Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 5 Issue 5 May 2021

Research Article

Amitriptyline: Physiological and Ethological Effects of a Largely Used Antidepressant Evaluated on Ants as Biological Models

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Received: March 01, 2021

Published: April 06, 2021

Abstract

The impact of the largely used ATC antidepressant drug amitriptyline on several ethological and physiological traits was examined on ants used as biological models. This drug appeared to affect the ants' food intake, general activity, locomotion, orientation ability, tactile perception, social interactions, cognitive abilities, as well as learning and memory skills. The ants slowly adapted themselves to the effect of amitriptyline on their locomotion, and became slightly habituated to the effect of the drug on their tactile perception. They developed no dependence on amitriptyline consumption though being at the limit of a significant threshold. After weaning, the effect of the drug slowly linearly decreased, becoming statistically lower than the initial level after about 15 hours and no longer differing from the control level after 36 hours. The effect rather rapidly decreased during the last 6 hours after weaning. Such a decrease accounted for the non dependence on the drug consumption. A large individual variability was observed for some of the examined traits. Though being safer than other antidepressants (among others, the IMAOs), the consumption of amitriptyline may cause health problems. Patients should consume it by taking widely spaced doses, and should be monitored as for their food intake, social interactions, cognition, memory, and habituation to the positive effect of amitriptyline.

Keywords: Cognitive Ability; Memory; *Myrmica sabuleti*; Redomex®; Social Interactions

Abbreviations

ang.deg.: Angular Degrees; ang.deg./cm: Angular Degrees per cm; mm/s: Millimeter per Second; χ2: Chi-Square; *vs*: Versus; n°: Number; cm: Centimeter; mm: Millimeter; ml: Milliliter; µl: Microliter; mg: Milligram; s: Second; min: Minute; h: Hour; t: Time; %: Percentage.

Introduction

Antidepressants are among the most consumed drugs in the world, especially in several middle-class communities and in very populated towns [1]. Such consumption is nowadays increasing [2]. There exist four main kinds of medicinal antidepressant drugs, plus several other drugs with specific mode of action (Table 1) [3]. Most of SSRI (inhibitors of the serotonin recapture) have fluoxetine as active substance; one example of commercialized product is Prozac. We have studied the effects of this drug on ants used as biological models six years ago, and found it was the most toxic one among the three antidepressant class of molecules we then examined (see here below) [4]. We have also studied the effects of another SSRI antidepressant drug, paroxetine, and found

that this drug impacts many important physiological and ethological traits of ants. Due to similarities in the functional mechanisms of basic neuronal processes in insects' neural system and human brain, we concluded that this antidepressant should be cautiously used, in small amount, during a short time and under medical supervision [5].The SRNI (inhibitors of the recapture of serotonin and noradrenalin) have venlafaxine as active substance; one example of commercialized product is effexor. We have studied the effects of this drug on ants as biological models and found it was less toxic than fluoxetine, though more toxic than the third kind of antidepressant class of molecules we examined (see here below) [6]. The TCA drugs (tricyclic antidepressants) inhibit several neurotransmitters including serotonin, noradrenalin and dopamine; the active substance of most of the drugs belonging to this class is clomipramine hydrochlorid; one example of commercialized products is anafranil. We have studied the effects of this drug, again on ants as biological models, and found it was the less toxic one among the three antidepressants we previously examined [6]. The MAOI drugs (inhibitors of monoamine oxidize), the fourth main class of antidepressant molecules, are given to severely depressed patients and only under strong monitoring [7].

The consumption of antidepressant drugs may still nowadays increase due to several humans' emotional and economical troubles resulting from the long lasting worldwide covid-19 pandemic. More and more antidepressant drugs are presently researched; efforts are made for obtaining efficient ones, with minimal side effects. Generally, they are used under prescription with short delay after their introduction in the market. However, the mode of action of some of these antidepressants is still not well known [8-10]. Their use is based on a hypothesis which has not yet been confirmed: depressions would be due to some loss of equilibrium between the activity of several neurotransmitters in the brain (e.g. serotonin, noradrenalin, and dopamine) [3,11-13]. Generally, antidepressants are not very efficient or at least not efficient over a long period of time. Their efficiency varies according to the patients [14]. Often, during clinical trials, one third of the patients are well cared for their depression thanks to the use of the examined antidepressant drug, one third feels only slightly better, and one third presents no health amelioration [15]. Ten actually used antidepressants appear to be the most efficient ones [14]. Their active compound, and one commercial denomination of them given as an example, are: amitriptyline (Laroxyl), mirtazapin (Norset,), duloxetin (Cymbalta), venlafaxine (Effexor), paroxetin (Deroxat), milnacipran (Ixel), fluvoxamine (Floxyfral), escitalopram (Seroplex), nefazodone (Serzone), vortioxétine (Brintellix). On the basis of this list, and the severity of their physiological and behavioral effects we estimated thanks to our previous studies [4,5,7], we hypothesis that the best option, in terms of ratio effectiveness *vs* risks of harmful side-effects, could be amitriptyline. It is the number one of the here above list of antidepressants ranked according to their efficientcy, and it is an TCA antidepressant, the less toxic antidepressant category found during our previous studies [4,5,7]. Furthermore, amitriptyline is not listed in the antidepressants presenting the more severe side effects [www.psychomedia.qc.ca>antidepresseurs>plus dangeureux]. Moreover, amitriptyline is given not only to adults but also to children, this being susceptible to hypothesize that this drug is not very toxic. We also took knowledge of the work of Cipriani A., *et al.* for making our choice [16]. We thus intended to examine, on ants used as biological models, the ethological and physiological effects of amitriptyline. Here below, we report some information available on this drug, we explained why we used ants as biological models, which species we used and summarize what we know on it, and we list the different physiological and ethological traits the effect of amitriptyline on which we aimed to examine. In the following 'Material and Methods' section, we briefly describe our experimental protocols since they are identical to those used in our previous studies [references given below]. We then relate and comment our results, compare them with those of other researchers and conclude.

Information available on amitriptyline

We affirm having looked to detailed information about side effects of amitriptyline only after having completed our experimental work which we thus conducted being blind to these potential effects. Most of useful information about these side effects can be found in the notice joined to the drug package and on internet sites [17-20]. In Belgium, the commercial drug the active substance of which is amitriptyline is labeled 'Redomex®'. The manufacturer is H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Danemark, and its sale was authorized by Lundbeck s.a. - Stephanie Square Centre - Avenue Louise 65/11 - 1050 Bruxelles. Amitriptyline is a tricyclic antidepressant (TCA - Table 1) which inhibits the recapture of several neurotransmitters including serotonin, noradrenalin and dopamine. It is used as an antidepressant (at doses of 75-200mg per day for adults and 30-100mg for children), as well as for treating

Kinds of antidepressants	Active substances	Name of medicines
SSRIs: selective serotonin reuptake inhibitors	Citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, sertraline …	Celexa, Lexapro, Prozac, Luvox, LuvoxCR, Paxil, Zoloff …
SRNIs: serotonine and noradrenaline reuptake inhibitors	Duloxetine, venlafaxine, desvenlafaxine, milmacipram, levo-milnacipran …	Pristiq, Cymbalta, Effexor, Savella, Fetzima …
TCAs: tricyclic antidepressants; several neurotransmitters including serotonine and noradrenaline reuptake inhibitors	Amitriptyline, desi-pramine, doxepin, nortriptyline, clomipramine, protriptyline …	Elavil, Redomex, Norpramin, Sinequan, Pamelor, Anafranil, Vivactil …
MAOIs: monamine oxidase inhibitors	Phenelzine, selegiline, tranylcypromine …	Nardil, Emsam, Parnate …
+ Atypical antidepressants, each one with a unique mechanism of action	Bupropion, mirtazopine, trazodone, vilazo-done …	Wellbutrin, Remeron, Desyrel, Viibryd …

Table 1: Kinds of antidepressants, their active substance, and some of such commercial medicines.

