World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Physiological and ethological effects of alprazolam, using ants as biological models

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Received: 15-04-2016 / Revised: 31-05-2016 / Accepted: 02-06-2016 / Published: 02-06-2016

ABSTRACT

Alprazolam is the most consumed anxiolytic drug. Its adverse effects have roughly been examined. Here we studied its effects on ants used as biological models. Alprazolam reduced the ants' food consumption, general activity, locomotion, precision of reaction, response to pheromones, audacity, brood caring, cognition, tactile perception, and middle term memory, though not impacting short term memory. It induced aggressiveness against nesmates. Habituation to alprazolam occurred for locomotion, but not for aggressiveness. Ants developed dependence on it. The effect of alprazolam on locomotion rapidly vanished after weaning. Its effect on aggressiveness rapidly decreased from 4h to 13h after weaning, then it slowly vanished in 60h. The rapid decrease of the drug effects may lead to dependence.

Key words: aggressiveness, cognition, dependence, locomotion, memory

INTRODUCTION

The most consumed drugs in the world are antidepressants, anxiolytics, soporifics, antibiotics and hormones. The two first ones may impact patients' general activity, nervous system functioning and behavior. We have already studied the effects of fluoxetine, a 'ISRS' antidepressant [1], anafranil (a 'ATC' antidepressant) and efexor, (a 'IRSNa' antidepressant) [2] using ants as biological models. Fluoxetine has many adverse effects and leads to dependence; efexor is less toxic though having yet some unwanted effects; anafranil has nearly no adverse effects. We then aimed to examine physiological and ethological effects of anxiolytics, i.e. of drugs not acting as antidepressants or soporifics, but efficiently reducing anxiousness and panic. These drugs are barbiturics and benzodiazepines. Nowadays, the most and nearly exclusively used anxiolytics are benzodiazepines, and the most used one is alprazolam (Figure 1) [3].

In the present work, we used again ants as biological models for studying the effects of alprazolam on 14 physiological and ethological traits. Here below, we explain why ants can be used as biological models, why we can efficiently use them, what is already presumed about the effects of alprazolam, and which traits we intend to examine.

(1) Why can ants be used as biological models?

Most biological processes are similar for all animals, including humans (i.e. genetics, metabolism, nervous cells functioning). Consequently, a lot of invertebrates and vertebrates are used as models for studying biology [4]. Invertebrates are more and more used because they offer scientists advantages, such as a short life cycle, a simple anatomy, and being available in large numbers [5]. Some species are largely used, for instance, the flatworm *Dendrocelium lacteum*, the nematode worm *Caenorhabdotes elegans*, the mollusk *Aplysia californica*, the beetle *Tribolium castaneum*, the fruit fly *Drosophila melanogaster*, and the domestic bee *Apis mellifera*. Among invertebrates, insects, especially social hymenoptera and among them, bees, are advantageously used as models [6], but ants too can be used. Indeed, colonies containing thousands of ants can be maintained in laboratories, at low cost and conveniently, throughout the entire year. Ants are among the most complex and social invertebrate animals as for their morphology, physiology, social organization and behavior. They

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are among the most morphologically evolved hymenoptera, having a unique resting position of their labium, mandibles and maxilla [7], as well as a lot of glands emitting numerous efficient pheromones [8]. Their societies are highly organized with a strong division of labor, an age-based polyethism and a social regulation [9]. 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, nest surroundings and foraging area [9]. They generally use an alarm signal, a trail pheromone, and a recruitment signal [10]; they are able to navigate using memorized visual and olfactory cues [11]; they efficiently recruit nestmates where, when and as long as it is necessary [12] and finally, they clean their nest and manage cemeteries at the limit of their foraging area [12]. 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 substances, treatments or situations.

(2) Why can we efficiently use ants as models?

We longly worked on ants belonging to the genus *Myrmica*, and among others, on *Myrmica ruginodis* (Nylander, 1846). We know some of its ecological traits, eye morphology [13], subtended angle of vision [14], visual perception [15], navigation system [16], visual and olfactory conditioning capabilities [17], and recruitment strategy [18]. The ontogenesis of cognitive abilities of *Myrmica* species has also been approached [19]. Studies on the impact of age, activity and diet on the conditioning capability of *M. ruginodis* [20] led to presume that ants could be good biological models. This was confirmed while studying the effects of caffeine, theophylline, cocaine and atropine, of nicotine [21], morphine and quinine, of fluoxetine [1], of anafranil and efexor [2], carbamazepine, as well as of buprenorphine and methadone [22]. Each time, we observed effects related to those observed on humans, and brought precision on them. In the present work, we used again the ant *M. ruginodis* for examining the effects of alprazolam.

We can easily assess the ants' sugar water and meat consumption, this food being given on the foraging area, at a visible place. We can also quantify the ants' general activity by looking to their movement in their nest, on their foraging area and food sites. We can assess the ants' locomotion (linear and angular speed), precision of reaction (orientation towards an alarm signal), response to a pheromone (trail following behavior), and audacity. We are accustomed to assess the ants' acquisition of visual conditioning and visual memory. We can quantify the ants' brood caring, cognition and aggressiveness against nestmates. We know how evaluating the ants' tactile perception which might be pain perception. We have set up an experimental protocol for assessing the ants' preference or aversion of a food. Knowing the effect of a drug on a given ants' trait, we can examine the ants' habituation to the drug and assess the decrease of the effect in the course of time after the end of drug consumption.

(3) What is already presumed about the effects of alprazolam?

Alprazolam has been observed to induce unwanted effects on humans such as decrease of appetite, sleepiness, decrease of watchfulness, difficulty in memorizing and thinking, aggressiveness, habituation and dependence. However, these effects are not yet clearly defined (for instance, some researchers state they depend on the time of the day at which the drug is consumed), and most of them are still debated (for instance, some researchers attribute them to other factors) [23, 24]. In spite of this, alprazolam goes on being largely used because more and more persons are suffering from anxiousness and because other similarly active substances, such as flunitrazepam, have far more adverse effects than alprazolam [25].

(4) Which traits did we intend to examine?

We intended to examine the ants'

- meat food and sugar liquid consumption, as well as general activity,
- speed of locomotion, sinuosity of locomotion, precision of reaction through their orientation towards an alarm signal, response to pheromone through their trail following behavior, audacity through their tendency in coming onto an unknown apparatus,
- brood caring behavior, cognition through their ability in crossing a way with twists and turns, aggressiveness against nestmates, tactile (possibly pain) perception,
- ability in acquiring visual conditioning, visual memory,
- habituation to the drug,
- dependence on the drug,
- and finally, the decrease of the effects of alprazolam after its consumption ended.

MATERIAL AND METHODS

Collection and maintenance of ants

The experiments were performed on four colonies of *Myrmica ruginodis* (Nylander, 1846) collected in an old quarry of the Aise Valley (Ardenne, Belgium), on the borders of a forest, the ants nesting under stones or in wood. The colonies were demographically similar, each containing a queen, brood and about 250 workers. 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. The glass tubes were deposited in a tray (34 cm x 23 cm x 4 cm), which internal sides were slightly covered with talc to prevent the ants from escaping. The trays served as foraging areas, food being delivered in them. The ants were fed with 30% sugar water provided *ad libitum* in a small glass tube plugged with cotton, and with pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) provided as meat three times a week on a glass slide. After having made all the control assessments, for making the subsequent experiments, the sugar water was replaced by an aqueous solution of alprazolam delivered to the ants as their usual sugar water. Laboratory temperature was maintained between 18°C and 22°C and the relative humidity was circa 80%. Lighting had a constant intensity of 330 lux while caring for the ants, training and testing them. During other time periods, lighting was dimmed to 110 lux. The ambient electromagnetic field had an intensity of 2-3 $\mu W/m^2$. All the members of a colony are here named nestmates, as commonly done by researchers on social hymenoptera.

