

Possible Harmful Effects of Sildenafil Citrate Examined Using Ants as Models

Marie-Claire Cammaerts^{1*} and Roger Cammaerts²

¹Independent Researcher, Retired from the Biology of Organisms Department, University of Brussels, Belgium

²Independent Researcher, Retired from the Natural and Agricultural Environmental Studies Department (DEMNA) of the Walloon Region, Belgium

***Corresponding Author:** Marie-Claire Cammaerts, Independent Researcher, Retired from the Biology of Organisms Department, University of Brussels, Belgium.

Received: June 28, 2018; **Published:** July 26, 2018

Abstract

Sildenafil citrate is nowadays largely consumed by men for solving erection problems. It is considered as safe by practitioners, though some adverse effects are sometimes reported. We studied its effect on ants as models, and found that it decreased meat food consumption, increased sinuosity of movement, decreased orientation ability, trail following behavior, audacity, aggressiveness against aliens, escaping ability, tactile perception, and, what seems to be the cause of most of these effects, decreased the accuracy of the sensitive perception. Sildenafil did not impact the ants' social relationship, cognition, and conditioning ability. It slightly improved the visual and olfactory memory. Ants never adapted themselves to the adverse effects of sildenafil citrate, and did not develop dependence on its consumption. The effects of this drug decreased slowly and linearly after weaning. We presume that the pointed out ethological and physiological effects of sildenafil citrate result on one hand from its inhibition of phosphodiesterases and probably of other enzymes allowing hydrolysis, and on the other hand from a small increase of the amounts of cGMP and probably cAMP.

Keywords: Behavior; cGMP; *Myrmica sabuleti*; Phosphodiesterase; Sensitive Perception; Viagra

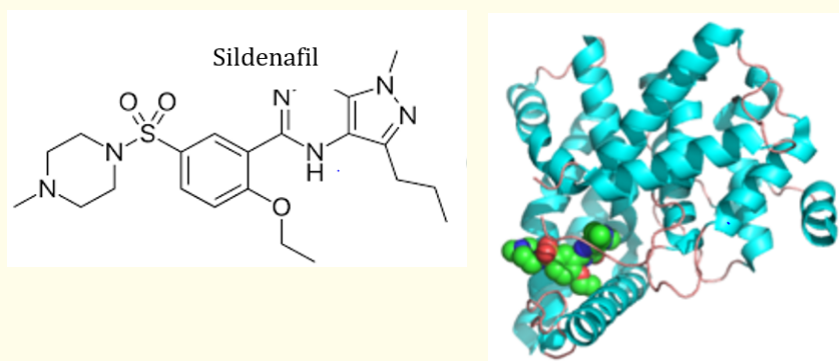
Abbreviations

ang.deg.: Angular Degrees; ang.deg./cm: Angular Degrees Per cm; mm/s: Millimeter Per Second; χ^2 : Chi Square; vs: Versus; n°: Number; cm: Centimeter; mm: Millimeter; ml: Milliliter; μ l: Micro Liter; mg: Milligram; s: Second; min: Minute; h: Hours; t: Time; %: Percentage

Introduction

The sildenafil molecule was discovered and patented in 1996, and became available on the market in 1998. This compound inhibits the type 5 phosphodiesterase (Figure 1), the function of the latter enzyme being to hydrolyze cyclic GMP. In presence of sildenafil, the level of cyclic GMP is thus maintained what leads, among others, to maintain the level of azote monoxides and thus to the relaxation of smooth muscles (<https://www.rxlist.com/viagra-drug.htm>, <https://en.wikipedia.org/wiki/Sildenafil>). Consequently, though it was at first deemed to treat hypertension and angina pectoris, sildenafil was repositioned to allow keeping a man's erection. The drug, in the form of sildenafil citrate and under trademarks (e.g. Viagra® or Verventi®), as well as nowadays under generic version, had rapidly success and was very lucrative. Patients, wanting to solve erection problems, do not generally take into account possible adverse effects of that drug. However, since sildenafil inhibits essentially type 5 phosphodiesterase and to a lower extend other phosphodiesterases, and since these enzymes are very important during several physiological functions, sildenafil citrate may have other effects than to keep men's erection. Indeed, in the directions for use joined to packages of sildenafil citrate, adverse effects following this drug consumption

