The word 'nestmates' is often used for ants of the same colony.

Ibuprofen given to the ants

Three tablets of ibuprofen 600 mg were furnished by the drugstore Wera (Bruxelles). Humans are advised to consume between 1,200 mg and 3,000 mg per day. We opted for a moderate dose of 2,400 per day. Humans drink approximately one liter of water each day. Humans consuming a moderate dose of ibuprofen ingest thus 2,400g of the drug together with one liter of water. Insects, and consequently ants, drink proportionally 10 less water than mammals. For being under an ibuprofen diet similar to that of humans moderately treated with that drug, the ants should be provided with a solution of 2,400g of ibuprofen in 100 ml of water, or 1,200g of the drug into 50 ml, that is two tablets of 600 mg into 50 ml. Two tablets of 600 mg were dissolved into 50 ml of sugar water. Then, 5 ml of the obtained solution were poured into the kind of tubes used for furnishing sugar water to the ants. The tap water used had a pH of 7.75 [29]. The stock solution of sugar water containing ibuprofen was kept at -25°C, and, when defrosted, was delivered to the ants in their usual feeder tubes (which had a capacity of 5 ml). Each day, we checked if the ants drunk the sugared solution of ibuprofen, and effectively, they did. We refreshed when necessary the cotton plug shutting the tubes, and renewed the content of the tubes each week.

Sugar water and meat consumption, general activity

The ants drinking the sugar water, eating the *T. molitor* larvae, and being active anywhere in their habitat were counted six times per day during six days, at the same times o'clock each time (Table 1, Daily counts), as done in previous works [28]. We established the mean of these daily counts (Table 1, Daily means), and compared the six daily means corresponding to ants consuming ibuprofen to those corresponding to ants under normal diet using the non-parametric test of Wilcoxon [30]. We also calculated the mean of the daily means (Table 1, last line).

Speeds of locomotion and orientation ability

As previously [25,26,28], the experiments were made on ants freely moving in their tray. Their linear and angular speeds were assessed without stimulation; their orientation was assessed by stimulating them with a worker of their own colony tied to a piece of white paper (Figure 2A). Such an attached ant emits the attractive mandibular glands pheromone. As in [25,26,28], for each variable, the trajectory of 40 workers was recorded and the ants' trajectories analyzed thanks to appropriate software [31]. The linear speed (in mm/s) of a trajectory is its length divided by the time spent to travel it. The angular speed (in ang. deg./cm) of a trajectory is the sum of the angles made by its successive adjacent segments, divided by its length. The orientation of a trajectory (in ang. deg.) towards a location is the sum of the successive angles between its direction and that towards the location, divided by the number of measured angles. If the obtained value is lower than 90°, the animal tends to orient itself towards the point; if it is larger than 90°, the animal tends to avoid the point. The median and quartiles of each distribution of 40 values were calculated (Table 2, lines 1, 2, 3). The distributions corresponding to ants under ibuprofen diet were compared to those corresponding to ants under normal diet, using the non-parametric χ^2 test [30].

Trail following behavior

This trait was quantified as previously [25,26,28]. The workers' poison gland produce the species' trail pheromone. Ten poison glands were set into 500 µl of hexane and this solution was kept during 15 minutes at -25°C. Then, 50 µl of this solution was deposited on a circumference (R = 5 cm, divided into 10 angular degrees arcs) drawn on white paper. This circular trail was presented to the ants, and their response was quantified by the number of arcs of 10 angular degrees 20 ants of each colony walked along the trail (Figure 2B). The distribution of the numbers obtained for the two colonies (40 numbers) was characterized by its median and quartiles (Table 2, line 4). The distribution corresponding to ants under ibuprofen diet was compared to that corresponding to ants under normal diet using the non-parametric χ^2 test.

Audacity

The method is identical to that used in previous works [25,26,28]. A tower standing on a platform, both being made of strong white paper (Steinbach®, height = 4 cm; diameter = 1.5 cm), was presented to the ants, and those moving on the apparatus were counted 12 times in the course of 12 minutes (Figure 2C). We established the mean and the extremes of the recorded values (Table 2). The values corresponding to the two colonies as well as those obtained in the course of two successive minutes were added (as in [32]), and the results for ants under ibuprofen and normal diets were compared using the Wilcoxon test.

Tactile perception

We quantified the displacement of ants on a rough substrate after they had consumed ibuprofen for 5 days. Ants perceiving the rough character of a substrate walk slowly and sinuously on it, while ants weakly perceiving such a character walk more quickly and less sinuously. As in previous studies [25,26,28], a folded piece (3 cm x 2 + 7 + 2 = 11 cm) of emery paper n° 280 paper was attached to the borders and the bottom of a tray (15 cm x 7 cm x 4.5 cm), the tray being so divided into a first zone 3 cm long, a second zone 3 cm long containing the emery paper (Figure 2D), and a third zone 9 cm long. Such an apparatus was built for each colony. Twelve ants of each colony were set in their apparatus, in the first zone. When leaving that zone, the ants moved on the emery paper. At that time, we assessed the linear and angular speeds of 12 ants of each colony (n = 24; Table 2, line 6). The values corresponding to ants consuming ibuprofen were compared to those corresponding to ants under normal diet using the non-parametric χ^2 test.

Brood caring

Several larvae of each colony were taken out of their nest and set in front of it. For each colony, five of these larvae were followed, as well as the ants' behavior in front of them (Figure 2E). The larvae among these five which still remained out of the nest after 5 seconds, 2, 4, 6, 8, and 10 minutes were counted, and the numbers obtained for each colony were pulled (Table 3, line 1). The numbers corresponding to ants under ibuprofen diet were compared to those corresponding to ants under normal diet using the non-parametric Wilcoxon test [30].

