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Ants as Biological Models for Studying Effects of Substances Used by Humans

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Abstract

In the course of studies on ants, it became obvious that they could be used as biological models. Going on with research on ants' behavior (among others on their cognitive abilities and the ontogenesis of some of these abilities), we increased the number of accessible ethological and physiological traits and improved our assessing methods in order to analyze as accurately as possible the effects of products used by humans. Until now, we set up experimental protocols allowing assessing 18 traits, and we have examined the effects of 20 products (alkaloids, antidepressants, analgesics, anxiolytics, flavor exhausters and sweeteners). The present paper summarizes these 18 protocols as well as the most important findings concerning the effects of these 20 examined products. It essentially gives qualitative data, while all the underlying quantitative data can be found in the given appropriate references. Briefly, effects observed in humans were confirmed and often explained, and unknown (or not divulgated) ones were revealed.

INTRODUCTION

Most biological processes are similar for all animals, including humans (i.e. genetics, metabolism, nervous cells functioning). A lot of invertebrates and vertebrates can thus be used as models for studying biological questions [1-3]. Invertebrates are more and more used as biological models because they offer scientists many advantages, such as a short life cycle, a simple anatomy, and being available in large numbers [4,5]. Some species are largely used as biological models, i.e. the flatworm *Dendrocelium lacteum*, the nematode worm *Caenorhabditis elegans*, the mollusk *Aplysia californica*, the beetle *Tribolim castaneum*, the fruit fly *Drosophila melanogaster*, and the domestic bee *Apis mellifera*. Among the invertebrates, insects, especially social hymenoptera and among them, bees, are advantageously used as biological models [6,7].

Ants also could be used. Colonies containing thousands of ants can easily be maintained in laboratories, at low cost, throughout the entire year. Ants are among the most complex and social invertebrate animals as for their morphology, physiology, social organization and behavior. They are among the most morphologically evolved hymenoptera, having indeed a unique resting position of their labium, mandibles and maxilla [8], as well as a lot of glands emitting numerous, efficient pheromones [9]. Their societies are highly organized with a strong division of labor, an age-based polyethism and a social regulation [10]. Their behavior is well developed: they care for their brood, build sophisticated nests, chemically mark the inside of their nest, and, differently, their nest entrances, their nest surroundings and their foraging area [11]. They generally use an alarm signal, a trail pheromone, and a recruitment signal [11]; they are able to navigate using memorized visual and olfactory cues [12 and references therein]; they efficiently recruit nestmates where, when and as long as it is necessary [11], and, finally, they clean their nest and provide their area with cemeteries [11]. So, according to the complexity of their society and their behavior, it looks reasonable to use ants as biological models for studying physiological and ethological effects of biologically active substances.

During many years, we have studied the biology of ants of the genus *Myrmica*, above all *M. rubra* (Linnaeus 1758), *M. ruginodis* (Nylander 1846), and *M. sabuleti* (Meinert 1861). We have now knowledge about their ecological traits, eye morphology, visual perception, navigation system, visual and olfactory conditioning capabilities, and recruitment strategy [12-15]. The ontogenesis of cognitive abilities of *Myrmica* species has also been approached [16]. Studies on the impact of age, activity and diet on *M. ruginodis*' conditioning capability [17] incited to presume that ants could be good biological models. This was confirmed by the study of the effects of 20 different substances such as alkaloids, drugs, medicines, food additives, and sweeteners (see below, the 'Results' section) [18-31]. Each time, effects similar to those observed for humans were observed, and many of them could be explained and/or detailed. Moreover, unknown or not divulgated ones were revealed, and are susceptible to exist in humans. On the

other hand, several of these largely used substances are released intact or nearly so into wastewater because they are poorly degraded by human's catabolism, and because wastewaters are inadequately treated. They can have harmful impacts on the biology of aquatic invertebrate or vertebrate animals [32,33]. Our studies on ants may allow deducing these impacts.