Aqueous solution of alprazolam

We used alprazolam produced by the manufacturer 'Pharmaceutical Society Pfizer' under the packaging Xanax®. Xanax® can be bought in any drugstore under the requirement of a practitioner's approval. It exists in pallets containing 0.25 mg, 0.5 mg, 1 mg or 2 mg of alprazolam. The usual humans' consumption varies from 0.25 mg to 0.5 mg of alprazolam three times per day, thus from 0.75 mg to 1.5 mg per day. The maximum amount allowed is 4 mg per day. In this work, we examined the effects of an amount of 1 mg per day, i.e. a commonly consumed amount. In general, humans drink about one litre of water per day. Insects consume proportionally ten times less water than mammals. It could be estimated that the most appropriate solution of alprazolam to be given to ants for being in agreement with a daily amount of 1 mg consumed by humans would be 1 mg into 100 ml of water, i.e. 0.5 mg into 50 ml. A half tablet of 1 mg Xanax® (i.e. 0.5 mg of alprazolam) was thus dissolved into 50 ml of the sugar water continuously provided to the ants. Five ml of that sugar solution with alprazolam were poured into tubes of the kind used for providing sugar water to the ants. The tubes were plugged with cotton which was refreshed each two days, while the entire solution was renewed every five or seven days. It was checked each day if ants actually consumed the aqueous sugar solution of alprazolam. Let us precise that alprazolam is stable in water.

All the assessments detailed below were made firstly while ants did not consume alprazolam, then, one month later, while they continuously consumed it. This time interval allowed using non-parametric χ^2 and Mann-Whitney tests for analyzing some of the results.

Sugar water and meat consumption, general activity

For assessing the ants' sugar water and meat food consumption, as well as their general activity, first under normal diet then under a diet with alprazolam, we counted during five days, four times per day, exactly under the same conditions (giving food or not, t°, humidity, light, time), the ants of the four colonies present on the sugar water, on the provided *T. molitor* larvae, as well as those moving at any place in their environment (food sites, foraging area, nest entrances and inside nest). For each colony and each kind of counts, we added the four numbers obtained (Table 1, Daily counts). We then established the mean of these daily counts and obtained thus, for each kind of counts, five mean values (Table 1, Daily means). These five mean values obtained for ants under alprazolam diet were compared to the corresponding five mean values previously obtained for the same ants under normal diet using the non-parametric test of Wilcoxon [26].We also established the average of these mean values (Table 1, Total means).

Linear and angular speed; orientation towards an alarm signal

The assessments were made on ants moving in their foraging area. For each assessment, the movement of ten ants of each colony (n = 4 x 10 = 40 ants) was analyzed. Ants' linear and angular speed was assessed without presenting any stimulus to the ants. Ants' orientation towards an alarm signal (which allowed examining the ants' precision of reaction) was assessed by presenting an isolated worker's head to the ants. Such a head is a source of alarm pheromone identical to that of an alarmed worker, in terms of dimensions of the emitting source (the mandibular glands' opening) and quantity of pheromone emitted [27].

Trajectories were manually recorded using a water-proof marker pen, on a glass slide horizontally placed above the tray 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 until the ant reached the stimulus or walked for about 6 cm. All the trajectories were then copied with a water-proof marker pen onto transparent polyvinyl sheets remaining affixed to a PC monitor screen due to their own static electricity charge. The trajectories were then analyzed using specifically designed software [28], each trajectory being entered in the software by clicking as many points as wanted with the mouse and by entering then the location of the presented worker's head. After that, the total time of the trajectory was entered, and the software was asked to calculate the ant's linear speed, angular speed and orientation. The linear speed (V, measured in mm/s) of an animal is the length of its trajectory divided by the time spent moving along this trajectory. The angular speed (S, measured in angular degrees/cm) of an animal's trajectory is the sum of the angles, measured at each successive point of the trajectory, made by each segment 'point i to point i – 1' and the following segment 'point i to point i + 1', divided by the length of the trajectory. The orientation (O, measured in angular degrees) 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 recorded trajectory, made by each segment 'point i of the trajectory - given point' and each segment 'point i - point i + 1', divided by the number of measured angles. When O is lower than 90°, the animal has a tendency to orient itself towards the source point and when it is larger than 90°, the animal has a tendency to avoid the source.

Each distribution of 40 values was characterized by its median and quartiles (Table 2, linear speed, angular speed, orientation) and the distributions obtained for ants consuming alprazolam were compared to those obtained for ants under normal diet, using the non-parametric χ^2 test [26].

Trail following behavior

The ants' response to their trail pheromone was assessed for examining the ants' general response to their pheromones. The trail pheromone of *Myrmica* ants is produced by the workers' poison gland. Ten of these glands were isolated in 500µl hexane and stored for 15 min at -25 °C. To perform one experiment, 50µl 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 (Figure 2 C). One minute later, the piece of paper with the artificial trail was placed in an ants' foraging area. The response of 40 ants, 10 for each colony, was recorded. When an ant came into contact with the trail, its response was assessed by the number of arcs of 10 angular degrees it walked without departing from the trail, even if it reversed its walking. 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 (Table 2, trail following, C = control, T = test). Each distribution of values was characterized by its median and quartiles. The distributions of values obtained for ants consuming alprazolam were compared to those obtained for ants under normal diet using the non parametric χ^2 test [26].

Audacity

This trait was assessed on the four colonies. A cylindrical tower built in strong white paper (Steinbach ®, height = 4 cm; diameter = 1.5 cm) was set in the ants' foraging area, and the ants present on it, at any place, were counted 12 times, in the course of 12 min. The mean and extremes of the obtained values were established (Table 2, audacity) and the values obtained under the two kinds of diet were compared using the non-parametric Mann-Whitney U test [26].

Brood caring behavior

This trait was assessed on colonies A and C containing numerous larvae. A few larvae were removed from the inside of the nest and deposited in front of the nest tube entrance. For each colony, five of them, as well as the ants' behavior in front of them, were observed (Figure 2 D). The larvae among the five observed still remaining out of the nest after 5 seconds, 2, 4, 6, 8, and 10 minutes were counted, and the numbers recorded for each colony were added (Table 3, brood caring). The results obtained for ants under alprazolam diet were compared to those obtained for these ants under normal diet using the non-parametric Wilcoxon test [26].

Cognition

This trait was assessed using ants of colonies A and C. Two apparatus schematically presented in [21] were used, one for each colony. They consisted in a small tray (15 cm x 7 cm x 4.5 cm) inside of which pieces of white extra strong paper (Steinbach ®, 12 cm x 4.5 cm) were inserted in order to create a way with twists and turns between an initial loggia too small for 15 ants and a larger free one. For each colony, 15 ants were collected and set all together, at the same time, in the initial loggia of the apparatus. Then, the ants located in the initial loggia and in the large one were counted after 5 seconds, 2, 4, 6, 8 and 10 minutes. The numbers obtained for the two colonies were added (Table 3, cognition), and the results obtained for ants under alprazolam diet were statistically compared to those previously obtained for ants under normal diet using the non-parametric Wilcoxon test [26].

Aggressiveness between nestmates

This trait was quantified using ants of colonies A and C. Ants' potential aggressiveness against nestmates was assessed in the course of dyadic encounters of five pairs of ants of each two colonies, the encountering being conducted in a small cylindrical cup (diameter = 2 cm, height = 1.6 cm), the borders of which had been slightly covered with talc. Each time (in total 5 x 2 = 10 encounters), one ant of the tested pair was observed for 5 minutes and its encounter with the other ant was characterized by the numbers of times it did nothing (level 0 of aggressiveness), touched the other ant with its antennae (level 1), opened its mandibles (level 2), gripped and/or pulled the other ant (level 3), or tried to sting or stung the other ant (level 4) (Figure 2 E). The numbers recorded for the two colonies were added (Table 3, aggressiveness against nestmates), and the results obtained for ants consuming alprazolam were compared to those previously obtained for ants under normal diet, using the non-parametric χ^2 test [26]. We set up a conceptual variable 'a' which equaled the total number of recorded aggressiveness levels 2 + 3 + 4 divided by the total number of levels 0 + 1. This variable 'a' was useful for examining the ants' habituation to alprazolam, and the decrease of its effects after its consumption ended.