Aggressive behavior against nestmates and aliens

Five dyadic encounters with a nestmate and with an alien ant were realized for each colony, as in previous studies [25,26,28]. Each encountering was performed in a cylindrical cup (diameter = 2 cm, height = 1.6 cm), the borders of which having been covered with talc. Each time, an ant of colony A or B was observed for 5 min and its behavior was assessed by the number of times it did nothing (level 0 of aggressiveness), contacted the other ant with its antennae (level 1), opened its mandibles (level 2), gripped its opponent (level 3), tried to sting or stung its opponent (level 4) (Figure 2F). The numbers obtained for the two colonies were pulled (Table 3, lines 2, 3), and the results corresponding to ants under ibuprofen diet were compared to those corresponding to ants under normal diet using the non-parametric χ^2 test. As in previous works [32], the ants' aggressiveness was also assessed by the variable 'a', which equaled the number of aggressiveness levels 2 + 3 + 4 divided by the number of levels 0 + 1.

Cognition

This protocol was used for the first time when examining the effects of nicotine [33]. Two pieces of white strong paper (Steinbach®, 12 cm x 4.5 cm) were folded and inserted in a tray (15 cm x 7 cm x 4.5 cm) in order to create a path with twists and turns between a first loggia and a second larger one into which a piece of wet cotton had been set. An apparatus was built for each colony (Figure 2G). Fifteen ants of each colony were set all together in the first loggia of their apparatus, then, those present in this loggia and in the larger one were counted after 30 s, 2, 4, 6, 8, 10 and 12 min. The numbers obtained for the two colonies were pulled (Table 3, line 4). The results for ants consuming ibuprofen were compared to those for ants under normal diet using the non-parametric Wilcoxon test.

Escaping from an enclosure

As in previous studies [25,26,28], for each colony, six ants were placed under a reversed polyacetate glass (h = 8 cm, bottom diameter = 7 cm, ceiling diameter = 5 cm) deposited in their tray. The rim of the glass bottom had a small notch (3 mm height, 2 mm broad) in order to give to the ants the opportunity of escaping (Figure 2H). For assessing the ants' escaping ability, those still enclosed and those escaped after 30 s, 2, 4, 6, 8, 10 and 12 min were recorded. The results obtained for the two colonies were pulled (Table 3, line 5), and the sums corresponding to ants consuming ibuprofen were compared to those corresponding to ants under normal diet using the non-parametric Wilcoxon test. As previously [25,26,28], we also evaluated the ants' ability in escaping by the variable "n° of ants escaped after 12 min/12".

Visual and olfactory conditioning and memory

Using a protocol many times employed (among others in [25,26,28]), we examined the impact of ibuprofen on conditioning and memorizing capabilities, after the ants had consumed ibuprofen for 6 days, and taking as control values those obtained on a colony during a previous study [34]. A yellow hollow cube was deposited above the sugar tube entrance for conditioning them to that visual cue. Another experiment consisted in depositing pieces of rosemary around the sugar water tube entrance to condition the ants to that olfactory cue. Tests were performed in the course of time, while ants should acquire conditioning and after removal of the cue, while they should lose it. The ants were individually tested in a Y-apparatus made of strong white paper, deposited in a tray (30 cm x 15 cm x 4 cm), a yellow hollow cube or pieces of rosemary being placed in one or the other branch of the apparatus (Figure 2IJ), the choice being random. Moving into the branch provided with a cue was considered as giving a correct response. To perform a test, 10 ants of each colony were experimented, the total number of ants equaling 20 for ants under ibuprofen diet and 10 for ants under normal diet. The proportion of correct responses was calculated for each test (Table 4). Those obtained for ants under one and the other diets were compared using the non-parametric Wilcoxon test.

Adaptation to ibuprofen consumption

After the ants consumed ibuprofen for 8 days, their sinuosity was again quantified (Table 5, line 1) as it had been before they consumed that drug and after they had consumed it for one day, for examining if ants became adapted to the effect of that compound on their locomotion. The distribution of values obtained after 8 days of ibuprofen consumption was compared to the control distribution and to that obtained after the ants had consumed ibuprofen for one day using the non-parametric χ^2 test.

Habituation to ibuprofen consumption

After the ants consumed ibuprofen during 9 days, their locomotion on a rough substrate (so, their tactile and pain perception impacted by that analgesic) was again quantified (Table 5, line 2) in the manner it had been before the ants consumed ibuprofen and after they had consumed it for 4 days, in order to know if ants habituated themselves to the analgesic effect of ibuprofen (Figure 2K). The numerical results obtained after 9 days of consumption were compared to the control results and to those obtained after 4 days of consumption using the non-parametric Wilcoxon test.

Dependence on ibuprofen consumption

After the ants had consumed ibuprofen for 10 days, an experiment was performed for examining if they developed dependence on that product, the protocol being similar to that used in previous studies [25,26,28,32]. For each of the two colonies, 15 ants were placed in a tray (15 cm × 7 cm × 5 cm) in which two tubes (h = 2.5 cm, diam. = 0.5 cm) had been set, one containing sugar water, the other containing a sugar solution of ibuprofen (the same as that used throughout this work) (Figure 2L). In one of the trays, the tube containing ibuprofen was set on the right, and in the other tray, it was set on the left. The ants drinking each liquid were counted 15 times in the course of 15 minutes, and the results added. The two obtained sums were compared to those expected if ants randomly went drinking each provided liquid, using the non-parametric goodness-of-fit χ^2 test [30].