Source: www.rxlist.com>drug-class; previously and here studied by us on ants as models

children's urinary-incontinency troubles (at doses of 25-50 mg per night), and also for treating some neuropathic pains. The most often observed adverse side effects are, among others: suicidal ideas, behavioral impairments, digestive problems, tiredness, confusion, dry mouth, agitation, headache, taste alteration, thirst [notice for use joined to the drug package]. Health problems may occur if amitriptyline is consumed together with several other drugs [again in the notice of the drug use]. Though the available lists of observed side effects are long and detailed, it could be of interest to gather more insight on the potential effects of amitriptyline on animals' ethological and physiological traits, as there are large similarities in the functional mechanisms of basic neuronal processes in animals' neural system and human brain. We aimed to do so, using ants as biological models, by exploring the effects of this drug on thirteen ants' physiological and ethological traits as well as by evaluating their adaptation to adverse effects, habituation to effects, and dependence on its consumption, and finally by studying the decrease of the effect of amitriptyline after weaning.

Why using ants as biological models

Most basic biological (cellular and inter-cellular) processes are quite similar in all animals including humans. Many vertebrates and invertebrates are thus used as biological model organisms [21,22]. Invertebrates are particularly used because they are small, are easily maintained in a laboratory, and have a short life cycle [23]. Among them, insects are often used, above all the fruit flies and the social hymenoptera [24]. Ants can thus be used as biological models, the more so since they present many sophisticated biological traits, e.g. they navigate using learned cues, recruit nestmates, differently mark parts of their territory, take care of their brood, build complex nests, clean them and manage cemeteries at the frontiers of their territory [25].

Which species we used and what we know on it

We are accustomed to work on *Myrmica sabuleti* Meinerts 1861, a species the biology of which we studied since a long time. We know its recruitment strategy, visual perception, navigation system, visual and olfactory conditioning ability [26], and the ontogenesis of several of their capabilities [27]. We have shown that they recognize themselves in a mirror, become imprinted at their emergence to the appearance of the front head of their congeners, learn their alarm reaction and trail following behavior during their first year of life in the presence of older congeners [27,28], natively possess a number line, acquire the notion of zero through experiences, and can acquire and use numerical symbolisms [29,30]. The distance and size effects as well as the Weber's law can be applied to their perception and reactions [31,32]. They can expect the time and the location of the next food availability [33,34], as well as if the amount of elements present aside this food will be larger or smaller [35]. All their cognitive abilities however always stay at a concrete level and never reach an abstract one.

Which traits we intended to examine

We intended to study the impact of amitriptyline on the ants' food consumption, general activity, locomotion, orientation ability, audacity, tactile perception, social interactions, cognitive capabilities, reaction in a stressing situation, learning ability and memory, as well as the adaptation to an adverse effect of the drug, the habituation to a positive effect, the dependence on its consumption, and the decrease of its effect after its consumption was stopped. Our experimental protocols were identical to those previously used [36-38]. Therefore, we here only briefly explain them, being however unable to avoid inevitable plagiarism. Let us recall that we made our experiments being blind to the available information on harmful side effects of amitriptyline.

Materials and Methods

Collection and maintenance of ants

We performed our experiments on two colonies of *M. sabuleti* collected in September 2019 in an abandoned quarry located at Olloy-sur-Viroin (Belgium, 50° 04' north, 4° 36' east). Each of these colonies contained about 500 workers, a queen and brood. They were maintained in the laboratory in one to three glass tubes, half-filled of water, a cotton plug separating the ants from the water. The nest tubes of each colony were deposited in a tray (34cm x 23cm x 4cm) which served as foraging area. In them, a cotton-plugged tube containing a sugar water solution was permanently provided as a source of water and sugared food, and pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) were delivered three times per week as a protein food source. The lighting of the laboratory was *ca* 330 lux, the temperature *ca* 20°C, the humidity *ca* 80%, and the electromagnetic field *ca* 2 μWm2, these environmental conditions being adequate for the used species. The ants were here often named 'workers' or 'nestmates' as do researchers on social insects.

Solution of amitriptyline given to the ants

A package of 100 tablets of Redomex® 10mg (BE048736), (produced by H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Danemark; authorized by Lundbeck s.a. - Stephanie Square Centre - Avenue Louise 65/11 - 1050 Bruxelles), was furnished by the pharmacist Wera (Brussels, Belgium). Each tablet contained 10mg of the active substance, amitriptyline. Adults patients offered to consume this drug are advised to intake 25 - 150mg of Redomex® per day. Let us consider that they intake 100mg Redomex® per day. As mammals, they daily consume about one liter of water (not considering water included in food items). Due to their morphology and physiology, insects drink about ten times less water than mammals. Consequently, to set ants under a Redomex® diet similar to that of humans, they must be provided with a solution of 100mg Redomex® in 100ml of water. Having only two experimental nests and thus two tubes which could contain 5ml of sugar water, we prepared a solution of 30mg Redomex®, i.e. of 3 drug tablets, in 30ml of this sugared liquid. Three tablets of Redomex® 10mg were crushed before being dissolved into these 30ml sugar water and the obtained solution was delivered to the ants in their usual cotton-plugged tubes (Figure 1). The plug of these tubes was refreshed every 2-3 days, and the entire solution was renewed every 7 days. It was checked two to three times per day if the ants drunk the provided solution, and they effectively did so. We firstly made the control experiments on the two colonies maintained under normal diet (proteins and sugared water, without added antidepressant drug). Then, we replaced the ants' sugar water by the aqueous sugared solution of Redomex®, and we started conducting the test experiments after the ants had consumed the drug during one day.

Figure 1: Successively chemical structure of amitriptyline (A), Redomex® package (B), realization of the solution given to the ants and ants drinking this solution (C to G).