Tactile (pain) perception

This trait was assessed on ants of colonies A and C using two apparatus, one for each colony, 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 n° 280 paper (Figure 2 F) was duly folded (11 cm: 2 cm + 7 cm + 2 cm) and tied to the bottom and the borders of the tray, dividing the tray in three zones: a small initial smooth one, 3 cm long, too small for 12 ants at a time, a 3 cm long zone on which ants' walking should be uncomfortable, and a large smooth zone 9 cm long. For each colony, 12 ants were set, all together, at the same time, in the small initial zone, and the linear as well as the angular speeds of these ants (n = 2 x 12 = 24) moving on the rough paper were assessed (Table 3, tactile (pain) perception). The linear and the angular speeds of ants consuming alprazolam were statistically compared to those of ants having never consumed that drug using the non-parametric χ^2 test [26].

Acquisition of visual operant conditioning, and visual memory

This was examined on the four colonies. At a given time, a yellow hollow cube under which ants could enter was set above pieces of *T. molitor* larvae as food, the ants undergoing so visual operant conditioning. These cubes were made of strong paper (Canson ®) according to previously published instructions [17]. The wavelengths reflections of the yellow paper have been determined [29]. Tests were performed while the ants were expected to acquire conditioning (i.e. the study of conditioning ability), and after removal of the yellow cube, while they were expected to partly lose their conditioning (i.e. the study of visual memory). Ants were individually tested in a Y-apparatus constructed of strong white paper, and set in a small tray (30 cm x 15 cm x 4 cm) as previously explained [17]. Each colony had its own Y-apparatus, the sides of the apparatus were slightly covered with talc, and the floor was changed between tests. The Y-apparatus was provided with a yellow hollow cube in one of its branches, half of the tests being conducted with the cube in the left branch and the other half with the cube in the right branch. Choosing the way with the yellow cube was considered as giving the 'correct' choice. Control experiments had previously been made on never conditioned ants as well as on trained ants consuming no alprazolam [17]. This had to be done because, once an animal is conditioned to a given stimulus, it becomes no longer naïve for such an experiment. To conduct a test on a colony, 10 workers were transferred one by one onto the area at the entrance of the Y-apparatus. Each ant was observed until it turned either to the left or to the right in the Y-apparatus, and this first choice was recorded when the ant was beyond a pencil drawn line indicating the entrance of the branch (Figure 2 G). After that, the ant was transferred into a polyacetate cup, until 10 ants were tested, this avoiding testing twice the same ant. All the tested ants were then returned in their foraging area. For each test, the number of ants choosing the "correct" way with the yellow cube was recorded, and the percentage of correct responses established (Table 4; n = 4 x 10 = 40). The numbers of correct responses obtained for ants consuming alprazolam were compared to those obtained for ants having never consumed that drug [17], using the non-parametric Wilcoxon test [26].

Habituation to alprazolam consumption

For evaluating if ants developed habituation to the drug, their linear and angular speeds were assessed after they consumed alprazolam during 11 and 17 days, and their aggressiveness against nestmates was assessed after they consumed the drug for 12 and 18 days (Table 5). These assessments were made in the same way and on the same colonies as the control ones and those made after 1 day (for the locomotion) and 4 days (for the aggressiveness) of alprazolam consumption. The results of these assessments were compared using the non-parametric χ^2 test [26].

Dependence on alprazolam consumption

After the ants of colonies A and C had continuously consumed alprazolam for 19 days, an experiment was performed for examining if they had acquired some dependence. Fifteen ants of each two colonies were transferred into a small tray (15 cm × 7 cm × 5 cm), the borders of which had been covered with talc, and in which two tubes (h = 2.5 cm, diam. = 0.5 cm) were laid, one containing sugar water, the other a solution of sugar water and alprazolam (the same solution as that used in the course of the whole experimental work), each tube being plugged with cotton. In one of the trays, the tube containing the drug was located on the right; in the other tray, it was located on the left (Figure 2 H). The ants drinking each liquid food were counted 12 times in 15 min, and the mean values were established for each kind of food. 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 [26].

Loss of the effects of alprazolam after its consumption ended

Habituation to alprazolam consumption having been detected, the initial values (those for t = 0 h) were those obtained after 17 days (linear speed) and 18 days (aggressiveness) of alprazolam consumption. After the ants had consumed alprazolam during 19 days, they no longer received it during two days (at days 20 and 21), and received it again at the turn of days 21 and 22 (at 24:00) in order to reduce their habituation to the drug. The weaning started at 9:30 on day 22 when the liquid food containing the drug was replaced by drug-free sugar water. After that, the ants' linear speed and aggressiveness against nestmates were assessed in the course of time, in the way they had been previously assessed, except that, for linear speed, 20 instead of 40 trajectories were used. The results (Table 6, Figure 3) were compared to one another and to the control ones using the non-parametric χ^2 test [26].

RESULTS

Sugar water and meat consumption; general activity

The observation as well as the numerical results (Table 1) showed that, under alprazolam diet, ants drunk less sugar water, eat less meat, and were less active than while they were under normal diet. The difference for sugar water was statistically significant (N = 5, T = 15, P = 0.031); that for meat was at the limit of significance (N = 5, T = 14, P = 0.062). Under alprazolam, the ants often stayed motionless at any place of their area, or slept with antennae folded in 'W' (Figure 2 A), and the active individuals (i.e. those moving, eating, guarding the entrance, relocating larvae …) were less numerous than those counted under normal diet (N = 5, T = 15, P = 0.031).

As a matter of fact, at the end of the experiment, meanly 1.30 ants drinking sugar water, 1.24 ants eating meat, and 8.32 active ants have been counted when the ants continuously consumed alprazolam, while 3.72, 2.72, and 17.96 ants had previously been counted when they did not consume it. Alprazolam reduced thus about by half the individuals' food consumption and general activity.

Linear and angular speed

The ants' locomotion was affected by alprazolam consumption (Table 2, linear speed, angular speed). Under drug consumption, the ants walked significantly less rapidly, moving at about 9 mm/s instead of 13.6 mm/s (χ^2 = 36.90, df = 3, P < 0.001), and consequently somewhat more sinuously, turning about 144 angular degrees/cm instead of 116 angular degrees/cm (χ^2 = 13.78, df = 3, 0.001 < P < 0.01).

Orientation towards an alarm signal

Alprazolam affected this ability (Table 2, orientation). While ants under normal diet oriented themselves well towards an isolated worker's head (a source of alarm pheromone; O = 35.3 angular degrees, Figure 2B), one day after they consumed alprazolam, they less excelled in doing so (O = 66.0 angular degrees). The difference was significant: χ^2 = 21.34, df = 2, P < 0.001. This decrease in precision was obvious: the ants under alprazolam moved aside or beyond the presented head, while those under normal diet often reached such a head.

Trail following behavior

This ability to respond to a pheromone and to move along a line traced with it was affected by alprazolam (Table 2, trail following). Under normal diet, ants followed a circular trail along meanly 9 arcs of 10°. Ants consuming alprazolam followed only 3 arcs of 10°. They detected the presence of the trail pheromone, but were poorly able to follow the trail and soon departed from it (Figure 2 C). The difference of behavior between the two kinds of diet was significant: χ^2 = 33.47, df = 2, P < 0.001.

Audacity

This trait was impacted by alprazolam (Table 2, audacity). The ants consuming that drug avoided to move on the unknown apparatus (they turned back on their way when approaching it) or moved on it but went soon away from it. No ant climbed to the top of the tower, contrary to what occurred for ants under normal diet. A few ants gripped the white paper of the apparatus, a behavior examined later on (see 'aggressiveness towards nestmates'). In 12 min, 0.50 ants consuming alprazolam were meanly counted on the apparatus while 0.85 ants under normal diet had previously been counted. The difference between the two counts was significant: U = 840, Z = 2.526, P = 0.011.