MATERIAL AND METHODS

Maintenance of ants, and their feeding with a substance to be examined

The ants were collected from different sites and maintained in laboratory in artificial nests made of glass tubes half filled with water, a cotton plug separating the ants from the water. The glass tubes devoted to one experimental colony were deposited in a tray (34 cm x 23 cm x 4 cm), which internal sides were slightly covered with talc to prevent the ants from escaping. The trays served as foraging areas and food was delivered in them. This food consisted of an aqueous solution of sugar (30%) provided *ad libitum* in a small glass tube plugged with cotton, and of pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) provided as meat three times a week on a glass slide. After having made all the control assessments and so checked the ants' good health, for making the subsequent experiments, the sugar water was replaced by an aqueous sugared solution of the substance to be examined, delivered to the ants as their usual sugar water i.e. in a small tube plugged with cotton. Laboratory temperature was maintained at 18°C – 22°C, the relative humidity at circa 80%. Lighting had an intensity of 330 lux while caring of the ants and testing them. During other time periods, it was dimmed to 110 lux. The ambient electromagnetic field had an intensity of 2-3 $\mu W/m^2$.

The concentration of the product given to the ants was established on the basis of the amount of that product usually consumed by humans and on the fact that insects proportionally drink about ten times less than mammals. Humans consume about one liter of water per day. The amount of the studied product consumed by humans per day (i.e. with one liter of water) was thus dissolved into 100 ml of sugared water (the one used for feeding the ants) and a stock solution was kept at – 25°C. This solution was given to the ants as their usual sugar water i.e. in small tubes containing 5 ml of the solution. The tubes were plugged with cotton which was refreshed each two days while the entire solution was renewed every seven days. It was checked each day if ants drunk the provided solution of a substance under study, and they effectively did so, for each of the 20 studied substances.

Assessment of ants' traits

We could precisely assess 18 ants' physiological and ethological traits. Here below, we list and very briefly explain them.

The ants' sugar water and meat consumption as well as their general activity were assessed by counting during five days, four times per day, the ants present on the sugar water, on the *T. molitor* larvae, as well as those moving at any place in their environment (food sites, foraging area, nest entrances and inside nest). Daily counts, daily means, and total mean for these three kinds of counts were then established.

Linear and angular speeds of freely moving ants were assessed without presenting them a stimulus, and their orientation towards an alarm signal was assessed while presenting them an isolated worker's head. Each time, 40 ants' trajectories were recorded and analyzed using specifically designed software [34], each trajectory being entered in the software by clicking as many points as wanted with the mouse and by entering then the location of the presented isolated head. The linear speed (V, here measured in mm/s) of an animal is the length of its trajectory divided by the time spent moving along this trajectory. The angular speed (i.e. the sinuosity, S, here measured in angular degrees/cm) of an animal's trajectory is the sum of the angles, measured at each successive point of the trajectory, made by each segment 'point i to point i – 1' and the following segment 'point i to point i + 1', divided by the length of the trajectory. The orientation (O, here measured in angular degrees) of an animal towards a given point (here an isolated head) is the sum of the angles, measured at each successive point of the recorded trajectory, made by each segment 'point i of the trajectory - given point' and each segment 'point i - point i + 1', divided by the number of measured angles. Each distribution of variable was characterized by its median and quartiles.

The ants' trail following behavior was quantified by presenting them with a circular artificial trail (R = 5 cm, 1 poison gland/trail; the poison gland producing the trail pheromone) deposited on their foraging area, and by counting the arcs of 10° followed without discontinuity by 40 ants. Again, the distribution of the obtained numbers was characterized by its median and quartiles.

The ants' audacity was evaluated by counting, 12 times in the course of 12 min, the ants present on a cylindrical tower built in strong white paper (Steinbach ®, height = 4 cm; diameter = 1.5 cm) and set on their foraging area. The mean and extremes of the obtained values were established.

The ants' brood caring behavior could be assessed by removing larvae from the inside of the nest and by counting then the larvae among ten given ones still not re-entered in the course of 10 minutes.

The ants' cognition was assessed by counting, in the course of time, those leaving a small area lying in front of a way with twists and turns, and those crossing this difficult way and reaching an area located beyond.

The ants' aggressiveness against nestmates was examined in the course of dyadic encounters conducted in a small cylindrical cup (diameter = 2 cm, height = 1.6 cm). The behavior of each ant was characterized by the numbers 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). The ants' aggressiveness was also assessed by a variable 'a', equal to the number of recorded aggressiveness levels 2 + 3 + 4 divided by the number of levels 0 + 1.

The ants' tactile (pain) perception was evaluated through their locomotion on a rough substrate (emery n° 280 paper)

on which ants moved with difficulties (pain?) presenting thus a small linear speed and a large angular one.

