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Ethological and physiological effects of paroxetine, the most consumed antidepressant nowadays: A study on ants as models

Marie-Claire Cammaerts[1,*,#] and Roger Cammaerts[2,$]

[1]Département de Biologie des Organismes, Faculté des Sciences, Université Libre de Bruxelles, 50, Av. F. D. Roosevelt, 1050 Bruxelles; [2]27, Square du Castel Fleuri, 1170 Bruxelles, Belgium.

ABSTRACT

Paroxetine, a selective serotonine recapture inhibitor (SSRI), is an antidepressant used since 1995. Its potential adverse effects have not been divulgated at that time and they were debated only in 2007. In the present study, we examined its effects on ants. This drug impacted their food intake and general activity. The ants became fairly excited, moved sinuously and erratically, presented abnormal trembling, and stayed motionless from time to time. Paroxetine also reduced the ants' orientation ability and their trail-following behavior. It reduced their tactile perception, brood-caring behavior, cognition and the ability of escaping from an enclosure. Under this drug diet, the ants had a larger audacity, but became aggressive against nestmates. Their middle- and long-term memory was drastically reduced, though their short-term memory stayed intact. The ants showed adaptation to some effects of paroxetine, but not to all of them (e.g. not to those affecting their cognitive abilities and their social behavior). They became somewhat habituated to the only favorable effect of the drug, the slight audacity increase. After paroxetine consumption was stopped, the effects of the drug, studied through the ants' aggressiveness, decreased rapidly during the first few hours, and then slowly vanished in 50-60 hours. Most of the effects observed in the ants agree with those described in humans nowadays. In the present study, besides precisely quantifying some effects, we relate other ones that may exist in humans. We conclude that paroxetine should be cautiously used, in small amounts, for very short time periods and under medical supervision.

KEYWORDS: aggressiveness, cognition, memory, dependence, nutrition, *Myrmica sabuleti*

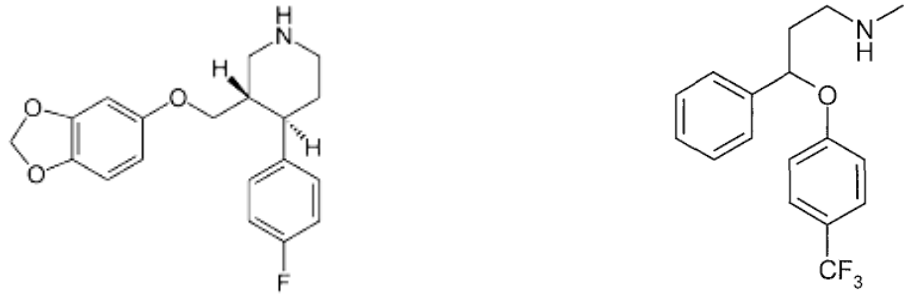
INTRODUCTION

Antidepressants are among the most consumed drugs by people, together with anxiolytics, antibiotics, hormones, and carbamazepine. The active ingredient in the presently most consumed antidepressant worldwide is paroxetine (Figure 1). It is an SSRI i.e. it selectively inhibits the recapture of serotonin. We have previously examined the effects of fluoxetine, the active ingredient in SSRI antidepressants largely consumed until a few years ago [1], and have found that this substance has several adverse effects on ants used as biological models (such as aggressiveness against nestmates). We presumed that the currently used paroxetine could also have adverse effects. As was the case with fluoxetine, the effects of paroxetine also may not have been sufficiently divulgated. We thus aimed to study the potential adverse

*Corresponding author: mtricot@ulb.ac.be

[#]Current address: 27, square du Castel Fleuri, 1170 Bruxelles, Belgium; mccammaerts@gmail.com

[$]Retired from Département de l'Etude du Milieu naturel et agricole, Service Public de Wallonie, 23 Av. Maréchal Juin, B-5030 Gembloux, Belgium.



Paroxetine Fluoxetine

Figure 1. Chemical structure of paroxetine, the active ingredient in the presently most consumed antidepressants, the effects of which are here examined, and of fluoxetine, the active ingredient in the previously most consumed antidepressant, the effects of which have been examined in a previous work [1].

effects of paroxetine in the same way we studied those of fluoxetine and of two other somewhat less harmful antidepressants [2]. 'In the same way' signifies that we used a well known species of ants as a biological model, and aimed to examine the 22 ethological and/or physiological traits we usually assess.

Below, we explain why ants can be used as biological models, summarize what we know about the chosen ant species, report easily available information on the effects of paroxetine, and list the 22 traits we wanted to examine. After that, we explain our methods, detail our results, discuss them, report recent scientific information on the drug, and conclude.

Most biological processes (i.e. genetics, metabolism, nervous cells functioning etc.) are similar for all animals, including humans. Hence, a lot of invertebrates and vertebrates are used as models for studying biology [3]. Invertebrates are increasingly used due to their short life cycle, simple anatomy, and availability in large numbers [4]. Some species are widely used, for instance, the flatworm *Dendrocelium lacteum*, the nematode worm *Caenorhabditis 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 [5], but ants too can be used. Ants are among the most complex social invertebrates as for their morphology, physiology, social organization and behavior. They are among the most morphologically evolved hymenoptera, having a unique resting position of their labium, mandibles and maxilla [6], as well as a large number of glands emitting numerous pheromones [7]. Their societies are highly organized with a strong division of labor, an age-based polyethism and a social regulation [8]. Their behavior is well developed: they care for their brood, build sophisticated nests, chemically mark the inside of their nest and in a different way, their nest entrances, nest surroundings and foraging area [8]. They generally use an alarm signal, a trail pheromone, and a recruitment signal [9]; they are able to navigate using memorized visual and olfactory cues [9]; they efficiently recruit nestmates [8, 9], and they clean their nest and manage cemeteries situated at the boundaries of their foraging area [8, 9]. Considering the complexity of their biology, it looks reasonable to use ants as biological models for studying effects of substances, treatments or situations. Moreover, colonies containing thousands of ants can be maintained in laboratories at a low cost throughout the year.

For the past many years, we had worked on ant species of the genus *Myrmica*, in particular on *Myrmica sabuleti*, Meinert 1861. We have studied some of its ecological traits, its eye morphology [10], subtended angle of vision and visual perception [11], navigation system [12], visual and olfactory conditioning capabilities [13] and recruitment strategy [14]. The ontogenesis of some of the cognitive abilities of *Myrmica* species has also been studied [15]. Studies on the impact of age,

activity and diet on the conditioning capability of *M. ruginodis* [16] led us to presume that ants could be good biological models. This was confirmed while studying, among others, the effects of caffeine, theophylline, cocaine, and atropine, of nicotine, of morphine and quinine, of fluoxetine (a SSRI antidepressant), of anafranil (a TCA, i.e. a tricycle antidepressant) and efexor (a SNRI, i.e. an antidepressant inhibitor of serotonin-noradrenalin recapture), of carbamazepine, of buprenorphine and methadone, and of alprazolam [17]. Each time, we observed effects which had also been observed on humans and brought precise information on these effects. Furthermore, we revealed other effects which may exist for humans. Here we used *M. sabuleti* again as a model for examining the effects of paroxetine, the active ingredient in the most consumed antidepressant all over the world at present.

What are the information one can get about paroxetine? Leaflets attached to drugs containing paroxetine (Paroxetine Sandoz®, Deroxat®, Aropax®, Seroxat®) inform us that this substance is an inhibitor of the recapture of serotonin, suitable for treating persons suffering from nervous depression, anxiousness, social phobia, stress, and panic. These leaflets also inform that precautions must be taken when driving a vehicle, that the drug might induce some suicidal thought in young people and that several symptoms may appear at weaning. Thus, from this information one could infer that paroxetine is efficient in case of depression and associated illnesses, and does not have very severe adverse effects. This information is similar to what we read on leaflets attached to fluoxetine package when we examined the effects of that substance. In the course of the latter study we discovered that fluoxetine has far more adverse effects than those written on the leaflet of the drug [1]. We were thus highly motivated for examining the effects of paroxetine in the same way we studied those of fluoxetine, using ants as biological models.