Brood caring behavior

This social trait was somewhat impacted by alprazolam consumption (Table 3, brood caring). Under normal diet, the ants readily took between their mandibles the larvae experimentally removed from the nest and transported them inside the nest (Figure 2 D). In ten minutes, all the observed larvae had been re-entered. Under alprazolam consumption, the ants had some difficulties in finding the larvae, and they sometimes presented slight aggressiveness. They took the larvae in their mandibles, but had difficulties in finding the nest entrance. After ten minutes, four larvae, among the ten observed, were still outside the nest. The difference of ants' behavior between the two kinds of diet was at the limit of significance: N = 4, T = 10, P = 0.06, seemingly due to the small sample.

Cognition

This trait was affected by alprazolam consumption (Table 3, cognition). Under normal diet, after the ten experimental minutes, 14 ants among the 30 tested ones were in the initial loggia, 12 ones moved in the twists and turns, and four ones had reached the large loggia beyond these twists and turns. Such a score is usual [24]. Under alprazolam diet, ants often stayed in the initial loggia either motionless or slowly moving. A few ones went into the twists and turns, moved along 5 to 10 cm, then came back in the initial loggia. Such a behavior was in agreement with that in front of an unknown apparatus (see above: audacity). Finally, 26 ants were still in the initial loggia, four ones were in the twists and turns, and none had reached the large loggia. The difference between the two diets as for the ants still in the initial loggia was significant: N = 6, T = 21, P = 0.016; the difference concerning the ants present in the large loggia was not significant due to the too small samples: N = 3, NS.

Aggressiveness against nestmates

Alprazolam affected this trait (Table 3, aggressiveness against nestmates; Figure 2 E). During dyadic encounters, comparatively with what occurred with ants under normal diet, those consuming alprazolam more often opened their mandibles in front of nestmates. The difference of aggressiveness between the two diets was highly significant: χ^2 = 130.32, df = 2, P < 0.001. The variable 'a' depicting the aggressiveness equaled 0.10 for ants under normal diet and 0.91 for ants consuming alprazolam. The ants were thus nine times more aggressive during dyadic encounters with nestmates when they consumed alprazolam. Supplementary qualitative observations were also made. During encounters, ants under normal diet did not try to avoid nestmates but often stayed near them; those consuming alprazolam often avoided nestmates, turning back on their way, generally with opened mandibles. Also, ants consuming alprazolam abnormally presented signs of aggressiveness while being on their foraging area. Moreover, while under normal diet, each time an ant was removed from its nest for experimental purpose, other ants moved towards the far end of the nest and stayed there. In the same circumstances, ants consuming alprazolam moved out of the nest, left their head and antennae, opened their mandibles, and re-entered after several minutes.

Tactile (pain) perception

Unexpectedly, this trait was affected by alprazolam (Table 3, tactile (pain) perception; Figure 2 F). Under normal diet, ants presented difficulties in moving on the rough substrate. They moved slowly (6.2 mm/s) and sinuously (230 angular degrees/cm). While consuming alprazolam, the ants were obviously less affected by the uncomfortable character of the rough substrate. They moved rather quickly (9.8 mm/s) and less sinuously (154 angular degrees/cm). The difference of locomotion between the two diets was significant (linear speed: χ^2 = 23.31, df = 2, P < 0.001; angular speed: χ^2 = 26.49, df = 2, P < 0.001).

Visual conditioning and memory

Under alprazolam consumption, the ants quickly acquired visual conditioning, doing so better than while under normal diet (Table 4). At first, they hesitated several seconds before choosing the correct way in the Y apparatus, and then moved slowly under the yellow cube (Figure 2 G). They presented some aggressive behavior in the Y apparatus, but even so they reached 67.5% of conditioning after only 31 training hours, and 80 % after 72 hours. The difference in ants' conditioning capability according to their diet was significant (N = 6, T = 20, P = 0.031). Thus, alprazolam did not affect the ants' short term memory.

Contrary to visual conditioning, visual memory was affected by alprazolam (Table 4). After only 7 hours without seeing the visual cue, the ants consuming alprazolam presented a score of 65% instead of 70% under normal diet. After 31 hours without seeing the visual cue, the ants consuming alprazolam had lost all their visual conditioning and presented a score of 50% while under normal diet they still presented a score of 62%. Finally, after 72 hours without training, the ants under alprozolam presented a score of 47.5% instead of 60%. The difference in visual memory between the two diets was significant: N = 6, T = - 21, P =

0.016. Thus, the ants consuming alprazolam lost the knowledge of a visual cue more quickly than usually: their middle term visual memory was affected.

Conclusively, the ants' short term memory was not affected by alprazolam (since they remembered the visual cue as long as it was present on their area), but their middle term memory was seriously impacted (since they rapidly lost their acquired conditioning).

Habituation to alprazolam consumption

The ants' locomotion was assessed after 1 day (see above, section 'Linear and angular speed'), 11 days and 17 days of alprazolam consumption (Table 5). After 11 days, the ants' linear speed was still lower than the control one (χ^2 = 14.52, df = 3, 0.001 < P < 0.01) but already somewhat higher than that observed after one day of consumption (χ^2 = 9.56, df = 3, 0.02 ~ P < 0.05). After 17 days, the ants' linear speed remained different from the control one (χ^2 = 8.16, df = 2, 0.01 < P < 0.02), but was this time significantly higher than that observed after one day of drug consumption (χ^2 = 24.70, df = 2, P < 0.001). According to their locomotion speed, the ants presented thus a progressive habituation to alprazolam. After 11 and 17 days of drug consumption, the ants' angular speed was similar to the control one (χ^2 = 2.90, df = 3, NS and χ^2 = 2.83, df = 2, NS respectively) and similar to that presented after one day of consumption (χ^2 = 6.11, df = 3, NS and χ^2 = 2.19, df = 2, NS respectively). According to the sinuosity of their movement, the ants thus progressively developed habituation to alprazolam. By extrapolation, it can be presumed that ants will be fully habituated to alprazolam after about 20 days of consumption.

Concerning the ants' aggressiveness against nestmates (Table 5), no habituation occurred after 12 days of alprazolam consumption: the results then obtained differed from the control ones (χ^2 = 99.99, df = 3, P < 0.001) and remained similar to those obtained after four days of consumption (χ^2 = 3.65, df = 3, NS). After the ants had consumed alprazolam during 18 days, their aggressiveness against nestmates was still obvious; it differed from the control one (χ^2 = 122.86, df = 3, P < 0.001) and was similar to that presented after four days of consumption (χ^2 = 1.89, df = 3, NS). No habituation occurred thus as for the ants' aggressiveness, at least after 18 days. The variable 'a' confirmed this fact.

Dependence on alprazolam

Confronted to sugar water and to sugar water containing alprazolam, the ants chose the latter solution (Figure 2 H). Indeed, 8 ants of colony A were counted on the pure sugar water while 26 ones were counted on the sugar water containing alprazolam, and in the same way, 56 ants of colony C were counted on the sugar water containing the drug while 3 ones were counted on the natural sugar water. In total, 88.17% (n = 82) of the ants were seen drinking the solution of alprazolam and 11.83% (n = 11) the drug-free solution. Thus, the ants preferred sugar water with alprozolam to pure sugar water, a result highly different from that expected under randomness (χ^2 = 33.54, df = 1, P < 0.001). The ants thus developed a physiological dependence on alprazolam after having consumed it at least for 19 days.

Decrease of the effect of alprazolam after its consumption ends

As previously shown ('habituation' section), under alprazolam diet, the workers' linear speed was slower than under normal diet (Table 6; t = 0h *vs* control: U = 408.5, Z adjusted = 3.764, P = 0.0002). After the ants stopped consuming alprazolam, their linear speed continuously increased in the course of time (Table 6, Figure 3) and became not different from the control one as soon as after 2h (U = 296.5, Z adjusted = 1.616, P = 0.106). The values obtained at t = 2h, 6h and 11h differed from that at t = 0h with P = 0.089, 0.026 and 0.0005 respectively. These levels of statistical significance and that of the difference between the values obtained at t = 2h and 11h (P = 0.063) revealed the rapid decrease of the effect of alprazolam on the ants' speed of locomotion after weaning. Such a rapid loss of effect may lead to dependence [21].

