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Physiological and ethological effects of antidepressants: a study using ants as biological models.

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ABSTRACT: *After having shown that fluoxetine, the active substance of the ISRS antidepressants, has severe adverse effects, we examined the effects of an ACT (anafranil, the active substance being clomipramine hydrochlorid) and an IRSNa (efexor, the active substance being venlafaxine) antidepressants, using identical biological models (= ants) and experimental protocols for comparative purpose. Anafranil appeared to be the less toxic. It reduced the individuals' 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, brood caring, visual and olfactory perception. It increased the ants' ability in acquiring visual and olfactory conditioning, as well as their visual and olfactory memory. Ants developed no habituation and no dependence on anafranil consumption. The effects of that drug linearly vanished in the course of time, in about 28 hrs after its consumption ended. Efexor had more adverse effects. It increased the individuals' jerking movements, and aggressiveness between congeners. 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. It did not impact ants' audacity, brood caring, and visual perception. There was no habituation to and no dependence on efexor consumption. The effects of this drug vanished in 12 h (linear speed), 28 h (sinuosity of movement) and 32 h (precision of response). All the observed effects are either in agreement with those already known, or more precisely described, or newly revealed. In fact, the ACT antidepressants are the less toxic, the IRSNa are somewhat dangerous, while the ISRS have severe adverse effects. Attention should also be paid to the presence of such drugs, largely consumed and eliminated intact, in natural water, impacting then the physiology and the behavior of all the living organisms.*

KEY WORDS: *aggressiveness, cognition, dependence, habituation, locomotion, memory*.

I. INTRODUCTION

Antidepressants are currently among the most consumed drugs in the western societies. They are eliminated intact or nearly intact, through the kidneys, and are then transported into natural water via sewerage systems, contaminating rivers and aquatic ecosystems [1]. In rivers contaminated by high concentrations of antidepressants, fishes appeared to present aberrant behavior: for instance, trouts in St Laurent [2] or minnows in Wisconsin [3]. These drugs probably not only impact the behavior and the physiology of fishes, but may also affect the entire aquatic fauna, especially the aquatic macro invertebrates [1, 4]. These last organisms play important roles in the ecology of water bodies, occupying a variety of ecological niches and performing a huge variety of behaviors, so that any modifications of these behaviors could lead to significant changes in the trophic relationship between organisms of the aquatic fauna. It is thus important to know the effects of antidepressants on these invertebrates to prevent dramatic changes in the behavioral and/or developmental processes occurring in aquatic ecosystems. Complex equipment is required for studying the behavior of aquatic invertebrates in laboratory conditions, so it could be useful to have an "easy to maintain - easy to study" biological model (of invertebrate organisms, among others) for developing such kind of studies. We have already studied the adverse affects of the actually most used antidepressant active substance, fluoxetine, using ants as biological model [5]. As detailed here below, ants could be a good biological model for studying physiological and ethological effects of antidepressants. Concerning fluoxetine, the observed adverse effects are severe, as aggressiveness between congeners, lost of olfactory perception, decrease of food consumption, and decrease of brood caring behavior. Fortunately, fluoxetine is actually becoming progressively less used, and other antidepressants are now becoming more and more often employed for helping depressive persons. We so intended to examine, in this paper, the potential harmful effects of antidepressants free of fluoxetine on invertebrates animals, using ants as experimental organisms.

There exist four kinds of antidepressants [6, 7]. The 'IMAO' group of antidepressants inhibit the monoamine oxidize and are now given only in hospitals, thus in limited amounts. The 'ATC' antidepressants are tricycle molecules which inhibit several neurotransmitters. The 'ISRS' group inhibit the recapture of serotonin; the most used active substance is fluoxetine, i.e. the substance of which we have already examined the adverse effects (reference here above). The 'IRSNa' antidepressants inhibit the recapture of serotonin and noradrenalin. Acting on the recapture of neurotransmitters, all the antidepressants may have unwanted effects. Effectively, several studies, among others Parent [8], Simon [9], Cipriani et al. [10], and Lane [11] have revealed such adverse effects on patients. On the basis of these studies, we can presume that not all potential adverse effects have been examined for the humans, and much less for other living organisms, and, concerning the humans, it is possible that not all the obtained results have been revealed. Adverse effects on invertebrate developmental processes have been studied by Nentwig [1] and Pery et al [4], but negative effects of antidepressants on behavioral processes of invertebrates remain poorly studied. Using ants as "test organisms", we have found several harmful effects for fluoxetine (reference here above). Of course, we cannot make direct conclusions about adverse effects of fluoxetine on humans and other organisms than ants on the basis of our results, but it can be assumed that potential adverse effects exist, for both invertebrate and vertebrate organisms, consequently to that drug consumption. It is the reason why, in the present work, after having examined the effects of the 'ISRS' [5], we study ethological and physiological effects of an 'ATC' and an 'IRSNa' antidepressant, once more using ants as "test organism".

As most of the biological processes are quite similar for all animals, including humans (i.e. genetics, metabolism, nervous cells functioning), a lot of invertebrates and vertebrates can be used as models for studying biological questions [12, 13, 14]. Invertebrates are more and more used as biological models because they offer scientists many advantages, among others a short life cycle, a simple anatomy, and being available in large numbers [15, 16]. Some species are largely used as biological models, for instance, the flatworm *Dendrocelium lacteum*, the nematode worm *Caenorhabdotes 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 [17, 18].

Ants also could be used. Colonies containing thousands of ants can easily be maintained in laboratories, at low cost and very conveniently, throughout the entire year. Ants are among the most complex and social invertebrate animals as for their morphology, their physiology, their social organization and their behaviors. They are among the most morphologically evolved hymenoptera, having indeed a unique resting position of their labium, mandibles and maxilla [19], as well as a lot of glands emitting numerous, efficient pheromones [20]. Their societies are highly organized with a strong division of labor, an age-based polyethism and a social regulation [21]. 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 [22]. They generally use an alarm signal, a trail pheromone, and a recruitment signal [22]; they are able to navigate using memorized visual and olfactory cues [23 and references therein); they efficiently recruit nestmates where, when and as long as it is necessary [24], and, finally, they clean their nest and provide their area with cemeteries [25]. 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 neuronal active substances.

During many years, we have studied species of ants belonging to the genus *Myrmica*, and above all *Myrmica sabuleti* Meinert 1861. We know some of its ecological traits, eye morphology, visual perception, navigation system, visual and olfactory conditioning capabilities, and recruitment strategy [26, 27, 28, 29]. The ontogenesis of cognitive abilities of *Myrmica* species, including *M. sabuleti*, has also been approached [30, 31, 32, 33, 34]. Studies on the impact of age, activity and diet on *M. ruginodis*' conditioning capability [35] leaded to presume that ants could be good biological models. This was confirmed by the study of the effects of caffeine, theophylline, cocaine, and atropine [36], of nicotine [37], of morphine and quinine [38], and of fluoxetine, an 'ISRS' antidepressant [5] on *M. sabuleti*. Each time, we observed effects related to those observed for humans, and brought information as well as precision about effects of the examined substance, of course on *M. sabuleti*, but clearly leading to presume similar effects for humans and other living organisms. We thus aimed to presently conduct a similar analysis (employing identical methods for comparative purpose) about potential adverse physiological and ethological effects generated by an 'ACT' and an 'IRSNa' antidepressants, using *M. sabuleti* as a model.

II. EXPERIMENTAL PLANNING

The 19 following traits were assessed on two colonies of *M. sabuleti* before and after they consumed anafranil (an ACT antidepressant), and on two other similar colonies before and after they consumed efexor (an IRSNa antidepressant). Two other colonies were used to provide 'alien workers'. These traits have previously been examined for fluoxetine, the active substance of ISRS antidepressants [5] using similar experimental design and methods, what will allow comparison between the three kinds of antidepressants studied (see the discussion section).

1 – the locomotion (and thus the general activity) through the ants' linear and angular speed,
2 – the precision of reaction through the orientation towards a source of their alarm pheromone,
3 – the response to pheromones through the trail following behavior,
4 – food consumption through the numbers of ants coming onto meat food,
5 – the "audacity" through the numbers of ants coming onto a test apparatus,
6 – the tactile sensation (or "pain" perception) through the ants' behavior in an uncomfortable situation,
7 – cognition through the ability in performing a task requiring cognition (moving through chicanes),
8 – the potential aggressiveness against nestmates through ants' behavior in the course of dyadic encountering,
9 – the expected aggressiveness against alien ants through ants' behavior in the course of dyadic encountering,
10 – the caring behavior through the behavior in front of larva removed from the nest,
11 – the visual perception through the distinguishing of two colors,
12 – the olfactory perception through the distinguishing of two odors,
13 – the visual learning ability through the acquisition of a visual conditioning,
14 – the visual memory through the duration of the remembering a learned visual cue,
15 – the olfactory learning ability through the acquisition of an olfactory conditioning,
16 – the olfactory memory through the duration of the remembering a learned olfactory cue,
17 – the habituation to the drug consumption through the speed of movement and the orientation to an alarm signal, fourteen days after continuous drug consumption,
18 – the dependence on drug consumption through the numbers of ants choosing food containing the drug,
19 – the decrease of the effects of the consumed antidepressant after its consumption ended, through assessment, in the course of time, of the ants' speed and sinuosity of movement, and of their orientation to an alarm signal.

III. MATERIAL AND METHODS

3.1, Collection and maintenance of ants

The study was made on four colonies of *M. sabuleti* (labeled 1, 2, 3 and 4), two other ones furnishing 'alien workers' required in one experiment. The colonies 1 and 2, collected in the Aise valley (Ardenne, Belgium), served for studying the effects of the antidepressant anafranil; the colonies 3 and 4, collected at Audregnies (on the terril of Ferrand, Hainaut, Belgium), were used for studying the effects of the antidepressant efexor; one colony collected at Treignes (Ardenne, Belgium) and one collected in the Aise valley served for providing alien workers. The colonies were collected in abandoned sandstone quarries or on terrils. The ants were nesting under stones, in field covered with small plants and brushes. The collected colonies were demographically similar, containing about 600 workers, one or two queens and brood at larval and nymphal stages. They were maintained in the laboratory in artificial nests made of one to three glass tubes half-filled with water, a cotton-plug separating the ants from the water. These glass tubes were deposited in trays (34 cm x 23 cm x 4 cm), the sides of which were covered with talc to prevent the ants from escaping. The trays served as foraging areas, food being delivered into them. The ants were fed with sugar-water provided *ad libitum* in a small glass tube plugged with cotton, and with pieces of *Tenebrio molitor* (Linnaeus 1758) larva provided twice a week on a glass slide. Temperature was maintained between 18°C and 22°C, humidity at about 80%, these conditions remaining constant over the course of the study. Lighting had a constant intensity of 330 lux while caring for the ants, training and testing them. During other time periods, the lighting was dimmed to 110 lux. The ambient electromagnetic field had an intensity of 2-3 $\mu W/m^2$. These conditions were optimum for the used species, so the present work presented no ethic problems.