are mentioned. These effects include digestive problems, headache, abnormal vision, respiratory difficulties, cardiovascular problems, auditory troubles, as well as other very severe ones occurring only seldom. Nothing is reported about a potential impact of sildenafil citrate on the individuals' food consumption, general activity, displacement, social relationships, cognition, memory, adaptation to the adverse effects of the drug, habituation to its beneficial effects, dependence on its consumption, and time during which adverse effects remain after this drug consumption was stopped. According to the large use of sildenafil citrate all over the world, it is essential to know as much as possible its effects on health, and to correctly divulgate them. This is why we intended to research, using ants as models, the possible effects of sildenafil citrate on 23 physiological and ethological traits, in the same manner as we examined the effects of numerous other products consumed by humans [1,2]. Here below, we explain why ants can be used as biological models, which species was used and what we know on it, and which physiological and ethological traits we aimed to consider. Then, after having related our methods and results, we come back to the functioning of some phosphodiesterases and of sildenafil citrate, we summarize the harmful and beneficial effects of that drug, and we conclude.



Phosphodiesterase (blue structure) + sildenafil (red and green structure) in its active site

Figure 1: Chemical structure of sildenafil and its fixation on a type 5 phosphodiesterase [<https://fr.wikipedia.org/wiki/Sildenafil>]. Sildenafil inhibits the type 5 phosphodiesterase, the function of which is to hydrolyze cyclic GMP. In humans, the resulting increase of cyclic GMP allows the relaxation and vasodilatation of smooth muscles, what leads among others to the maintenance of blood affluence into the corpus cavernosum. Other phosphodiesterases than those located in that corpus are also inhibited by sildenafil, and the amount of cyclic GMP (and perhaps of cyclic AMP) increases. These events may cause adverse effects, the study of which is the aim of the present work, but using ants as biological models.

Why using ants as models and which species was chosen?

Most biological functions are common to nearly all the animals and to humans. Animals can thus be used as models to examine the physiological and ethological effects of substances or treatments devoted to humans [3]. Invertebrates are often used due to their short life cycle, simple anatomy and availability in large numbers [4]. Insects, in particular social hymenoptera, are often used [5]. As explained in [6], ants can be good models. They are among the most complex social invertebrates in terms of their morphology, physiology, social

organization and behaviors. They have several glands that emit numerous pheromones [7]. Their societies are highly organized, with a division of labor, an age-based polyethism and a social regulation [8]. Ants care for their brood, build sophisticated nests, mark their nest inside, entrances, surroundings and their foraging area with different odors [8]. They use several chemical signals to alarm, to trail and to recruit nestmates [8,9], and are able to navigate using learned visual and olfactory cues [8,9]. They clean their nest and manage cemeteries at the boundaries of their foraging area [8,9]. Based on the complexity of their biology, ants can thus be used as biological models. Moreover, colonies with hundreds of ants can be maintained in a laboratory at low cost year-round. Having largely studied ants belonging to the genus *Myrmica*, including *M. sabuleti*, we know some features of their ecology, eye morphology, angle of vision, visual perception, navigation, conditioning abilities, recruitment [10], and the ontogenesis of some of their cognitive abilities [11]. We have also defined the extent of these abilities [12-14]. Doing so, we became conscious that ants represent useful models, what enabled us to examine the effects of several products consumed by humans. We here used once more the species *Myrmica sabuleti* Meinert 1861.

Which traits can we examine in ants?

Generally, we examined the effects of a product on: the ants' meat and sugar water consumption, general activity, linear speed, angular speed, orientation towards an alarm signal, trail following behavior, audacity, tactile (pain) perception, brood caring behavior, aggressiveness against nestmates, aggressiveness against aliens, cognition, ability to escape from an enclosure, visual and olfactory conditioning ability and memory, adaptation to the product consumption, habituation to its effect, dependence on its consumption, and the decrease of its effect after its consumption was stopped. In the present paper, we again considered all these traits together with a new one, the sensitive perception, for studying the impact of sildenafil citrate on them. For avoiding long descriptions, we only briefly relate protocols already used many times, and refer the readers to previous works [15,16].