Loss of the effects of ibuprofen after its consumption was stopped

This was examined using a previously set up protocol [34]. A fresh solution of ibuprofen was provided to the ants 12 hours before the start of weaning, and a first assessment was made after these 12 hours, i.e. at t = 0. Weaning began when the solution of ibuprofen was removed from the ants' tray and when an aqueous solution of sugar, free of the drug, was provided. Since then, the ants' sinuosity was quantified as it had been before the ants consumed the drug (= the control), after they consumed it during one day and after they had consumed it during 8 days, except that 20 and not 40 ant's trajectories were analyzed in order to make the assessments over the experimental time. We opted for this ants' trait because the ants never adapted themselves to the impact of ibuprofen on their sinuosity, while they became somewhat habituated to the analgesic effect. The assessments of the ants' sinuosity since weaning were done at intervals of 2 hours. The distributions of the values were compared to that obtained at t = 0 and to the control using the non-parametric χ^2 test, as well as, using Statistica V.10 software, the non-parametric two-tailed Kruskal-Wallis ANOVA (K-W test) for multiple comparisons, taking account of the number of groups of values to be compared [30]. Table 6 gives the numerical results, figure 3 presents them graphically. The end of the experiment occurred when the ants' sinuosity became similar to the control one.

Results and Discussion

Food consumption, general activity

Ants consuming ibuprofen eat less meat and drank less sugar water than ants living under normal diet (Table 1). This was statistically significant (meat as well as sugar water consumption: N = 6, T = -21, P = 0.016). These ants were also less active than when under normal diet (Table 1) but this result was not significant (N = 6, T = -17, P = 0.109). Ibuprofen reduced thus valuably the ants' food consumption, and might slightly impact their activity.

Days	Colonies	Under normal diet			Under ibuprofen diet		
		meat	sugar water	activity	meat	sugar water	activity
		Daily counts					
I	A	1 1 1 1 1 0	6 7 6 6 6 7	9 10 9 8 9 9	0 0 1 1 0 0	1 1 0 1 1 2	7 7 8 13 14 13
	B	2 2 1 1 1 2	4 4 3 4 4 5	10 9 10 10 12 13	1 0 0 0 0 1	0 0 1 1 0 0	9 9 10 10 11 10
II	A	1 1 1 1 1 1	3 4 5 4 4 3	9 9 10 10 11 10	1 1 0 0 1 1	6 6 7 6 6 5	12 10 11 10 11 12
	B	1 2 2 3 3 2	6 6 7 8 7 7	10 9 9 11 10 11	0 0 1 1 0 0	4 4 5 3 3 4	6 6 7 5 5 6
III	A	1 1 0 0 0 1	4 5 4 4 4 3	8 9 9 10 11 11	1 1 0 0 0 1	3 3 4 3 3 4	10 11 10 10 10 11
	B	0 0 1 1 1 1	4 3 4 3 4 4	8 8 9 9 10 10	1 1 0 1 0 0	5 5 3 4 4 3	7 7 8 8 8 7
IV	A	0 0 1 0 0 1	5 6 6 6 6 5	6 6 7 8 8 9	1 1 0 1 1 1	2 2 2 2 3 2	9 10 9 14 15 14
	B	3 3 2 2 2 1	3 3 2 2 2 4	8 8 7 8 8 9	0 1 1 0 0 1	1 1 1 1 2 2	15 14 14 12 13 15
V	A	2 2 1 1 1 0	5 5 4 6 6 5	9 10 9 14 13 14	0 0 1 1 1 0	1 2 2 2 2 2	9 10 10 12 11 12
	B	1 0 1 1 1 1	3 3 2 4 4 5	10 10 9 11 11 10	1 0 1 1 0 0	1 2 2 1 1 1	8 9 9 10 9 9
VI	A	2 2 1 2 1 2	5 5 6 5 6 6	7 7 6 14 14 13	0 1 0 0 0 1	1 1 0 0 1 1	9 9 10 10 10 9
	B	0 0 1 1 0 0	5 6 6 5 5 7	12 11 9 15 14 15	1 1 0 1 0 0	0 0 1 1 0 0	10 10 9 12 9 9
		Daily means					
I	A + B	1.17	5.17	9.83	0.33	0.67	10.08
II	A + B	1.58	5.33	9.92	0.50	4.92	8.42
III	A + B	0.58	3.83	9.33	0.50	3.67	8.92
IV	A + B	1.25	4.17	7.67	0.67	1.75	8.17
V	A + B	1.00	4.33	10.83	0.50	1.58	9.83
VI	A + B	1.00	5.58	11.42	0.42	0.50	9.50
		Average of daily means					
		1.09	4.73	9.93	0.48	2.18	9.15

Table 1: *Effects of ibuprofen on ants' food consumption and general activity.*
The ants of colonies A and B eating meat, drinking sugar water and being active were counted six times, during six days (Daily counts); the mean of these 12 counts was established each day (Daily means), and the average of the six daily means was finally calculated for each ants' trait and diet. Ibuprofen decreased significantly the ants' food consumption, and slightly their activity.

Locomotion

Ants consuming ibuprofen walked at a lower linear speed and a higher angular speed than ants living under normal diet (Table 2, lines 1 and 2). This change in locomotion was statistically significant (linear speed: $\chi^2 = 36.57$, df = 2, P < 0.001; angular speed: $\chi^2 = 49.35$, df = 2, P < 0.001). A following experiment examined if this adverse effect persisted or decreased in the course of ibuprofen consumption (see below, section 'Adaptation to ibuprofen adverse effects').

Orientation towards an alarm signal

Under ibuprofen diet, the ants oriented themselves less well towards an alarm signal than while under normal diet (Table 2, line 3; Figure 2A). Such a decrease of orientation capability (Figure 2A) was statistically significant ($\chi^2 = 19.69$, df = 2, P < 0.001). This may be due to some decrease of the ants' perception (through their antennae) caused by the analgesic effect of ibuprofen.

Trail following behavior

Ants consuming ibuprofen followed a circular trail along meanly 4.5 angular arcs of 10° while they followed such a trail along meanly 9.5 angular arcs of 10° when not consuming this drug (Table 2, line 4). The difference of trail following behavior between ants under the two kinds of diet was obvious while experimenting (Figure 2B) and statistically significant ($\chi^2 = 40.76$, df = 3, P < 0.001). As for the previous experiment, this may be due to a decrease of the ants' olfactory perception caused by the analgesic effect of ibuprofen.