3.2, Acquisition of the two antidepressants, realization of aqueous solutions for ants.

The two antidepressants were provided by the pharmacist J. Cardon (1050 Brussels). Anafranil is produced by the manufacturer Sigma Tau (France); it is an ATC which active substance is clomipramine hydrochlorid. Efexor is produced by the manufacturer MEPHA PHARMA (Switzerland); it is an IRSNa which active substance is venlafaxin. The amounts of these antidepressants given to depressive persons are about 100 – 150 mg of drug per day. People drink about one litter of water per day (excluding liquid contained in food, thee or coffee). So, the concentration of drug in water consumed by humans is about 0.1 g of drug in 1,000 g of water that is 1/10,000. Insect are known to consume far less (about ten times less) water than mammals. For obtaining equivalent amounts (= an equivalent dose) of drug consumed by persons and by ants, the most appropriate

concentration of antidepressant which should be given to ants would be 1/1,000, so 1 mg of drug into 1,000 mg of water. Therefore, 25 mg of anafranil (= one dose) were scratched then dissolved in 25 ml of a saturated solution of brown sugar, the ants' usual liquid food, while 75 mg of efexor (= one dose) were removed from one capsule then dissolved in 75 ml of such a sugar water. The concentration in drug of the final solution was thus 25 mg in 25 ml of water, as well as 75 mg in 75 ml of water, so 1/1,000. This solution was given to the ants, like their usual liquid food, in a small glass tube plugged with cotton, the cotton being refreshed each two days and the entire solution renewed each 14 days. It was checked each day if ants actually consumed the given liquid food containing the antidepressant.

In is important to quote that the exposition to full dose of the drug was immediate, rather than progressive, and so, did not correspond to the procedure actually followed by patients under medical treatment. Indeed, current medical prescriptions of an antidepressant establish a progressive increase of the dose, until the patient reaches the nominal dose for his treatment. Similarly, the end of the treatment implies a progressive decrease of the dose, rather than an abrupt stop of drug consumption as it occurred in our experimental design.

3.3, Orientation, linear and angular speed; *1, 2.*

Ants' linear and angular speed was assessed for detecting excitation or sleepiness in the animals. Ants' orientation towards an isolated congener's head allows measuring the ants' precision of reaction. An isolated worker's head, with widely opened mandibles, is a source of alarm pheromone identical to that of an alarmed worker, in terms of the dimensions of the emitting source (the mandibular glands' opening) and of the quantity of pheromone emitted (the intensity of the stimulus) [39]. These assessments were made on ants freely moving on their foraging area. Each time, assessment was made on ants of two nests having never consumed antidepressant, then on ants of these two nests having consumed an antidepressant during two days. For each assessment, the movement of ten ants of each nest (n = 20 ants) was analyzed.

Trajectories were recorded manually, using a water-proof marker pen, on a glass slide placed horizontally 3 cm above the area where the tested individuals were moving. A metronome set at 1 second was used as a timer for assessing the total time of each trajectory. Each trajectory was recorded during 5 to 10 seconds or until the ant reached the stimulus. All the trajectories were then traced, with a water-proof marker pen, onto transparent polyvinyl sheets (Fig. 1A) using the glass slide as the reference model, and the polyvinyl sheets were affixed to a PC monitor screen. The trajectories were analyzed using specifically designed software [40] so that the trajectory parameters could be quantified.

The three used parameters were defined as follows:

The linear speed (V) of an animal is the length of its trajectory divided by the time spent moving along this trajectory. It was measured in mm/s.

The angular speed (S) (i.e. the sinuosity) of an animal's trajectory is the sum of the angles, measured at each successive point of the trajectory, made by the segment 'point i - point i – 1' and the segment 'point i - point i + 1', divided by the length of the trajectory. This variable was measured in angular degrees/cm (= ang. deg. / cm).

The orientation (O) of an animal towards a given point (here an ant's head) is the sum of the angles, measured at each successive point of the registered trajectory, made by the segment 'point i of the trajectory - given point' and the segment 'point i - point i + 1' divided by the number of measured angles. This variable was measured in angular degrees (= ang. deg). When such a variable (O) equals 0°, the observed animal perfectly orients itself towards the stimulus; when O equals 180°, the animal fully avoids the stimulus; when O is lower than 90°, the animal has a tendency to orient itself towards the stimulus; when O is larger than 90°, the animal has a tendency to move in a direction that deviate from the stimulus.

Each distribution of 20 measurements was characterized by its median and its quartiles (since being not Gaussian; TABLE 1, table lines 1, 2, 6, 7), and the distribution of values obtained for ants having consumed an antidepressant was statistically compared to that previously obtained for the ants of the same colonies having never consumed that drug, using the non-parametric χ^2 test [41]. The significance threshold was set to $\alpha = 0.05$.

3.4, Trail following behavior; *3.*

This behavior was assessed for examining the ants' response to a pheromone. The trail pheromone of *Myrmica* ants is produced by the workers' poison gland. Ten of these glands were isolated in 0.5 ml (500 µl) hexane and stored for 15 min at -25 °C. To perform one experiment, 0.05 ml (50 µl) of the solution was deposited, using a metallic normograph pen, on a circle (R = 5 cm) pencil drawn on a piece of white paper and divided into 10 angular degrees arcs (= ang. deg.). One minute after being prepared, the piece of paper with the artificial trail was placed in the ants' foraging area. When an ant came into contact with the trail, its movement was observed (Fig. 1G). Its response was assessed by the number of arcs of 10 angular degrees it walked without departing from the trail, even if it turned back while walking on the trail. If an ant turned back when coming in front of the trail, its response was assessed as "zero arc walked"; when an ant crossed the trail without following it, its response equaled "one walked arc". Before testing the ants on a trail, they were observed on a

"blank" circumference imbibed with 50µl of pure hexane, and the control numbers of walked arcs were so obtained (Table 1, TABLE lines 3 and 8, C = control, T = test). On such experimental trails, *Myrmica* workers do not deposit their trail pheromone because they do so only after having found food or a new nest site. Each time, these manipulations were made firstly on ants having never consumed antidepressant, then on ants of the same colony having consumed an antidepressant for three days. For each control and test experiment, 20 individuals of each two used colonies were observed (n = 40). Each distribution of values was characterized by its median and its quartiles (since being not Gaussian). The distributions of values obtained for ants having consumed an antidepressant were compared to the corresponding ones obtained for ants having never consumed this drug, by using the non parametric χ^2 test [41].

3.5, Ants' food consumption; *4.*

Before the ants consumed an antidepressant, and after they had consumed this drug for three days, the workers present on the meat food (pieces of *T. molitor* larva) at a time ants must be fed were counted 10 times in the course of 10 min. The numbers obtained for the two kinds of food intake (with no drug, then with drug) were statistically compared using the Mann-Whitney U test [41], and the mean as well as the extreme values of the recorded numbers were established (TABLE 1, table lines 4 and 9).

3.6, Ants' "audacity"; *5.*

Before the ants consumed an antidepressant, and three days after they had consumed that drug, a cylindrical tower built in strong white paper (Steinbach ®, height = 4 cm; diameter = 1.5 cm) was set on the ants' foraging area (Fig. 1B), and the ants present on it, at any place, were counted 10 times, in the course of 10 min. The mean and the extreme values of the obtained values were established each time and the two series of values were compared using the non parametric Mann-Whitney U test (same reference as above; TABLE 1, table lines 5 and 10).

3.7, Ants'tactile sensation (presumed "pain sensation"); *6.*

It was tempted to assess this physiological trait by setting ants in an experimental apparatus made of a small tray (15 cm x 7 cm x 4.5 cm) into which a piece (3 cm x 11 cm) of rough emery paper (number 280) was duly folded (11 cm: 2 cm + 7 cm + 2 cm) and tied to the bottom and the border of the tray, so dividing the tray in three zones: a small initial smooth zone (3 cm long), a zone (3 cm long) on which ants' walking should be uncomfortable, and another smooth zone, large (9 cm long) for inciting the ants crossing the uncomfortable zone. Two such apparatus were used, one for each used colony. The ants were tested before they consumed antidepressant, then five days after they had continuously received an antidepressant. Each time, 12 ants were set, all together, at the same time, in the small initial zone. The ants present in each of the three zones of the apparatus were counted after 0, 2, 4, 6, 8, 10 min, and the linear as well as the angular speed of 12 ants for each two tested colonies (so n = 24) moving on the rough paper were assessed using the method briefly explained above (Fig. 1H; TABLE 2). The numbers of ants obtained for ants having consumed an antidepressant were statistically compared to those previously obtained for ants of the same colonies having never ingested that drug, using the non parametric Wilcoxon test [41], while the linear and angular speed of such ants were statistically compared using the non parametric χ^2 test (same reference as above).

3.8, An ants' cognitive ability requiring no memory; *7.*

This ability was assessed on ants having never received antidepressant, then seven days after they had continuously an antidepressant in their liquid food. The assessment was made using an adequate experimental apparatus schematically presented in the figure 3 of a previous article [37] and here shown in Fig. 1 C. This apparatus consisted in a small tray (15 cm x 7 cm x 4.5 cm) inside of which two pieces of white extra strong paper (Steinbach ®, 12 cm x 4.5 cm), duly twice folded, were inserted in order to create a way with four chicanes between a narrow (too narrow for 15 ants) initial space (initial loggia) and a larger area (free loggia). Two such experimental apparatus were used, each one for one of the two nests. Each time, for each nest and each feeding situation, 15 ants were collected from their colony and set all together, at the same time, in the initial loggia of the apparatus, and those located in this loggia as well as in the free loggia were counted after 0, 5, 10, 15 and 20 min (TABLE 3). The numbers obtained for ants consuming an antidepressant were statistically compared to those previously obtained for these ants having never received this substance using the non parametric Wilcoxon test [41].