The effect of alprazolam on ants' aggressiveness against nestmates vanished otherwise (Table 6, Figure 3). Let us recall (see 'habituation' section) that ants consuming the drug were far more aggressive than those having never consumed it (Table 6; t = 0 h *vs* control: χ^2 = 121.60, df =2, P < 0.001). From weaning, aggressiveness continuously but irregularly decreased, the diminishing being significant and the most rapid between 4 and 13½ h (χ^2 = 11.85, df =2, 0.0005 < $P_{one\text{-}tailed}$ < 0.005). After 13h, the decrease slowed down. Even 52 hours after weaning, the aggressiveness remained stronger than that of ants having never consumed the drug (χ^2 = 38.35, df = 2, $P_{one\text{-}tailed}$ < 0.001). We visually checked that ants were no longer aggressive against nestmates about 60h after they stopped consuming alprazolam.

DISCUSSION

Alprozolam, the active substance of Xanax® as well of Apotex® and Alprox® is the most consumed anxiolytic substance all over the world.

At first sight, on basis of the directives for use of these medicines, its potential adverse effects appear to be not very important. On basis of other information, adverse effects should be examined in details and divulgated [30]. According to the alarming effects we detected on ants in the present work, we looked for more information on the subject and found that, effectively, some important adverse effects exist, four of them being briefly reported in several studies [e.g. 31, 32, 33]: sleepiness, paradoxal reaction (excitation, aggressiveness), antero-grade amnesia, dependence. Here below, we underline the information our work gives about these four adverse effects, and report other effects observed on ants and not yet known or divulgated.

Concerning sleepiness, ants often stopped to move, and stayed motionless, in a resting position, at any moment and anywhere in their environment. Sleepiness induced by alprazolam may thus lead to true sleeping.

Concerning the paradoxal reaction, ants were sometimes excited, nervous, moving sinuously, and they presented aggressive reactions either alone (for instance against the apparatus used to assess their audacity or in the Y-apparatus used to study their conditioning ability) or against their nestmates. They left their head and antennae, contacted nestmates with their antennae, opened their mandibles; they may even grip a nestmate' leg. In the literature, it is reported that some persons may suffer from sleepiness while other ones may have paradoxal reactions. In the present work, we observed that the same ant may be sleepy, then excited and aggressive, and thereafter again inactive. The two adverse effects can thus occur in the same individual. Note that aggressiveness induced by alprazolam is not as strong as that induced by newly used antidepressants, the active substance of which is fluoxetine. Under a diet with the latter substance, the ants killed their nymphs and finally died [1]. Let us add that, while experimenting, we observed that ants consuming alprazolam seemed to be less social, spent less time aside their nestmates, and stayed more often alone than when they did not consume the drug. Such a change in behavior was not easy to detect at its beginning but became obvious a few days after alprazolam consumption. It lasted until the drug was removed from the ants' food.

Concerning the antero-grade amnesia (an effect we did not know while experimenting, i.e. we worked 'being blind'), our experiments allowed to precise that short term memory is not affected since the ants remembered the visual cue as long as it was present above their meat food, but that middle term memory was largely affected since the ants lost the memory of a learned cue as soon as after a few hours without seeing it. However, the ants still recognize their alarm and trail pheromone, their foraging area odor, nest entrance odor, location of food. We can thus conclude that the ants' long term memory was not affected. Only events having occurred several hours before could not be memorized.

The three here above commented adverse effects of alprazolam must be taken into account when the drug is consumed by drivers. Indeed, alprazolam is, together with cannabis, the second substance after alcohol which severely impacts driving quality [34].

The fourth adverse effect of alprazolam reported in some studies [31, 32, 33] is dependence, potentially occurring after several weeks of consumption. This fact is briefly explained in [35]. Our study on ants showed that a strong physiological dependence on alprazolam soon occurred. We obtained 88% of ants preferring food with alprazolam than food without it. Dependence on alprazolam is not a potential, accessory unwanted effect. It is a real, always occurring, and dangerous effect leading to use alprazolam as a true drug (such as cocaine, morphine, nicotine). This must be known by persons advised to care of themselves using alprazolam. Practitioners should take this effect into account when proposing alprazolam to their patients, and should follow them in the course of treatment. Let us add that physiological problems occur during sewerage, and that women are known to consume alprazolam more often than mans, essentially for supporting domestic problems [35].

Our study also revealed seven not yet known or not divulgated effects. Under alprazolam consumption, ants eat less meat and less sugar water: this was obvious and statistically significant. They were less active: they not only at times slept, but also often stopped to move, to care of their brood, to collect food or recruit nestmates. They moved more slowly than usual. They reacted less precisely and inefficiently to their pheromones. They were less inclined to move on an unknown apparatus. They took less care of their brood. Their cognitive ability (requiring no memory) was reduced. Their tactile (pain) perception was reduced. All these effects might exist in humans treated with this drug. Note that some of these effects are similar to those induced by strong drugs such as morphine, quinine, and methadone [36, 22].

Another important point revealed by our study is that ants became finally habituated to alprazolam as for their locomotion, and thus probably general activity. This may also occur in humans, who may thus want to consume more alprazolam. Such an effect is similar to that of strong drugs such as cocaine, morphine, methadone, nicotine. On the contrary (and this is also important), no habituation occurred as for the ants' aggressiveness against nestmates. It is not ruled out that this induced aggressiveness even increases in the course of the drug consumption. Practitioners should pay attention to this potential danger in humans.

The study of the decrease of the effects of alprazolam on ants after its consumption ended also brought interesting information. A rapid decrease was observed for the impact on locomotion, and during a few hours for aggressiveness. Rapid decrease of the effects of a drug after its consumption ends leads to physiological dependence [21]. When humans stop consuming alprazolam, they will perceive a rapid decrease of its effects, and will suffer from physiological symptoms (somewhat described in [35]) which may incite to consume again alprazolam. Humans wanting to stop using alprazolam must thus be helped and cared of. It must also be pointed out that after weaning, the effect of alprazolam on ants' aggressiveness (and excitation), although rapidly decreasing for a time, persisted for at least about fifty hours.

Finally, ants have been a good model for examining the effects of alprazolam (i.e. ants allowed sufficient samples, exact observations, precise quantifications and underscoring yet unknown effects). Other known benzodiazepines have more adverse effects than alprazolam. Other anxiolytic substances, for instance extracts of plants, should thus be considered by practitioners and pharmacists.

CONCLUSION

Using ants as models, we revealed several adverse impacts of alprazolam, some ones being already known though not divulgated as much as they should have been, other ones having not been divulgated. This drug decreases food consumption, general activity, locomotion, precision of reaction, response to pheromones, audacity, brood caring, cognition, tactile perception, and middle term memory. It leads to aggressiveness, habituation, and physiological strong dependence. After its consumption ends, the effect of alprazolam on activity rapidly vanishes, while its effect on aggressiveness slowly vanishes in about 60 hours, the decrease being however rapid 4 to 13 hours after consumption ended, what leads to dependence. Physiological health problems may thus occur in humans stopping using alprazolam. Other similar drugs (benzodiazepines) have more or stronger adverse effects. Research should be made for less problematic anxiolytic drugs.

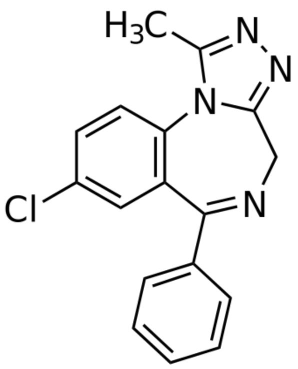

Figure 1. Chemical structure of alprazolam, the most used anxiolytic substance.

Table 1. Effect of alprozolam on ants' sugar water and meat food consumption as well as on their general activity. Experimental details and statistical analysis are given in the text. Assessments were made during five days, on four colonies, while ants were under a normal diet, then while they consumed the same diet together with alprazolam. Alprazolam reduced the ants' sugar water and meat consumption, as well as their general activity.