Audacity

Ants consuming ibuprofen were less inclined to move onto an unknown apparatus (Figure 2C) than ants living under normal diet. They stopped in front of this apparatus, or walked on it but went soon away from it (Table 2, line 5). The lower audacity of ants consuming ibuprofen compared with that of ants under normal diet was significant: N = 5, T = -15, P = 0.031. All happened as if ants consuming ibuprofen presented a lower straightforwardness.

Tactile perception

This trait was largely impacted by ibuprofen (Table 2, the two last lines) what was expected since this drug is used as an analgesic. Under normal diet, the ants moved slowly, cautiously and sinuously on a rough substrate, touching it, in front of them, with their antennae. While consuming ibuprofen, the ants moved far more frankly (Figure 2D), more quickly and less sinuously. This was statistically significant (linear speed: $\chi^2 = 34.28$, df = 1, P < 0.001; angular speed: $\chi^2 = 32.52$, df = 2, P < 0.001). It was later on examined if this analgesic effect of ibuprofen persisted over its consumption, or if ants progressively became habituated to it (see below, section 'Habituation to the analgesic effect of Ibuprofen').

Traits	Sugar water diet	Sugar water+ ibuprofen diet
Linear speed (mm/s)	12.5 (11.1 - 15.0)	9.2 (8.2 - 10.0)
Angular speed (ang.deg./cm)	119 (101 - 139)	178 (156 - 193)
Orientation (ang. deg.)	38.4 (33.6 - 50.7)	60.5 (47.8 - 85.9)
Trail following (n° arcs)	9.5 (8.0 - 16.3)	4.5 (3.8 - 6.0)
Audacity (n° ants)	1.40 [0 - 2]	0.50 [0 - 1]
Tactile (pain) perception:		
Linear speed (mm/s)	5.3 (4.7 - 5.8)	8.9 (8.2 - 9.5)
Angular speed (ang.deg./cm)	266 (235 - 307)	171 (154 - 191)

Table 2: *Effects of ibuprofen on six physiological and ethological traits.*
Ibuprofen decreased the ants' speed of locomotion and increased their sinuosity. It decreased their orientation and trail following ability, their audacity, and (of course) their tactile (pain) perception.

Brood caring behavior

This behavior was somewhat affected by ibuprofen (Table 3, line 1; Figure 2E). While living under normal diet, the ants quickly transported into the nest the larvae experimentally placed out of it. At the end of the experiment, the ten larvae had been transported inside of the nest. Under ibuprofen diet, the ants less well performed this social task. They delayed in finding the larvae removed from the nest, in holding them, in moving in the correct direction, and thus in replacing the larvae inside the nest. In the course of the ten experimental minutes, only 6 larvae among the 10 observed have been duly replaced. This result had a low significant value (N = 4, T = 10, P = 0.063) due to the smallness of the sample. The putative impact of ibuprofen of the ants' social relationship was again examined in the following experiment.

Aggressiveness against nest mates

Under normal diet, the ants were never aggressive against nestmates. While consuming ibuprofen, they appeared to be slightly but obviously aggressive towards their nestmates (Table 3, line 2; Figure 2F). This was statistically significant ($\chi^2 = 31.08$, df = 2, $P < 0.001$) and in agreement with the here above results on ants' brood caring. Ibuprofen impacted thus the ants' social relationship.

Aggressiveness against aliens

The ants living under normal diet were aggressive against aliens, opening their mandibles for a time, gripping the alien ant and finally, if they could (if the alien had not already prevented it doing so) stung it or tried to do so. Ants consuming ibuprofen were more aggressive again aliens. They opened their mandibles during a short time period, soon gripped the opponent and tried to sting it without delay (Table 3, line 3). Such a difference between ants under ibuprofen and normal diet was statistically significant ($\chi^2 = 8.66$, df = 3, $0.02 < P < 0.05$). *Myrmica* ants produce in their mandibular glands an alarm pheromone which also reduces the aggressiveness of other ants of the same species [35]. This pheromone is emitted by ants largely opening their mandibles. It may thus be presumed that ants consuming ibuprofen less well perceived that alarm pheromone (a presumption in agreement with the results on the ants' orientation towards an alarm signal, see above), and were consequently more aggressive than ants not consuming this drug.

Cognition

This trait was not statistically affected by ibuprofen consumption (Table 3, line 4; Figure 2G). During the 12 experimental minutes, the tested ants living under normal diet or under ibuprofen diet tried to leave the small area lying in front of the twists and turns path, and to navigate that difficult path. Ants consuming ibuprofen did so with a slightly longer delay than ants not consuming that drug, what was in agreement with the previous result on the ants' audacity (see above). At the end of the experiment, only a few more ants consuming ibuprofen were still in the small area in front of the difficult way (18 vs 14), the difference between ants under ibuprofen and normal diets being not significant (N = 6, T = 17.5, P = 0.133). Also, a little less ants had reached the large area beyond the difficult way (5 vs 6), the difference between ants under the two kinds of diet being again not significant (N = 6, T = 10.5, P = 0.50). The fact that ibuprofen seemed to not impact the ants' cognition was again examined thanks to the two following experiments (see below).

Escaping ability

Ants under normal diet as well as under ibuprofen diet tried to escape from the enclosure into which they had been experimentally placed, but they did so somewhat less efficiently while consuming ibuprofen (Table 3, line 5; Figure 2H). In fact, while consuming ibuprofen, the ants hesitated before moving through the exit of the enclosure, what was in agreement with their lower tendency in moving onto an unknown apparatus (see above, the section 'Audacity'). Finally, somewhat more ants consuming ibuprofen were still in the enclosure after the 12 experimental minutes (but this difference was not significant: N = 6, T = 18.5, P = 0.063), and somewhat less ants were out of the enclosure after the same time period (but this difference was again not significant: N = 6, T = 13.5, P = 0.313). Ibuprofen impacted thus the ants' straightforwardness, but not their state of stress (stressed ants fail in escaping [19]) and cognition, the latter deduction being checked once more through the following experiment on the ants' conditioning ability (see below).