For approaching the effect of a substance on the ants' state of stress, we worked on a few colonies and for each of them, imprisoned 5 ants under a reversed polyacetate glass (h = 8 cm, bottom diameter = 7 cm, ceiling diameter = 5 cm) set on the foraging area. The rim of the bottom had been provided with a small notch (3 mm height, 2 mm broad) what allowed the ants escaping from the reversed glass. The calmest, less stressing ants moved rather quietly essentially along the rim of the reversed glass, and could escape. The most stressing ants went on turning all round and failed in discovering the exit. For quantifying the ants' state of stress, we described their behavior, we assessed their linear and angular speed, and we counted the ants still under the glass at the end of 10 successive minutes. We also set up a variable expressing the calming effect of the studied substance: V/S which equaled the linear speed value (in 10^{-1} mm/s) divided by the angular speed value (in angular degrees/cm).

The ants' acquisition of operant visual or olfactory conditioning, as well as their visual or olfactory memory could also be assessed. At a given time, a visual or an olfactory cue was set above or aside the ants' food, and ants were then individually tested, in the course of time, in an Y-apparatus provided with the visual or the olfactory cue in one of its branches. Choosing the way with the cue was considered as giving the 'correct' choice. The percentage of correct responses obtained in the course of time assessed the ants' acquisition of operant conditioning, then after removal of the cue, the ants' memory.

Knowing the adverse effects of a product, we could assess the ants' adaptation to an unwanted effect of that product, i.e. the ants' tolerance of the product, the fact that they no longer suffered from its adverse effects. In the same way, knowing a beneficial effect of a product, we could assess the ants' habituation to the product, i.e. the fact that they became accustomed to it, the beneficial effect of which becoming lower and lower in the course of time, what corresponds, for humans, to a potential increase, in the course of time, of the amount of consumed product.

The ants' preference or aversion of a food containing the studied product (what can reveal potential dependence on the product, i.e. addiction on it, impossibility to live without it) was evaluated thanks to choice tests. Ants were transferred into a small tray (15 cm × 7 cm × 5 cm), the borders of which having been covered with talc, and in which two tubes (h = 2.5cm, diam. = 0.5 cm) were laid, one containing sugar water, the other a sugared solution of the studied substance, each tube being plugged with cotton. The ants drinking each liquid food were counted 12 times in 15 min. The mean values were established for the two kinds of food, and these means were statistically compared to those expected if ants went randomly drinking the two kinds of food.

The decrease, in the course of time, of the effect of a substance after its consumption ended could be assessed by giving again pure sugar water to the ants instead of the sugared solution of the substance, and quantifying a given effect of the substance in the course of time.

Each time, the results were statistically analyzed using appropriate non-parametric tests.

Most important ethological and physiological effects of 20 examined substances

Although the present paper summarizes 14 published papers, each one based on numerous quantitative data, it gives essentially qualitative data. Readers are invited to find all the underlying quantitative data, as well as the statistical results, in the given appropriate references [18-31].

Caffeine [18] increased ants' speed of locomotion, decreased their precision of reaction, response to pheromones, and food consumption, and somewhat increased their conditioning ability and memory (they retained 20% instead of 10% of their learning). There was no dependence at all on that substance, and its effects vanished slowly in 16 hours after consumption ended.

Theophyline [18] had effects similar to those of caffeine. It largely decreased food conqumption (mean 1.8 instead of 2.3 ants were counted on the food), its effects slowly vanished in 28 hours. Again, no dependence occurred on this alkaloid consumption.

Cocaine [18] decreased the ants' speed of locomotion, precision of reaction, response to pheromones, food consumption (meanly 1.6 instead of 2.2 ants were counted on the food), and above all their learning and memory (they ants reached only a score of 50% (= no learning) instead of 80%). It largely increased their audacity (4.5 instead of 1.3 ants were meanly counted on a risky apparatus); it leaded to habituation and to strong dependence (Figure 1A). Its effects rapidly vanished in 4-5 hours.

Atropine [18] had few adverse effects. It decreased the ant's speed of locomotion and food consumption; it leaded to no dependence and its effects slowly vanished in 8-10 hours.

Nicotine [19] had complex effects. It increased the ants' speed of locomotion. It decreased their precision of reaction, response to pheromones and food consumption (meanly 1.4 instead of 3.7 ants were counted on the food). It did not impact the ants' cognition, but learning became possible only when the reward was nicotine. When rewarded by meat, ants learned nearly nothing; rewarded by nicotine, they reached a score similar to the control one. In other words, nothing but nicotine could act as a reward. No habituation occurred. No dependence occurred as long as ants felt well, but strong dependence developed as soon as some problems appeared, such as lack of food. In the latter case, 73 – 100% of the ants chose the food containing nicotine. The effect of this alkaloid rapidly decreased between 8 and 11 hours after its consumption ended. The similarity between these effects and those observed in humans was obvious.