Days	**colonies**	**Normal diet** sugar water	meat	activity	**Diet with alprazolam** sugar water	meat	activity
		Daily counts					
1	A	0,0,1,1, 2	0,2,1,1 4	1,5,2,7 15	1,1,2,2 6	1,1,0,0 2	6,4,5,5 20
	B	0,1,1,2 4	0,0,0,1 1	1,2,2,4 9	0,0,0,0 0	0,0,0,0 0	1,0,0,1 2
	C	0,1,4,2 7	0,0,0,1 1	7,5,6,3 21	0,0,0,0 0	0,0,0,0 0	2,0,0,0 2
	D	0,1,2,1 4	1,1,1,1 4	5,7,4,6 22	2,2,2,2 8	0,0,0,1 1	3,3,2,4 12
2	A	1,2,3,2 8	1,0,0,0 1	8,6,5,6 25	0,0,2,0 2	0,2,0,0 2	5,5,4,4 18
	B	1,1,1,1 4	1,1,1,1 4	2,2,4,2 10	0,0,0,0 0	0,0,0,0 0	0,0,0,0 0
	C	0,0,0,0 0	1,1,2,2 6	4,4,3,4 15	0,0,0,0 0	0,0,0,0 0	2,3,2,1 8
	D	0,3,2,2 7	0,0,0,0 0	3,3,4,5 15	0,1,1,0 2	1,0,0,1 2	3,2,2,2 9
3	A	0,1,1,0 2	0,0,1,3 4	6,5,4,7 22	1,0,1,0 2	0,1,1,1 3	3,5,4,3 15
	B	1,1,1,1 4	0,1,1,0 2	5,2,2,2 11	0,0,0,0 0	0,0,0,0 0	0,1,1,1 3
	C	3,2,1,1 7	0,1,1,1 3	7,6,5,5 23	0,0,0,0 0	0,0,0,0 0	1,0,0,1 2
	D	0,0,0,1 1	1,2,1,1 5	3,5,6,5 19	0,0,0,0 0	1,1,1,1 4	2,2,2,2 8
4	A	0,0,1,0 1	0,0,0,0 0	3,4,5,5 17	0,0,1,0 1	1,0,1,1 3	4,4,4,4 16
	B	1,2,1,1 5	0,0,0,0 0	2,2,2,3 9	1,1,0,1 0	0,0,0,0 0	1,1,1,2 5
	C	3,3,2,3 11	1,1,1,1 4	10,5,7,6 28	0,0,0,0 0	0,0,0,0 0	2,1,1,1 6
	D	2,1,1,1 5	1,1,0,0 2	5,5,6,3 19	0,0,0,0 0	2,0,1,1 4	2,2,1,1 6
5	A	0,0,0,1 1	0,2,2,0 4	5,4,4,7 20	1,0,0,0 1	0,0,0,1 1	3,2,3,3 11
	B	0,0,0,0 0	0,0,1,1 2	0,1,3,3 7	0,0,1,0 1	0,0,0,0 0	2,1,3,2 8
	C	1,1,2,3 6	1,0,0,1 2	7,5,4,9 25	0,0,0,0 0	0,0,0,0 0	2,3,2,2 9
	D	2,1,2,1 6	1,2,0,2 5	7,5,3,5 20	0,0,0,0 0	0,1,0,1 2	2,2,1,2 7
		Daily means					
1		4.3	2.5	16.8	3.5	0.8	9.0
2		4.8	2.8	16.3	1.0	1.0	8.8
3		3.5	3.5	18.8	0.5	1.8	7.0
4		3.0	1.5	19.9	1.0	1.8	8.0
5		3.0	3.3	18.0	0.5	0.8	8.8
		Total means					
		3.72	2.72	17.96	1.30	1.24	8.32

Table 2. Effect of alprazolam on five ants' ethological traits. For each trait, 40 values were recorded. The table gives their median (and quartiles) or their mean [and extremes]. Experimental methods, statistics and results are detailed in the text. C = control (a blank circumference); T = test (a circumference traced with trail pheromone).

Traits	**Normal diet**	**Diet with alprazolam**
linear speed (mm/s)	13.6 (12.2 – 14.8)	9.0 (8.2 – 11.1)
angular speed (angular degrees/cm)	116 (106 – 135)	144 (120 – 166)
orientation (angular degrees)	35.3 (29.2 – 44.2)	60.0 (39.6 – 90.1)
trail following (n° of walked arcs)	C: 1.0 (1.0 – 1.0) T: 9.0 (6.0 – 15.3)	C: 1.0 (1.0 – 1.0) T: 3.0 (1.8 – 5.0)
audacity (n° of ants)	0.85 [0 – 2]	0.50 [0 – 1]

Table 3. Effect of alprazolam on ants' brood caring behavior, cognition, aggressiveness against nestmates and tactile (pain) perception. Ants of two colonies were tested while under normal diet, then while under the same diet with alprazolam. Experimental methods, statistics and results are detailed in the text. Aggressiveness levels: 0 = no reaction, 1 = antennal contact, 2 = mandibles opening, 3 = grip, 4 = sting. The variable 'a' (= n° levels 2 + 3 + 4 / n° levels 0 + 1) summarizes the aggressiveness level.

Trait		**Normal diet**		**Alprazolam diet**	
Brood caring:					
n° of not re-entered larvae after:	5 sec	10		10	
	2 min	8		8	
	4 min	6		7	
	6 min	4		6	
	8 min	2		5	
	10 min	0		4	
Cognition:					
n° of ants in front and beyond the twists and turns after:		in front	beyond	in front	beyond
	5 sec	28	0	29	0
	2 min	25	0	28	0
(in front = in the small	4 min	23	0	26	0
initial loggia	6 min	17	2	25	0
beyond = in the large	8 min	17	3	28	0
free loggia)	10 min	14	4	26	0
Aggressiveness against nestmates:					
levels:	0	112		18	
	1	65		59	
	2	18		66	
	3	0		4	
	4	0		0	
variable 'a'		0.10		0.91	
Tactile (pain) perception:					
linear speed (mm/sec)		6.2 (5.5 – 7.5)		9.8 (8.2 – 10.6)	
angular speed (ang. deg./ cm)		230 (200 – 259)		154 (127 – 173)	

Table 4. Effect of alprazolam on ants' visual conditioning and memory. The ants were trained to a yellow cube and tested in a Y apparatus provided with such a cube in one of its branch. The table gives the numbers of ants giving the 'correct' response and the proportion of correct responses for the tested population. Obviously, alprazolam did not reduce and even enhanced the ants' ability in acquiring visual conditioning, thus the ants' short term memory, but drastically reduced their middle term visual memory.

Traits		**Diet with alprazolam**					**Normal diet**
		nest 1	**2**	**4**	**5**	**%**	**%**
Visual conditioning							control: 50%
after:	7 hrs	7	5	8	5	62.5	47.0
	24hrs	7	7	6	6	65.0	60.0
	31hrs	6	7	7	7	67.5	63.3
	48hrs	8	7	7	7	72.5	65.0
	55hrs	7	9	7	8	77.5	75.0
	72hrs	8	8	8	8	80.0	81.7
Visual memory							
after:	7 hrs	7	6	7	6	65.0	70.0
	24hrs	5	6	5	6	55.0	65.0
	31hrs	5	5	5	5	50.0	62.0
	48hrs	5	5	5	3	45.0	50.0
	55hrs	5	5	6	3	47.5	62.0
	72hrs	5	5	4	5	47.5	60.0

Table 5. Habituation to alprazolam consumption. Three traits affected by alprazolam were assessed while ants did not consumed it, and after they had consumed the drug during 3 different time periods. Details are given in the text. Briefly, habituation to alprazolam occurred for locomotion, but not for aggressiveness. The aggressiveness levels 0 to 4 and the variable 'a' are defined in the legend of Table 3.