Material and Methods

Collection and maintenance of ants

The experiments were performed on two colonies of *M. sabuleti* collected in 2016, in an abandoned quarry located in the Aise valley, in the Ardenne region (Belgium) and on another colony of the same species also collected at the same time in the same site, which served as a control for the ants' conditioning experiment and which provided the 'aliens' used during the study of the ants' aggressiveness. Each of these colonies were maintained in glass tubes containing water in a cotton plugged compartment, these nest tubes being as usual set in a tray (34 cm x 23 cm x 4 cm) [8]. The ants were fed *ab libitum* with pieces of *Tenebrio molitor* (Linnaeus, 1758) larvae, and sugar water delivered in cotton plugged small tubes. The ambient temperature equaled 19 - 21°C, and the air humidity about 80%. The lighting equaled about 330 lux while working on ants, and the electromagnetism 2 μWm^2 . These conditions are optimum for the species. The ants belonging to the same colony are often here referred as 'nestmates', as commonly done by researchers on social insects.

Solution of sildenafil citrate given to the ants

Humans are advised to meanly use a tablet of 50 mg of sildenafil citrate per day, at their convenience. Generally, humans drink one liter of water per day. So, when they consume sildenafil citrate, they meanly consume an amount of 50 mg of that drug together with one liter of water. Insects, so ants, drink about 10 times less water than mammals. Consequently, to set ants under a diet similar to that of humans consuming sildenafil citrate, they should receive a solution of 50 mg of that drug in 100 ml of saturated sugar water. A package of 'sildenafil citrate 25 mg' was furnished by the drugstore Wera (Bruxelles). We made a stock solution of 25 mg of sildenafil citrate in 50 ml of saturated sugar water, and kept it at -25°C. The tap water had a pH of 7.75. After having defrosted and homogenized the stock solution, 5 ml of it were poured in a small tube (one for each colony) of the kind used to provide ants with sugar water and which was set in the colony's tray. We checked if the ants drunk the provided sugared sildenafil citrate solution and they did. The cotton plug of the tubes was refreshed every 2 - 3 days, and the content of the tubes was renewed every seven days.

Food consumption, general activity

The ants present on the sugar water, on the *T. molitor* larvae, and being active at any place in their habitat were counted six times per day during six days, at the same times o'clock each day (Table 1, Daily counts) [15,16]. The mean of these daily counts was calculated (Table 1, Daily means), and the six daily means obtained for ants consuming sildenafil citrate were compared to the six daily means obtained for ants under normal diet using the non-parametric test of Wilcoxon [17]. The averages of the daily means were also calculated (Table 1, last line).

Linear and angular speed, orientation

These traits were assessed on ants moving in their foraging area, the linear and angular speeds without stimulating the ants, the orientation by stimulating them with a nestmate tied to a piece of paper (Figure 3A). This tied nestmate emitted its attractive mandibular glands alarm pheromone. As in previous works [15,16], for each variable, the trajectory of 40 workers was recorded and analyzed with appropriate software [18]. The linear speed (in mm/s) is the length of a trajectory divided by the time spent to travel it. The angular speed (in ang. deg./cm) is the sum of the angles made by successive adjacent segments, divided by the length of the trajectory. The orientation (in ang. deg.) towards a given location is the sum of the successive angles made by the direction of the trajectory and that towards the location, divided by the number of measured angles. When the value of orientation is lower than 90°, the animal tends to orient itself towards the location; when it is larger than 90°, the animal tends to avoid the location. The median and quartiles of each distribution of 40 values were established (Table 2, lines 1, 2, 3). The distributions obtained for ants consuming sildenafil citrate were compared to those obtained for ants under normal diet, using the non-parametric χ^2 test [17].