Fluoxetine [20], the active substance of the most consumed antidepressants, increased the ants' sinuosity of movement, and decreased their precision of reaction, food consumption, and brood caring behavior (they even killed their nymphs). It induced aggressiveness against nestmates (Figure 1B). The variable 'a', assessing the aggressiveness, equaled 3.2 instead of 0) but decreased the normal aggressiveness against alien ants. Fluoxetine decreased the ants' cognition, learning and memory (the ants reached a score of 50 – 45% instead of 65 –

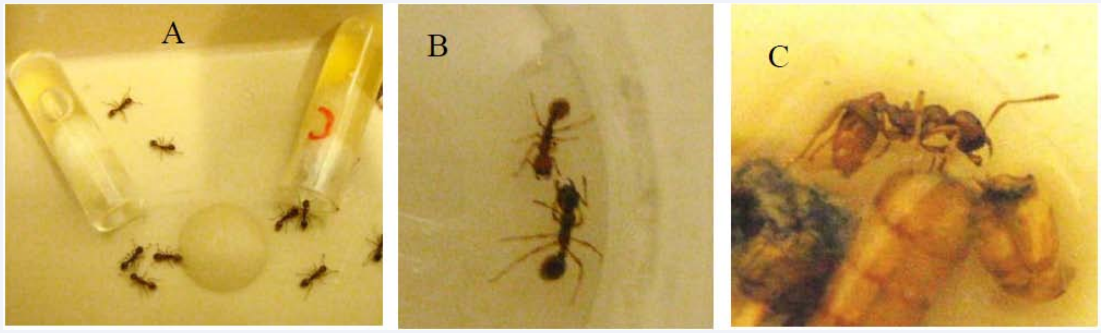

Figure 1 A: Some views of the experiments: A: ants choosing sugar food containing cocaine (C), having thus acquired addiction to that substance (copied from [18]). B: Ants of the same colony (nestmates), consuming fluoxetine, a large used antidepressant, aggressing one another (they opened their mandibles) (copied from [20]). C: As testifying by its swollen gaster, an ant having too much eaten food containing monosodium glutamate, a largely used food additive enhancing savor of not sugared meal; the ant died the following day (copied from [27]).

80%). It leaded to no habituation and to no dependence, and its effects slowly vanished in 2 - 2½ days. Anafrani [21], an ACT antidepressant, reduced the ants' activity, precision of reaction, response to pheromones, audacity, tactile perception, cognitive ability, and aggressiveness towards aliens. It did not impact food consumption, acceptance of congeners (the variable 'a' equaled 0.03 instead of 0), brood caring, visual and olfactory perception. It increased the ants' ability in acquiring visual and olfactory conditioning (the ants reached a score of 80 – 90% instead of 70 – 80%), as well as their visual and olfactory memory. It leaded to no habituation and no dependence. Its effects vanished in about 28 hours after its consumption ended. Efexor [21], an IRSNa antidepressant, increased the ants' jerking movements, and some aggressiveness against nestmates (the variable 'a' equaled 0.72 instead of 0). It decreased their precision of reaction, response to pheromones, food consumption, tactile perception, cognitive ability, aggressiveness towards aliens, olfactory perception, ability in acquiring visual and olfactory conditioning, and memory (the ants reached scores of 60 and 50% instead of 70 and 80%). No habituation to and no dependence on that drug occurred. Its effects vanished in 12 hours (linear speed), 28 h (sinuosity of movement) and 32 hours (precision of response).

Carbamazepine [22] is the drug given to persons suffering from epilepsies or pain in their cerebral nervous system. It decreased the ants' speed of locomotion, precision of reaction, audacity, research for food, cognition, and slightly their olfactory and tactile perception. It induced aggressiveness against nestmates (the variable 'a' equaled 10 instead of 0) while slightly reducing that against aliens. Carbamazepine increased the ants' ability in acquiring visual and olfactory conditioning (the ants reached a score of 80 and 85% instead of 70 and 80%), as well as their visual and olfactory memory. Ants never developed dependence on, or habituation to, that drug consumption. After its consumption ended, its effects vanished in 20 hours (for the linear speed), 26 hours (for the sinuosity), and 32 hours (for the orientation towards an alarm signal). This drug is eliminated intact by the kidneys and affects macroinvertebrates and vertebrates depending on natural water (see the 'Discussion' section).