We intended to examine the following 22 ethological and/or physiological traits, assessing them first while ants were under normal diet, and then while the ants were under paroxetine diet. Among these 22 traits, only four were examined on ants that have consumed paroxetine, namely the ants' adaptation to, habituation to, and dependence on the drug, and the decrease in the effects of the drug after its consumption was stopped. The 22 traits are:

- sugar water consumption
- meat consumption
- general activity
- linear speed
- angular speed
- orientation towards an alarm signal
- trail-following behavior
- audacity
- tactile (pain) perception
- brood-caring behavior
- cognition
- aggressiveness against nestmates
- aggressiveness against aliens
- ability of escaping from a trap
- visual conditioning ability
- visual memory
- olfactory conditioning ability
- olfactory memory
- adaptation to the drug consumption
- habituation to the drug effects
- dependence on the drug use
- decrease in the effects of the drug after its consumption was stopped

MATERIALS AND METHODS

Collection and maintenance of ants

The experiments were conducted on two colonies of *M. sabuleti*, collected in the Aise Valley (Ardenne, Belgium) in June 2016. The ants nested under stones; the colonies contained 500-800 workers, 1-2 queens and brood. They were maintained in the laboratory in artificial nests made of 2-3 glass tubes half filled with water, a cotton plug separating the ants from the water. The nest tubes of each colony were deposited in a tray (34 cm × 23 cm × 4 cm), the internal sides of which were slightly covered with talc to prevent the ants from escaping. The trays served as foraging areas and food was delivered in them. Food consisted of an aqueous solution of sugar (30%) provided *ad libitum* in a small glass tube (diameter: 1.5 cm, length: 7 cm) plugged with cotton, and of cut *Tenebrio molitor* larvae (Linnaeus, 1758) provided as meat three times a week on a glass slide. Laboratory temperature

was maintained at 18 °C-22 °C, and the relative humidity at circa 80%. Lighting had an intensity of 330 lux during caring of the ants and testing them. During other time periods, lighting was provided by natural light and varied from 5 to 120 lux according to the time of the day. The ambient electromagnetic field had an intensity of 2-3 $\mu W/m^2$. The members of a colony are herein named nestmates, as commonly done by researchers on social hymenoptera.

Solution of paroxetine given to the ants

A package of paroxetine Sandoz® was furnished by the pharmacy Wera (Bruxelles, Belgium). Humans are advised to consume 20 mg paroxetine per day. Humans, on an average, consume about one litre of water per day. Thus, under paroxetine diet, they consume, per day, 20 mg of the drug and one litre of water. Insects, and thus ants, drink proportionally about ten times less water than mammals. Consequently, a solution of 20 mg of paroxetine in 100 ml water must be given to ants so that they lived under a paroxetine diet similar to that lived by humans daily. A tablet of 20 mg paroxetine was thus dissolved in 100 ml of sugar water commonly given to the ants. This solution was delivered to the ants in small tubes used for providing them with sugar water. It was checked daily whether ants effectively drunk the solution of paroxetine, and they did. The cotton plugging the tubes was refreshed every 2-3 days, while the entire solution was renewed every 7 days. Experiments on ants under paroxetine diet started 24 hours after the colonies had received the adequate solution.

Sugar water and meat consumption, and general activity

For assessing these traits, we counted the ants drinking the sugar water, eating the *T. molitor* larvae, and moving at any place in their environment (food sites, foraging area, nest entrances and inside the nest) for six successive days, three times between 12:00 to 15:00 hrs, and three times between 21:00 to 24:00 hrs (West European winter time = UTC + 1), (Table 1, Daily counts). For each day, we established the mean of these counts (Table 1, Daily means). The six daily means obtained for ants consuming paroxetine were compared to the six corresponding ones previously obtained for the same ants under normal diet, using the non-parametric test of Wilcoxon [18]. Moreover, we established the average of the daily means (Table 1, Average of daily means).

Linear and angular speed; orientation towards an isolated worker's head

The assessments were made on ants moving on their foraging area, and the movement of 20 ants of each colony was analyzed for each variable (n = 20 ants × 2 colonies = 40 trajectories). The linear and angular speeds of ants were assessed without presenting a stimulus to the ants. The orientation of ants towards an alarm signal was assessed by presenting to the ants, on their foraging area, an isolated worker's head on a piece of paper (1 cm × 1 cm). The worker's head had the mandibles widely opened and it emitted the alarm pheromone of the species.

Trajectories were manually recorded on a glass slide horizontally placed above the ants' tray. A metronome set at 1 second allowed assessing the running time of each trajectory. Each trajectory was recorded until the ant reached the stimulus or walked along about 6 cm. They were then copied with a water-proof marker pen onto transparent polyvinyl sheets which could remain affixed to a PC monitor screen due to their own static electricity charge. The trajectories were analyzed using specifically designed software [19]. Each trajectory was entered in the software by clicking as many points as wanted with the mouse and then entering the location of the presented isolated 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 here in mm/s) of an animal is the length of its trajectory divided by the time spent moving along this trajectory. The angular speed (i.e. the sinuosity, S, measured here 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 here in angular degrees) of an animal towards a given point (here an isolated head) is the sum of the angles, measured at each successive point of the recorded trajectory,

Table 1. Effect of paroxetine on the consumption of sugar water and meat by ants and on their general activity. Ants of two colonies (A and B) drinking sugar water, eating meat, and moving (i.e. not resting; labeled as 'activity') anywhere in their nest and foraging area were counted 6 times per day (3 times between 12:00 and 15:00 hrs and 3 times between 21:00 and 24:00 hrs) (Daily counts), for 6 days, first while under normal diet, then while consuming paroxetine. Daily means, and the average of the daily means were established. Statistical results are given in the text. Briefly, ants consuming paroxetine eat less and were less active.

	Normal diet			**Diet with paroxetine**		
Days	Sugar water	Meat	Activity	Sugar water	Meat	Activity
			Daily counts			
I A	3 6 7 4 5 5	0 1 1 2 2 2	4 4 4 8 8 9	3 3 3 6 6 7	0 0 0 1 1 1	0 2 2 2 2 3
B	8 8 7 8 9 9	1 1 1 3 3 3	4 4 4 10 10 11	9 8 9 17 15 16	0 0 0 0 0 0	2 2 3 7 8 6
II A	5 5 4 8 7 8	0 0 1 2 2 1	3 3 2 5 3 3	4 3 4 5 4 4	0 0 0 2 2 1	2 1 2 6 6 6
B	5 7 6 5 6 6	2 1 2 1 2 1	3 4 5 6 6 5	7 8 7 8 8 7	0 1 0 0 0 0	1 1 1 7 7 8
III A	5 4 4 4 3 2	1 1 0 2 2 2	2 2 3 9 7 8	4 3 4 2 2 1	0 1 0 1 1 1	0 1 0 3 4 4
B	1 1 2 13 12 14	1 0 0 2 1 2	3 3 2 6 9 9	2 2 2 2 2 2	0 0 0 1 0 0	2 3 2 5 2 3
IV A	4 4 4 5 7 6	0 0 1 1 1 1	3 4 5 4 5 4	0 0 0 2 2 2	1 1 1 1 0 0	2 2 3 6 5 4
B	10 9 9 8 8 8	1 1 1 1 1 1	4 3 4 4 5 6	8 7 8 6 6 6	0 0 0 0 0 0	2 3 3 4 3 2
V A	6 5 6 4 5 6	1 0 1 2 2 2	2 2 2 7 8 7	1 1 1 2 1 2	1 1 1 0 0 0	2 1 2 3 4 3
B	3 3 3 8 8 8	0 0 1 2 2 2	4 2 3 6 7 7	0 0 1 1 0 1	0 0 0 1 0 1	1 1 0 2 3 3
VI A	4 4 4 4 4 3	0 0 1 2 1 1	2 1 1 6 4 3	5 4 4 4 5 4	0 0 0 1 1 1	3 4 3 4 4 5
B	7 6 7 6 5 4	0 1 0 2 2 2	3 2 3 7 7 7	1 1 1 6 5 6	0 0 0 1 0 1	2 3 3 2 3 2
			Daily means			
I	6.58	1.67	6.67	8.50	0.25	3.25
II	6.00	1.25	4.00	5.75	0.50	4.00
III	5.42	1.17	5.25	2.33	0.42	2.42
IV	7.58	0.83	4.25	3.92	0.33	3.25
V	5.42	1.25	4.75	0.92	0.42	2.08
VI	4.83	1.00	2.75	3.83	0.42	3.17
			Average of daily means			
	5.97	1.20	4.61	4.21	0.39	3.03

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 point; when it is larger than 90°, the animal has a tendency to avoid the point. Each distribution of 40 values was characterized by its median and quartiles (Table 2) and the distributions obtained for ants consuming paroxetine were compared to those obtained for ants under normal diet using the non-parametric χ^2 test [18].