Trail following

The trail pheromone of *Myrmica* ants is produced by the workers' poison gland. As in previous works [15,16], 10 poison glands were isolated into 500 μ l of hexane, the solution being then placed in a freezer, at -25°C. Fifteen minutes later, 50 μ l of this hexane solution was deposited on a circumference (R = 5 cm) pencil drawn on white paper and divided into arcs of 10 angular degrees. That circular trail was presented to the ants, in their foraging area, and the behavior of 20 ants of each colony was quantified by the number of arcs of 10 angular degrees they walked along the trail (Figure 3B). The values obtained for ants consuming sildenafil citrate were compared to those obtained for ants living under normal diet using the non-parametric χ^2 test, and each distribution of 40 values was characterized by its median and quartiles (Table 2, line 4).

Audacity

As previously [15,16], a tower tied to a platform, both made of strong white paper (Steinbach®, tower height = 4 cm, tower diameter = 1.5 cm), was presented to the ants in their foraging area, and the workers moving on this apparatus were counted 10 times over 10 min (Figure 3C). The mean and the extremes of the recorded values were established (Table 2, line 5). The values obtained for the two colonies during successive periods of two minutes were added, and the five sums obtained for ants consuming sildenafil citrate were compared to the five sums previously obtained for these ants under normal diet using the non-parametric Wilcoxon test [17].

Tactile (pain) perception

When acutely perceiving the rough character of a substrate, the ants walk slowly and sinuously on it. When weakly perceiving such an uncomfortable character, they walk more quickly and less sinuously. Consequently, the ants' walking on a rough substrate allows assessing their tactile and pain perception. This is why, as previously [15,16], a folded piece (3 cm x 2 + 7 + 2 = 11 cm) of emery paper n° 280 paper was tied to the borders and the bottom of a tray (15 cm x 7 cm x 4.5 cm), which became so divided in a first zone 3 cm long, a second zone 3 cm long containing the emery paper, and a last zone 9 cm long. Such an apparatus was constructed for each colony. To conduct the experiment, 12 ants of each colony were transferred into the first zone of their apparatus, and their linear and angular speeds were assessed as usually (see here above 'Linear and angular speed, orientation') when they walked on the rough substrate (n = 24; Table 2, line 6). The values corresponding to ants consuming sildenafil citrate were compared to those corresponding to ants under normal diet using the non-parametric χ^2 test.

Brood caring

A few larvae or nymphs of each colony were removed from the nest and set in front of the entrance. Ten of them as well as the ants' behavior towards them were observed (Figure 3E). Those not yet replaced in the nest after 5 s, 2, 4, 6, 8, and 10 minutes were counted (Table 3, line 1). The numbers corresponding to ants consuming sildenafil citrate were compared to the numbers corresponding to ants under normal diet using the non-parametric Wilcoxon test [17].

Aggressiveness against nestmates and aliens

Five dyadic encounters for each colony were performed, either with a nestmate or with an alien ant, as in previous works [15,16], in a cylindrical cup (diameter = 2 cm, height = 1.6 cm), the borders of which were covered with talc. Each time, an ant of colony A or B was observed for 5 min, and its reaction in front of the opponent was assessed by the number of times it did nothing (level 0 of aggressiveness), contacted the opponent with its antennae (level 1), opened its mandibles (level 2), gripped the other ant (level 3), tried to sting or stung the other ant (level 4) (Figure 3F, 3G; Table 3, lines 2, 3). The numbers obtained for ants consuming sildenafil citrate were compared to those corresponding to ants under normal diet using the non-parametric χ^2 test. The ants' behavior was also assessed by the variable "a" which is the number of aggressiveness levels 2 + 3 + 4 divided by the number of levels 0 + 1, as in previous studies [15,16].

Cognition

This trait was examined using a protocol set up while studying the effects of nicotine [19]. Two duly folded pieces of white paper (Steinbach®, 12 cm x 4.5 cm) were inserted in a tray (15 cm x 7 cm x 4.5 cm) which was so divided into a first small loggia, a path with twists and turns, and finally a large loggia containing a piece of wet cotton. Such an apparatus was built for each colony. To conduct the experiment, 15 ants of each colony were transferred in the first loggia of their apparatus, and those present there as well as in the large loggia were counted after 30s, 2, 4, 6, 8, 10 and 12 minutes. The numbers obtained for the two colonies were added (Table 3, line 4), and the results corresponding to ants consuming sildenafil citrate were compared to those corresponding to ants under normal diet using the non-parametric Wilcoxon test.