Morphine [23] decreased the ants' activity, precision of reaction, response to pheromones, audacity, and largely tactile sensation at least at the beginning of its consumption. It also decreased the ants' cognition, conditioning ability (the ants reached a score of 50 and 45% instead of 70 and 80%) and memory. Habituation to morphine soon occurred as for the ants' activity, and slowly developed as for the tactile sensation. A strong dependence on morphine appeared. Its effects quickly vanished in about eight hours after its consumption ended. An obvious similarity exists between these effects and those observed in humans.

Quinine [23], the substance used for caring of persons suffering from malaria, had few adverse effects. It however decreased the ants' tactile perception. No habituation, as well as no dependence, occurs even after 14 days. After its consumption ended, effects of quinine linearly decreased in the course of time, vanishing in about ten hours, what was followed by a recovery period of about ten hours. Such a decrease leads to an important discovery concerning the impact of quinine on the propagation of the malaria illness (see the 'Discussion' section).

Buprenorphine [24], an analgesic also used for treating addicted persons, decreased the ants' speed of locomotion, precision of reaction, response to pheromones, food consumption, audacity (1.1 instead of 2.2 ants were counted on a risky apparatus), brood caring, cognition, largely the tactile (pain) perception and induced some aggressiveness against nestmates (the variable 'a' equaled 0.5 instead of 0.1). This drug did not impact visual and olfactory conditioning and memory. Adaptation occurred for its effect on locomotion and precision of reaction, but no habituation occurred for its decrease of tactile perception. No dependence developed on buprenorphine. The analgesic effect of this substance persisted intact during four hours after its consumption ended, and disappeared after ca 15 hours.

Methadone [24], a substance used as an analgesic and for treating addicted persons, decreased the ants' speed of locomotion, precision of reaction, response to pheromones, food consumption, audacity, brood caring, cognition, and tactile (pain) perception. It induced some aggressiveness against nestmates (the variable 'a' equaled 0.51 instead of 0.16), and prevented any conditioning. Adaptation to this substance occurred as for its impact on locomotion, but not for their precision of reaction.

No habituation occurred for its decrease of tactile perception. No dependence developed on methadone. The analgesic effect of methadone quickly decreased during three to five hours, then stayed at level for *ca* four hours, and after that, again decreased during two to three hours, vanishing thus in 10 – 13 hours.

Alprazolam [25] is nowadays the most consumed anxiolytical drug, being the active substance of Xanax®, Apotex®, and Alprox®. This substance reduced the ants' food consumption, general activity, locomotion, precision of reaction, response to pheromones, audacity, brood caring behavior, cognition, tactile (pain) perception, and middle term memory, though not impacting their short term memory (the ants reached a score of 80% but lost their learning in 24 hours). It induced aggressiveness towards nesmates (the variable 'a' equaled 1.17 instead of 0.10). Habituation to alprazolam occurred as for locomotion, but not as for aggressiveness. Ants developed a strong physiological dependence on that drug. The effect of alprazolam on locomotion rapidly vanished after weaning, becoming not significant after 2 hours. The effect on aggressiveness vanished in about 60 hours, but the decrease was rapid from 4 hours to 13 hours. After the here above summarized study, we could not but look for an alternative, and examined a mixture of four plants' extracts [26], *Valeriana officinalis* L., *Humulus lupulus* L., *Passiflora incarnata* L., and *Ballota nigra* L., easily available in any drugstore, e.g. under the label 'Sedinal Plus®'. This product did not significantly alter the ants' brood caring, cognition, behavior in front of nestmates (the variable 'a' equaled 0.04 instead of 0.02), tactile perception, learning and memory (the ants reached a score of 80% and retained 15% like during the control). It efficiently calmed the ants which often rested but not fully slept, avoided risky tasks and quietly reacted in a stressing situation. Adaptation occurred for the few adverse effects of this product (large sinuosity of movement, what had an effect on some behavior). No habituation occurred for its calming effect and it did not lead to dependence. After its consumption ended, its calming effect slowly vanished in 29 hours, step by step. We think that an extract of balm *Melissa officinalis* L. could advantageously be added to the examined mixture. An extract of *M. officinalis* allows no stressing though staying active, and has a nice taste. With such a supplementary extract, the examined mixture of four plants' extract might no longer induce some drowsiness, and may have a more pleasant taste.