Table 2. Effects of paroxetine on six ethological traits of ants. The assessments, detailed in the text, were made on two colonies first under normal diet, then under a diet with paroxetine. Median (and quartile) values or mean [and extremes] are given. Statistical results can be found in the text. Briefly, paroxetine affects all the examined traits, except the locomotion speed.

Traits	Normal diet	Diet with paroxetine
Linear speed (mm/s)	14.1 (12.9 - 15.8)	13.9 (12.8 - 16.1)
Angular speed (ang. deg./cm)	132 (111 - 151)	190 (170 - 212)
Orientation (ang. deg.)	37.1 (27.8 - 60.1)	69.3 (55.8 - 86.2)
Trail following (n° arcs)	9.0 (6.0 - 16.0)	2.0 (1.0 - 4.0)
Audacity (n° ants)	1.20 [0 - 2]	1.80 [1 - 3]
Tactile (pain) perception:		
Linear speed (mm/s)	6.1 (5.5 - 6.9)	8.8 (7.5 - 10.2)
Angular speed (ang. deg./s)	270 (239 - 303)	199 (175 - 240)

Trail-following behaviour

The trail pheromone of *Myrmica* ants is produced by the workers' poison gland. Ten of these glands were isolated in 500 µl hexane and stored for 15 min at -25 °C. To perform one of the experiments, 50 µl of the solution was deposited, using a metallic normograph pen, on a circle (R = 5 cm) drawn with a pencil on a piece of white paper and divided into arcs of 10 angular degrees. One minute later, the piece of paper with the artificial trail was placed in the ants' foraging area. The response of 20 ants of each colony to the trail was assessed by the number of arcs of 10 angular degrees they walked along the trail without departing from it (Figure 2A), even if they reversed their 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'. Each distribution of values was characterized by its median and quartiles (Table 2), and the distribution obtained for ants consuming paroxetine was compared to that obtained for ants under normal diet using the non-parametric χ^2 test.

Audacity

For assessing this trait, a cylindrical tower built in strong white paper (Steinbach®, height = 4 cm, diameter = 1.5 cm) was set on the ants' foraging area, and the ants present on it, at any place, were counted 12 times, in the course of 12 min (Figure 2B). The mean and extremes of the obtained values were established (Table 2) and the values obtained for ants under the two kinds of diet were compared to one another using the non-parametric Mann-Whitney U test [18].

Tactile (pain) perception

This trait was assessed for each colony using an apparatus made of a small tray (15 cm × 7 cm × 4.5 cm) into which a duly folded piece (3 cm × 11 (i.e. 2 + 7 + 2) cm) of rough emery n° 280 paper was tied to the bottom and the borders of the tray. The tray was divided into a small smooth zone 3 cm long, 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 in the small zone. Most of them moved away from the small zone and walked for a time on the rough paper. At that time, their linear and angular speeds were assessed (n = 12 trajectories × 2 colonies = 24; Table 3). The values obtained for ants consuming paroxetine were compared to those obtained for ants that have never consumed that drug using the non-parametric χ^2 test.

Brood-caring behavior

For each colony, a few larvae were removed from the inside of the nest and deposited in front of the nest entrance. The ants' behavior in front of five larvae was observed (Figure 2C), and the larvae among the five observed still remaining out of the

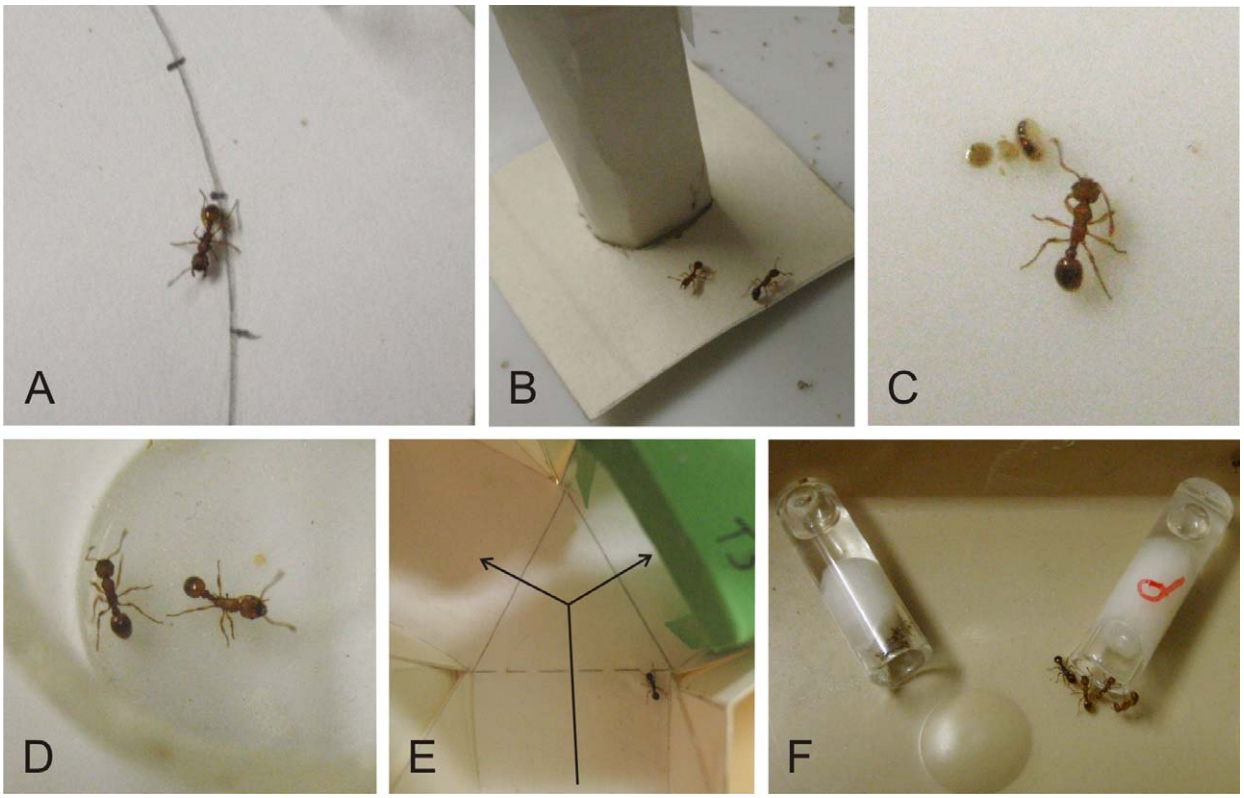


Figure 2. Some photographs of the experiments. **A**: an ant, under paroxetine diet, following an artificial circular trail and abnormally presenting an aggressive posture. **B**: ants under paroxetine diet coming onto an unknown apparatus, one of them opening its mandibles. **C**: an ant under paroxetine diet, not immediately taking care of a larva. **D**: two nestmates under paroxetine diet, avoiding one another in the course of a dyadic encounter. **E**: an ant, under paroxetine diet and conditioned to a hollow green cube, hesitating to respond to that cue when tested in a Y apparatus provided with such a cube in one branch. The arrows indicate the two possible directions in the Y apparatus. **F**: ants under paroxetine diet preferring such a diet (the tube with a P written in red) instead of pure sugar water (the tube on the left).

nest after 30 s, 2, 4, 6, 8, and 10 min were counted. The numbers recorded for each colony were then added (Table 3). The results obtained for ants consuming paroxetine were compared to those obtained for these ants under normal diet using the non-parametric Wilcoxon test.

Cognition

This trait was assessed for each colony using an apparatus which consisted of a small tray (15 cm × 7 cm × 4.5 cm) with two duly folded pieces of white extra-strong paper inserted inside (Steinbach®, 12 cm × 4.5 cm) in order to create a path with twists and turns between a loggia too narrow for 15 ants (the initial small loggia) and a larger one (the large loggia) [17]. For each colony, 15 ants were set all together in the initial loggia. Then, the ants present in this initial loggia and in the large one were counted after 30 s, 2, 4, 6, 8, 10 and 12 min. The numbers obtained for the two colonies were added (Table 3). The sums obtained for ants consuming paroxetine were compared to those obtained for these ants under normal diet using the non-parametric Wilcoxon test.

Aggressiveness against nestmates and aliens

The ants' aggressiveness against nestmates was assessed in the course of five dyadic encounters for each colony. The ants' aggressiveness against aliens was assessed in the course of five similar encounters, in which the alien ant belonged to another colony of *M. sabuleti* collected at some distance from colonies A and B. Each encountering was conducted in a small cylindrical cup

Table 3. Effects of paroxetine on five ethological and physiological traits of the ants. Ants of two colonies were tested when under normal diet, and then while consuming paroxetine. Experimental details and statistical results are given in the text. Briefly, paroxetine affected brood-caring behavior, cognition and the ability to escape from an enclosure; it induced aggressiveness against nestmates and reduced that against aliens. Levels: 0 = no reaction, 1 = antennae movement, 2 = mandibles opening, 3 = gripping, 4 = stinging; 'a' = n° levels (2 + 3 + 4)/(0 + 1).