3.9, Ants' aggressiveness towards congeners or alien workers; *8, 9.*

This trait was quantified before the ants consumed an antidepressant, then eight days after they had continuously consumed that antidepressant. Ants' potential aggressiveness towards nestmates as well as expected aggressiveness towards alien workers (i.e. belonging to another colony) was assessed in the course of dyadic encounters of five ants of each of the two used colonies (for each studied antidepressant), the encountering being conducted in a small glass (base diameter = 3 cm, top diameter = 4 cm, height = 5 cm), the borders of which had been slightly covered with talc. Each time (in total ten encounters with nestmates and ten encounters with alien workers, each time before then under an antidepressant consumption), the ant of the presently used colony was observed for 3 minutes and its meetings with the other ant was characterized by the numbers of times it did nothing (level 0 of aggressiveness), it touched the other ant with its antenna (level 1), it opened its mandibles in front of the other ant (level 2; Fig. 1D), it gripped and/or pulled the other ant (level 3), and it tried to sting or stung the other ant (level 4). The numbers recorded for each two used colonies were added (TABLE 4), and the results obtained for ants consuming an antidepressant were compared to those obtained for the same ants having never consumed that drug, using the non parametric χ^2 test [41].

3.10, Ants' brood caring behavior; *10.*

This trait was examined, for the two used colonies for each antidepressant studied, before the ants consumed an antidepressant, then ten days after they had consumed that drug. Each time, a few larva were removed from the inside of the nest and deposited in front of the nest tube entrance. Five of them were carefully observed, as well as the ants' behavior in front of the larva (Fig. 1I). The numbers of the observed larva still remaining out of the nest were counted after 0, 2, 4, 6, 8, 10 minutes, and the numbers recorded for each two colonies were added (TABLE 5). The results obtained for ants consuming an antidepressant were compared to those similarly obtained for these ants having never consumed that drug using the non parametric Wilcoxon test [41].

3.11, Ants' visual perception; *11.*

For examining this sensorial ability, ants from each colony were trained with their own training apparatus and then tested using another similar outfit, each colony having also its own test apparatus. The apparatus consisted of a glass slide (2.6 cm x 7.6 cm) with a cube (2 cm x 2 cm x 2 cm) made of extra strong white paper (Steinbach ®) placed at an end. On one face of the cube was a blue square (1.5 cm x 1.5 cm) and on the opposite face a yellow identical square. The squared cues were cut from strong colored paper (Canson ®) the colors of which had previously been analyzed for their wavelengths reflection [42, 43]. During training, a cut *T. molitor* larvae was tied to the end of the glass slide where there was no cube. In this way, the larvae (= the reward) was located 4 cm to the right of the blue visual cue, as well as 4 cm to the left of the yellow visual cue. During the tests, no meat was placed on the apparatus.Four tests were performed six days, as well as nine days, after the ants were exposed to (and so could see) the apparatus with the two visual cues and the reward, on their foraging area. During these tests, the apparatus was provided with a cube free of colored cues (first test), or a cube with a blue square (second test), or a cube with a yellow square (third test), or a cube with a blue and a yellow cues (fourth test, Fig. 1E). Each time, the ants present on the glass slide were counted 10 times, for each two colonies, and the mean value of the 20 counts was calculated (TABLE 6). The four kinds of counts were compared to one another using the non parametric test of Wilcoxon [41]. If the ants coming onto the glass slide in the presence of one, or the other, or the two colored cues were more numerous than those coming in the presence of a cube free of any cue, then the ants had been able to visually perceive the colored cues [28, 29].

3.12, Ants' olfactory perception; *12.*

For examining this sensorial ability, ants from each two colonies used for studying the effects of one antidepressant were trained by way of an own experimental apparatus. They were then tested using another, similar apparatus, each colony having also its own test apparatus. The apparatus consisted of a piece of extra strong white paper (Steinbach ®, 12 cm x 6 cm) orthogonally folded lengthwise to present a horizontal and a vertical part. A small glass tube (length: 7 cm; diameter: 1 cm) was inserted into a hole (diameter: 1.2 cm) cut in the middle of the vertical part very close to the base. The glass tube was placed in the foraging area with the opening in the middle of the apparatus. A schema of such an apparatus is given in [28]. During training, the glass tube was filled with sugared water (the reward) and closed with a cotton plug, while pieces of thyme and pieces of estragon were deposited respectively on the left and on the right horizontal ends of the experimental apparatus. The reward was so located 4 cm to the right of the thyme and 4 cm to the left of the estragon. The experimental apparatus used for testing was free of odorous plants, or provided with thyme, or with estragon, or with both cues, and the glass tube was empty (i.e., no reward was given during tests), but closed with a cotton plug to prevent entry.

Four tests were so performed six days, as well as nine days, after the ants were exposed to (and so could perceive) the two odors, together with their sugar food, on their foraging area, using the four here above cited experimental designs. During each series of tests, the ants present on the experimental apparatus were counted 10 times, for each two used colonies. Each time, the mean value of the 20 counts was calculated (TABLE 6), while the four kinds of counts were compared to one another using the non parametric test of Wilcoxon [41]. If the ants coming onto the apparatus in the presence of one, or the other, or the two olfactory cues were more numerous than those coming in the absence of any cue, then the ants had been able to olfactory perceive the odorous cues [28, 29].

3.13, Ants' visual and olfactory operant conditioning ability and memory; *13, 14, 15, 16.*

Briefly, at a given time, either a green hollow cube or pieces of dried shallots were set above or aside pieces of *T. molitor* larva tied to a supporting piece of glass. The ants so underwent either visual or olfactory operant conditioning. Each time, tests were performed, in the course of time, while the ants were expected acquiring conditioning, then, after having removed the green cube or the pieces of shallots, while the ants were expected to partly lose their conditioning.

In detail, ants were collectively visually trained to a hollow green cube constructed of strong paper (Canson ®) according to the instructions given in [44] and set over the meat food which served as a reward. The color has been analyzed to determine its wavelengths reflection [42]. Only the ceiling of each cube was filled, this allowing ants entering the cube. Choosing the green cube was considered as giving the 'correct' choice when ants were tested as explained below. The ants were olfactory conditioned by setting pieces of dried shallots aside the tied pieces of *T. molitor* larva. Choosing the pieces of shallots was considered as giving the 'correct' choice when ants were tested as explained below.

Ants were individually tested in a Y-shaped apparatus (Fig. 1F) constructed of strong white paper according to the instructions given in [29], and set in a small tray (30 cm x 15 cm x 4 cm), apart from the experimental colony's tray. Each colony had its own testing device. The Y apparatus had its own bottom and the sides were slightly covered with talc to prevent the ants from escaping. In the Y-apparatus, the ants deposited no trail since they were not rewarded. However, they could utilize other chemical secretions as traces. As a precaution, the floor of each Y-apparatus was changed between tests. The Y-apparatus was provided with either a green cube, or pieces of dried shallots, in one or the other branch. Half of the tests were conducted with the cube, or the odorous plant, in the left branch and the other half with the cube, or the odorous plant, in the right branch of the Y maze, and this was randomly chosen. Control experiments had previously been made on never conditioned ants [29], and on trained ants of colonies having never received antidepressant [37]. This must be done because, once an animal is conditioned to a given stimulus, it becomes no longer naïve for such an experiment. It was so impossible to perform, on the same ants, conditioning without then with an antidepressant in the ants' food. The only solution was to use previous results obtained in the course of identical experiments made on very similar colonies never fed with any drug.

To conduct a test on a colony, 10 workers – randomly chosen from the workers of that colony – were transferred one by one onto the area at the entrance of the Y-apparatus. Each transferred ant was observed until it turned either to the left or to the right in the Y, and its choice was recorded. Only the first choice of the ant was recorded and this only when the ant was entirely under the cube, i.e. beyond a pencil drawn thin line indicating the entrance of a branch (Fig. 1F). Afterwards, the ant was transferred into a polyacetate cup, the border of which was covered with talc, until 10 ants were so tested, this avoiding testing the same ant twice. All the tested ants were then placed back on their foraging area. For each experiment, the numbers of ants, among n = 10 + 10 = 20, which turned towards the "correct" green cube or pieces of shallots, or went to the "wrong" empty branch of the Y were recorded. The percentage of correct responses for the tested ant population was so established (Fig. 2). The results obtained for ants that have consumed an antidepressant were compared to previous results obtained for ants that had never consumed that substance, using the non parametric Wilcoxon test [41]. The value of N, T, and P, according to the nomenclature given in the here above reference, are defined in the results section.

3.14, Ants' habituation to the drug consumption; *17.*

Fourteen days after the ants had continuously consumed an antidepressant, their linear and angular speed, as well as their orientation towards an isolated worker's head, were assessed (TABLE 7). The results were compared to the control ones and to those obtained after two days of the drug consumption using the non parametric χ^2 test [41].

3.15, Ants' dependence on the drug consumption; *18.*

After the ants had continuously consumed an antidepressant during five days, an experiment was performed for examining if they acquired some dependence on the consumed drug. Fifteen ants of each two used colonies were transferred into a small tray (15 cm x 7 cm x 5 cm), the borders of which had been covered with talc and in which two tubes (h = 2.5cm, diam. = 0.5 cm) were laid, one containing sugar water, the other sugar water and the antidepressant consumed by the ants (at the concentration 1/1,000, the same as during the whole experimental process), each tube being plugged with cotton. In one tray, the tube containing the drug was located on the right; in the other tray, it was located on the left. Photographs of such an experimental design can be seen in [37, 38, 39]. The ants drinking each liquid food were counted 12 times (Figs J, K), the mean values being then established for each kind of food (TABLE 7). They were statistically compared to the values expected if ants randomly went drinking each kind of food, using the non parametric goodness of fit χ^2 test [41].

3.16, Decrease of the effects of the drug, after its consumption ended; *19.*

Four weeks after that the ants had continuously consumed an antidepressant, the liquid food containing the drug was removed from the ants' tray and replaced by sugar water free of the drug. This change was made at a given recorded time. After that, the ants' linear speed, angular speed, and orientation to an isolated worker's head, were assessed after successive given time periods (Fig. 3). The results revealed the decrease of the effects of the drug, on ants. Their statistical significance could be estimated via the non parametric χ^2 test [41].

IV. RESULTS

4.1, Concerning anafranil, an ACT antidepressant, the active substance being clomipramine hydrochlorid

4.1.1, Locomotion and general activity

This trait was affected by the antidepressant anafranil. The ants' linear speed was statistically lower than the control one (9.9 *vs* 14.4 mm/sec; $\chi^2 = 29.3$, df = 3, $P < 0.001$), while and consequently, their angular speed was somewhat larger (137 *vs* 104 ang. deg./cm; $\chi^2 = 12.15$, df = 2, $0.001 < P < 0.01$; TABLE 1, table line 1, Fig. 1A). Effectively, only one day after the ants consumed that antidepressant, they were observed moving more slowly than usually. They appeared very calm, doing everything calmly, slowly, and gently contacting one another, but those traits were not assessed during our experiment.

4.1.2, Orientation towards a source of alarm pheromone; precision of reaction.