Monosodium glutamate [27] is a food additive largely consumed since 1909. We found that it largely increased the ants' meat food consumption (Figure 1C), and largely decreased their precision of reaction, response to pheromones, cognition as well as their learning and memorization abilities, the ants simply learned nothing. Having the choice between intact meat and meat imbibed with glutamate, the ants prefer the latter. This resembled to dependence on the product, or at least may lead to over consume not sugared food. Thus, briefly, glutamate affected ants' food consumption because it has a nice 'umami' taste, and it impacted the ants' behavior requiring cognition and memory, so their brain and nervous system functioning, because it can hydrolyze into glutamic acid, which may act as a neurotransmitter and may also be excitotoxic.

Aspartame [28] is probably the most consumed sweetener in the world. It appeared having unwanted ethological and physiological effects on ants. It increased the ants' food consumption (meanly 2.97 instead of 1.30 ants were counted on the food), speed of locomotion, and audacity. It drastically impacted visual and olfactory memory the ants learned nothing, reduced the precision of reaction, response to pheromones, brood caring behavior and cognition. When having the choice between a solution of aspartame and one of sugar, the ants largely chose the latter one. The perturbations caused by aspartame may be due to the fact that though being not a glycoside, aspartame gives to the brain the 'presence of sugar' information. Moreover, aspartame may hydrolyze and give rise to aspartic acid, phenylalanine and methanol, and finally to formaldehyde, three of these substances being toxic for the nervous system.

The glycoside rebaudioside A is a sweetener extracted from the plant *Stevia rebaudiana* and commercialized under the label 'Stevia' [29]. This product did not affect the ants' food consumption, locomotion, precision of reaction, response to pheromones, brood caring, cognition, as well as visual and olfactory conditioning and memory. It slightly increased their audacity, and when having the choice between it and saccharose, the ants somewhat preferred the later. In fact, the ants were not inclined in consuming stevia and looked everywhere for their usual preferred sugar. Stevia appeared thus to be a safe sweetener (except that it may give rise to a small amount of steviol); it did not impact general health, behavior and cognition, but it was perceived as less pleasant than saccharose.

We then examined if a solution of stevia/aspartame 91/9 [30] might have both a pleasant taste (like aspartame) and nearly no adverse effects (like stevia). Effectively, this mixture (not commercialized) did not change the ants' food consumption, locomotion, precision of reaction, response to pheromones, audacity, brood caring behavior, cognition, and conditioning ability and memory (the ants reached scores similar to the control ones). Moreover, confronted to sugar water and a stevia/aspartame 91/9 solution, the ants equally drunk the two solutions. Very probably aspartame enhanced the taste of stevia, and as the latter is a true glycoside, so a stevia/aspartame 91/9 solution did not affect the ants' physiology and ethology as pure aspartame did. In front of saccharose and a stevia/aspartame ca 96/4 solution, the ants chose the saccharose. Thus, the mixture 9% aspartame (and no less) + 91% stevia (and no more) constitutes a safe and tasty sweetener which could be used instead of only aspartame or stevia.

Another sweetener, sucralose, is nowadays authorized in more than hundred countries. However, since it is not a true glycoside though having a strong sugared taste, it has a high probability of inducing perturbations similar to that of pure aspartame. A mixture of saccharose/sucralose 99.5/0.5 [31] exists under the label 'Ti-light®'. We thus examined its effects on ants. This mixture increased sugar water consumption, decreased general activity, precision of reaction, audacity, brood caring, cognition and ability in acquiring visual conditioning (short term memory; the ants reached a score of 57.5 instead of 81.7), induced some aggressiveness against nestmates (the variable 'a' equaled 0.71 instead of 0.04), and slightly reduced tactile perception. It did not affect middle and long term memory. Its adverse effects

may be related to its strong sugared taste (indeed, in presence of saccharose and the saccharose/sucralose mixture, the ants slightly preferred the mixture) though it contains little sugar, as well as to the toxic chloropropanols sucralose may produce. Theoretically, a mixture of saccharose/sucralose 99.9/0.1 (not available) may have very few adverse effects, but it would be a less 'light' product than the presently available mixture.

DISCUSSION

No addiction exists on caffeine and theophyline consumption. These alkaloids have not a pleasant taste and are present at a very low concentration in drinks (i.e. 1/1,000). People may be dependent on drinks containing caffeine or theophyline without being dependent of the alkaloids themselves.