Traits	Normal diet	Diet with paroxetine
Brood caring: n° of larvae out of the 10, not transported into the nest in the course of 10 min	t: 30 s 2 4 6 8 10 min n° 10 8 6 4 3 0	t: 30 s 2 4 6 8 10 min n° 10 10 10 8 7 4
Cognition: ants in front of and beyond twists and turns in the course of 12 min	t n° in front n° beyond 30 s 26 0 2 21 0 4 18 0 6 15 0 8 12 1 10 10 3 12 9 4	t n° in front n° beyond 30 s 30 0 2 23 0 4 20 0 6 19 0 8 16 0 10 17 0 12 19 0
Aggressiveness against nestmates	levels 0 1 2 3 4 var 'a' n° 91 60 14 0 0 0.09	levels 0 1 2 3 4 var 'a' n° 23 66 76 0 0 0.85
Aggressiveness against aliens	levels 0 1 2 3 4 var 'a' n° 0 20 60 78 31 8.45	levels 0 1 2 3 4 var 'a' n° 5 33 42 5 5 1.24
Escaping from an enclosure: ants in and out of the enclosure in the course of 12 min	t: 30 s 2 4 6 8 10 12 n° in 12 10 10 10 8 7 7 n° out: 0 2 2 2 4 5 5 variable = 5/12 = 0.41	t: 30 s 2 4 6 8 10 12 n° in : 12 12 11 11 9 9 9 n° out: 0 0 1 1 3 3 3 variable = 3/12 = 0.25

(diameter = 2 cm, height = 1.6 cm), the borders of which had been slightly covered with talc (Figure 2D). Each time (5 × 2 = 10 encounters with nestmates, 5 × 2 = 10 encounters with aliens), one ant of colony A or B was observed for 5 min and its encounter with the opponent was characterized by the number 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). The numbers recorded for the two colonies were added (Table 3). The results obtained for ants consuming paroxetine were compared to those obtained for ants under normal diet using the non-parametric χ^2 test. The ants' aggressiveness was also assessed by a variable a (a = n° of recorded aggressiveness levels 2 + 3 + 4 divided by n° of recorded levels 0 + 1).

Escaping from an enclosure

This trait was assessed, on each colony, by enclosing six ants in a reversed polyacetate glass (h = 8 cm, bottom diameter = 7 cm, ceiling

diameter = 5 cm) set on the ants' foraging area. The ants were introduced into the reversed glass through a hole (diameter = 3 mm) in the center of the ceiling. The lower part of the inner surface of the reversed glass was slightly covered with talc to prevent ants climbing on it. The rim of the bottom was provided with a small notch (3 mm height, 2 mm broad) for giving the ants the opportunity to escape from the enclosure. For quantifying the ants' escape, we counted those still under the glass and those escaped after 30 s, 2, 4, 6, 8, 10 and 12 min. The results obtained for the two groups of six ants were added (Table 3) and the sums obtained for ants consuming paroxetine were compared to those previously obtained for ants under normal diet using the non-parametric Wilcoxon test. We also calculated the variable "n° of ants that escaped in 12 min/12" for each kind of diet, 12 being the initial number of imprisoned ants (Table 3).

Visual and olfactory conditioning, and visual and olfactory memory

At a given time, a green hollow cube under which ants could walk was set above the cut *T. molitor* larvae, and thus the ants underwent visual operant conditioning. One week later, fragments of rosemary were set near those of *T. molitor* larvae, and thus the ants underwent olfactory operant conditioning. The cubes were made of strong paper (Canson®). Tests were performed while the ants were expected to acquire conditioning, and after removal of the green cube or the rosemary, while they were expected to partly lose their conditioning. Ants were individually tested in a Y-apparatus, constructed of a strong white paper, set in a small tray (30 cm × 15 cm × 4 cm). 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 green hollow cube or fragments of rosemary in one branch; half of the tests were conducted with the cue in the left branch and the other half with the cue in the right branch. Moving towards the cue was considered as giving the correct response (Figure 2E). Control experiments had previously been made on never-conditioned ants as well as on trained ants not consuming paroxetine [13]. This had to be done because, once an animal is conditioned to a 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 its first choice was recorded when it was beyond a pencil-drawn line indicating the entrance of a branch (Figure 2E). After that, the ants were transferred to a polyacetate cup, until 10 ants were tested, for avoiding testing of the same ant twice. All the tested ants were then returned in their foraging area. For each test, the number of ants (n = 10 ants × 2 colonies = 20 choices) which gave the correct response was recorded, and the percentage of correct responses was established (Table 4). Numerical results obtained for ants consuming paroxetine were compared to those previously obtained for ants that have never consumed that drug using the non-parametric Wilcoxon test.

Adaptation to paroxetine consumption

For evaluating if ants adapted themselves to some negative effects of paroxetine, we again assessed their angular speed and their orientation to an alarm signal after they had consumed the drug for 10 days (Table 5). This assessment was made exactly as the control assessment and the assessment made after 2 days of paroxetine consumption. The results of these assessments were compared using the non-parametric χ^2 test.

Habituation to paroxetine

To evaluate if ants developed habituation to paroxetine (i.e. became accustomed to that drug which would thus be less efficient in the course of time), we assessed the ants' audacity after they had consumed the drug for 12 days (Table 5). This assessment was made in the same way as the control assessment and the assessment made after 2 days of consumption. The results of these assessments were compared using the non-parametric Mann-Whitney test.

Dependence on paroxetine

After the ants had consumed paroxetine for 15 days, an experiment was performed for examining if they had acquired dependence on that drug. For each colony, 15 ants were transferred into a small tray (15 cm × 7 cm × 5 cm), the borders of which

Table 4. Effects of paroxetine on visual and olfactory conditioning and memory. Ants were trained to a visual or to an olfactory cue, and were tested in a Y apparatus, one branch of which was provided with the visual or the olfactory cue. The table gives the number of correct responses given by 10 ants of each colony in the course of time, and the total percentage of correct responses obtained each time. More details and statistical results are given in the text. Briefly, paroxetine did not affect the ants' ability in acquiring conditioning (thus, their short-term memory) but drastically impacted their middle- and long-term memory. *: results previously obtained [13].

Assessment	**Normal diet**	**Diet with paroxetine**		
Time (hours)	**% correct responses***	**colony A**	**colony B**	**% correct responses**
Visual conditioning				
7	51	6	4	50
24	57	5	6	55
31	57	6	7	65
48	63	6	7	65
55	63	6	6	60
72	67	7	6	65
Visual memory				
7	73	5	5	50
24	77	5	5	50
31	69	5	5	50
48	67	5	6	55
55	65	5	5	50
72	62	5	6	55
Olfactory conditioning				
7	60	5	7	60
24	63	6	6	60
31	68	6	7	65
48	70	7	7	70
55	75	7	7	70
72	79	8	8	80
Olfactory memory				
7	63	4	5	45
24	62	4	6	50
31	63	5	5	50
48	60	5	5	50
55	60	4	5	45
72	53	5	5	50

Table 5. Adaptation and habituation to paroxetine diet. Tests were made on ants that have never consumed the drug (control), and have consumed it for two days and for 10 days (Adaptation) or 12 days (Habituation). Experimental details and statistics are given in the text. Briefly, ants adapted themselves to the impact of the drug with respect to the sinuosity of their movement, but not as for their ability in orienting. They became slightly habituated to the effect of the drug on their audacity, and such a habituation could lead humans to consume larger amounts of the drug. Median (and quartile) or mean [and extremes] values are given.

Traits	Control	After 2 days	After 10 or 12 days
Adaptation			
linear speed	14.1 (12.9 - 15.8)	13.9 (12.8 - 16.1)	13.1 (11.8 - 14.9)
angular speed	132 (111 - 151)	190 (170 - 212)	154 (140 - 174)
orientation	37.1 (27.8 - 60.1)	69.3 (55.8 - 86.2)	71.0 (55.9 - 90.9)
Habituation			
audacity	1.20 [0 - 2]	1.80 [1 - 3]	1.45 [1 - 3]

were covered with talc, and in which two tubes (h = 2.5 cm, diam. = 0.5 cm) were placed, one containing sugar water, and the other a sugared solution of paroxetine (the same solution as that used in the course of the whole experimental work). Each tube was plugged with cotton (Figure 2F). 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. The ants drinking each liquid food were counted 12 times in 15 min, and the mean value was established for each kind of food. The sums of the values obtained for each colony were statistically compared to the values expected if ants randomly drank each kind of food, using the non-parametric goodness of fit χ^2 test [18]. For comparative purpose, an identical experiment was performed using 15 ants of the other colony collected at some distance from colonies A and B (see the section 'Aggressiveness against nestmates and aliens' above), and which had never received paroxetine.