This trait appeared to be somewhat affected by anafranil consumption during two days. The ants went on reacting to the alarm signal, and moving towards it. But they were less efficient in precisely orienting themselves towards such a signal. The numerical results reflected this observation (TABLE 1, table line 2: 46.2 ang. deg *vs* 35.8 ang. deg; $\chi^2 = 5.0$, df = 2, $0.05 \approx P < 0.01$).

4.1.3, Trail following behavior; response to pheromones

This behavioral trait was affected by anafranil consumption. The ants went on perceiving the trail pheromone. They came onto the trail but followed it only along short distances: they moved slowly, went to the right and to the left of the trail, and soon deviated from it. The numerical results reflected this observation: the number of arcs walked along the trail was three times less numerous than before the ants consumed the antidepressant during three days (TABLE 1, table line 3: 4 *vs* 12; $\chi^2 = 15.48$, df = 3, $0.001 < P < 0.01$).

4.1.4, Food consumption

This trait was not affected by anafranil consumption during four days. The ants were seen coming onto the food site and eating effectively the meat food as usual, each day, staying on the site a usually long time period. The numerical results confirmed this observation: meanly 2.6 ants were counted on the meat site at the time anafranil was present in the ants' liquid food, while 3.5 ants were there counted at the time no antidepressant was provided, these two results being not statistically different (TABLE 1, table line 4: U = 164.5, Z = 0.95, P = 0.34).

4.1.5, "Audacity"

This trait was affected by anafranil consumption. Indeed, under such consumption, the ants became reluctant in coming onto the "risky" experimental apparatus. In front of it, they stopped, moved slowly, turned back on their way, or cautiously came onto it but never climbed on the tower, and soon, slowly, moved away from the apparatus (Fig. 1 B). The numerical results confirmed this observation: while under normal diet meanly

2.5 were counted on the apparatus, under anafranil consumption, only 0.75 were so counted. The difference between the two counts was statistically significant (TABLE 1, table line 5: Z = 105, Z = 2.88, P = 0.003).

4.1.6, Tactile (« pain ») sensation

Unexpectedly, this trait appeared to be affected by anafranil consumption. Indeed, under such consumption, the ants scarcely hesitated to cross the rough zone, and moved there nearly as on a normal substrate. The numerical results reflected these observations (TABLE 2, anafranil). The numbers of ants present, in the course of time, in the initial zone as well as in the large final one statistically differed from those observed before anafranil consumption during six days (initial zone: N = 6, T = -21, P = 0.016; large zone: N = 5, T = 15, P = 0.031). The number of ants in the rough zone only slightly and not statistically differed (N = 6, T = 17.5, $0.078 < P < 0.109$). Under anafranil consumption, the ants' linear and angular speed on the rough substrate largely differed from those occurring under normal diet (linear speed: $\chi^2 = 44.57$, df = 2, $P < 0.001$; angular speed: $\chi^2 = 41.60$, df = 3, $P < 0.001$). In other words, under anafranil consumption, the ants moved more quickly and less sinuously than under normal diet (! the inverse than on a normal substrate, see results related above, in the first paragraph of the section), appearing so to be less uncomfortable than commonly, on a rough substrate. However, the ants under anafranil consumption did not move on a rough substrate exactly just like on a normal one. Their linear and angular speed still differed from those presented (under anafranil consumption) on a normal substrate (linear speed: $\chi^2 = 13.92$, df = 2, $P < 0.001$; angular speed: $\chi^2 = 17.72$, df = 2, $P < 0.001$). Thus, the ants still but very slightly and statistically less, perceived the rough substrate as an uncomfortable one.

4.1.7, A cognitive ability requiring no memory

This trait was, unexpectedly, impacted by anafranil consumption during seven days. Under normal diet, only 13 ants were still in the small initial loggia after 20 min, and six ones could reach the free loggia through the chicanes. Under anafranil consumption, the ants moved slowly, with hesitation, and often turned back on their way. After 20 experimental minutes, 17 ants were still in the small initial loggia while only one ant could navigate the chicanes up to the free loggia (Fig. 1C; TABLE 3, anafranil). This result was statistically significant: initial loggia: N = 5, T = 15, P = 0.031; free loggia: N = 4, T = 10, P = 0.063.

4.1.8, Aggressiveness towards congeners or alien workers

Under normal diet, ants of nests 1 and 2 never presented aggressiveness towards nestmates, but immediately and strongly aggressed alien workers. Under anafranil consumption, these ants also fully accepted congeners during dyadic encountering (TABLE 4, anafranil; $\chi^2 = 0.21$, df = 2, $P \approx 0.90$). On the contrary, they were less aggressive towards alien workers: they seldom presented the stinging behavior, essentially only gripping the aliens or even only widely opening their mandibles (Fig. 1D). A tested ant was seen going behind, and avoiding meeting the alien worker. These observations leaded to numerical results statistically different from the control ones (TABLE 4, anafranil; $\chi^2 = 19.86$, df = 2, $P < 0.001$).

4.1.9, Brood caring behavior

The ants of the two used colonies identically behaved. After anafranil consumption during ten days, these ants took in their mandibles the larva experimentally removed from the nest, just like before such consumption. Each ant holding a larvae moved without dropping down the larvae; it tempted to re-enter the nest, but did so somewhat more slowly, and so less efficiently than under normal diet. The numbers of not re-entered larva after given time periods were so slightly different from those previously obtained under normal diet (TABLE 5, anafranil; N = 4, T = 10, P = 0.063).

4.1.10, Visual and olfactory perception

Under anafranil consumption, ants having continuously found their meat food aside a blue and a yellow squares reacted to each of these two cues presented separately or together (Fig. 1E; TABLE 6, anafranil). This result was statistically significant (after 6 days: blue: N = 9, T = 45, P = 0.002; yellow: N = 8, T = 36, P = 0.004; the two cues: N = 9, T = 45, P = 0.002; after 9 days: each time: N = 10, T = 55, P = 0.001). The numbers of ants reacting to the two cues were similar to those of ants reacting to one or the other cue, what means that ants 'learned' (so saw) each of the two cues. The same event occurred for the two olfactory cues presented to the ants (TABLE 6, anafranil; after 6 as well as 9 days, thyme or estragon or the two cues: N = 10, T = 55, P = 0.001). Once more, the obtained numbers allowed stating that the ants 'learned' (so perceived) each of the two olfactory cues. The workers reacted to the olfactory cues slightly more rapidly than to the visual ones ($1.25 > 1.00$), what is in agreement with the visual perception, the eye morphology, and the essentially use of olfactory cues for navigating by the studied species, *M. sabuleti* (references in the introduction section).

4.1.11, Visual and olfactory operant conditioning ability and memory

Under anafranil consumption, the ants very quickly and statistically more quickly than usually, acquired visual conditioning; they also reached a better score than under normal diet (Fig. 2, anafranil, black circles *vs* empty ones; N = 6, T = 21, P = 0.016). After removal of the visual cues, the ants consuming anafranil went on giving 80% of correct responses (Fig. 1F), even after 72 h, instead of 70% under normal diet. Their visual memory was so increased under that drug consumption, this being statistically significant (N = 6, T = 21, P = 0.016). The ants' olfactory conditioning was also largely enhanced by anafranil consumption (Fig. 2, anafranil, black squares *vs* empty ones). After only 7 h of conditioning, the ants reached a score of 85%, which is a higher score than that reached under normal diet after 55 h of conditioning. Under anafranil consumption, the ants' final score varied between 85% and 90% while under normal diet, it equaled 80%. This was statistically significant (N = 6, T = 21, P = 0.016). After removal of the olfactory cues, the ants under anafranil consumption went on giving the correct response, exhibiting a score of 80% after 79 h instead of 55% under normal diet. This increase of olfactory memory was statistically significant (N = 6, T = 21, P = 0.016).

4.1.12, Habituation to the drug consumption

Ants did not acquire any habituation to the effects of anafranil, on the contrary (TABLE 7, anafranil, upper part). Indeed, after fourteen days of drug consumption, they went on presenting a low linear speed (and so a rather large angular speed), and an orientation to an alarm signal of poor quality (comparison with what occurred before anafranil consumption: linear speed: $\chi^2 = 29.56$, df = 1, $P < 0.001$; angular speed: $\chi^2 = 10.80$, df = 1, $P < 0.001$; orientation: $\chi^2 = 7.10$, df = 2, $P \approx 0.02$). The effect of anafranil on these traits was even stronger after fourteen days than after 2 days, but this was not always significant: linear speed: $\chi^2 = 5.83$, df = 2, $P \approx 0.05$; angular speed: $\chi^2 = 2.03$, df = 2, NS; orientation: $\chi^2 = 2.66$, df = 1, NS.

4.1.13, Dependence on the drug consumption

Ants presented no dependence at all on anafranil consumption, on the contrary. Indeed, after five days of drug consumption, for each of the two tested nests, no ants stayed on the liquid food containing anafranil, and nearly all of them went drinking the liquid food free of anafranil (Fig. 1J, TABLE 7, anafranil, lower part). Meanly, 1.43% of ants were seen on the food containing anafranil, while 98.57 were seen on that free of that drug. This result was of course highly significant ($\chi^2 = 93.34$, df = 2, $P < 0.001$), and allows stating that if a person presents dependence on anfranil consumption, he may present only a psychological dependence, under the assumption that physiological dependence could be explained by the same mechanisms for insects and humans.