Firstly revealed while studying effects of cocaine, and later on confirmed while studying effects of several other substances, dependence on a substance occurs when the effects of this substance rapidly decrease, at least for a time. This rapid decrease is perceived by consumers who want then to further consume the substance. Before using any product for caring of humans, the decrease in the course of time of the effect of that product should be analyzed. If the decrease is rapid, or is rapid during a few hours, then the product may cause addiction, and experiments must be performed to check this potentially harmful addiction. On basis of our studies, we can advance that a drug containing only one active substance may have a high probability of being rapidly eliminated or becoming inefficient, and of leading to addiction, while a drug made of several differently active substances might lose its efficiency more slowly, progressively, probably step by step, and consequently may not lead to addiction. In other words, for caring of humans and animals, using a mixture of different active compounds, each one having a moderate and somewhat different effect, may be more judicious than using only one specifically, highly active substance.

Nicotine appeared to replace any rewarding cue, and leaded to dependence as soon as any problem occurred. This explains the behavior and the dependence of nicotine consumers who require help and care.

Attention must be paid to the use of fluoxetine, the most consumed antidepressant substance. Indeed, in ants, this product induced aggressiveness against nestmates. Also, this adverse effect has been observed in fishes (see below in the present 'Discussion' section). Consequently, this induced aggressiveness is susceptible to occur in humans. Among the antidepressants we studied, the firstly used ones, the ACT the active substance of which is clomipranine hydrochlorid, are the less toxic, with few adverse effects. The following ones, the IRSNa the active substance of which is venlafaxine, are rather toxic since they have several adverse effects. The last used ones, the ISRN the active substance of which is fluoxetine, are highly toxic; they present several severe adverse effects. They should be given to humans in very small amount, only when absolutely necessary, and under medical supervision.

Attention should be paid to persons cared of with carbamazepine because this drug appeared to have several adverse effects. Moreover, this substance is nowadays present in natural water, and depuration systems should be perfected for eliminating it (as well as several other pharmaceutical pollutant substances) from the waste waters (see below in the present 'Discussion' section).

Morphine acts as a strong drug, and inevitably soon leads to severe physical addiction. Since it efficiently reduces pain, it should be exclusively used in case of severe pain. Quinine has nearly no adverse effects. However, an important point must be pointed out. In ants, its effects vanished in about 10 hours, and this was followed by a recovery period of 10 hours. Consequently, for caring of persons suffering from malaria, only one dose of quinine could be given each 24 hours. However, 10 hours after the end of consumption, quinine is no longer active. Before the following dose of quinine, schitzontes of *Plasmodium* cannot develop in red blood cells, having not enough time to do so, and humans do not suffer from a malaria episode. On the contrary, gamontes can develop in these blood cells, having time enough to do so, and in presence of mosquitoes of the genus *Anopheles*, the illness can be transmitted to other humans. It is thus urgent to develop medicinal drugs allowing stopping the development of *Plasmodium* gamontes. The large use of quinine, all over the zones where malaria exists, explains the large expansion of the illness, with about 10^6 deaths per year.

Our study on buprenorphine and methadone allowed concluding that 1) buprenorphine should be preferred to methadone for an analgesic use (example: for animals), and should be preferred to morphine for such a use, 2) methadone should be used only for caring of addicted persons; it should be given to them as a substitute of morphine or heroine since it induces effects similar to those of these drugs, but without inducing the exhilarating effect of heroine, and it leads to no addiction.

The anxiolytical substance alprazolam, the most used over the world, appeared to have several harmful effects (e.g. it induced aggressiveness, even 60 hours after its consumption ended), and to soon lead to strong dependence. On the contrary, an adequate mixture of the extract of four plants appeared to have an efficient anxiolytical effect and nearly no adverse effects. Moreover, it does not lead to dependence. This mixture may even be of better quality if an extract of a fifth plant, balm, was added. Practitioners should thus revise their use of chemically synthesized drugs which have precise and strong effects, but also adverse ones, and look to natural substances potentially able to have beneficial effects. Of course, experimental works on biological models must be done before using any new substances, natural or synthetised, for the humans' care.

The food additive glutamate may hydrolyze into glutamic acid, a potential neurotransmitter; the sweetener aspartame may lead to the presence of aspartic acid in the body, a substance which may also act as a neurotransmitter. Attention should thus be paid when glutamate and aspartame are consumed during the same food intake, e.g. glutamate in a meal and aspartame in a drink. Glutamic acid might become excitotoxic.