Food consumption and general activity assessment

While ants were under normal diet and in a second phase of the experimental process, while they were under a diet with Redomex®, we separately counted the nestmates present on their

meat food, at the entrance of their sugar water tube, and being active at any place of their environment (the foraging area, the nest entrance, the inside the nest). We made these punctual count four times during the day and two times during the night (total = 6 counts x 2 colonies = 12 counts per day) during 6 days, each day at the same time o'clock. For each kind of diet and each raw of count, we established the daily mean of these 12 counts (Table 2, lines 1 - 6). At the end of this assessment, we calculated the mean of the six daily means for each kind of diet and each raw of count (Table 2, last line). For each kind of count (meat intake, sugar water intake, general activity), the six daily means obtained for ants consuming Redomex® were compared to the six daily means obtained for ants under normal diet using the non-parametric test of Wilcoxon, the level of significance being set at P = 0.05 [39].

Locomotion analysis, orientation assessment

This analysis and assessment was performed on ants freely moving in their foraging area. We recorded their linear and angular speeds without stimulating them, and their orientation while stimulating them with a nestmate tied to a piece of paper (Figure 2, A). A nestmate placed in such a stressful situation emits its attractive mandible glands alarm pheromone, thus stimulating the observed nestmate. For assessing the ants' speeds as well as for assessing their orientation, 40 trajectories were recorded and analyzed using appropriate software [40] and using the following definitions. The linear speed (in mm/s) is the length of a trajectory divided by the time spent to travel it; the angular speed (in ang.deg./cm) is the sum of the angles made by successive adjacent segments, divided by the length of the trajectory; the orientation (in ang. deg.) to a location is the sum of successive angles made by the direction to the location and the direction of the trajectory, divided by the number of measured angles. A value lower than 90° indicates that the animal tends to orient itself toward the location; a value higher than 90° indicates that it tends to avoid the location. For the linear speed, the angular speed, and the orientation, the median and quartiles of the 40 obtained values were established (Table 3, lines 1, 2, 3). The distributions obtained for ants consuming Redomex® were compared to those obtained for ants under normal diet (= control) by using the non-parametric χ^2 test [39].

Audacity assessment

This assessment was made by depositing, in the ants' foraging area, a cylindrical tower (height = 4cm; diameter = 1.5cm) tied to a squared platform (9 cm2), both in Steinbach® white paper, and by counting 10 times over 10 minutes the ants present on this apparatus (Figure 2, B). The mean and the extremes of the recorded numbers were established (Table 3, line 4). The numbers obtained for the two colonies were pooled and those obtained during every two successive minutes were added. The five sums obtained for ants under amitriptyline diet were compared to those obtained for ants under normal diet (= control) by using the non-parametric test of Wilcoxon [39].

Tactile perception evaluation

When perceiving the rough character of a substrate, the ants change their locomotion and walk with difficulty, slowly, sinuously, and often touch the substrate with their antennae (Figure 2, C1). They thus modify their behavior in response to a tactile stimulus. When poorly perceiving such a rough character, e.g. being affected by a neuronal active drug, the ants walk on the substrate more easily, more quickly and less sinuously. Consequently, for assessing the ants' tactile perception, we quantified their linear and angular speeds while they walked on a rough substrate. A duly folded piece (3cm x 2 + 7 + 2 = 11cm) of n° 280 emery paper was set in a tray (15cm x 7cm x 4.5cm) in order to divide it into three zones, i.e. a 3cm long one, a 3cm long one covered with the emery paper, and a 9cm long zone. Experimenting on a colony consisted in depositing 12 nestmates in the 3cm long zone of the apparatus, and in assessing their speeds when they moved onto the rough substrate (as explained in the subsection relative to linear and angular speeds). Since two colonies were used, 24 values of linear and angular speeds were obtained, and for each kind of speed, the median and quartiles of the recorded values were calculated (Table 3, lines 5, 6). The distributions obtained for ants consuming amitriptyline were compared to those obtained for ants under normal diet (= control) by using the non-parametric χ^2 test [39].

Brood caring

For each colony, a few larvae or nymphs were taken out of the nest and deposited near the nest entrance. For each colony, five of these taken out larvae were observed during five minutes (the total number of observed larvae equaled 10). We worked only on five larvae for each colony because we had to be able to track them simultaneously during 5 minutes. We did not repeat the experiment because the latter largely perturbed the colony. We cautiously observed the ants' behavior in front of these 2 x 5 tracked larvae (Figure 2, D). For each colony, the larvae among the five tracked

ones not re-entered in the nest after 30 seconds, then 1, 2, 3, 4 and 5 minutes were counted, and the six corresponding numbers obtained for the two colonies were added (Table 4, line 1). The six sums obtained for ants consuming amitriptyline were compared to the six sums obtained for ants under normal diet (= control) using the non-parametric test of Wilcoxon [39].

Social interactions estimation

For assessing the potential impact of amitriptyline on the usually peaceful social interactions between individuals belonging to the same colony, five ants' dyadic encounters were conducted for each colony (total of encounters = 10). Each encounter occurred in a cup (diameter = 2cm, height = 1.6cm, the borders being lightly covered with talc to prevent the ants from escaping). During each dyadic encounter, one ant of the pair was cautiously observed during 5 minute. Its behavior 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), or tried to sting or stung the other ant (level 4) (Figure 2, E). The corresponding numbers obtained for each ants and each used colony were added (Table 3, line 2). The five added numbers obtained for ants consuming amitriptyline were compared to those obtained for ants not consuming this drug (= control) using the non-parametric χ^2 test [39]. In addition, for each kind of diet, the ants' social relationship was also characterized by a variable, 'a', equaled to the number of aggressiveness levels 2 + 3 + 4 divided by the number of aggressiveness levels 0 + 1 (Table 4, line 2).

Cognition estimation

For assessing this trait on one colony, 15 ants were transported into a tray (15cm x 7cm x 4.5cm) into which two duly folded pieces of paper (Steinbach®, 12cm x 4.5cm) had been inserted, creating so a twists and turns path between two spaces, a 2cm long one in front of the "difficult" path materialized by the device, and a 8 cm long one beyond the device. The 15 ants were deposited in the area lying in front of the twists and turns path (= the device) (Figure 2, G). Since the precise moment at which the nestmates were deposited in front of the device, for each two colonies, the ants still in front of the difficult path and those having reached the area lying beyond it were counted after 2, 4, 6, 8, 10 and 12 minutes. The corresponding numbers obtained for the two colonies were added (Table 4, line 3). For the ants counted in front the difficult path as well as for those counted beyond this path, the numbers obtained for ants consuming amitriptyline were compared to those obtained for ants under normal diet (= control) thanks to the non-parametric Wilcoxon test [39].

Escaping ability assessment

For making this assessment, for each colony, six ants were enclosed under a reversed polyacetate cup (height = 8cm, bottom diameter = 7cm, ceiling diameter = 5cm, the inside surface being slightly covered with talc) deposited in their foraging area. A notch (3mm height, 2mm broad) had been managed in the rim of the bottom of this enclosure so that the ants could escape (Figure 2, F). Being imprisoned triggers stress. The enclosed ants should thus regain their calm, inspect the rim of the enclosure, discover the notch and use it for escaping. The here made assessment concerned thus the ants' stressing and a cognitive ability. For each colony, the ants escaped after 2, 4, 6, 8, 10 and 12 minutes were counted and the corresponding numbers obtained for the two colonies were added (Table 4, line 4). The six sums obtained for ants consuming amitriptyline were compared to those obtained for ants living under normal diet using the non-parametric Wilcoxon test [39].