4.1.14, Decrease of the effects of the drug, after its consumption ended

The effects of anafranil on the ants' behavior vanished in about 28 h after its consumption ended. The numerical results (Fig. 3, upper part) confirmed what could be observed during these 28 hours. More precisely, the ants' linear speed initially affected by anafranil (t = 0 h: 9.9 mm/sec) progressively increased until reaching the control value (14.4 mm/sec): this trait equaled 10.4, 11.4, 12.3, 12.9, 13.4 and 14.4 mm/sec after 4, 8, 12, 16, 24 and 28 h respectively. Sixteen hours after anafranil consumption ended, ants' linear speed was still statistically affected by the drug ($\chi^2 = 6.5$, df = 2, $P < 0.05$) while after 24 h, this trait became only slightly and not statistically affected ($\chi^2 = 2.15$, df = 2, NS). The same statistical estimation occurred for the ants' sinuosity. This trait decreased from 166 ang. deg./cm at t = 0 to 99 ang. deg./cm at t = 28 h, equaling 163, 151, 141, 133 and 121 ang. deg./cm after 4, 8, 12, 16 and 24 h respectively. The value 133 was still statistically different from the control one ($\chi^2 = 12.22$, df = 3, $P < 0.01$) while that existing at t = 24 h (121 ang. deg./cm) was only slightly and not statistically different from the control one ($\chi^2 = 1.88$, df = 2, NS). As for the ants' orientation towards a source of alarm pheromone, initially affected by anafranil consumption (at t = 0: 65.1 ang. deg.), it successively equaled 60.2, 57.0, 47.2, 44.8, 40.1 and 36.4, respectively 4, 8, 12, 16, 24 and 28 h after anafranil consumption ended. Again, the values occurring at T = 16 h were statistically different from the control ones ($\chi^2 = 4.91$, df = 1, $P < 0.05$) while those existing at t = 24 h slightly and not statistically differed from the control ones ($\chi^2 = 0.92$, df = 1, NS). The decrease of anafranil effects, in about 28 h, revealed by the ants' locomotion and precision of response, appeared to be either a linear function of the time (T), or more probably a polynomial one. The equation should be, for the ants' linear speed: $V = 10.05 + 0.156\ T$ ($r = 0.98$, $P = 0.00007$) or $V = 9.79 + 0.22\ T - 0.0023\ T^2$, for the ants' angular speed: $S = 169{,}25 - 2.29\ T$ ($r = 0.98$, $P = 0.00005$) or $S = 166.83 - 1.67\ T - 0.022\ T^2$, and for the ants' orientation: $0 = 63.63 - 1.029\ T$ ($r = -0.977$, $P = 0001$) or $O = 65.889 - 1.60\ T + 0.02\ T^2$. In other words, anafranil effects vanished in about 28 h, decreasing nearly linearly, though a little more rapidly at the beginning and a little more slowly at the end.

4.2, Concerning efexor, an IRSNa antidepressant, the active substance being venlafaxin

4.2.1, Locomotion and general activity

Efexor largely affected the ants' locomotion (TABLE 1, table line 6) and general activity. More precisely, the ants' sinuosity largely and statistically increased ($\chi^2 = 26.14$, df = 2, $P < 0.001$), while their linear speed somewhat yet statistically decreased ($\chi^2 = 9.50$, df = 2, $P < 0.01$). In fact, the ants seemed agitated, moving erratically and turning nearly continuously. Sometimes, they had difficulties in correctly moving their legs; they stopped, moved again, turned and so on. Apparently, they walked as quickly as usually, but their jerks and large sinuosity decreased the length of their trajectories, i.e. their speed of locomotion. They moved with hesitation near the food sites, did not stay long time periods on them; they re-entered their nest with some difficulties and moved again erratically inside of it.

4.2.2, Orientation towards a source of alarm pheromone; precision of reaction

This trait was affected by efexor consumption. In the vicinity of an alarm signal, the workers obviously perceived the emitted pheromone, but then presented a pronounced positive klino kinesis and positive taxis (as usual) of poor quality. The numerical results reflected this observation (TABLE 1, table line 7): the ants' orientation towards an isolated worker's head largely and statistically differed from the control one (77.5 ang. deg *vs* 58.4 ang deg.; $\chi^2 = 19.26$, df = 1, $P < 0.001$).

4.2.3, Trail following behavior; response to pheromones

This trait was largely impacted by efexor consumption. On a blank circumference, neither ants under normal diet nor those consuming efexor followed the circular line (TABLE 1, table line 8, C: $\chi^2 = 2.81$, df = 1, NS). Under normal diet, ants very well followed a trail (concentration of one poison/trail; median value: 12 arcs of 10°). After having consumed efexor for three days, ants no longer correctly moved on the trail (median value: 2 arcs of 10°; TABLE 1, table line 8, T). Generally, the ants only crossed the trail (Fig. 1G); sometimes, they followed it along a few arcs; among 40 tested workers, only one could moved along 15 arcs of 10°. This lack in capacity of trail following was highly significant ($\chi^2 = 51.10$, df = 2, $P < 0.001$).

4.2.4, Food consumption

This trait was heavily affected by efexor consumption. Nearly no ant came onto the meat food site at a time they should have come since starved for two days. The mean number of ants counted on the meat site equaled 0.20 while before ants consumed efexor, it equaled 2.1 (TABLE 1, table line 9). This result was statistically highly significant (U= 12, Z = 5.07, $P < 10^{-6}$). After the experiment, we artificially set a few ants on the meat: they walked on the meat; they crossed the meat site several times, and then went away, always presenting their erratic locomotion. Food intake was thus obviously decreased by efexor consumption.

4.2.5, "Audacity"

This trait was not affected by efexor consumption (TABLE 1, table line 10). The ants went on being reluctant in coming onto the presented experimental apparatus, and so, similar numbers of ants were counted on that apparatus whatever the ants' diet. This lack of difference was validated by statistical analysis (U= 142, Z = 1.56, P = 0.12).

4.2.6, Tactile ("pain") sensation

This trait was somewhat yet statistically impacted by efexor consumption (TABLE 2, efexor). More precisely, under normal diet, few ants crossed the rough zone. So, after 10 min, 18 ants among 24 were still in the initial zone, 6 ones were in the uncomfortable zone and no one moved in the large zone. After having consumed efexor for five days, the ants were less reluctant in crossing the uncomfortable zone. So, only 12 ants were still in the initial zone after 10 min, 9 ones were on the rough bottom, and 4 ones were moving in the large zone. This result was statistically significant: initial and rough zone: N = 6, T = 21, P = 0.016; large zone: N = 5, T = 15, P = 0.031.

Also, in the uncomfortable zone, the ants' locomotion appeared to be less affected by the rough bottom after efexor consumption than before, this revealing some decrease of the ants' tactile perception due to efexor consumption (Fig. 1H). Numerically, on the rough bottom, the ants' linear and angular speed equaled 7.5 mm/sec and 193 ang. deg./cm respectively, while before efexor consumption, these variables equaled 3.8 mm/sec and 255 ang. deg./cm respectively. These differences were statistically significant: linear speed: $\chi^2 = 24.0$, df = 1, $P < 0.001$; angular speed: $\chi^2 = 16.67$, df = 2, $P < 0.001$. However, even under efexor consumption, the ants went on perceiving some difficulties in moving on the rough bottom since they moved there more slowly. Indeed, on the foraging area, the linear and the angular speed of ants under efexor consumption equaled 11.1 mm/sec and 181 ang.deg./cm respectively, while these variables equaled 7.5 mm/sec and 193 ang.deg./cm

respectively on the presented rough bottom (linear speed: $\chi^2 = 21.29$, df = 2, $P < 0.001$; angular speed: $\chi^2 = 0.15$, df = 1, NS).

4.2.7, A cognitive ability resuiring no memory

Under efexor consumption, the ants were less able to find their way through the presented chicanes (TABLE 3, efexor). Under normal diet, only 8 ants among 30 were still in the small loggia after 20 min, while 8 ones could navigate the chicanes and reach the free loggia. On the contrary, after having consumed efexor for seven days, 16 ants among 30 were still in the small loggia after 20 min, while only one could reach the free loggia through the chicanes. This result was statistically significant: small loggia as well as free loggia: N = 5, T = + or – respectively 15, P = 0.031.

4.2.8, Aggressiveness towards congeners or alien workers

The observations made on nest 3 and on nest 4 were in agreement, and were confirmed by the obtained numerical results (TABLE 4, efexor). In presence of a congener, an ant under normal diet never exhibited any aggressive behavior, while after having consumed efexor for eight days, such an ant became a little bit aggressive, making rough contacts and largely opening its mandibles. This change of behavior was statistically significant: $\chi^2 = 103.79$, df = 2, $P < 0.001$. In presence of an alien worker, an ant under normal diet was immediately very aggressive, gripping and even stinging the alien. Under efexor consumption, an ant less attacked an encountered alien worker. It often only opened its mandibles, sometimes gripped the alien, but was in most cases gripped itself by the alien. This decrease in aggressiveness was statistically significant: $\chi^2 = 32.08$, df = 3, $P < 0.001$. However, the ants consuming efexor were still (though less than before consuming that drug) more aggressive towards alien workers than towards congeners ($\chi^2 = 70.56$, df = 2, $P < 0.001$).

4.2.9, Brood caring behavior

Under efexor consumption, the ants went on caring of their brood (TABLE 5, efexor; Fig. 1I). Indeed, before consuming efexor, the ants of the two tested nests re-entered the entire pool of larva artificially removed from the nest, in about 10 min, while after having consumed that drug for ten days, the same ants re-entered the entire larva except only one. This difference could be explained by the erratic movement of the ants, and their excitation. But, apparently, efexor did not impact the ants' caring behavior, and this was statistically validated (N = 5, T = -9, P = 0.406, NS).

4.2.10, Visual and olfactory perception

Briefly, under efexor consumption, ants kept their visual and their olfactory perceptions, but the latter trait was somewhat weaker than usually. In details (TABLE 6, efexor, visual perception), after six days, as well as nine days, of finding food near visual cues, ants reacted to each of the two cues as well as to the two ones presented at the same time. These reactions were statistically significant (each time: N = 10, T = +55, P = 0.001). The ants distinguished the two presented colors since their mean number in front of the two colors was lower than the sum of their mean in front of each color ($1.45 < 1.75 + 1.10$; $1.70 < 1.60 + 1.65$).

As for the ants' olfactory perception (TABLE 6, efexor, olfactory perception), after six days, as well as after nine days, of finding their food near two odors, the ants reacted to each of these two odors as well as to the two ones presented all together. These reactions were statistically significant: thyme: after six days: N = 7, T = 28, P = 0.008; estragon as well as thyme + estragon: N = 8, T = 36, P = 0.004; after nine days: thyme or estragon: N = 5, T = 15, P = 0.031; thyme + estragon: N = 9, T = 45, P = 0.002. However, on basis of our observations and of numerical results, it can be stated that the ants' olfactory perception was lower than their visual one (1.25 and $1.25 < 1.45$ and 1.70 respectively), what is not usual for *M. sabuleti* which uses essentially odors for its navigation [28]. The ants' olfactory perception was thus affected by efexor consumption, though still existing, the ants going on distinguished the two presented odors. Indeed their mean number in front of the two odors was lower than the sum of the mean number in front of each odor ($1.25 < 1.10 + 1.25$; $1.25 < 0.95 + 1.05$).