Concerning the examined sweeteners aspartame and a mixture of sugar/sucralose, their adverse effects are essentially due to the fact that they are not true glycosides though giving to the brain the information of a large supply of sugar, *via* their

nice and strong sugared taste. The expectation of sweet food leads to the use of glucose still available in the organism. If the organism receives a small and not sufficient amount of glycoside, it will search for glycosides. This is reported in several papers for instance [35,36]. As a consequence of this physiological reaction, the consumption of tasty sweeteners which are not glycosides lead to eat more than usually, essentially carbohydrates, and does not allow losing weight. This is particularly true for pure sucralose since this sweetener is not a glycoside, but has a sugared taste about 600 times stronger than that of sugar.

Concerning the two here above mentioned sweeteners, they may give rise to toxic elements. Aspartame may give rise to aspartic acid, as well as to methanol and finally to formaldehyde; sucralose may hydrolyze into chloropropanols. All these compounds are neurotoxic. Moreover, sucralose may affect the insuline responses in obese people, although it is used for losing weight [37]. As a matter of fact, the history of the discovery and the use of sweeteners is a succession of discoveries, of use by people without attention to potential adverse effects, of progressive information about harmful effects, then with some delay, of decrease of their use, and finally of a shift to other products. This is obvious while reading reviews about sweeteners for instance: [38]. On basis of our studies, at our mind, the nowadays best sweetener should perhaps be a mixture of a fair amount of stevia and of a very small amount of an artificial sweetener having a nice taste.

It is not impossible that when a substance (sweetener, food additive, medicine) having a wanted effect is discovered, some of its adverse effects are at least partly observed but are not divulgated. For commercial purpose, the discovered substance might be stated as being safe. Indeed, for nearly all the substances we examined and found adverse effects, previous works existed, stating their safety. Such works may have not been done without some conflict of interest. For example, aspartame is largely used in Europe and glutamate salt is very largely used in Asian countries. Researchers from these respective regions have shown through their works the safety of their product; they may have worked with some conflict of interest. We claim that any product devoted to humans or animals must imperatively previously be studied, on biological models, and without conflict of interest. Let us add that differences between colonies of ants were observed in the course of our studies. This incites us to underline that individual and/or ethnic differences may exist in humans about the tolerance of some drug or food additive. For example, persons suffering from phenylketonuria cannot consume aspartame, and Asian people have a high probability of suffering when cared of with carbamazepine because they may present a genetic mutation.

A last point to mention again is that many artificial substances produced and consumed by humans are nowadays present in natural water, and affect the physiology and the behavior of animals. This is the case for fluoxetine [39], carbamazepine [40], sucralose [41], three substances here shown to affect ants, and proved by other researchers to impact aquatic animals' biology. It is also the case for hormonal derivates which disrupt endocrinal processes, change the sex-ratio, affect sexual and reproductive behavior, or cause impairment of development resulting in physical malformations in populations of aquatic invertebrates [42,43]. Controlling consumed water and improving depurative methods must be a present objective all over the world.

CONCLUSION

Humans need potions, food additives, food complements for caring of themselves or enhancing their life style. However, these 'new' substances may not only have the researched wanted effect but also other *a priori* unknown adverse ones. Any such substance, even if appearing inoffensive, should be examined without conflict of interest, by using biological models, before being proposed for caring of humans or animals. Our studies on ants showed that these insects, easily maintained in laboratory, at low cost and without interruption, can be good biological models. They offer large samples, precise assessments, plenty of analyzable physiological and ethological traits, and no ethic problems. Studies on ants should be a valuable first step in the study of a substance, before experimenting on other animals such as rats, mice, monkeys, and finally humans. During our studies, we could confirm already known harmful effects of several substances, we could even more precisely describe them (example: the dependence on nicotine), and we could explain some of them (example: behavioral disruptions due to the fact that sweeteners are not true glycosides though having nice sugared taste). We could also reveal previously unknown (or not divulgated) adverse effects of substances (example: aggressiveness under fluoxetine or alprazolam diet; decrease of middle and long term memory under alprazolam diet), and brought new information on some substance (example: the duration of the effect of quinine allows the sexual reproduction of *Plasmodium*, and so the transmission of malaria to other humans). Science and knowledge progress step by step, and every even small discovery, on any model, may be useful and sometimes very important if honestly obtained.

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