Decrease of the effects of paroxetine in the course of time after its consumption ended

The weaning started when the liquid food containing the drug was removed from the ants' tray and replaced by pure sugar water. Since that time, a trait among those examined here had to be assessed in the course of time in the way it had previously been assessed. This trait ought to be affected by paroxetine, should not induce adaptation, habituation and death, and has to be quantified in less than half an hour. We opted to use the ants' abnormal aggressiveness against nestmates induced by the drug. This trait was assessed after different time periods following weaning, just like it had previously been quantified, except that only three instead of five encounters were conducted for each colony. The results obtained for each colony were added and results obtained at different times (Table 6) were compared to one another or to the control ones using the non-parametric χ^2 test. The successive values of the variable 'a' are graphically presented in figure 3. A polynomial regression curve which provided an optimum fit with the experimental values was obtained using Statistica® v10 software (StatSoft Inc., Tulsa, OK).

RESULTS

Sugar water and meat food consumption, and general activity

Paroxetine affected the ants' food consumption (Table 1). Under the drug diet, ants drank slightly less sugar water than when under normal diet. This result was at the limit of significance (n = 6, T = 5.97, P = 0.078), probably because the sample was small. Under paroxetine diet, ants largely ate less meat than when under normal diet, which is a significant result (n = 6, T = 21, P = 0.016). We observed the ants present on the sugar water and the *T. molitor* larvae throughout the entire study, i.e. until the ants received pure sugar water again. They were obviously less frequent in drinking and

Table 6. Decrease in the effects of paroxetine after its consumption was stopped. The examined trait was the aggressiveness against nestmates, induced by the drug. The table gives the number of times each level of aggressiveness (see Table 3) was observed, and the values of the variable 'a' (n° levels (2 + 3 + 4)/(0 + 1); with levels 3 and 4 = null) obtained in the course of time. The last mentioned values are plotted, in figure 3, in relation with the time elapsed since weaning. Briefly, the effects of the drug rapidly decreased during 9 hours (what may induce dependence), then slowly disappeared in a total of 60 hours.

Time after weaning (hours)	Levels of aggressiveness			Variable 'a'
	0	1	2	
0	7	29	56	1.55
3	12	25	44	1.20
6	11	26	43	1.16
9	16	39	37	0.67
12	14	49	43	0.67
15	9	32	22	0.53
18	13	31	20	0.45
25	21	41	23	0.37
29	29	46	25	0.33
35	23	46	19	0.27
38	26	57	23	0.27
49	28	33	13	0.21
54	30	56	14	0.16
62	34	35	8	0.11

eating than when under normal diet. Paroxetine thus affected the ants' food intake, and this effect persisted in the course of time.

As for the ants' general activity, we observed, while performing the control assessments, that ants were on an average more active during the evening than during midday. Indeed, in total, 109 ants were counted during the day, while 162 ones were counted during the evening. This circadian rhythm was not perturbed by paroxetine consumption: in total, 67 ants were counted during the day, and 151 ones during the evening (from the data in Table 1).

On the other hand, irrespective of time, the ants were less active while they consumed paroxetine than while they were under normal diet (Table 1). The difference between the assessments made under the two kinds of diet was significant (n = 5, T = 15, P = 0.031). Paroxetine thus affected the ants' general activity and this seemed to persist as long as ants consumed the drug. Indeed, we often observed ants resting in their foraging area or near the sugar water, with their antennae folded in a 'U' shape. Then, suddenly, they became very active, moving erratically all around their foraging area, opening their mandibles, being aggressive against nestmates, not performing social tasks, and finally they stopped being active and rested again.

Linear and angular speeds

The paroxetine diet impacted the ants' sinuosity of movement, but not their speed of locomotion (Table 2). Indeed, ants consuming this drug since two days moved at a speed similar to the control one ($\chi^2 = 0.30$, df = 4, P = 0.99) but with a larger sinuosity ($\chi^2 = 20.63$, df = 3, P < 0.001). This was also obvious by simply observing the ants. Moreover, some ants presented abnormal, rapid and inefficient movements of their legs, their antennae, and seldom their entire body. This effect

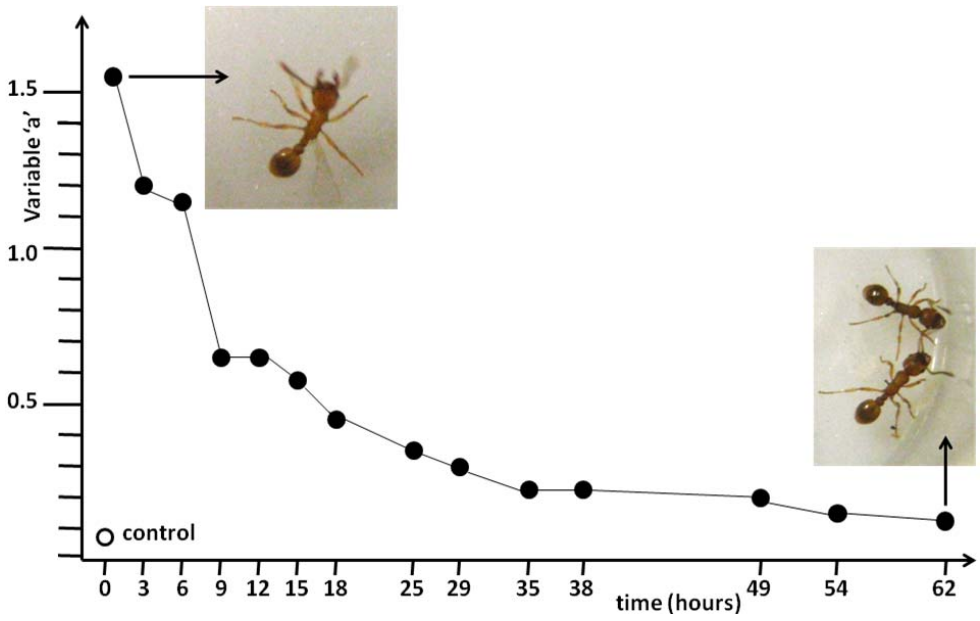


Figure 3. Decrease in the effects of paroxetine after its consumption was stopped. The examined trait was the aggressiveness against nestmates induced by the drug. The values of the variable 'a' are numerically detailed in table 6. The effects of the drug rapidly decreased during 9 hours (what may lead to dependence), then slowly disappeared in a total of 60-65 hours. Upper photo: a young ant presenting abnormal rapid movements of a leg and of an antenna; lower photo: two nestmates showing normal behavior towards one another.

seemed to be more pronounced in young ants (upper photo of figure 3). In the course of the ants' consumption of the drug, this increase of sinuosity and these abnormal movements seemed to become less pronounced, a fact we checked after the ants had consumed paroxetine for 10 days (see the section 'Adaptation to paroxetine' below).

Orientation towards an isolated worker's head

This trait was affected by the paroxetine diet (Table 2). Ants under normal diet very well oriented themselves towards an isolated worker's head, but they poorly did so after having consumed the drug for two days. The difference in ants' orientation under the two diets was significant ($\chi^2 = 31.10$, df = 2, $P < 0.001$). One week later, we examined if this impact remained intact or decreased in the course of the drug consumption (see the section 'Adaptation to paroxetine' below).

Trail-following behavior

This behavior was affected by the paroxetine diet (Table 2, Figure 2A). Under normal diet, ants on an average followed a circular trail along 9 arcs of 10°. While consuming paroxetine, they on an average followed such a trail along only 5 arcs. The difference between these two scores was significant ($\chi^2 = 38.65$, df = 2, $P < 0.001$). It may be due to the increase of sinuosity induced by paroxetine (see above), but also due to some impact of the drug on the ants' cognitive capabilities, a presumption we examined later (see below).