4.2.11, Visual and olfactory operant conditioning ability and memory

Under efexor consumption, ants acquired some visual conditioning after a longer latency period (2 days *vs* one day) and reached a lower score (60% *vs* 70%) comparatively with what occurred under normal diet (Fig. 2, efexor, acquisition, black circles *vs* empty ones). This result was statistically significant (N = 6, T = - 21, P = 0.016). After the removal of the visual cues, the ant under efexor consumption presented a loss of 15%, 0%, 10%, 5%, 5%, and 5% of their conditioning, after 7 hrs, 24 hrs, 30 hrs, 48 hrs, 55hrs and 72hrs respectively (they so retained nearly nothing), while under normal diet, they presented a loss of only 5%, - 5%, 0%, 0%, 0% and 0% after the same time periods (Fig. 2, efexor, loss, black circles *vs* empty ones). We made such a comparison of the values obtained under the two kinds of diet because the initial values (i.e. 60% and 70%) were not identical. The observed impact of efexor consumption on the individuals' visual memory was

statistically significant (N = 6, T = 21, P = 0.016). As for the ants' olfactory conditioning, after 7, 24, 30, 48, 55, and 72 training hours respectively, the ants under efexor consumption exhibited a conditioning score of only 55%, 45%, 55%, 50%, 50% and 50% while under normal diet, a score of 55%, 65%, 70%, 75%, 80% and 80% had been observed (Fig. 2, efexor, acquisition, black squares *vs* empty ones). The ants 'olfactory conditioning was thus severely impacted by efexor consumption. This result was statistically significant (N = 5, T = 15, P = 0.031). According to this lack of olfactory conditioning, we could admit that the individuals no longer developed any detectable olfactory memory (same figure as above, loss, black square).

4.2.12, Habituation to the drug consumption

Ants presented no habituation to efexor consumption (TABLE 7, efexor, habituation). Indeed, after having consumed that drug for 14 days, the ants went on moving more sinuously ($\chi^2 = 29.57$, df = 1, $P < 0.001$), and so more slowly ($\chi^2 = 25.86$, df = 1, $P < 0.001$) than usually. Their locomotion was yet somewhat more impacted after 14 days than after two days of efexor consumption, but this fact was only slightly statistically significant (linear speed: $\chi^2 = 7.01$, df = 3, $0.05 < P < 0.10$; angular speed: $\chi^2 = 5.58$, df = 1, $0.01 < P < 0.02$). The ants' orientation towards an alarm signal was still affected by efexor consumption after 14 days (*vs* control: $\chi^2 = 19.80$, df = 1, $P < 0.001$), just like it was after two days of that drug consumption (*vs* values at two days: $\chi^2 = 0.19$, df = 2, NS). Such numerical and statistical results are in agreement with the appearance of the ants consuming efexor, and clearly showed that no habituation occurred to that drug.

4.2.13, Dependence on the drug consumption

No dependence on efexor consumption occurred (TABLE 7, efexor, dependence). During the adequate experiment, only two ants among 38 for nest n° 3 and among 33 for nest n° 4 were counted on the food containing efexor, while 36 and 31 ants for nests 3 and 4 respectively were counted on the food free of that drug (Fig. 1K). In total, 5.66% of ants choose the food containing the drug and 94.34% that free of it. This result was statistically significant (4 *vs* 67: $\chi^2 = 53.39$, df = 1, $P < 0.001$). We can thus state that efexor consumption leads to no physical dependence at all.

4.2.14, Decrease of the effects of the drug, after its consumption ended

Briefly, the effects of efexor vanished in about 12 h, 28 h and 32 h according to the examined physiological trait, and each time following a polynomial function of the time (T) (Fig. 3, efexor).

More specifically, the effect on the ants' linear speed (V) vanished in about 12h according to the function: $V = 10.68 + 0.23T - 0.004\ T^2$. After 0, 4, 8 and 12 h, the ants' speed of movement equaled 10.7, 11.2, 12.5, and 13.1 mm/sec respectively. After 16, 24 and 28 h, it equaled 13.5, 13.8 and 13.8 mm/sec respectively, i.e. values not statistically different from the control one ($\chi^2 = 2.66$, 0.10 and 0.10 respectively, df = 1, NS). After 36 h, it reached the control value, so 14.2 mm/sec. These numerical results confirmed the observation on the ants' movement. As for the ants' sinuosity of movement (S), it vanished in about 28 h according to the function: $S = 185 - 2.96T + 0.03T^2$. After 4, 8, 12, 16 and 24 h, this trait equaled 175, 164, 154, 144 and 136 ang. deg./cm respectively. After 28 h, it equaled 125 ang. deg./cm, a value statistically not different from the control one (120 ang. deg./cm; $\chi^2 = 1.45$, df = 2, NS). After 36 h, the ants' sinuosity was identical to the control one, observed before efexor consumption. All this was in agreement with the ants' observed locomotion. Concerning the ants' orientation towards an alarm signal (O), this ability became again similar to the control one after about 36 h, varying from 74.6 to 37.9 ang. deg. according to the function: $O = 73 - 1.2T + 0.009T^2$. After 4, 8, 12, 16, 24 and 28 h, the ants' orientation equaled 68.1, 62.9, 57.5, 54.0, 51.4 and 47.0 ang. deg., and was so not as good as without consuming efexor (38.4 ang. deg.). After 32 h, this trait equaled 43.2 ang. deg., being so yet slightly, but not statistically, different from the control one ($\chi^2 = 1.61$, df = 1, NS). At last, after 36 h, it equaled 37.9 ang. deg., reaching so the control efficiency ($\chi^2 = 0.42$, df = 1, NS). Once more, all this was obvious while testing and observing the ants.

As a matter of fact, after efexor consumption ended, the ants recovered in about 12h as for their speed of movement, in about 28 h as for their sinuosity, their excitation, and in about 36 h as for the precision of their reaction, this latter physiological trait being the most 'long lasting', the most difficult to restore.

V. CONCLUSION, DISCUSSION

Antidepressants are among the most consumed drugs in the world, the most consumed antidepressant being fluoxetine. This substance is released almost intact in the rivers and streams, through the sewage pipes and waste water treatment plants. So, it could potentially affect aquatic organisms, as for instance aquatic invertebrates [1]. This concern is compounded by the fact that this substance has severe adverse effects on ants in laboratory conditions [5]. But fluoxetine is not the only one consumed antidepressant. In the present work, using experimental protocols identical to those used in our previous study on fluoxetine [5], we examined the effects of two others, different antidepressants, anafranil and efexor, which active substances are clomipramine

hydrochlorid and venlafaxin respectively, on ants, used as biological models, for estimating if these effects are less severe than those of fluoxetine. Anafranil, an 'ATC' antidepressant (see introduction section), decreased the ants' general activity, their precision of reaction, their response to pheromones, their 'audacity', their tactile (or 'pain') perception, their cognition, and their aggressiveness towards alien individuals. It only slightly decreased their brood caring behavior, did not impact their food consumption, and did not affect their visual and olfactory perception. It increased their conditioning ability as well as their visual and olfactory memory. Individuals never developed habituation to, or dependence on, that drug consumption. After its consumption ended, the effects of anafranil nearly linearly vanished in about 28 hrs. Efexor, an 'ISRS' antidepressant (see introduction section), increased the individuals' general activity and their sinuosity of movement. It largely decreased their precision of reaction, their response to pheromones, their food consumption, and somewhat reduced their tactile perception and their cognitive ability. It did not impact their 'audacity', nor their brood caring behavior; it induced aggressiveness between congeners, and diminished that towards alien ants. This drug did not affect the ants' visual perception but somewhat impacted their olfactory one. Under efexor consumption, ants could acquire some visual conditioning but no olfactory one, and their memory was affected. The ants developed no habituation to, as well as no dependence on efexor consumption. The effects of that drug vanished in 12 h to 32 h, according to a polynomial function of the time. Fluoxetine, an 'IRSNa' antidepressant (see introduction section and [5]), increased the individuals' general activity, decreased their precision of reaction, their response to pheromones, their food consumption, their brood caring, their cognition, their olfactory perception, and their conditioning as well as their memorization abilities. It induced aggressiveness between congeners, and decreased that towards aliens. There was no habituation to, and no dependence on that drug, which effects vanished in two to two and a half days. So, on basis of our experiments, anafranil has the less adverse effects while efexor has more and stronger adverse effects, and fluoxetine has the strongest adverse impacts of all three antidepressants.

Our results are in agreement with the primary and secondary effects actually known (and accessible to public) for humans for these antidepressants. Anafranil is known for having some sedative effect, for leading to gain of weight and for somewhat reducing pain perception [45, 46, 47]. It is admitted that efexor induces uncontrolled locomotors movements, trouble with visual, auditory and gustatory perception, as well as some loss of appetite [48]. As for fluoxetine, only the more anxious individuals are sensitive to its action, and under that drug consumption, fewer individuals stay motionless [49]. Persons consuming fluoxetine may be perturbed, impatient, agitated, and might be exposed to suicidal behaviors [6 and references therein, 50].

A few remarks must now be made about our experimental methodology. The samples we used were not very large, generally equaling 20 individuals, but are not too small for physiological and ethological observations [41]. The ants consumed only small quantities of the provided drug each day, but we are sure that they effectively consumed some drugs because they had no other liquid and sugar food at their disposal than that containing the drug. We observed that the ants under anafranil consumption, being calm, moving slowly, and often resting, acquire very quickly visual as well as olfactory conditioning, both of excellent quality, while those consuming efexor and appearing rather 'nervous' acquired conditioning of poor quality. This observation is in agreement with a previous result [35]: the ants working the less, reached better conditioning score; the ants working the most, reached conditioning score of poor quality. It is also in agreement with what is commonly known for dogs and horses training sessions: the more calm the animals are, the better they could acquire wanted behavior. On ants, we observed no dependence (no physical dependence, very probably, rather than psychological one) on the studied antidepressants consumption. So, if humans become dependent on such antidepressants consumption, this dependence may be more a psychological one than a physiological one. We here found, as in our previous similar works (references in the introduction section), that there was no dependence on substance consumption when there was no habituation to that substance, and when its effects rather slowly vanished in the course of time. Some general remarks can now be emitted. Ants appeared to be excellent biological models. Insects react similarly to humans when exposed to similar stimuli [51]; ants have a nicotinic receptor [52]. Ants are highly evolved and eu-social (see the introduction section). Many results (i.e. about tactile perception, memory, aggressiveness, habituation, dependence, and decrease of effects after the end of consumption) give an objective, precise, and detailed idea of the effects that could be presumed on humans. Experiments on ants offer numerous advantages to researchers: there are low expensive, possible at any period of the year, rapid, and reproducible. However, these kinds of experiments must be only a first step in the study of the potential adverse effects of drugs for humans. Further experimentation is required on mammals (rats, mice, monkeys; for instance: [53]) before stating any strong conclusions for humans. As a matter of fact, on basis of what we observed for drugs largely consumed by humans, we underline the importance of making experiments on ants or other insects, as well as on rats, mice or monkeys before giving newly elaborated drugs to humans [54]. On the other hand, ants could be excellent biological models for analyzing adverse effects of various substances on aquatic invertebrates, which are effectively actually impacted by drugs (references in the introduction section, [55]). Finally, we estimate that appropriate wastewater treatment systems should be set up

for avoiding contamination of natural water by artificial drugs, this not only for antidepressants but also for any artificial substances employed by humans and not destroyed during water treatment processes, but eliminated intact or nearly intact in the natural watercourses. One of these numerous substances is carbamazepine, an anti-epileptic drug, largely present in natural water of western countries [55, 56]. We thus intend to study the adverse effects of carbamazepine on ants in a future work.