Conditioning acquisition and memory assessment

For each colony under amitriptyline diet, at a given time, a green hollow cube made in strong paper (Canson®) was deposited above the entrance of the sugar water tube and the pieces of *T. molitor* larvae provided as protein food source were set near that tube. Having done so, the ants underwent operant visual conditioning, as described in previous studies [28-30]. For this test, the experiment on ants under normal diet (= the control experiment) had been previously made on another colony because when an individual becomes conditioned to a stimulus, it kept its conditioning during a rather long time (in the present case, two to three days) and even after it has lost its conditioning, it more quickly than initially acquires it again. It can thus no longer be used for assessing its conditioning acquisition under another experimental condition (here, a diet with amitriptyline) before a very long time (the time needed for complete disappearance of the memory of the cues presented during the initial conditioning process). The results of this previously made control experiment can be found in [41]. After the deposit of the green cube, the ants were tested over their conditioning acquisition, then the cubes were removed from the ants' foraging area, and the ants were again tested this time over their loss of conditioning. The tests were conducted in a Y-apparatus made of strong white paper, its sides having been slightly covered

with talc, and deposited in a separated tray for each colony. Each Y-apparatus was provided with a green hollow cube in one of its branch. Half of the tests were made with the cube in the left branch and the other half with the cube in the right branch. For making a test on a colony, 10 ants were transferred one by one in the area of the Y-apparatus lying in front of its division into two branches. The ants' first choice of one or the other branch of the Y-apparatus was recorded (Figure 2 H). Choosing the branch containing the green cube was considered as giving the correct response. After having been tested, each ant was deposited into a cup until 10 ants of its colony were tested. After the test, the 10 ants were transferred back into their foraging area, near their nest entrance. The responses of the ants of the two colonies were added (n = 10 x 2 = 20 responses) and the proportions of correct responses (= the conditioning scores) obtained over time were established (Table 5). The numbers of correct responses obtained during the six tests made while the ants consuming amitriptyline acquired conditioning, and those obtained while they loss their conditioning were compared to the corresponding previously obtained numbers of responses of ants belonging to another colony maintained under normal diet by using the non-parametric Wilcoxon test [39].

Figure 2: Some views of the experiments. 1: under normal diet (control), 2: under amitriptyline diet; A: ants moving to a tied nestmate, doing so poorly under the drug diet. B: ants coming onto an unknown apparatus, somewhat less while consuming amitriptyline. C: ants walking on a rough substrate with difficulty under normal diet, more frankly under the drug diet. D: an ant under normal diet holding a larva, and an ant under the drug diet not doing so. E: two nestmates presenting some aggressive posture while consuming amitriptyline, and not while living under normal diet. F: an enclosed ant escaping while living under normal diet, and not doing so while consuming amitriptyline. G: ants having crossed a twists and turns path while living under normal diet, and unable to do so while consuming the drug. H: ants trained to a green cube giving the wrong response (left photo) and the right one (right photo). I: ants under amitriptyline not preferentially choosing the tube containing this drug (red dot) and presenting thus no strong dependence on amitriptyline consumption.

Adaptation to physiological and ethological effects

An individual adapt itself to a drug or a situation when it becomes less and less affected by this drug or situation over its use / over the time. For studying adaptation toward amitriptyline, a trait impacted by the drug must be examined after one or two days of consumption, then again after several days of consumption and the results of the two tests must be compared. Here, a trait largely impacted by amitriptyline consumption and easily precisely assess-

able was the ants' locomotion. Therefore, for each colony, the ants' linear speed and angular speeds were again assessed after the ants consumed the drug during eight days, exactly in the same way as they had been assessed after two days of consumption. For each speed, the median and quartiles of the recorded values were established (Table 6, upper part), and the distributions of the values were compared to the corresponding ones obtained after two days of amitriptyline consumption as well as obtained for ants living under normal diet (= control) by using the non-parametric Wilcoxon test [39].

Habituation to expected effects

An individual adapts itself to a drug or a situation when it becomes less and less sensitive to the expected effect of the drug or the situation over time. For studying habituation, a trait favorably affected by the drug or the situation must be examined one or two days after that the individual uses the drug or experiences the situation, and then again after it uses/experiences them for several days, and the results of the two exams must be compared. In the present work on ants, amitriptyline appeared to decrease the tactile perception what may be an expected effect of the drug. Consequently, this trait was assessed after the ants consumed amitriptylyne for 4 days, then again for 12 days, and the results of the two assessments were compared (Table 6, middle part).

Dependence on amitriptyline consumption

An individual becomes dependent on a drug or a situation when it wants continuing using/experiencing this drug or situation, when it enjoys doing so, and finally when it can no longer live without trying to use/experience them. Here, the ants' dependence on amitriptyline was studied after the ants had consumed the drug during 10 days. Then, on the basis of this first study, a second test was made after the ants had consumed the drug for 13 days. At the 10^{th} and the 13^{th} days, for each two colonies, 15 ants were transferred into a tray (15cm ×7 cm × 5cm) in which two cotton-plugged tubes (h = 2.5 cm, diam. = 0.5 cm) had been set, one filled with sugar water, the other filled with the sugar solution of amitriptyline used all over the experimental work. The tube containing the drug was set on the right in one tray and on the left in the other tray (Figure 2 I). During 15 minutes, the ants of each colony present at the entrance of each tube were counted 15 times, and the 15 corresponding counts obtained for the two colonies were added (Table 6, lower part). These sums allowed calculating the proportion of ants who choose the drug-free solution and who choose the solution containing the drug. In addition, the total numbers obtained for one and the other solutions were compared to the two numbers expected if the ants randomly visited the two presented tubes, using the non-parametric χ^2 goodness-of-fit test [39].

Decrease of the effect of amitriptyline after its consumption was stopped

This decrease was examined after the ants had consumed amitriptyline during 14 days, and the study of the decrease of effects was based on the impact of the drug on the ants' angular speed. The ants received a fresh solution of amitriptyline 12 hours before starting the study of the decrease of the drug effect after weaning. After these 12 hours under amitriptyline diet, the ants' angular speed was assessed just like it had been assessed after one and eight days of the drug consumption, except that not 40 but only 20 trajectories were analyzed in order to be able to finalize the assessments all along the loss of the impact of amitriptyline on the ants' angular speed. After this assessment made at t = 0h, weaning started, i.e. the tubes filled with the sugared solution of amitriptyline were replaced by tubes filled with the usual drug-free sugared solution. From this time, the ants' angular speed was assessed every three hours until it became similar to that of ants maintained under normal diet. The distribution of the values of angular speed obtained over time were compared to that obtained at t = 0 as well as to that previously obtained for ants under normal diet using the non-parametric χ^2 test for independent samples [39]. The numerical and statistical results are given in Table 7, and are also illustrated in Figure 3. The mathematical function describing the best the change of angular speed over the loss of the effect of amitriptyline was also approximately established. It is given in the results section.