Audacity

This trait was slightly but advantageously affected by the paroxetine diet (Table 2, Figure 2B). Under such a diet, the ants were more inclined to coming onto an unknown and risky apparatus than when they were under normal diet. The difference in behavior under the two diets was significant (U = 120, adjusted Z = -2.3583, P = 0.018). The audacity of ants consuming paroxetine was not excessive (compared, for example, with that exhibited under cocaine diet [17]). All happened as if ants consuming paroxetine were less attentive to dangers, were more prompt to do risky tasks, and were more self-confident. This might correspond, for humans, to an antidepressant effect. It was checked if such

an impact of the drug persisted in the course of its consumption or if it somewhat decreased with time (see the section 'Habituation to paroxetine' below).

Tactile (pain) perception

This physiological trait was unexpectedly affected by the paroxetine diet (Table 2). Under normal diet, ants moved with difficulty (with pain?) on a rough substrate. Their linear speed was very low, and their angular speed very high. While consuming paroxetine, ants moved more freely on a rough substrate. Their linear speed was somewhat higher, and their angular speed somewhat lower than when under normal diet. These differences in locomotion on a rough substrate under the two diets were significant: linear speed: $\chi^2 = 18.74$, df = 2, $P < 0.001$; angular speed: $\chi^2 = 16.34$, df = 2, $P < 0.001$.

Brood-caring behavior

This trait was affected by the paroxetine diet (Table 3). Under normal diet, the ants transported all the 10 larvae (artificially removed from the nest) into the nest in 10 minutes. On the contrary, ants consuming the drug transported only 6 larvae out of the 10 within the same time period. This difference in efficiency was significant: N = 5, T = 15, P = 0.031. Ants under paroxetine diet presented difficulties in moving while transporting a larva, and in finding the nest entrance, and also delayed in taking a larva in their mandibles (Figure 2C). Consequently, not only the ants' locomotion and orientation, but also their cognition and relation with the members of their colony could be affected by paroxetine. These two presumptions were examined in the following two experiments.

Cognition

This physiological trait was affected by the paroxetine diet (Table 3). Under normal diet, after the 12 minutes of the experiment, only 9 ants out of 30 were still present in the small loggia in front of the twists and turns while four ones had reached the large loggia beyond that path. Under paroxetine diet, after the same time period, 19 ants among 30 were still present in the small loggia while no one had reached the large loggia beyond the twists and turns. The difference in ants' cognition when under one or the other diet was significant (for the small loggia: n = 7, T = 28, P = 0.008; for the large loggia: n = 3, not significant (NS) due to the smallness of the sample). This impact of the drug partly explains the impact on the brood-caring behavior (see above).

Aggressiveness against nestmates and aliens

These two ethological traits were impacted by the paroxetine diet (Table 3).

Ants under normal diet were very seldom aggressive against their nestmates. In the course of the conducted encountering, they often stayed near their nestmates, touching them with their antennae, or they moved in their vicinity. The variable 'a' assessing the ants' aggressiveness had a very low value (0.09). While consuming paroxetine, in the course of similar encountering, ants appeared to avoid their nestmates (Figure 2D), and when they were in front of them, they often opened their mandibles. The variable assessing the ants' aggressiveness had a high value (0.85). The difference in behavior between ants under each kind of diet was significant ($\chi^2 = 63.26$, df = 2, $P < 0.001$). We observed that such abnormal aggressiveness between nestmates also occurred in the ants' foraging area, and that it did not disappear and even slightly increased in the course of paroxetine consumption.

As for the aggressiveness against alien ants, ants under normal diet immediately (i.e. just when encountering the alien) opened their mandibles, gripped the alien and, if possible, tried to sting it. The variable 'a' assessing such aggressiveness had a high value (8.45). While consuming paroxetine, ants were less aggressive towards aliens. They opened their mandibles and seldom gripped the alien. In fact, they were soon gripped by the alien, and even stung. All happened as if, under paroxetine diet, ants reacted too late to the presence of the alien, did not correctly estimate the imminent danger, and when doing so, simply ran away instead of attacking. The variable assessing their aggressiveness had a value far lower than that noted while not consuming paroxetine (1.24). The difference in behavior between ants under one or the other kind of diet was significant ($\chi^2 = 73.88$, df = 2, $P < 0.001$).

Escaping from an enclosure

This ethological trait was affected by the paroxetine diet (Table 3). Under a normal diet, ants moved all around the area under the reversed glass as well as all along its rim. Five ants out of 12 could escape in 12 minutes. The first few ants that were able to escape returned to the exit hole and helped their congeners in finding the exit. The variable assessing the ants' ability of escaping equaled 0.41. While consuming paroxetine, ants were less skillful in doing so. They ran all around the area lying under the reversed glass. They also walked along the rim but they often failed in perceiving the exit notch. After 12 minutes, only three ants could escape and nine were still captive. Also, the escaped ants never returned to help their captive nestmates. The variable assessing such evasion was fairly low: 0.25. The difference in ants' behavior under the two diets was significant: for the ants still captive as well as for the evaded ones, the statistical result was N = 6, T = +/– 21, P = 0.016. Such a difference may be due to the erratic movement showed by ants under paroxetine diet, as well as probably due to some impact of the drug on the ants' cognitive abilities (see the sections 'Brood-caring behavior' and 'Cognition').

Visual and olfactory conditioning, and visual and olfactory memory

Briefly, the ants' ability in acquiring conditioning was not affected by paroxetine diet, but their memory was drastically impacted by this drug (Table 4).

In detail, ants consuming paroxetine acquired visual conditioning at a speed nearly similar to that at which they acquired conditioning when they were under normal diet, and reached a similar score (Figure 2E). The difference in conditioning scores while being under the two kinds of diet was not significant (N = 6, T = +9, -12, P = 0.42). Seven hours after the visual cue was removed from the ants' sugar water site, ants under normal diet still presented a conditioning score of 73%, and after 72 hours without seeing the cue, they went on responding to it with a score of 62%. On the contrary, ants consuming paroxetine lost all their conditioning as soon as after seven hours, and probably a little earlier. The difference in ant's memorization ability under the two diets was significant (N = 6, T = 21, P = 0.016).

The same events occurred for the olfactory conditioning and memory. Ants under normal diet reached a score of 60% and 79% after 7 and 72 hours of training, respectively. Ants consuming paroxetine reached similar scores after these two time periods. The difference in conditioning ability of ants under the two kinds of diet was not significant: N = 4, T = -9, P = 0.125. After removal of the olfactory cue, ants under normal diet presented a score of 63% 7 hours later, and a score of 60% 55 hours later. Under paroxetine diet, ants lost their olfactory conditioning as soon as the cue was removed, and presented low scores of 45% to 50% from 7 hours until 72 hours after the cue removal. The difference in ant's memory while under the two kinds of diet was significant: N = 6, T = -21, P = 0.016.

Adaptation to paroxetine

After having consumed paroxetine for 10 days, the ants had a linear speed that was still similar to the control one ($\chi^2 = 3.22$, df = 3, NS) and to that found after two days of consumption, but their angular speed was lower than that found after two days ($\chi^2 = 21.83$, df = 3, $P < 0.001$), though still different from the control one ($\chi^2 = 12.33$, df = 3, $0.001 < P < 0.01$) (Table 5). Also, the ants' difficulties in moving (revealed in the section 'Linear and angular speeds') seemed less pronounced, and uncontrolled abnormal movements occurred less often in the course of the drug consumption. Thus adaptation to paroxetine to some extent occurred for this trait. On the contrary, no adaptation was detected as for the ants' orientation towards an alarm signal. The values obtained after ten days of consumption still differed from the control ones ($\chi^2 = 31.10$, df = 2, $P < 0.001$) and were nearly identical to those obtained after two days of consumption ($\chi^2 = 1.86$, df = 2, NS) (Table 5). Furthermore, the experimental work concerning memory, which lasted 4 × 72 h = 12 days, was conducted on the same days as the experiments on the ants concerning their adaptation to paroxetine as well as after these experiments. These experiments on ant's memory showed an impact of the drug on the ants' memory. We can thus conclude that adaptation to paroxetine did not

occur for traits relevant to cognition and brain functioning. We also observed that the aggressive behavior of ants under paroxetine diet against nestmates persisted in the course of this drug consumption. Hence adaptation to paroxetine as for its impact on this behavioral trait of ants did not occur.

Habituation to paroxetine

Ants may present habituation to paroxetine diet to some extent (Table 5). Audacity was the only assessed trait which was slightly but advantageously affected by paroxetine, thus approaching the antidepressant effect required for humans. Indeed, ants' audacity slightly increased after two days of paroxetine consumption, but this effect was lower ten days later. The difference between the control values and those obtained after twelve days of consumption was not significant: U = 165, adjusted Z = -1.0776, P = 0.28. However, the effect of paroxetine was still detectable: the difference between the values obtained after two and twelve days of consumption was not significant: U = 150, adjusted Z = 1.48, P = 0.14. Hence, habituation to the beneficial effect of the drug occurred to some extent. This may lead humans in consuming more amount of drug for obtaining an equivalent effect.