Traits	normal diet	diet with ibuprofen
Brood caring: numbers of larvae not re-entered in the nest over 10 minutes	time: 30" 2 4 6 8 10 n° : 10 8 6 4 2 0	time: 30" 2 4 6 8 10 n° : 10 8 8 6 6 4
Aggressiveness against nestmates	levels 0 1 2 3 4var 'a' 0.09 n° 64 44 10 0 0	levels 0 1 2 3 4var 'a' 0.55 n° 32 47 44 0 0
Aggressiveness against aliens	levels 0 1 2 3 4 var 'a' 3.26 n° 6 31 45 47 29	levels 0 1 2 3 4 var 'a' 6.29 n° 4 20 43 56 52
Cognition: ants in front of and beyond twists and turns in the course of 12 min	t n° in front n° beyond 30s 29 0 2 28 0 4 24 0 6 21 2 8 18 4 10 15 5 12 14 5	t n° in front n° beyond 30s 30 0 2 25 1 4 24 1 6 22 1 8 22 2 10 18 3 12 18 6
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 8 7 5 3 2 n° out: 0 2 4 5 7 9 10 variable = 10/12 = 0.83	t: 30s 2 4 6 8 10 12 n° in: 12 11 10 9 8 7 7 n° out: 0 1 2 3 4 5 5 variable = 5/12 = 0.42

Table 3: *Effects of ibuprofen on five ethological and physiological traits.*
Ants' brood caring and aggressiveness against nestmates and aliens were impacted by ibuprofen. This drug affected thus the ants' social relationship. Ants' cognition was not impacted by ibuprofen. Due to their lower audacity, ants consuming the analgesic were less able to escape from an enclosure.

Visual and olfactory conditioning and memory

These traits were not or only very slightly impacted by ibuprofen (Table 4, Figure 2IJ).

Traits Time (hrs)	Under normal diet		Under ibuprofen diet		
	Colony C	%	Colony A	Colony B	%
Visual conditioning					
7	7	70	6	5	55
24	8	80	5	7	60
31	8	80	7	6	65
48	8	80	7	9	80
55	8	80	8	7	75
72	8	80	9	7	80
Visual memory					
7	8	80	9	7	80
24	7	70	8	7	75
31	8	80	8	8	80
48	7	70	9	7	80
55	7	70	8	7	75
72	7	70	7	7	70
Olfactory conditioning					
7	7	70	7	6	65
24	8	80	7	9	80
31	8	80	8	8	80
48	9	90	9	9	90
55	9	90	8	9	85
72	9	90	8	10	90
Olfactory memory					
7	9	90	9	10	95
24	8	80	9	8	85
31	8	80	8	8	80
48	7	70	8	9	80
55	8	80	7	10	85
72	8	80	8	8	80

Table 4: *Impact of ibuprofen on ants' conditioning ability and memory.*
The table gives the numbers of ants among 10, and the proportion of ants, which gave the correct response in a Y-apparatus. The important examined traits were not at all impacted by ibuprofen, what is favor of this drug use.

Concerning the visual conditioning, the difference between ants consuming and not consuming ibuprofen was statistically not significant (N = 4, T = 10, P = 0.063). After 72 hours of training, the ants consuming ibuprofen reached the same score (80%) than ants living under normal diet. However, ants consuming ibuprofen acquired visual conditioning somewhat more slowly than ants living under normal diet. Indeed, observation showed that the ants consuming ibuprofen often hesitated to move into the branch of the Y-apparatus containing the yellow hollow cube, and stopped just in front of the cue, and finally went into the other branch. It could thus be concluded that the conditioning ability of ants consuming ibuprofen was not really impacted by that drug, but that their straightforwardness was affected, a deduction already done in this work (see above, the sections 'Audacity', 'Cognition', 'Escaping ability'). After removal of the visual cue, ants consuming ibuprofen lost nothing of their conditioning during at least 48 hours, and lost only 10% of it in 72 hours, just like ants living under normal diet. The ants' short and middle term visual memory was thus not affected by ibuprofen consumption.

As for the olfactory conditioning, ants consuming ibuprofen could acquire in 24 hours the excellent score of 80%, and thereafter of 85 and 90%, behaving thus as ants under normal diet. Observation showed that the ants consuming ibuprofen were simply somewhat less inclined to move into the branch of the Y apparatus containing the cue, and often stayed in front of it for a time, exhibiting thus a low straightforwardness just like when being visually trained. After having removed the olfactory cue, the ants under ibuprofen diet lost nothing of their conditioning; they even better responded than ants under normal diet, a result at the limit of significance (N = 4, T = 10, P = 0.063).

Thus, ibuprofen did not impact the ants' ability in acquiring visual or olfactory conditioning, and in memorizing the learned cues. This analgesic did not affect the ants' short and middle term memory, what was is favor of its use.

Adaptation to ibuprofen adverse effects

The ants presented no adaptation to the impact of ibuprofen on their locomotion (Table 5, upper part). After having consumed that drug for 8 days, they still walked at a lower linear speed and a higher angular speed than while living under normal diet. Their locomotion speeds were statistically similar to those presented after one day of ibuprofen consumption (linear speed: $\chi^2 = 0.35$, df = 2, $0.80 < P < 0.90$; angular speed: $\chi^2 = 0.85$, df = 3, $0.80 < P < 0.90$). This is not in favor of ibuprofen use as a safe drug.