Results and Discussion

Meat food consumption, sugar water intake, general activity

These three traits were impacted by amitriptyline consumption (Table 2). While consuming this drug, the ants eat less meat, drunk less sugar water, and were in general less active than when living under normal diet. These three decreases were statistically significant (p-value for significant effect fixed at p = 0.05) (each time: N = 6, T = -21, P = 0.016). Amitriptyline appeared thus to have a marked effect on ant's basic physiological processes.

Days	Normal diet			Diet with amitriptyline		
	Food intake	Sugar water intake	Activity	Food intake	Sugar water intake	Activity
1	1.75	1.75	8.00	0.75	-0.50	7.25
2	1.25	1.25	7.75	0.50	0.50	7.00
3	1.75	1.50	7.25	0.75	0.25	6.50
4	1.50	1.75	7.25	0.25	0.80	6.25
5	1.50	2.00	7.50	0.50	0.50	6.75
6	1.25	1.75	8.00	0.50	0.25	7.50
1 - 6	1.50	1.67	7.63	0.54	0.42	6.86

Table 2: The table gives the daily mean numbers of ants eating meat, drinking sugar water and being active, as well as the overall means. Impact of amitriptyline on the ants' food consumption and general activity. Amitriptyline affected these three traits. Details and statistics are given in the text.

Linear and angular speeds

These traits were impacted by amitriptyline consumption. While consuming this drug, the ants walked more slowly and more sinuously than usually (Table 3, lines 1, 2). This was quite obvious to the observers and was confirmed by the statistical analysis of the results: linear speed: $\chi^2 = 55.00$, df = 2, $P < 0.001$; angular speed: $\chi^2 = 50.73$, df = 1, $P < 0.001$. On the basis of visual observations only, i.e. observations having not been numerically assessed, it seemed that the young ants, easily recognizable by their light color, were more affected than the old ones by the pointed out impact of amitriptyline on their locomotion. After the ants had consumed amitriptyline during eight days, it was examined if they adapted themselves, at least to some extent, to the here revealed effect of the studied drug (see the subsection relative to adaptation).

Orientation

This trait was impacted by amitriptyline consumption: ants consuming this drug did not well orient themselves towards a tied nestmate. This was quite obvious to the observers (Figure 2 A1, A2), and was confirmed by the numerical results (Table 3, line 3) and the statistical analysis ($\chi^2 = 41.10$, df = 2, $P < 0.001$). Such an effect probably resulted at least partly from the ants' large sinuosity while consuming amitriptyline, but may also be due to a decrease of their "olfactory" (= chemical) perception (or their overall perception) under that drug diet, a presumption checked partly through a following experiment (see the subsection relative to tactile perception).

Audacity

This trait was slightly (but not significantly) impacted by amitriptyline consumption (Table 3, line 4; Figure 2 B1, B2), the recorded values being at the limit of significance level (N = 4, T = 10, P = 0.063), maybe due to the smallness of the samples. During this experiment, a non assessed behavior was observed: some ants appeared to be reluctant in coming onto the risky unknown apparatus while a few other ones walked onto it, climbed on the tower with no hesitation, apparently 'ignoring' the 'danger' of the situation. Some variability may thus exist between the individuals' reaction to amitriptyline consumption. For better approaching this presumption, the ants were very carefully observed during the following experiments relative to tactile perception, brood caring behavior, social interactions, and cognition capabilities (see below the subsections concerning these four traits).

Traits	Normal diet	Diet with amitriptyline
Linear speed (mm/s)	10.7 (10.0 - 11.7)	7.1 (6.4 - 8.2)
Angular speed (ang.deg./cm)	116 (101 - 131)	226 (202 - 250)
Orientation (ang.deg.)	36.5 (28.4 - 45.7)	67.8 (58.9 - 83.2)
Audacity (n°)	2.10 [1 - 3]	1.15 [0 - 2]
Tactile perception:		
Linear speed	4.1 (3.6 - 4.7)	9.4 (8.1 - 10.3)
Angular speed	271 (245 - 310)	181 (145 - 195)
on a rough substrate		

Table 3: Effect of amitriptyline on five ants' physiological and ethological traits. The table gives the median (and quartiles) or the mean [and extremes] of the recorded values. Amitriptyline decreased the ants' linear speed, increased their sinuosity of movement, their orientation, somewhat their audacity, and largely their tactile perception. More information and statistics can be found in the text.

Tactile perception

Amitriptyline impacted this trait. The ants consuming the drug walked on a rough substrate more rapidly and less sinuously (i.e. anthropomorphically speaking, more easily, more frankly) than ants living under normal diet. This change was quite obvious to observers (Figure 2 C1, C2), pointed out by numerical results (Table 3, lines 5, 6), and confirmed by the statistical analysis (linear speed: $\chi^2 = 46.16$, df = 1, $P < 0.001$; angular speed: $\chi^2 = 25.04$, df = 2, $P < 0.001$). Such a result was in agreement with that relative to the ants' orientation ability (see the subsection concerning this trait): amitriptyline may thus affect (i.e. reduce) the individuals' sensitive perception, at least the perception of chemical signals and of the roughness of a substrate.

Brood caring

Amitriptyline impacted the ants' brood caring behavior. While ants under normal diet (instinctively) rapidly re-entered the larvae removed from the nest, those consuming the drug did not do so, or largely delayed in doing so. They approached the larvae with delay, they seldom immediately hold them, they walked with difficulty while holding a larva, they not easily found the nest entrance, all this leading to a poorly efficient rate of re-entering the larvae in the nest (Table 4, line 1; Figure 2 D1, D2). The difference between the numbers of not re-entered larvae over time by ants living under drug-free diet and drug diet was statistically significant (N = 6, T = -21, P = 0.016).

An event never observed in ants under drug-free diet occurred several times during the present experiment: two ants belonging to a colony under drug diet tried to hold the same larva, pooling each one on it. This may indicate some impairment of the normally peaceful social relationships/interaction between nestmates, a presumption the following experiment examined (see the following subsection).

Social interactions

Amitriptyline impacted the ants' social interaction. This was quite obvious to the observers during the experimental dyadic encountering (Figure 2 E1, E2). While ants living under normal diet were never aggressive against their nestmates, stayed aside doing nothing, contacted each other with their antennae, and seldom slightly opened their mandibles, the ants consuming amitriptyline often largely opened their mandibles when encountering an opponent nestmate. The numbers of the different levels of aggressiveness occurrence (as well as the value of the variable 'a') differed between the ants maintained under one and the other kinds of diet (Table 4, line 2). This difference was statistically significant: $\chi^2 = 66.49$, df = 2, $P < 0.001$. Such a result was in agreement with the impact of amitriptyline on the ants' brood caring behavior (see the above subsection).