Dependence on paroxetine

Dependence on paroxetine was assessed after ants consumed this drug continuously for 15 days. For colony A, 19 ants were counted that preferred sugar water free of drug, and 17 ones that preferred sugar water containing paroxetine. For colony B, 13 ants were counted that chose paroxetine solution and only 1 that chose pure sugar water. In total, 30 ants chose the paroxetine solution and 20 ones preferred the drug-free sugar water (Figure 1F). Such a result statistically differed from that expected if ants randomly went drinking each kind of liquid: $\chi^2 = 16.16$, df = 1, $P < 0.001$. The difference in numbers of ants was not very high. We thus submitted ants of a colony which never received paroxetine (i.e. the colony which provided aliens in a former experiment) to the present experiment. These ants behaved completely otherwise: eighteen ants were counted that chose pure sugar water, and only five ones were very briefly seen on the paroxetine solution (they went rapidly away). We can thus conclude that dependence to paroxetine consumption exists to some extent. Since adaptation to the drug to some extent seemed to occur and since habituation to some adverse effects seemed also to exist, humans may be tempted to go on consuming the drug, ignoring (or doing so) its 'hidden' adverse effects, and may even be tempted to increase their consumed amount.

Decrease in the effects of paroxetine after its consumption was stopped

The ethological trait examined, since weaning until the fading of the effects of paroxetine, was the ants' aggressiveness against nestmates, assessed in the course of dyadic encounters. Numerical results are given in table 6, and figure 3 shows the decrease in the aggressiveness variable 'a', step by step with time. On the whole, the decrease in aggressiveness (y) with respect to time (x) can be best fitted by a fourth-power regression curve ($y = 1.56 - 0.12x + 0.04x^2 - 6.49x^3 + 3.7x^4$, with $r^2 = 0.98$; not shown in the figure), meaning that it is not a simple decrease as would be shown by a quadratic curve. In details, in the course of the first three hours, an obvious decrease occurred, but was not significant ($\chi^2 = 2.36$, df = 2, NS). During the next three hours, nearly no decrease occurred. Thereafter, from 6 to 9 hours after weaning, an obvious decrease occurred again but was not significant $\chi^2 = 3.21$, df = 2, NS). However, from the start of weaning to 9 hours later, the decrease was significant (0 - 9 h: $\chi^2 = 8.87$, df = 2, $P < 0.02$), and this may account for the dependence developed on the drug. After that, the decrease in the effects slowed down; the difference in the effects between 9 and 18 hours, for instance, was not significant ($\chi^2 = 1.31$, df = 2, NS). After 18 h, the decrease continued to be very slow, and there was no statistical difference between consecutive experiments. After 35 h, we compared the results to the control ones, until they were no longer different: 35 h *vs* control: $\chi^2 = 21.65$, df = 2, $P < 0.001$; 54 h *vs* control: $\chi^2 = 15.95$, df = 2, $P < 0.001$; 62 h *vs* control: $\chi^2 = 2.54$, df = 2, $0.20 < P < 0.30$, NS. Thus the drug ceased to have adverse effects only after a weaning period of about 60-65 hours, although an obvious decrease occurred during the first few hours following the start of the weaning. For humans, such an initial decrease may give them

the temptation to consume the drug again and thus to depend on it.

DISCUSSION

Working on ants as models, we observed that paroxetine decreased food intake, general activity (even leading to some resting periods), orientation ability, trail-following behavior, tactile perception, brood-caring behavior, cognition, and ability to escape from an enclosure. Paroxetine slightly increased audacity (the only favorable effect), increased the sinuosity of movement (even leading to some excitation), and induced abnormal movements. We often observed that young ants were more affected than old ones. Paroxetine induced aggressiveness against nestmates, but reduced that against aliens. This drug did not affect the ants' conditioning ability, but drastically reduced their middle- and long-term memory. Adaptation occurred for some adverse effects (e.g., locomotion, abnormal movements), but not for traits requiring cognition and not for the abnormal aggressiveness against nestmates. Habituation to the drug occurred to some extent as for the only favorable effect, the slight increase in audacity. Physical dependence was also observed to some extent. The effects of paroxetine disappeared as per the mathematical function of a fourth-power regression curve, revealing that t he degradation and the elimination of the drug was not a simple process. The decrease was rapid during the first nine hours following weaning (transposed to humans, it may lead to dependence and make the weaning painful), then went on disappearing slowly in about 50 more hours (a time period during which adverse effects still exist). All this looks far more adverse than what can be guessed on the basis of easily available information on the drug.

Pharmaceutical internet links [e.g. santecheznous.com/drug/getdrug/co-paroxetine; www.doctissimo.fr/medicament-DEROXAT.htm; sante.canoe.ca/drug/getdrug/mylan-paroxetine; patient.info/medicine/paroxetine-seroxat] laud the use of paroxetine in case of nervous depression, anxiousness, and phobia, but do not advise against an increase in the amount consumed, and diminish the importance of the potential adverse effects. On the contrary, internet sites created without conflict of interest [e.g. https://fr.wikipedia.org/wiki/Paroxetine] relate that paroxetine may lead to some sleepiness, some excitation, appetite problems, aggressive behavior, oppositional behavior, psychomotor defection, trembling, akathisis (i.e. the impossibility of stopping the movements of some parts of one's body [20, 21]), alteration of long-term memory but not of the short-term one, and decrease in cognitive abilities. It is reported that adaptation to the drug occurs in a few weeks but not for all the adverse effects and that severe symptoms occur at weaning, sometimes persisting after years. The half-life of paroxetine is about 24 hours. Elimination by liver and kidneys is long-lasting because, among others, the substance ties to proteins of the plasma.

The initial development and history of paroxetine explains the above dilemma. The drug was commercialized and approved by the FDA in 1992. Since 1995, it had a large success due to the divulgation of studies showing its favorable and efficient effects and neglecting its adverse effects [https://patient.info/medicine/paroxetine-seroxat, www.anti-depressants.com/drugs/ssri/paroxetine/, www.healthline.com/drugs/paroxetine/oral-tablet]. Subsequent works still recommended the use of paroxetine, but in the years 2000, new studies and among them, a thesis of sociology [22] revealed that paroxetine has a very weak effect as an antidepressant (i.e. nearly no difference in comparison with a placebo) while its adverse effects are numerous and important. Its history can be found in many web links [such as www.slate.fr/story/107101/verite-enfin-revelee-sur-antidepresseur-paroxetine]. These links, as well as the above cited one [https://fr.wikipedia.org/wiki/Paroxetine, and references therein] also inform that, since then, new information concerning paroxetine has been divulgated (in scientific reports, in television programs) and that a court trial occurred concerning the fraudulent promotion of the benefits of paroxetine by a well-known pharmaceutical company. However, all this could not erase the initial opinion about paroxetine, and the drug went on being largely used until now.

Since studies on paroxetine may have been done with conflict of interest, one can wonder where the truth is: is it in the initial studies about the substance, or in the last ones? The present study

was effectively done without any conflict of interest since we work as ethologists, do not consume any antidepressant, and are not remunerated for performing our studies. Below, we compare our present results with those obtained in the past.

Briefly, our results agree with the most recent information on the effect of paroxetine in humans, and even emphasize the harmfulness of the product. Indeed, under paroxetine diet, ants presented a decrease in food intake, general activity and cognitive abilities (orientation, trail-following, moving through twists and turns, escaping from an enclosure etc.). They exhibited resting, excitation, large sinuosity of movement, trembling and abnormal movements (akathisis). We observed avoidance and aggressiveness against nestmates, lower brood-caring behavior, loss of middle- and long-term memory, but increase in audacity to some extent. In addition, we detected some decrease in aggressiveness against aliens and a decrease in tactile perception. Adaptation to the drug was observed with regard to the sinuosity of movement, but not for the abnormal aggressiveness and for the traits requiring nervous system functioning. Habituation was also revealed, and such an effect may lead humans to increase the amount of the drug consumed. Dependence was also pointed out, and this may cause severe problems at weaning. In ants, the adverse effects of paroxetine persisted up to 60 hours after consumption was stopped, but a rapid decrease in the effects occurs in the first few hours, which in humans may lead to dependence [17].