Habituation to the analgesic effect of ibuprofen

Ants developed habituation to the decrease of pain perception induced by ibuprofen (Table 5, lower part; Figure 2K). After nine days of that drug consumption, they moved on a rough substrate, not as frankly as after four days of consumption, though not yet exactly as before consuming ibuprofen. In details, their linear speed was statistically lower than that after four days ($\chi^2 = 17.72$, df = 2, $P < 0.001$) but still higher than that under normal diet ($\chi^2 = 16.44$, df = 1, $P < 0.001$), while their angular speed was higher than that after four days of consumption ($\chi^2 = 12.80$, df = 1, $P < 0.001$) and was similar to that presented under normal diet ($\chi^2 = 3.22$, df = 2, $P = 0.20$). Such a habituation, although incomplete after 9 days, to the wanted analgesic effect of ibuprofen is not in favor of the use of that drug since more amounts of it will be required for obtaining the same analgesic effect in the course of its consumption.

Adaptation	Control	After 1 day	After 8 days
Linear speed (mm/s)	12.5 (11.1 - 15.0)	9.2 (8.2 - 10.0)	9.5 (8.3 - 10.0)
Angular speed (ang. deg./cm)	119 (101 - 139)	178 (156 - 193)	171 (150 - 188)
Habituation	Control	After 4 days	After 9 days
Linear speed (mm/s)	5.3 (4.7 - 5.8)	8.9 (8.2 - 9.5)	6.9 (6.1 - 7.6)
Angular speed (ang.deg./cm) on a rough substrate	266 (235 - 307)	171 (154 - 191)	246 (216 - 260)

Table 5: *Adaptation and habituation of ants to ibuprofen after respectively 8 and 9 days of this drug consumption. The ants did not adapt themselves to the impact of ibuprofen on their locomotion (upper part of the Table). They became somewhat habituated to the analgesic effect of the drug (lower part of the Table). These two results are not in favor of the use of ibuprofen.*

Dependence on ibuprofen consumption

Ants did not develop dependence on ibuprofen consumption (Figure 2L). Confronted to sugar water and to sugar water containing this drug, 9 ants of colony A went drinking the former solution and 12 ones the latter solution, while 11 ants of colony B went drinking the former solution and 15 ones the latter solution. In total, 23 ants were seen on the sugar + ibuprofen solution and 24 ants were seen on the sugar solution free of the drug. Thus, ibuprofen did not lead to physical dependence, what is in favor of that drug use.

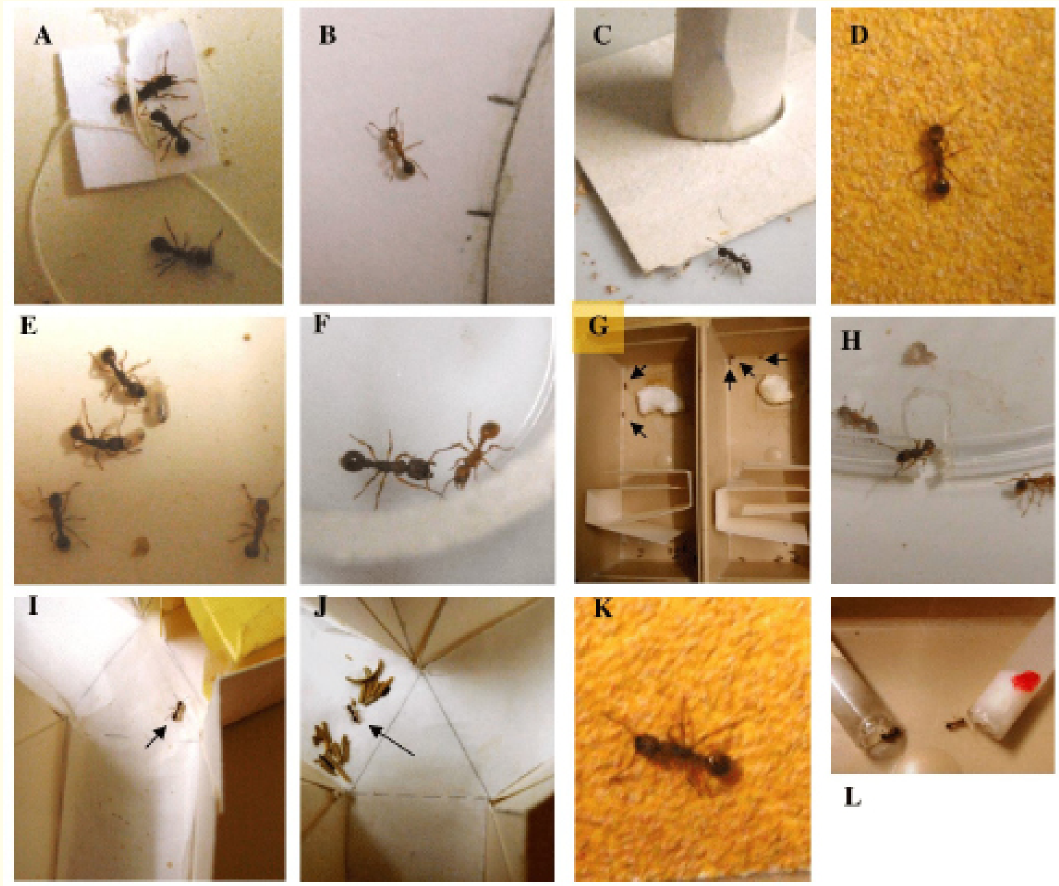

Figure 2: *Some views of the experiments. All the photographed ants were under ibuprofen diet. A: an ant having reached a tied nestmate, and an ant being unable to do so. B: an ant departing from a circular trail. C: an ant hesitating in coming onto an unknown apparatus. D: an ant moving frankly on a rough substrate, its tactile (pain) perception being reduced. E: an ant taking care of a larva, an ant hesitating to do so, and two ants staying motionless. F: two mestmates being somewhat aggressive towards one another, i.e. opening their mandibles. G: ants having to cross a twists and turns path for reaching a large area provided with wet cotton; arrows show two (on the left) and three ants (on the right) having been able to do so. H: an ant escaping from an enclosure through a notch in the rim, and an ant, on the left, having been unable to do so. I: an ant (arrow) trained to a yellow cube giving the correct response in a Y-apparatus, i.e. moving towards such a visual cue. J: an ant (arrow) trained to rosemary giving the correct response in a Y-apparatus, i.e. moving towards such an olfactory cue. K: after 9 days of ibuprofen consumption, an ant moving with some difficulty on a rough substrate (touching it with its antennae, moving its legs with difficulties), being thus somewhat habituated to the analgesic effect of ibuprofen. L: ants going similarly drinking sugar water (left tube) and sugar water containing ibuprofen (right tube with a red spot): they are not dependent on that drug consumption.*