Traits	**Diet**	**Assessments**
Linear speed (mm/s)	without alprazolam	13.6 (12.2 – 14.8)
	+ alprazolam during 1 day	9.0 (8.2 – 11.1)
	+ alprazolam during 11 days	11.4 (9.8 – 12.5)
	+ alprazolam during 17 days	12.1 (11.1 – 13.1)
Angular speed (angular degrees/cm)	without alprazolam	116 (106 – 135)
	+ alprazolam during 1 day	144 (120 – 166)
	+ alprazolam during 11 days	129 (105 – 148)
	+ alprazolam during 17 days	125 (107 – 143)
Aggressiveness against nestmates		levels 0 1 2 3 4 ; 'a'
	without alprazolam	112 65 18 0 0 ; 0.10
	+ alprazolam during 4 days	18 59 66 4 0 ; 0.91
	+ alprazolam during 12 days	7 54 50 5 0 ; 0.90
	+ alprazolam during 18 days	14 62 85 4 0 ; 1.17

Table 6. Decrease of the effects of alprazolam after its consumption ended. Ants were again fed with sugar water free of alprazolam at t = 0, then their linear speed and aggressiveness against nestmates were quantified after several time periods. Details are given in the text. The results are partly illustrated in Figure 3.

Time (hours)	**Linear speed (mm/s)**	**Aggressiveness towards nestmates**					
		Levels 0	**1**	**2**	**3**	**4**	**variable 'a'**
control	13.6 (12.2 – 14.8)	112	65	18	0	0	0.10
0	12.1 (11.1 – 13.1)	14	62	85	4	0	1.17
2	12.8 (11.6 – 13.9)						
4		19	55	81	2	0	1.12
6	13.3 (11.5 – 15.1)						
8 ½		19	51	52	2	0	0.77
11	14.3 (12.3 – 15.5)						
13 ½		20	54	33	0	0	0.45
16		26	55	30	0	0	0.37
25		25	64	28	0	0	0.31
30		25	88	29	0	0	0.26
36		22	74	20	0	0	0.21
52		38	88	18	0	0	0.14

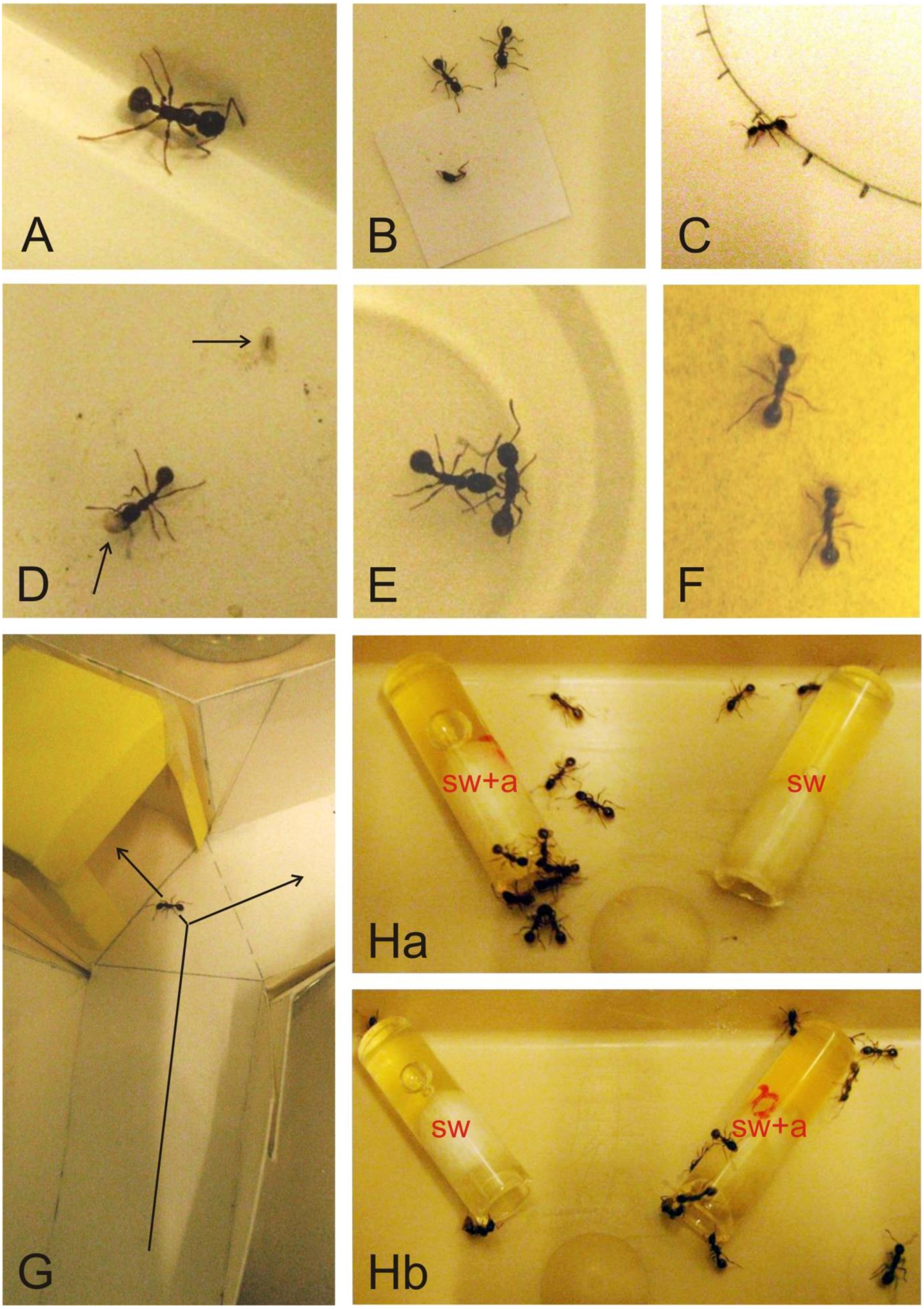

Figure 2. Some view of the experiments. **A**: an ant under alprazolam diet resting on its foraging area. **B**: two ants under normal diet moving towards a worker's head. **C**: an ant under alprazolam diet departing from a

circular trail. **D**: an ant under normal diet transporting a larva (arrow), while another larva (arrow) is still on the foraging area. **E**: two ants of the same colony under alprazolam consumption, the one located on the left gripping a leg of its nestmate. **F**: two ants under alprazolam consumption moving rather easily on a rough substrate, their tactile perception seeming reduced. **G**: an ant trained to a yellow cube in the way of correctly responding to that cue when tested in a Y-apparatus; the arrows indicate the two possible ways. **H**: ants of colonies A (**Ha**) and C (**Hb**), confronted to pure sugar water (sw, in red) and sugar water containing alprazolam (sw + a, in red), drinking preferentially sugar water containing alprazolam; they are dependent on the drug.