Cognition

The ants' ability in crossing a twists and turns path was impacted by amitriptyline consumption (Figure 2 G1, G2; Table 4, line 3). Under normal diet, 7 ants could cross such a path over 12 experimental minutes. While consuming amitriptyline, none could do so over the same time period. The difference between the ants living under either the one or the other kind of diet was significant: numbers of ants in front of the difficult path: N = 6, T = +21, P = 0.016; numbers of ants beyond this path: N = 4, T = 10, P = 0.063). Such an impact of the drug on the ants' physiology was also examined thanks to the two following experiments, i.e. those relative to the ants' escaping ability and conditioning acquisition.

Escaping ability

Amitriptyline impacted the ants' ability to escape from an enclosure (Table 4, line 4; Figure 2 F1, F2). Under normal diet, enclosed ants firstly walked erratically all round the enclosure, then became calmer and walked essentially along the rim of the enclosure. They found the notch managed in the rim and went out of the enclosure using it. While consuming amitriptyline, the ants also firstly walked everywhere erratically, then became calmer and essentially walked along the rim, moving thus in front of the notch. However, they very often walked in front of the notch without turning and going through it for escaping. It seems that the ants did not perceive the notch or did not "guess" that, thanks to it, they could escape. The number of ants escaped over time statistically differed between the ants consuming or not amitriptyline (N = 6, T = -21, P = 0.016). This result was somewhat in agreement with that obtained for the ants' cognition (see the above subsection): the two results revealed some cognitive impairment possibly caused by amitriptyline consumption. This conclusion was again checked thanks to the following experiment relative to conditioning acquisition and memory (see the following subsection).

Traits	Normal diet	Diet with amitriptyline
Brood caring: n° of larvae among 10 not re-entered over time	30s 1 2 3 4 5 min 8 7 6 5 2 0	30s 1 2 3 4 5 min 12 12 10 10 8 6
Level of aggressiveness against nestmates and n° of cases	0 1 2 3 4 'a' 56 47 11 0 0 0.10	0 1 2 3 4 'a' 16 26 64 1 0 1.55
N° of ants in front and beyond a twists and turns path over time	time: 2 4 6 8 10 12min in front: 21 19 16 14 12 11 beyond: 0 0 1 3 5 7	time: 2 4 6 8 10 12 min in front: 29 23 21 19 17 16 beyond: 0 0 0 1 1 1
N° of ants among 12 escaped over time	time: 2 4 6 8 10 12 min n° ants: 2 4 5 7 9 12	time: 2 4 6 8 10 12 min n° ants: 0 1 1 1 3 3

Table 4: Impact of amitriptyline on four ants' physiological and ethological traits.

Details are given in the text. Briefly, amitriptyline affected the ants' social interactions (lines 1, 2), and their cognition (lines 3, 4).

6.10. Conditioning acquisition, memory

Amitriptylyne largely reduced the ants' ability in acquiring operant conditioning (Table 5). Under normal diet, ants easily acquired conditioning, reaching a score of 70% as soon as after 31 hours and of 80% after 72 hours of training. In contrast, the ants consuming amitriptyline never acquired conditioning, a statistically significant observation (N = 6, T = -21, P = 0.016). Their cognitive skills and/or short-term memory were thus perhaps impacted by the drug. Since the ants learned nothing over 72 training hours, we could not assess their middle and their long-term memory. However, we observed that ants walked for very long time all around their foraging area before finding their nest entrance: their long-term memory might thus be somewhat affected by amitriptyline consumption (personal observation, not assessed).

Adaptation to physiological and ethological effects

Some slight and slow adaptations seemed to occur to the impact of amitriptyline on the ants' locomotion (Table 6, upper part). After 8 days on that drug diet, the ants still walked more slowly than usually, and this difference was significant ($\chi^2 = 30.80$, df = 2, $P < 0.001$). However, the ants did not then walk as slowly as after 2 days of amitriptyline consumption, and the difference between their linear speed after 8 and 2 days of the drug consumption was significant ($\chi^2 = 7.87$, df = 2, $0.01 < P < 0.02$). Some slight adaptation has thus perhaps occurred over 8 days of the drug consumption. More pronounced adaptation may perhaps occur later in the course of the drug consumption. Concerning the ants' sinuosity of movement, that presented after 8 days on the drug diet was higher

Time	Normal diet % correct responses	Diet with amitriptyline n° of correct *vs* wrong responses: colony A, B score
7h	50%	3 versus 7, 5 versus 5 40%
24h	65%	5 versus 5, 5 versus 5 50%
31h	70%	5 versus 5, 5 versus 5 50%
48h	70%	6 versus 4, 3 versus 7 45%
55h	75%	4 versus 6, 5 versus 5 45%
72h	80%	5 versus 5, 5 versus 5 50%
cue removal		
7h	70%	
24h	70%	
31h	70%	could not be examined
48h	70%	
55h	70%	
72h	70%	

Table 5: Impact of amitriptline on the ants' conditioning acquisition and memory. Amitriptyline impacted the ants' conditioning ability and thus their short and middle-term memory. Their long-term memory could not be examined since they have here learned nothing. More information can be found in the text.

Traits	Normal diet	Amitriptyline diet since 2 or 4 days	Amitriptyline diet since 8 or 12 days
Adaptation (after 8 days)			
Linear speed (mm/s)	10.7 (10.0 - 11.7)	7.1 (6.4 - 8.2)	8.4 (7.2 - 9.9)
Angular speed (ang.deg./cm)	116 (101 - 131)	226 (202 - 250)	216 (193 - 244)
Habituation (after 12 days)			
Linear speed (mm/s)	4.1 (3.6 - 4.7)	9.4 (8.1 - 10.3)	8.3 (7.7 - 8.8)
Angular speed (ang.deg./cm) on a rough substrate	271 (245 - 310)	181 (145 - 195)	166 (144 - 191)
Dependence	n° of ants on the drug-free solution; n° of ants on the drug solution		
After 10 days	colony A: 44, colony B: 36 -> 61.5%; colony A: 40, colony B: 10 -> 38.5%		
After 13 days	colony A: 81, colony B: 14 -> 61.3%; colony A: 37, colony B: 23 -> 38.7%		

Table 6: Ants' adaptation to the impact of amitriptyline on their locomotion; ants' habituation to the impact of amitriptyline on their tactile perception; ants' dependence on amitriptyline consumption. The ants slowly slightly adapted themselves to the impact of the drug on their locomotion. They also slowly slightly became habituated to the effect of amitriptyline on their tactile perception. They did not develop strong dependence on the drug consumption. Details can be found in the text.

than the normal one, what was statistically significant (χ^2 = 72.38, df = 1, P < 0.001), but was a little lower than that presented after 2 days under the drug diet, the latter slight difference being however not significant (χ^2 = 0.56, df = 2, 0.70 < P < 0.80). On the basis of these results, we chose the trait 'angular speed' for studying the loss of the effect of amitriptyline after its consumption was stopped.