Decrease of the effect of ibuprofen after its consumption was stopped

The effect of weaning on the ants' sinuosity was monitored after the ants had consumed ibuprofen for 22 days. The numerical results of this experiment are given in table 6 and a graphic presentation of them is shown in figure 3. The decrease was slow. Four to six hours after weaning, the sinuosity was similar to that observed after one or eight days of ibuprofen consumption. Eight hours after weaning, the ants' sinuosity was lower than that before weaning (χ^2 = 10.4, df = 2, 0.001 < P < 0.01; K-W: P = 0.0024), but still somewhat different from the control one (χ^2 = 11,77, df = 3, 0.001 < P < 0.01; K-W: P = 0.134). Ibuprofen still had thus some slight effect. Ten hours after weaning, the ants' sinuosity was very different from that before weaning (χ^2 = 25.62, df = 2, P < 0.001; K-W: P = 0.0006), and statistically not different from the control (χ^2 = 7.67, df = 4, 0.10 < P < 0.20; K-W: P = 1) though being slightly higher. Twelve hours after weaning, the ants' sinuosity was similar to the control one, i.e. to that presented before consuming ibuprofen. Consequently, the effects of ibuprofen slowly vanished in a total of about 10 hours, a decrease of effects which allowed not developing dependence on that drug.

Experiments and times		Ants' sinuosity (ang.deg./cm)	Statistics			
			vs control		vs t = 0h	
			χ^2	K-W	χ^2	K-W
Control		119 (101 – 139)				
After 1 day of consumption		178 (156 – 193)				
After 8 days of consumption		171 (150 – 188)				
After 22 days: weaning						
time after weaning :	t = 0h	193 (178 – 216)	P < 0.001	P < 0.001		
	t = 2h	186 (174 – 197)	P < 0.001	P < 0.001	NS	P = 1
	t = 4h	177 (164 – 217)	P < 0.001	P < 0.001	NS	P = 1
	t = 6h	174 (145 – 190)	P < 0.001	P = 0.001	NS	P = 0.58
	t = 8h	149 (134 – 177)	P < 0.01	P = 0.27	P < 0.01	P = 0.005
	t = 10h	139 (124 – 147)	P > 0.10	P = 1	P < 0.001	P = 0.001
	t = 12h	115 (102 – 132)	NS	P = 1	P < 0.001	P < 0.001

Table 6: *Decrease of the effects of ibuprofen after weaning.*
The trait used for examining this decrease was the ants' sinuosity. This revealed that the effect of ibuprofen slowly decreased over time after weaning according to a quadratic function, and fully vanished in about 10 hours. Details are given in the text and a graphic presentation of the results is shown in figure 3. In the third and fourth columns, the left values result from non-parametric χ^2 *tests, and the right values from a two tailed non-parametric Kruskal-Wallis ANOVA for multiple comparisons. ang.deg./cm = angular degree per centimeter; t = time.*

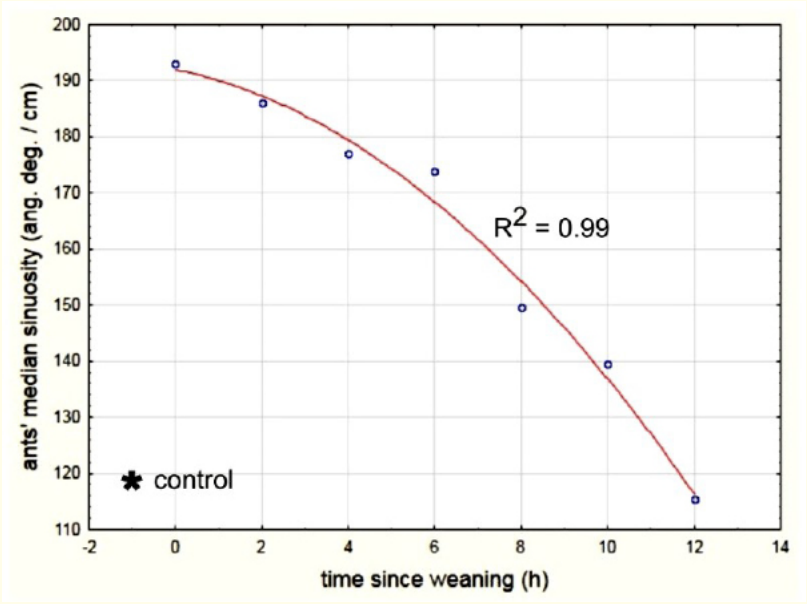

Figure 3: *Decrease of the effects of ibuprofen after weaning. The numerical results and statistics are summarized in table 6 and detailed in the text ('Results and Discussion' section). The effects of ibuprofen decreased slowly after weaning and vanished in about 10 hours, what allowed no dependence on that drug. The decrease occurred according to a quadratic function of the time. Ang.deg./cm = angular degrees per cm; h = hour.*

The decrease of the effect of ibuprofen on the ants' sinuosity after weaning followed the following quadratic function, in which E = effect, t = time and 191,976 = the calculated initial sinuosity value:

E = -0.396 t2 – 1.553 t + 191,976

The determination coefficient of such a regression is R^2 = 0.99.