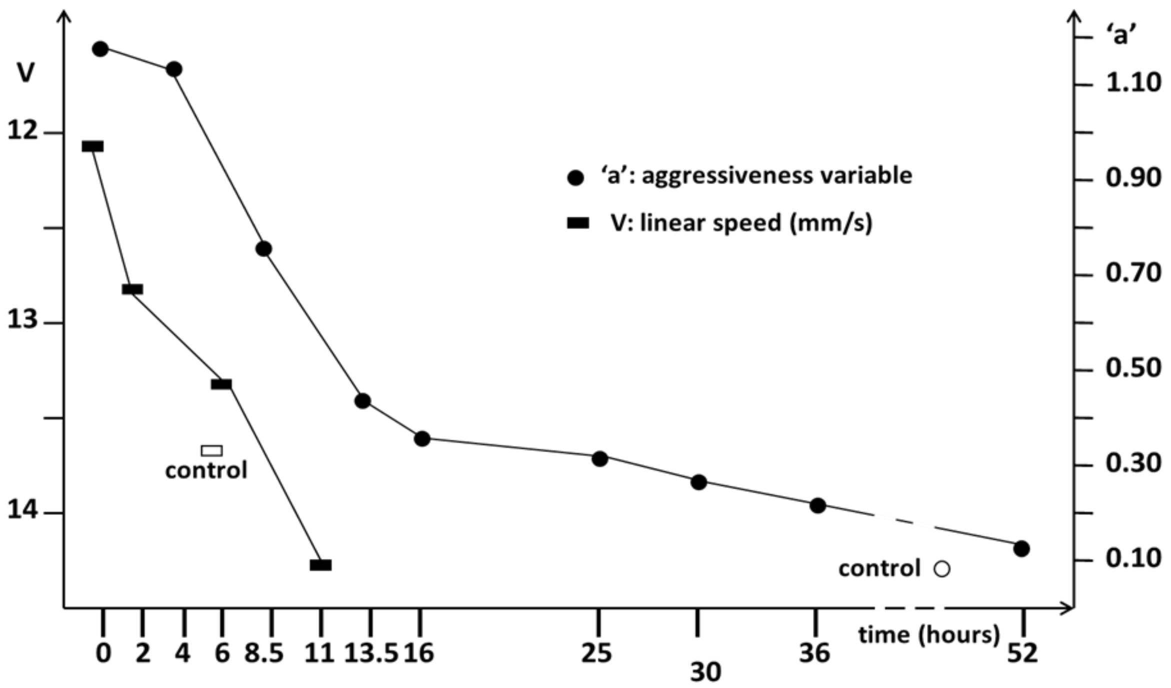

Figure 3. Decrease of the effects of alprazolam after its consumption ended. Experimental details and statistical information are given in the text. Briefly, the effect of the drug on locomotion vanished in about 9 - 10 hours, becoming not different from the control as soon as after 2 hours of weaning; the effect on aggressiveness rapidly decreased from 4 to 13 hours, then slowly vanished in a total of about 60 hours.

REFERENCES

[1] Cammaerts MC, Cammaerts D. Physiological and ethological effects of fluoxetine, on ants used as a biological model. Int J Biol 2015; 7: 1-18. doi:10.5539/ijb.v7n2p1.
[2] Cammaerts MC, Cammaerts D. Physiological and ethological effects of antidepressants: a study using ants as biological models. Int J Pharmac Science Invention 2015; 4 (2): 4-24. ANED 27.6718/04204024.
[3] Qu'est ce que le Xanax (alprazolam) ? http://www.psychomedia.qc.ca/medicaments/qu-est-ce-que-le-xanax-alprazolam (Accessed February 10, 2016).
[4] Wehner R, Gehring W. Biologie et Physiologie Animales; De Boek Université, Thieme Verlag: Paris, Bruxelles, 1999.
[5] Wolf FW, Heberlein U. Invertebrate models of drug abuse. J Neurobiol 2003; (54): 161-178. http://dx.doi.org/10.1002/neu.10166.
[6] Andre RG et al. Insect Models for Biomedical Research. In: Woodhead AD, ed. Non mammalian Animal Models for Biomedical Research, Boca Raton, FL: CRC Press, 1989; pp. 61-72.
[7] Keller RA. A phylogenetic analysis of ant morphology (Hymenoptera: Formicidae) with special reference to the Poneromorph subfamilies. Bull Am Museum Nat Hist. 2011; 355: 99pp.
[8] Billen J, Morgan ED. Pheromone communication in social insects - sources and secretions. In: Vander Meer RK, Breed MD, Espelie KE, Winston MLK editors. Pheromone Communication in Social Insects: Ants, Wasps, Bees, and Termites; Westview Press, Boulder, Oxford, 1998: 3-33.
[9] Passera L, Aron S. Les fourmis: comportement, organisation sociale et évolution; Les Presses Scientifiques du CNRC: Ottawa Canada, 2005.

[10] Hölldobler B, Wilson EO. The ants. Harvard University Press, Springer-Verlag: Berlin, 1990.

[11] Cammaerts MC. Navigation system of the ant *Myrmica rubra* (Hymenoptera, Formicidae). Myrmecol News 2012; 16: 111-121.

[12] Passera L. La véritable histoire des fourmis; Librairie Fayard: Paris, 2006.

[13] Rachidi Z, Cammaerts MC, Debeir O. Morphometric study of the eye of three species of Myrmica (Formicidae). Belg J Entomol 2008; 10: 81-91.

[14] Cammaerts MC. Visual vertical subtended angle of *Myrmica ruginodis* and *Myrmica rubra* (Formicidae, Hymenoptera). Bull Soc r belg Ent 2011; 147: 113-120.

[15] Cammaerts MC. The visual perception of the ant *Myrmica ruginodis* (Hymenoptera – Formicidae). Biologia 2012; 67: 1165-1174.

[16] Cammaerts MC et al. Use of olfactory and visual cues for traveling by the ant *Myrmica ruginodis* (Hymenoptera, Formicidae). Myrmecol News 2012; 16: 45-55.

[17] Cammaerts MC, Nemeghaire S. Why do workers of *Myrmica ruginodis* (Hymenoptera, Formicidae) navigate by relying mainly on their vision? Bull Soc r belg Ent 2012; 148: 42-52.

[18] Cammaerts MC, Cammaerts R. Food recruitment strategies of the ants *Myrmica sabuleti* and *Myrmica ruginodis*. Behav Proc 1980; 5: 251-270.

[19] Cammaerts MC, Cammaerts R. Ontogenesis of ants' cognitive abilities (Hymenoptera, Formicidae). Advanced Studies in Biology 2015; 7: 335-348 + synopsis: 349-350.

[20] Cammaerts MC, Gosset G. Impact of age, activity and diet on the conditioning performance in the ant *Myrmica ruginodis* used as a biological model. Int J Biol 2014; 6: 10-20.

[21] Cammaerts MC et al. Some physiological and ethological effects of nicotine; studies on the ant *Myrmica sabuleti* as a biological model. Int J Biol 2014; 6: 64-81.

[22] Cammaerts MC et al. Effects of buprenorphine and methadone, two analgesics used for suppressing humans' addiction to morphine; a study using ants as biological models. Int J Pharmac Science Invention 2015; 4: 1-19.

[23] Alprazolam-Wikipédia. https://fr.wikipedia.org/wiki/Alprazolam/html (Accessed March 5, 2015).

[24] Médicaments psychiatriques et effets secondaires. http://www. worldlingo.com/med/enwiki/fr/Alprazolam/html (Accessed March 5, 2016)

[25] Flunitrazepam (Rohypnol). http://www.cesar.umd.edu/cesar/drugs/rohypnol.asp (Accessed May 29, 2016).

[26] Siegel S, Castellan NJ. Nonparametric statistics for the behavioural sciences; McGraw-Hill Book Company: Singapore, 1989.

[27] Cammaerts-Tricot MC. Phéromones agrégeant les ouvrières de *Myrmica rubra*. J Ins Physiol 1973; 19: 1299-1315.

[28] Cammaerts MC et al. An easy and cheap software-based method to assess two-dimensional trajectories parameters. Belg J Zool 2012; 142: 145-151.

[29] Cammaerts MC. Colour vision in the ant *Myrmica sabuleti* Meinert, 1861 (Hymenoptera: Formicidae). Myrmecol News 2007; 10: 41-50.

[30] Dossier thématique sécurité routière n°4 - Drogues et medicaments.pdf. https://www.ibsr.be (Accessed February 15, 2016).

[31] Alprazolam- indications-posologie-et-effets-secondaires. http:// sante.journaldesfemmes.com/30898-alprazolam-indications-... (Accessed March 17, 2016).

[32] Xanax. http://www.vulgaris-medical.com (Accessed March 17, 2016).

[33] Les benzodiazépines : un traitement risqué ? http://passeportsante.net/fr/Actualités/Dossiers/Dossier complexe.aspx (Accessed March 5, 2016).

[34] Legrand SA et al. Prevalence of alcohol and other psychoactive substances in injured drivers: comparison between Belgium and the Netherlands. Forensic Science International 2012; 220 (1-3), 224-231.

[35] Les benzodiazepines: plus dangereuses qu'on ne le pense. http://www.benzo.org.uk/waf2.htm (Accessed March 5, 2016)

[36] Cammaerts M-C, Cammaerts R. Physiological and ethological effects of morphine and quinine, using ants as biological models. J. Pharmac Biol 2014; 4: 43-58