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TABLE 1. Effects of two antidepressants on five physiological and ethological traits.

Experimental details are given in the text. The table gives, for the three first traits, the median and quartiles of the obtained values, and for the two last traits, the mean and extreme values. It also gives, for the three first traits, the result of non parametric χ^2 tests, and for the two last ones, the result of Mann-Whitney U tests. Briefly, anafranil reduced the individuals' linear speed, somewhat decreased their precision of reaction, and impacted their response to pheromones. It did not affect their food consumption, but reduced their 'audacity'. Efexor increased the ants' sinuosity, somewhat reducing so their linear speed; it reduced their precision of reaction, their response to pheromones, and their food consumption; it did not impact their 'audacity'.

Examined traits	No drug consumed	Under drug consumption	statistics
ANAFRANIL			
Locomotion Linear speed (mm/sec) Sinuosity (ang.deg./cm)	 14.4 (13.1-15.5) 104 (85-113)	 9.9 (8.8-11.1) 137 (122-151)	 P < 0.001 0.001<P<0.01
Orientation to an alarm signal (ang. deg.)	35.8 (30.2-44.4)	46.2 (35.2-57.6)	P ≈ 0.05
Trail following (nber of arcs walked)	C: 1.0 (1.0 – 2.0) T: 12.0(6.0-18.0)	C: 1.0 (1.0 – 1.3) T: 4.0 (2.0-9.3)	NS 0.001<P<0.01
Food consumption	3.5 (1 – 7)	2.6 (2 – 4)	P = 0.34 NS
"audacity"	2.5 (0 – 6)	0.75 (0 – 2)	P = 0.003
EFEXOR			

Locomotion			
Linear speed (mm/sec)	14.2 (12.7 – 15.2)	11.1 (10.2 – 12.9)	P < 0.01
Sinuosity (ang.deg./cm)	120 (108 – 127)	181 (164 – 212)	P < 0.001
Orientation to an alarm signal (ang. deg.)	38.4 (27.9 – 48.0)	77.5 (64.5 – 86.2)	P < 0.001
Trail following (nber of arcs walked)	C: 1.0 (1.0 – 1.0) T: 12.0(8.0-18.0)	C: 1.0 (1.0 – 1.0) T: 2.0 (1.0 – 3.0)	NS P < 0.001
Food consumption	2.1 (1 – 4)	0.2 (0 – 1)	P < 10-6
"audacity"	1.6 (0 – 4)	1.1 (0 – 3)	P = 0.12 NS

TABLE 2. Effect of two antidepressants on tactile perception.

Experimental and statistical details are given in the text. Briefly, under anafranil or efexor consumption, the tactile perception was reduced: a few ants crossed the uncomfortable zone changing only slightly (though yet statistically) their locomotion (linear speed: mm/sec; angular speed: ang.deg./cm).

Variables assessed	Under normal diet				Under antidepressant			
ANAFRANIL	T	small	rough	large zones	T	small	rough	large zones
$N^{ber}$of ants in the three zones	0	24	0	0	0	21	3	0
	2	19	5	0	2	14	3	7
	4	18	5	1	4	12	7	5
	6	16	5	3	6	8	8	8
	8	20	3	1	8	8	5	11
	10	19	5	0	10	9	6	9
in the rough zone:								
linear speed	3.7 (3.2 – 4.1)				8.2 (7.4 – 8.3)			
angular speed	318 (277 – 378)				154 (129 – 185)			
on usual substrate								
linear speed	14.4 (13.1 – 15.5)				9.9 (8.8 – 11.1)			
angular speed	104 (85 – 113)				137 (122 – 151)			
EFEXOR	T	small	rough	large zones	T	small	rough	large zones
$N^{ber}$of ants in the three zones	0	24	0	0	0	21	3	0
	2	22	2	0	2	14	8	2
	4	18	4	2	4	10	9	5
	6	18	4	2	6	10	8	6
	8	16	5	3	8	14	6	4
	10	18	6	0	10	12	9	4
in the rough zone:								
linear speed	3.8 (3.1 – 4.1)				7.5 (6.9 – 9.1)			
angular speed	255 (209 – 302)				193 (161 – 208)			
on usual substrate								
linear speed	14.2 (12.7 – 15.2)				11.1 (10.2 – 12.9)			
angular speed	120 (108 – 127)				181 (164 – 212)			

TABLE 3. Effect of two antidepressants on a cognitive ability requiring no memory.

Ants were set in an initial loggia from which they could escape towards a free one, by navigating through chicanes. Details and statistics are given in the text. Briefly, anafranil as well as efexor decreased this ability.

ANAFRANIL	Under normal diet		Under anafranil consumption	
N^{ber} of ants in the:	initial loggia	free loggia	initial loggia	free loggia
after 0 min	21	0	30	0
5 min	12	3	22	1
10 min	17	3	20	1
15 min	15	4	20	1
20 min	13	6	17	1
EFEXOR	Under normal diet		Under efexor consumption	
N^{ber} of ants in:	initial loggia	free loggia	initial loggia	free loggia
after 0 min	14	1	22	0
5 min	8	3	21	0
10 min	10	2	21	0
15 min	7	3	19	1
20 min	8	8	16	1

TABLE 4. Effects of two antidepressants on aggressiveness.

Potential aggressiveness towards congeners and expected one towards alien workers were assessed, during dyadic encountering, giving the levels 0, 1, 2, 3, 4 for meetings with no reaction, antennal contacts, mandibles opening, gripping, and stinging respectively. Experimental and statistical details are given in the text. Briefly, anafranil did not change the full acceptance between congeners, and decreased the usual aggressiveness towards alien workers, while efexor induced aggressiveness towards nestmates and decreased that in front of aliens.

Drug	levels of aggressiveness	Congeners' meetings		Aliens' meetings	
		normal diet	+ drug	normal diet	+ drug
ANAFRANIL	0	71	91	9	
	1	38	39	17	42
	2	4		49	67
	3			102	95
	4			22	7
EFEXOR	0	73	19	2	
	1	36	60	25	24
	2	56		50	75
	3	1		91	37
	4			26	7

TABLE 5. Effect of two antidepressants on brood caring behavior.

Five larva of the two used colonies were removed from their nest and observed. Those still remaining out of the nest (not returned inside the nest by workers) were counted in the course of time. Anafranil only slightly affected this ethological trait, while efexor scarcely and not statistically impacted it. Statistical precision is given in the text.

ANAFRANIL	under normal diet	under anafranil consumption
N° of larva not re-entered after	0 2 4 6 8 10 min 10 8 5 3 1 0	0 2 4 6 8 10 min 10 8 6 5 4 2
EFEXOR	under normal diet	under efexor consumption
N° of larva not re-entered after	0 2 4 6 8 10 min 10 9 7 5 2 0	0 2 4 6 8 10 min 10 8 6 4 3 1

TABLE 6. Effect of two antidepressants on visual and olfactory perception.

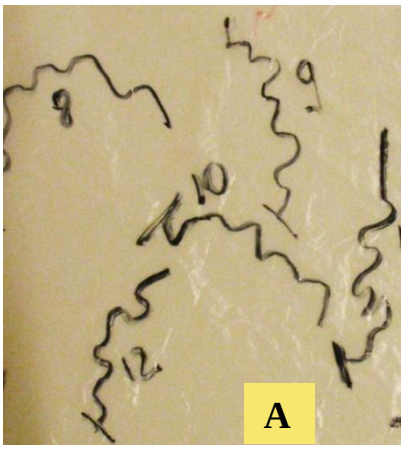
Ants of two colonies could find their food in presence of a blue square and a yellow one, as well as pieces of thyme and of estragon. Six and nine days later, they were tested in presence of no cue, one or the other visual or olfactory one, and the two visual or olfactory ones. Reacting ants were counted 10 times, and the mean values calculated. Experimental details and statistical analysis are given in the text. Briefly, anafranil did not reduce the ants' visual or olfactory perception, while efexor slightly decreased the ants' olfactory perception.

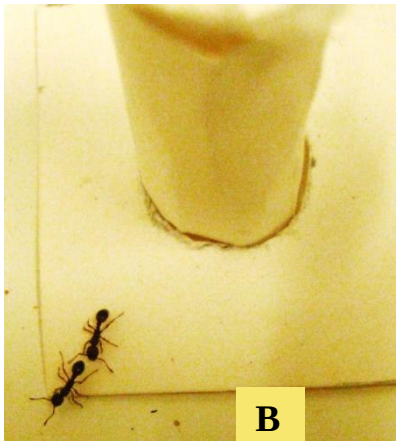
Drug Examined trait	Training time period	Mean n^{bers} of ants reacting to no cue, one cue, the other one, or the two ones			
ANAFRANIL					
		no cue	blue	yellow	blue + yellow
Visual perception	6 days	0.30	1.00	0.80	1.00
	9 days	0.00	2.80	1.90	2.60
		no cue	thyme	estragon	thyme + estragon
Olfactory perception	6 days	0.00	1.10	1.25	1.25
	9 days	0.20	2.40	2.00	2.60
EFEXOR					
		no cue	blue	yellow	blue + yellow
Visual perception	6 days	0.50	1.75	1.10	1.45
	9 days	0.50	1.60	1.65	1.70
		no cue	thyme	estragon	thyme + estragon
Olfactory perception	6 days	0.75	1.10	1.25	1.25
	9 days	0.50	0.95	1.05	1.25

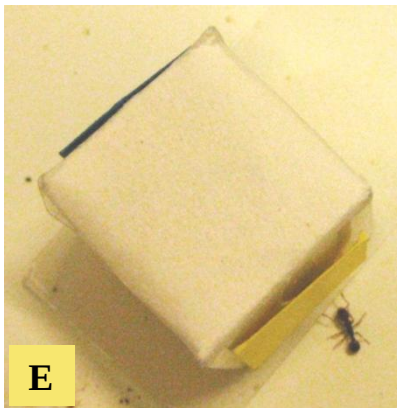
TABLE 7. Habituation to (1), and dependence on (2), two antidepressants consumption.
(1) Ants' locomotion and orientation to an alarm signal were assessed before, after two (see Table 1), and after fourteen days of drug consumption: the effect of anafranil, as well as of efexor, persisted and even became stronger. (2) Ants had experimentally the choice between liquid food containing an antidepressant and identical liquid food free of that drug: nearly no ant chose the food containing either anafranil or efexor. Experimental details and statistical precision are given in the text.