Habituation to expected effects

Numerical results are given in Table 6, middle part. Such ants' potential habituation was examined after the ants had consumed amitriptyline during 12 days. At that time, the ants' linear speed on a rough substrate was still higher than the control one (χ^2 = 40.34, df = 1, P < 0.001). However, after 12 days on amitriptylyne diet, the ants' linear speed on a rough substrate was slightly but significantly lower than that presented after 4 days of the drug consumption (χ^2 = 5.40, df = 2, 0.02 < P < 0.05). The ants have thus acquired some slight habituation to the decrease of their tactile perception induced by the drug. After 12 days on amitriptyline diet, the ants' angular speed on a rough substrate was still lower than that under normal diet (χ^2 = 42.99, df = 1, P < 0.001). Also, at that time, this angular speed was similar to that presented after 4 days of amitriptyline consumption (χ^2 = 0.96, df = 2, 0.50 < P < 0.70). Thus, the ants developed no habituation to the impact of the drug on their sinuosity of movement on a rough substrate, at least after 12 days of the drug consumption. In summary, some slight, slow habituation to the drug reduction of tactile perception seems to have occurred

Dependence on amitriptyline consumption

Numerical results are given in table 6, lower part. During the first assessment of this dependence made after the ants had consumed amitriptyline for 10 days, 44 ants of colony A went to the tube free of drug and 40 ones to that containing the drug, while 10 ants of colony B went to the tube containing the drug and 36 ones to that free of drug. There was thus a large variability among the ants' response, i.e. as for their potential dependence on amitriptyline consumption. Globally, the proportion of ants having chosen the tube containing the drug equaled 38.5% and that of ants having

chosen the drug-free tube equaled 61.5%. Statistically, this result was at the limit of significance in favor of the drug-free tube (χ^2 = 2.81, df = 1, 0.05 < P < 0.10) making difficult to have a clear opinion of it. More precisely, based on that statistical analysis, we can suggest that the ants did not preferentially visit the tube containing the drug (they thus probably presented no strong dependence on amitriptylin consumption), but they also did not preferentially visit the tube free of this drug (they perhaps potentially experienced some dependence, though with no direct evidence). A second assessment of this trait was made after the ants had consumed the drug during 13 days. Variability between ants and colonies was again observed and the obtained numerical results were similar to those obtained after the ants consumed the drug during 10 days, i.e. ants sighted at the entrance of the drug-free tube: 81 + 14 = 95 (61.3%); ants sighted in front of the tube containing the drug: 37 + 23 = 60 (38.7%). These numerical results did not statistically differ from those expected if ants randomly visited the two kinds of diet: χ^2 = 3.56, df = 1, 0.05 < P < 0.10. Consequently, like after 10 days of the drug consumption, the ants did not prefer the solution containing the drug but they also did not prefer that free of drug. It is known that dependence occurrence on a substance can be accounted for the kinetic of the loss of the effect of the substance after weaning [42]. The decrease of the effect of amitriptyline (on the ants' angular speed: see the subsection relative to this study in the 'Materials and Methods' section) was thus carefully examined after the ants had consumed the drug during 14 days (see the following subsection).

Time h = hours	Angular speed (ang.degfcm) median (quartiles)	Versus t = 0			Statistics versus control		
		X^2	df	p	χ^2	df	p
t = 0	205(182-238)						
3h	203(182-224)	0		NS	45.00	1	< 0.001
6h	197(188-221)	1.71	2	0.30 < P < 0.50	45.00	1	< 0.001
9h	183(155-201)	5.88	2	0.05 < P < 0.10	40.54	1	< 0.001
12h	182(169-207)	3.98	2	0.10 < P < 0.20	45.00	1	< 0.001
15h	178(168-206)	5.17	2	P ~ 0.05	36.94	1	< 0.001
18h	172(151-195)	7.06	2	P ~ 0.02	40.54	1	< 0.001
21h	169(148-184)	12.64	2	0.001 < P < 0.01	32.96	1	< 0.001
24h	162(149-177)	18.58	2	< 0.001	26.19	1	< 0.001
27h	149(134-184)	14.56	2	< 0.001	19.71	1	< 0.001
30h	143(135-166)	22.55	2	< 0.001	14.40	1	< 0.001
33h	126(118-151)	26.15	2	< 0.001	6.05	2	0.02 < p < 0.05
36h	114 (98-135)	32.64	2	< 0.001	0.25	2	0.80 < P < 0.90
Control	116(101-131)						

Table 7: Decrease of the effect of amitriptyline after its consumption was stopped. The effect of amitriptyline slowly, linearly decreased after weaning, becoming different from its initial one after about 15 hours, and not different from what occurred under normal diet after 36 hours. Such a slow, long lasting decrease was in agreement with the absence of dependence on the drug consumption, and the rather rapid final decrease (from about 33 to 36 hours) may explain that this absence of dependence was not very obvious but at the limit of significance level.

Decrease of the effect of amitriptyline after its consumption was stopped

Numerical results are given in Table 7 and graphically presented in figure 3. During the first twelve hours after weaning, the effect of amitriptyline stayed statistically similar to its initial one. Fifteen hours after weaning, its effect became at the limit of statistically differing from its initial one, and thereafter, it slowly, progressively became more and more different from its initial one. During this vanishing, a large individual variability was observed and confirmed while establishing the distribution of the recorded angular speed values. Globally, the drug appeared to have still an effect on the ants until 33 hours after weaning, i.e. the ants' angular speed was still different from their usual (control) one. The effect of amitriptyline was fully vanished only 36 hours after its consumption was stopped (the ants' angular speed was identical to the control one only after that time), decreasing rather rapidly (i.e. more rapidly than during the previous hours) from about 30h to 36h. The slow decrease of the effect of amitriptyline after weaning was in agreement with the absence of dependence on its consumption (see the subsection relative to dependence); the final rapid loss of effect during the last 6 hours may account for this not pronounced non-dependence on the drug consumption. On the basis of the ants' sinuosity of movement, the decrease of the effect of amitriptyline after weaning could mathematically be described by the function: E = -2.5 t, with E = effect and t = time in hour.

Figure 3: Decrease of the effect of amitriptyline after its consumption was stopped. This decrease was studied on the basis of the effect of the drug on the ants' mean angular speed. Amitriptyline kept an effect similar to its initial one during 13 - 15 hours after weaning. Then, this effect went on slowly, linearly decreasing for about 15 more hours, and became statistically not different from the control 36 hours after weaning. Numerical and statistical results are given in Table 6. More information is available in the text, sections 'Material and Methods', 'Results', and 'Discussion'.

Discussion and Conclusion

The use of antidepressants is nowadays increasing. Having previously studied on ants as biological models the physiological and ethological effects of fluoxetine [4], anafranil [5], effexor [5] and paroxetine [7], we here examined in the same way those of amitriptyline, an ATC largely consumed and considered as being rather safe [14].