The many strong adverse effects of paroxetine showed in the present study and in the literature warn us that this drug should be used only in case of absolute necessity. We suggest that pharmaceutical instructions attached to the drug should clearly mention all the known adverse effects. The amount of paroxetine consumed should never be increased in the course of time. Young people should never consume it. Paroxetine consumption must be stopped as soon as possible, and attention must be paid to persons beginning weaning. Whereas under fluoxetine diet ants kill their nymphs and most of their larvae, and then die [1], we estimate that paroxetine has less strong adverse effects than fluoxetine, and this is finally a positive statement. However, contrary to fluoxetine, paroxetine leads to addiction, and this is a negative point.

We have previously examined the effects of the most consumed anxiolytic 'alprazolam', and found that it has many adverse effects [17]. Then, we examined, in the same way, the effects of an extract from four plants acting as anxiolytic, and found it was efficient and did not have any adverse effects [17]. To care for depressive persons pharmaceutics should wisely research for plant extracts presenting no adverse effects. Indeed, several plants have been shown to have obvious antidepressant properties together with other effects such as helping in sleeping well, reducing stress, and increasing cognition. Examples of such plants are St. John's worst (*Hypericum perforatum*), saffron (*Crocus sativus*), ginseng (*Panax ginseng*), passionflower (*Passiflora incarnate*), balm (*Melissa officinalis*), skull cap (*Scutellaria lateriflora*), valerian (*Valeriana officinalis*), and ginkgo (*Ginkgo biloba*). St John's worst is very efficient [25]. It produces a substance which inhibits the recapture of serotonin, just like the SSRI antidepressants do. It has no adverse effects, but it is photosensitive and must not be consumed together with some particular drugs. Saffron also acts like an SSRI antidepressant; 30 mg of saffron is as efficient as 20 mg of fluoxetine [24]. Ginseng has been proved to act as an antidepressant with very few adverse effects; it is however not suitable for diabetic persons [25]. Passionflower is efficient, has nearly no adverse effect, and can be advantageously consumed together with other plant extracts [26]. Balm is efficient, has a nice taste, has a favorable effect on cognition, has no adverse effect and is advantageously used in association with other plants [27]. Skull cap is efficient thanks to the flavonoids it contains. It has no adverse effect and does not induce a decrease in activity or drowsiness [28]. Valerian is efficient in 80% of the patients (20% react inversely), on whom it has no adverse effect and is advantageously associated with hop, passionflower, balm and skull cap [29]. Ginkgo helps old persons suffering from nervous depression, but has some known adverse effects about which consumers should be informed [30]. No doubt that an adequate mixture of extracts of such plants could care for persons suffering from nervous depression, anxiousness, insomnia and panic.

One last point to mention is that most of the drugs largely consumed nowadays by humans, i.e., antidepressants, antibiotics, hormones and anxiolytics contaminate natural water because they are mainly eliminated intact by humans and are not sufficiently retrieved when waste water is purified. They affect living organisms, vertebrates and invertebrates, which are living in such contaminated water [31, 32, 33].

CONCLUSION

Paroxetine, largely consumed as an antidepressant nowadays, was shown to have several adverse effects in ants (among others it causes akathisis, affects cognition and memory, induces aggressiveness, and leads to dependence). The young individuals are the most affected. It is however less toxic than fluoxetine, the previously most used antidepressant, but it leads to addiction. Hence, paroxetine should be used only in case of absolute necessity, under medicinal supervision and for very short time periods. The alternative use of natural plant extracts should be researched.

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CONFLICT OF INTEREST STATEMENT

We affirm that there is no conflict of interest at all as for the use, the efficiency and the safety of the product examined herein, paroxetine.

REFERENCES

1. Cammaerts, M.-C. and Cammaerts, D. 2015, Int. J. Biol., 7(2), 1-18.
2. Cammaerts, M.-C. and Cammaerts, D. 2015, Int. J. Pharmaceut. Sci. Invent., 4(2), 4-24.
3. Wehner, R. and Gehring, W. 1999, Biologie et physiologie animales, De Boek Université, Thieme Berlag, Paris, Bruxelles.
4. Wolf, F. W. and Heberlein, U. 2003, J. Neurobiol., 54, 161-178.
5. Andre, R. G, Wirtz, R. A. and Das, Y. T. 1989, Insect Models for Biomedical Research. In: Non mammalian Animal Models for Biomedical Research, A. D. Woodhead, Boca Raton, FL: CRC Press.
6. Keller, R. A. 2011, Bull. Am. Museum Nat. Hist., 355, 1-90.
7. Billen, J. and Morgan, E. D. 1998, Pheromone communication in social insects - sources and secretions. In: Editors, Pheromone Communication in Social Insects: Ants, Wasps, Bees, and Termites; R. K. Vander Meer, M. D. Breed, K. E. Espelie and M. L. K. Winston, Westview Press, Boulder, Oxford.
8. Hölldobler, B. and Wilson, E. O. 1990, The ants, Harvard University Press, Springer-Verlag, Berlin.
9. Passera, L. and Aron, S. 2005, Les fourmis: comportement, organisation sociale et évolution, Les Presses Scientifiques du CNRC, Ottawa, Canada.
10. Rachidi, Z., Cammaerts, M.-C. and Debeir, O. 2008, Belg. J. Entomol., 10, 81-91.
11. Cammaerts, M-C. 2004, Physiol. Entomol., 29, 472-482.
12. Cammaerts, M.-C. and Rachidi, Z. 2009, Myrmecol. News, 12, 117-127.
13. Cammaerts, M.-C., Rachidi, Z. and Cammaerts, D. 2011, Bull. Soc. R. Belg. Ent., 147, 142-154.
14. Cammaerts, M-C. and Cammaerts, R. 1980, Behav. Processes, 5, 251-270.
15. Cammaerts, M.-C. and Cammaerts, R. 2015, Adv. Studies Biol., 7, 335-348 + synopsis: 349-350.
16. Cammaerts, M.-C. and Gosset, G. 2014, Int. J. Biol., 6, 10-20.
17. Cammaerts, M.-C. 2016, J. Anat. Physiol., 1(1), 1003.
18. Siegel, S. and Castellan, N. J. 1989, Nonparametric Statistics for the Behavioural Sciences, McGraw-Hill Book Company, Singapore.
19. Cammaerts, M.-C., Morel, F., Martino, F. and Warzée, N. 2012, Belg. J. Zool., 142, 145-151.
20. Leo, R. J. 1996, J. Clin. Psychiatry, 57, 449-454.
21. Schmitt, J. A., Kruizinga, M. J. and Riedel, W. J. 2001, J. Psychopharmacol., 15, 173-179.
22. Nelson, A. D. 2007, Adolescents and antidepressants: analyzing a social scientific controversy. Thesis of Sociology.

23. Shelton, R. C. 2009, J. Clin. Psychiatry, 5, 23-27.
24. Hausenblas, H. A., Saha, D., Dulyak, P. J. and Anton, S. D. 2013, J. Integr. Med., 11, 377-383.
25. Kim, N. H., Kim, K. Y., Jeong, H. J. and Kim, H. M. 2011, Behav. Med., 37, 42-46.
26. Akhondzadeh, S., Naghavi, H. R., Vazirian, M., Shayeganpoor, A., Rashidi H. and Khani, M. 2001, J. Clin. Pharm. Ther., 26, 363-367.
27. Kennedy, D. O., Little, W., Haskell, C. F. and Scholey, A. B. 2004, Psychosom. Med., 66, 607-613.
28. Brock, C., Whitehouse, J., Tewfik, K. and Towell, T. 2014, Phytother. Res., 28, 692-698.
29. Neamali, A., Chaman, F., Hosseini, M. and Boskabady, M. H. 2004, J. Pharm. Bioallied. Sci., 6, 97-103.
30. Woelk, H., Arnoldt, K. H., Kieser, M. and Hoerr, J. 2007, J. Psychiatr. Res., 41, 472-480.
31. Arnold, K. E., Boxall, A. B., Brown, A. R., Cuthbert, R. J., Graw, S., Hutchinson, T. H., Jobling, S., Madden, J. C., Matcalfe, C. D., Naidoo, V., Shore, R. F., Smits, J. E., Taqqart, M. A. and Thompson, H. M. 2013, Biology Letters, 9, 20130492.
32. Corcoran, J., Winter, M. J. and Tyler, C. R. 2010, Critical Reviews in Toxicology, 40, 287-304.
33. Ginebreda, A., Munoz, I, Lopez de Alda, M., Brix, R, Lopez-Doval, J. and Barcelo, D. 2009, Environment International, 10 pages.