Drugs; studied traits	Variables assessed	Numerical results
ANAFRANIL Habituation after 14 days (before and after 2 days: Table 1)	linear speed (mm/sec) angular speed (ang.deg./cm) orientation (ang. deg.)	8.7 (7.8 – 9.6) 153 (127 – 179) 60.5 (36.2 – 72.3)
Dependence	ants on food + anafranil *vs* on food free of that drug	*nest 1:* 0 *vs* 119 = 0% *vs* 100% *nest 2:* 3 *vs* 102 = 2.86% *vs* 97.14%
EFEXOR Habituation after 14 days (before and after 2 days: Table 1)	linear speed (mm/sec) angular speed (ang.deg./cm) orientation (ang. deg.)	11.1 (9.5 – 11.4) 206 (193 – 229) 75.1 (64.5 – 89.7)
Dependence	ants on food + efexor *vs* on food free of that drug	*nest 1:* 2 *vs* 36 = 5.26% *vs* 94.74% *nest 2:* 2 *vs* 31 = 6.06% *vs* 93.94%

ANAFRANIL







EFEXOR

DEPENDENCE ON

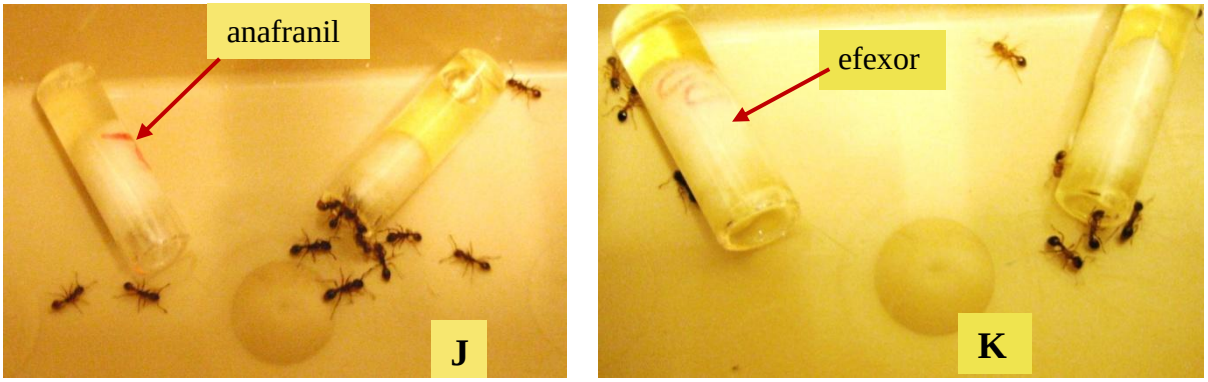
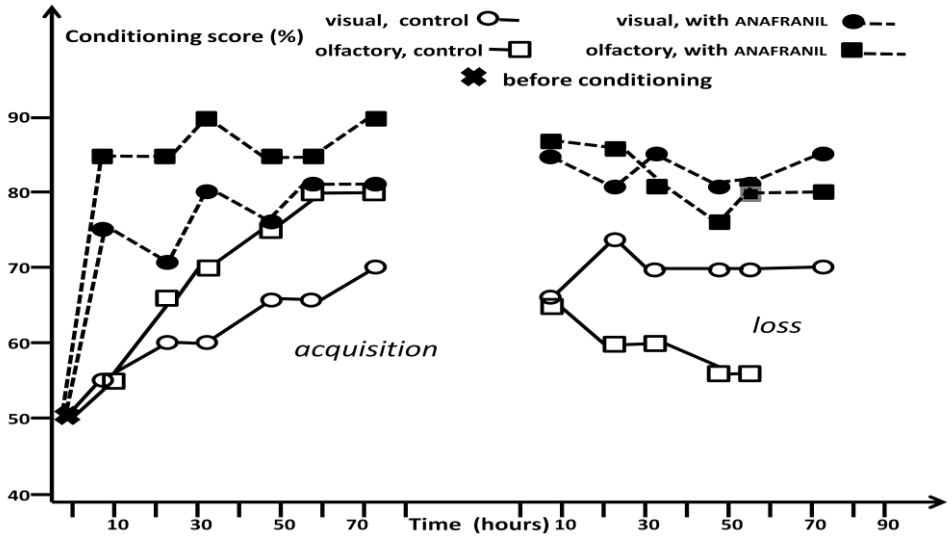


Figure 1. Some views of the experiments made with anafranil (A – F + J) and efexor (G – I + K). A: ants' trajectories; the trajectories are short; the ants moved slowly. B: ants in front of a 'risky' apparatus; they moved away from it. C: ants in a small loggia, in front of chicanes: no ants crossed the chicanes and so reached the large zone located behind. D: an ant in front of an alien worker: it only opened its mandibles. E: an ant reacted to a colored cue previously present on its foraging area for six days. F: an ant conditioned to a hollow green cube reacted to it (went under it) when tested in a Y maze apparatus. G: an ant under efexor consumption, tested on a trail, did not follow that trail but only crossed it. H: an ant under efexor consumption moved nearly normally on an uncomfortable, rough substrate; its tactile perception was somewhat decreased. I: an ant consuming efexor took care of a larvae as usually, though being more excited than usually. J: ants having continuously had anafranil in their liquid food preferred food free of that drug: they presented no dependence on anafranil consumption. K: ants receiving efexor chose food free of that drug: no dependence developed on efexor consumption.



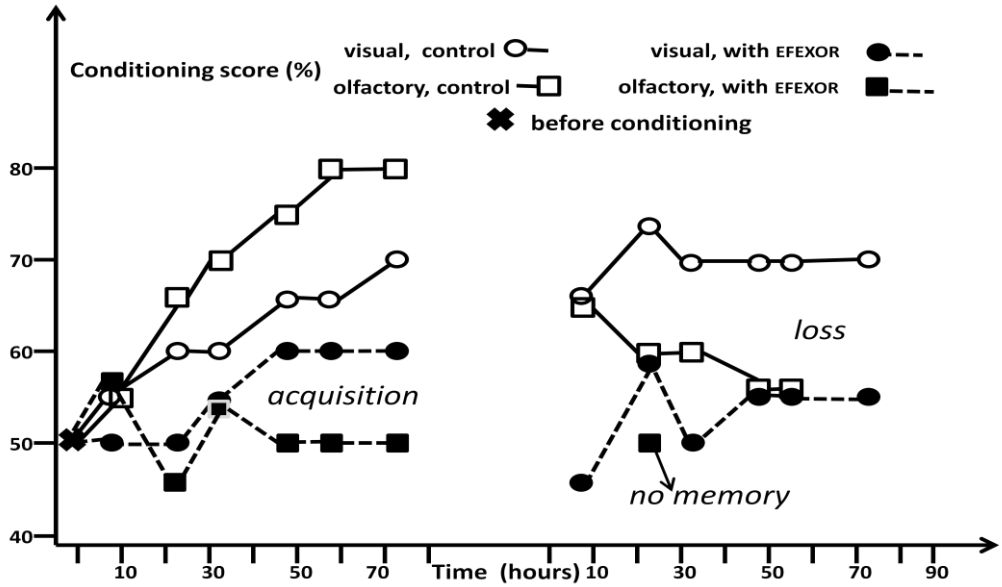


Figure 2. Effect of two antidepressants on ants' ability in acquiring visual and olfactory conditioning, and on their visual and olfactory memory. Experimental and statistical details are given in the text. Briefly, anafranil (upper graphs) largely increased these ants' traits, while efexor drastically reduced them.

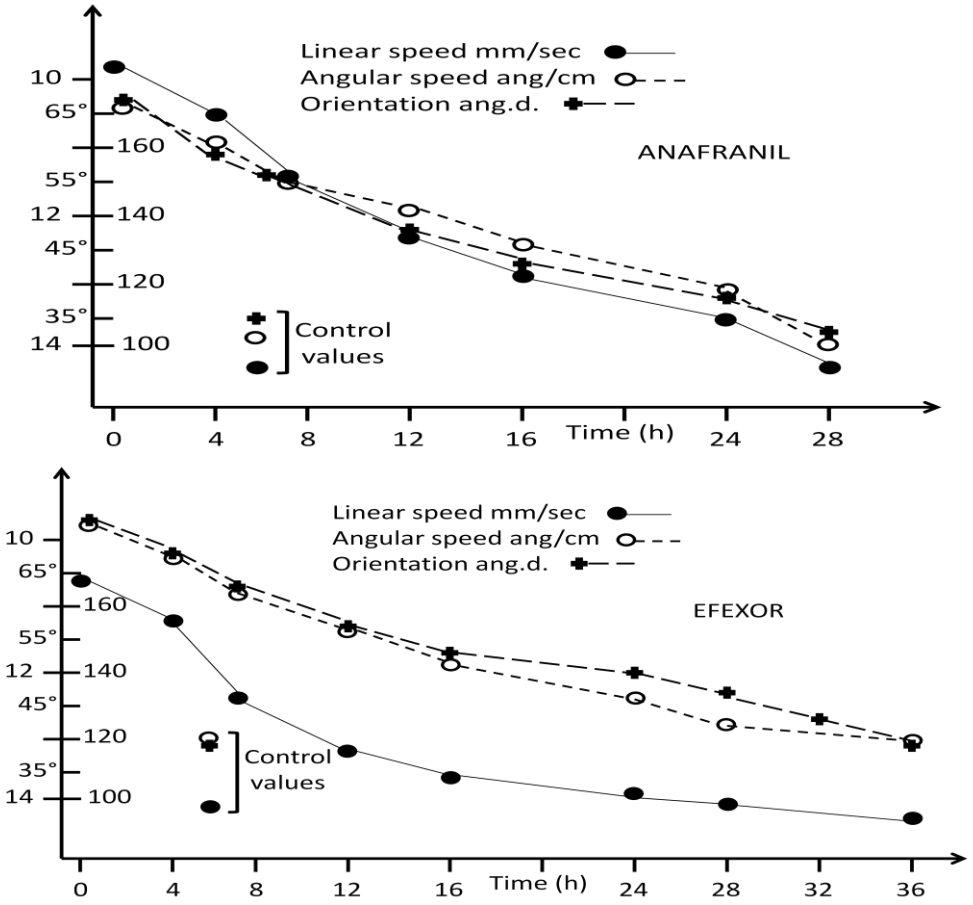


Figure 3. Decrease of the effects of two antidepressants, after their consumption ends. The effects of anafranil linearly vanished in about 28 h; those of efexor vanished in about 12, 28 and 36 h according to the examined trait, and following a polynomial decreasing curve.