Escaping ability

As in previous studies [15,16], for each colony, 6 ants were set under a reversed polyacetate glass (h = 8 cm, bottom diameter = 7 cm, ceiling diameter = 5 cm) deposited in their tray. The glass had a small notch (3 mm height, 2 mm broad) in the rim of its bottom, what gave to the ants the opportunity of escaping (Figure 3H). To assess the ants' escaping ability, those escaped and those still enclosed after 30 s, 2, 4, 6, 8, 10 and 12 minutes were counted. The numbers obtained for the two colonies were added (Table 3, line 5), and the results corresponding to ants consuming sildenafil citrate were compared to those corresponding to ants under normal diet using the non-parametric Wilcoxon test. The ants' ability in escaping was also assessed by the variable "n° of ants escaped after 12 minutes divided by 12".

Visual and olfactory conditioning and memory

After the ants had consumed sildenafil citrate for 8 days, their conditioning and memorizing abilities were examined using a protocol many times employed [15,16]. The control values had previously been obtained on a third collected colony [20]. A yellow hollow cube was set above the sugar water tube entrance, what allowed studying the visual conditioning and memorizing of the ants. After this study, pieces of rosemary were deposited in front of the sugar water tube entrance, what allowed studying the olfactory conditioning and memorizing of the ants. Tests were made over time while ants were expected to acquire conditioning, and after removal of the cue, while they were expected to lose it. For making a test, ten ants of colony A and of colony B were individually set in a Y-apparatus provided with a yellow hollow cube or pieces of rosemary in one of its branch (Figure 3I, 3J), randomly the right or the left one. Moving into the branch containing the cue was considered as giving the correct response. The response of the 20 ants was recorded, and the proportion of correct responses calculated (Table 4). These proportions corresponding to ants consuming sildenafil citrate were compared to those corresponding to ants under normal diet using the non-parametric Wilcoxon test.

Sensitive perception

The two nest entrances of each two colonies were marked, one with two thin blue spots (on the right and the left of the nest entrance, made with a blue pencil), the other with carrot odor (rubbing a piece of that vegetable on the right and the left of the nest entrance) (Figure 3K). After one as well as two days of habituation, 20 ants of each colony were tested, in a separate tray, in front of such marked entrances of two never used tubes and of the intact entrance of two other never used tubes (Figure 2). The ants tempting entering each kind of entrance were counted 20 times over 10 min, and the numbers obtained were separately added for each kind of entrance (in total: two not marked, one marked with thin blue spots, and one marked with carrot odor). These four sums were compared to those which should have been obtained if ants equally chose the four provided entrances using the non-parametric goodness of fit χ^2 test [17] (Table 5). An ants' similar choice of the different entrances would indicate a reduction of the ants' visual perception; an ants' preference for the blue marked entrance would on the contrary prove that the ants' visual perception was intact; an ants' preference for the entrance marked with carrot odor would be in favor of an intact ants' olfactory perception.

Adaptation to the adverse effects of sildenafil citrate

After the ants had consumed the drug for 7 days, a trait affected by it was again assessed (in the way it has previously been assessed) for examining if ants could adapt themselves to that impact, i.e. if the impact was of lower intensity. The trait here considered for making the present experiment was the ants' locomotion, i.e. their linear and their angular speeds. The distribution of the obtained values ($n = 40$) of the linear speed as well as of the angular speed were compared to the control ones and to those obtained after one day of the drug consumption using the non-parametric χ^2 test (Table 6).

Habituation to beneficial effects of sildenafil citrate

A beneficial effect of sildenafil citrate on an ants' trait here examined should have been assessed after several days of the drug consumption for examining if ants became habituated to this effect. However, we found no beneficial effect of sildenafil citrate on any physiological and ethological traits here examined. Habituation could thus not be studied.