Conclusions

The adverse effects of ibuprofen begin to be known, or to be at least correctly divulgated, but all its potential ones have not yet been examined in details. Using ibuprofen to care of humans was recently observed to induce, in some cases, gastritis, jaundice, cephalalgia, drowsiness, confusion, asthma, and skin allergy [37]. Available studies, including a critical review, made on the effects of ibuprofen are mentioned in the 'Introduction' section. In these studies, nothing is related about potential impacts of the drug on the individuals' food consumption, movement, cognition, memory, social relationship, adaptation, habituation and dependence. Drugs may have such impacts. For instance, paracetamol largely impacts the learning and the memory [2]; fluoxetine induces aggressiveness between congeners [36]. We thus examined which can be the different adverse effects of ibuprofen, and did so using ants as models as we did previously [19,32,33,34]. We have similarly previously studied the effects of paracetamol, the most used analgesic before ibuprofen [2]. So, after having achieved the present study, we could compare the efficiency and the safety of the two analgesics (Table 7 and below), and propose a rather safe use of them (see below).

Ants' Examined Traits	Ibuprofen	Paracetamol
Meat Consumption	Decreases	Does Not Impact
Sugar Consumption	Does Not Impact	Does Not Impact
Activity	Slightly Decreases	Does Not Impact
Linear Speed	Decreases	Decreases
Angular Speed	Increases	Increases
Orientation	Decreases	Decreases
Trail Following	Decreases	Decreases
Audacity	Decreases	Does Not Impact
Tactile Perception	Decreases	Decreases
Brood Caring	Decreases	Does Not Impact
Social Relationship	Impacts, Induces Aggressiveness	Does Not Impact
Escaping Behavior	Slightly Decreases	Does Not Impact
Cognition	Does Not Impact	Decreases
Visual Conditioning	Does Not Impact	Decreases
Visual Memory	Does Not Impact	Decreases
Olfactory Conditioning	Does Not Impact	Decreases
Olfactory Memory	Does Not Impact	Decreases
Adaptation	No Adaptation	Slight Adaptation
Habituation	Habituation	Habituation
Dependence	No Dependence	Dependence
Loss of Effect After Weaning	Slow, in 10 Hours	Rapid, in 4 Hours

Table 7: *Comparison between the effects of ibuprofen (present study) and paracetamol [2] on 22 physiological and ethological traits, the studies having been made on ants as models.*

Ibuprofen decreased the ants' food consumption, and slightly their activity, decreased their linear speed, increased their sinuosity, and decreased their orientation, trail following ability, audacity, tactile perception (this is the analgesic effect of ibuprofen), and somewhat their escaping ability. The drug impacted the ants' brood caring and social relationship (they were somewhat aggressive towards nestmates). However, ibuprofen did not affect the ants' cognition, conditioning ability and memory. Paracetamol did not affect the ants' food consumption and activity, it increased their sinuosity, decreased their linear speed, orientation and trail following abilities, and their tactile perception (its analgesic effect). Paracetamol did not affect the ants' audacity, escaping behavior, brood caring and social relationship. However, this drug largely affected the ants' cognition, conditioning ability and memory. The two analgesics had thus different adverse effects, resulting from their different mode of action: ibuprofen inhibits the production of an enzyme allowing the synthesis of prostaglandins; paracetamol acts on the central nervous system. Ants developed no adaptation to ibuprofen, and some slight adaptation to paracetamol. They became habituated to the analgesic effect of the two drugs, what is a natural physiological reaction of the sensitive cells. No dependence occurred on ibuprofen, the effects of which slowly vanished in 10 hours; dependence occurred on paracetamol, the effects of which rapidly vanished in 4 hours.

On the basis of the available information, it can be concluded that neither ibuprofen nor paracetamol are entirely safe. Each of them presented adverse effects, but these effects are different: ibuprofen impacts some physiological and ethological traits; paracetamol impacts other traits and leads to dependence. We presume that an alternative use of three analgesics 'ibuprofen, paracetamol, and acetylsalicylic acid' may be a rather safe solution in case of severe pain. On basis of the different toxicity of the three analgesics, we cautiously propose to first consume during 72 hours the less harmful and inducing no dependence analgesic, ibuprofen (at middle dose), then, if the analgesic treatment needs to be prolonged, during another, but maximum, 72 hours period, to consume paracetamol (at middle dose), which is a little more harmful and induces dependence, and perhaps thereafter, during 24 hours, acetylsalicylic acid (at middle dose), the most toxic of the three analgesics. Of course, the effects of such a treatment have to be examined on animals and humans before it should be applied. Practitioners have made clinical studies on humans similar to ours on ants. Their results differed in details, according to the examined effects of the analgesics, but finally, their conclusions agree and agreed with ours [38-42]. These practitioners finally stated that ibuprofen is similarly efficient and tolerated [38,39] or somewhat more efficient and better tolerated than paracetamol [9,39,40-42], and that these analgesics may be preferred to acetylsalicylic acid [9]. They observed that each of these analgesics somewhat impacted the humans' health or estimated that they may potentially do so. They thus advised to use them only for a time at a moderate dose. A clinical study made by 1,108 practitioners on 8,677 adults leaded to identical conclusions, and the authors advised to use a combination of the three analgesics with ibuprofen first since it is the less harmful, then paracetamol for a short time because it causes overdose, and finally, if an analgesic treatment is still required, acetylsalicylic acid which has the poorest tolerability [43]. We can thus conclude that the best solution in case of moderate to strong pain could be an ordered combination of the three analgesics, taking into account the degree of dangerousness of each of them.

Note that after one week of such a treatment with three analgesics and for a few following days, as well as in case of moderate pain, the use of the natural analgesic 'curcuma' could be a safe solution [32]. Let us add that the perception of moderate pain should not necessary be suppressed: it is a useful alarm signal of the organism, informing the individual about a causal anomaly or health problem which must be medically treated.

Conflict of Interest

We affirm having no conflict of interest concerning the production and the use of ibuprofen and paracetamol. We are independent researchers on ants, and receive no money for conducting our studies.

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