We found that this drug impacted the ants' food consumption, general activity, tactile perception, social interactions, cognition, learning and memory skills. The ants very poorly adapted themselves to the impact of amitriptyline on their locomotion, they became very slightly and slowly habituated to the effect of the drug on their tactile perception and they developed perhaps some very slight dependence on its consumption (no significant effect, but close to significance level). The effect of amitriptyline slowly, linearly decreased after weaning, becoming poorly marked after 15 hours, and the effect totally vanished in 36 hours after a rather quick decrease between about 30 hours and 36 hours. Such a decrease accounted for the perhaps slight absence of ants' dependence on that drug. Here below, we comment these observations, compare them them with those of other researchers, and conclude.

We made our entire experimental work being blind to what is known on amitriptyline side effects and found on ants effects rather in agreement with those observed in humans, with respect to obvious biological differences between insects' and humans' behaviors of course (see below). Due to these similarities in observed physiological and ethological effects of that drug in ants and humans, these social insects appeared to be good models for such pharmaceutical studies which can be performed at very low cost and in a short time, a statement also based on our similar previous studies [43]. We observed a rather large variability among the experimented ants including the loss of the effects of the drug after

weaning, and we here presented the global results. We planned our experimentation in order to finalize it in a total of 14 days in order to preserve the health of the colonies, their brood and their queens for future studies.

Amitriptyline is known for efficiently decreasing humans' pain perception. Here are three examples. Pain caused by temporomandibular disorder (which affects joint and/or muscles) was significantly reduced in patients treated with amitriptylyne [44]. Amitriptyline was superior to placebo in relieving pain (steady, burning and throbbing pains) in 29 patients with painful diabetic neuropathy, this effect regardless the patients' state of mood [45]. In general, ATC depressants are efficient in preventing migraines, though inducing adverse effects [46]. A comparative study of the efficiency of three antidepressants allowing preventing migraine proved that amitriptyline was the best alternative [47]. This effect of amitriptyline on humans' pain perception is somewhat in agreement with our observations on ants tested on a rough substrate: their tactile perception was reduced. Note that ants slowly developed some slight habituation to this expected effect of the drug. Such a habituation may occur in humans. This potential event must be monitored in patients treated with amitriptyline because in the case of its occurrence, these patients will be tempted to increase their dose intake in order to reach again their initial level of pain release.

Note that the generic drug amitriptyline has been medically proved to be as efficient and tolerated than its reference formulate [48]. Let us add that the combined use of amitriptyline and chlordiazepoxide appeared to be more efficient for reducing pain perception than the use of amitriptyline alone [49].

Even if considered as being a rather efficient antidepressant and an efficient pain releaser, and being even more efficient than other similar drugs, amitriptyline presents harmful side effects [50]. The most common adverse reported effects are tiredness, dry mouth, headache, blurred vision, and anxiousness etc. i.e. effects due to the anti cholinergic action of the ATC antidepressants. Our findings on ants are rather in agreement with some of these side effects observed in patients, such as impact on ants' food consumption, general activity, tactile and olfactory perception. In addition, we observed several effects not (or not yet) reported in patients such as impact on social interactions, cognition, learning, and memory skills. The latter adverse effects (or at least some ones) caused by amitriptyline and observed in ants might perhaps occur in patients treated with this drug, and attention should thus been paid to the consumers' social relationships, cognition, learning and memory skills among other traits. Also, the ants very poorly adapted themselves to the effects of amitriptyline on their locomotion skills, and they became very slightly and slowly habituated to the effect of the drug on their tactile perception. This should be examined in patients under treatment with amitriptyline, especially the potential habituation since if this occurs, patients will be tempted to increase their drug consumption.

Ants perhaps developed some very slight (not statistically significant) dependence on amitriptyline consumption, and exhibited a large variability between the individuals and the colonies. Let us recall that the development of some dependence on the use of a drug is correlated to the way the effect of this drug decreases and vanishes after its use was stopped. In our study on ants, the effect of amitriptyline slowly, linearly decreased after weaning; it became poorly marked after 15 hours, totally vanished in 36 hours, and rather rapidly decreased from *ca* 30 to 36 hours after weaning. All this accounted for the very slight, the nearly no dependence on amitriptyline consumption. Concerning the development of some dependence on amitriptyline consumption in patients, this occurs in some patients [51]. Furthermore, experiments on rats seem to show that some ATC depressants withdrawal may induce dependence through their super sensitization of muscarinic (cholinergic) systems. The occurrence of possible dependence on amitriptyline consumption should thus be monitored in patients treated with this drug. Also, according to the long lasting effect of amitriptyline (i.e. 36 hours), we cautiously proposed to advice patients consuming the drug only every 36 hours this allowing the drug to be eliminated from their body before they ingest a new dose.

A few more rare harmful effects induced by amitriptyline have been reported in patients. Among others, risks of fractures have been observed [52]. Such an effect could not been examined in our experiments. Weight gain and carbohydrate craving have also been mentioned [53], but the authors explain that this effect may result from the patients' depression itself or from their improved mood, and not from the amitriptyline consumption. We somewhat support the latter opinion since, during our work, the ants under this drug diet consumed less meat and less sugar water than when living under normal diet.

An important result of a study on patients under amitriptyline treatment is the possibility of predicting the occurrence of severe adverse effects due to amitriptyline consumption [54]. In fact, not all the patients are identical as for their ability in metabolizing and eliminating amitriptyline, and those poorly doing so largely suffer from the drug adverse effects. This fact may be in agreement with the large variability we observed among tested ants regarding their physiological and ethological responses to amitriptyline consumption, the young ants appearing to be the most affected (personal observation). A similar conclusion about an individuals' variability has been made about the occurrence of some dependence on amitriptyline use [51]. Once more, not all the patients react identically as for a potential development of dependence on the drug.

To conclude, our findings on ants are in agreement with observations made on patients under amitriptyline treatment (i.e., decrease of activity, decrease of pain perception, several physiological and ethological disorders, variability between the individuals, potential slight dependence on the drug consumption) but we found in addition a few other harmful effects (so-called side effects as regards patients) not described for patients (i.e. impact on orientation, audacity, social interactions, cognition, learning, and memory, very slight adaptation to impacts, very slight habituation to wanted effects, and at the statistical limit of developing some dependence). Our opinion is that even if having some adverse effects, amitriptyline is an efficient drug for treating depression and reducing pain perception, and is safer than the other ATC, the ISRS, IRSNa, ISSR, and IMAO antidepressants. It can thus be used for a time, with no abuse for avoiding excessive harmful side-effects. During its use, patients must be monitored for side effects similar to those mentioned in our study. They must also be monitored to evaluate if they at least partly adapt themselves to these side effect, if they become habituated to the wanted effect of the drug and if they do not develop dependence on its consumption. More studies should be performed in order to increase our knowledge about the side effects of nowadays used antidepressants as well as to produce novel, more efficient and safer ones, as advocated by other researchers [55,56].

Conflict of Interest

We affirm having no conflict of interest as for the use of amitriptyline, of e.g. Redomex®. We work on ants, on water supply in Southern countries and on ecological quality of watercourses and we receive no money to do so.

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