Dependence on sildenafil citrate consumption

We examined if ants developed some dependence on sildenafil consumption after they consumed this product during 17 days. The protocol was identical to that employed in previous studies [15,16,19,20]. For each colony, 15 ants were transferred into a tray (15 cm \times 7 cm \times 5 cm) in which two tubes ($h = 2.5$ cm, diam. = 0.5 cm) had been set, one containing sugar water, the other containing the sugar solution of sildenafil citrate used throughout the present work (Figure 3L). The tube containing the drug was located on the right side in one tray, and on the left side in the other tray. The ants coming onto each presented liquid were counted 15 times over 15 minutes, the counts corresponding to each kind of liquid were separately added, and the sums obtained for each colony were also added. These sums were compared to those expected if ants went randomly onto each kind of provided liquid, using the non-parametric goodness of fit χ^2 test.

Decrease of the effect of sildenafil citrate after its consumption was stopped

This decrease was examined, as in previous studies [15,16], after the ants had consumed sildenafil citrate during 24 days. They received a fresh solution of the drug 12 hours before its removal, and after these 12 hours, this solution was removed from the ants' tray and a usual solution of sugar was provided. This was the start of the weaning, i.e. $t = 0$. The ants' sinuosity was assessed just before $t = 0$, then every two hours (so, at $t = 2, 4, 6, 8, 10, 12, 14$ hours), like it had been when ants were under normal diet (= control), after they consumed sildenafil citrate for one day (Table 1), and after they consumed the drug during eight days (Table 6), except that 20 instead of 40 ant's trajectories were analyzed in order to make the assessments all along the experimentation. The distributions of the 20 values obtained after given time periods were compared to those obtained at $t = 0$ and to the control one (made of 40 values) using the non-parametric χ^2 test. Moreover, using Statistica V.10 software [17], a non-parametric Kruskal-Wallis one-tailed test (K-W test) for multiple comparisons was also used to make these comparisons with either the control values (made of 20 values out of 40 in such a way that the median and

quartiles were similar to those of the 40 values) or the values at $t = 0$, taking the two latter groups as control groups. A Bonferroni adjustment is incorporated. The experiment ended when the ants' sinuosity became similar to the control one. The mathematical function describing the regression of sinuosity over time after weaning was established using Statistica® V.10 software, and the choice between linear and polynomial regressions was made using the procedure described in [21].

Results and Discussion

Food consumption, general activity

Ants consuming sildenafil citrate appeared to eat less meat than those living under normal diet (Table 1) and this was statistically significant ($N = 6$, $T = -21$, $P = 0.016$). On the contrary, they consumed sugar water as usually (Table 1; $N = 6$, $T = +7.5$, -13.5 , $P = 0.313$). They were also as active as while under normal diet (Table 1; $N = 6$, $T = -15$, $+6$, $P = 0.219$). Sildenafil affected thus the consumption of solid food but not of liquid one, and did not impact the individuals' general activity.

Days	Colonies	Sugar water diet			Sugar water + sildenafil citrate diet		
		Meat	Sugar water	Activity	Meat	Sugar water	Activity
Daily counts							
I	A	1 1 2 2 1 1	2 2 2 3 2 3	12 13 12 13 12 12	0 1 0 1 1 0	4 4 3 4 4 4	10 10 11 11 11 10
	B	1 1 1 0 1 1	2 2 1 2 2 1	8 9 10 10 9 9	0 1 1 0 0 1	1 1 1 1 2 2	9 8 9 9 9 8
II	A	2 2 2 2 1 2	3 3 4 4 3 3	9 10 10 10 9 11	0 0 1 1 0 0	3 2 2 2 3 3	12 11 12 12 11 12
	B	0 1 0 1 1 0	1 2 1 2 1 1	9 8 9 9 9 10	1 1 0 0 1 1	2 3 2 3 3 2	8 9 9 9 9 8
III	A	1 0 1 1 1 0	2 2 3 3 2 2	12 10 9 10 10 11	1 1 1 1 1 0	3 3 2 3 3 3	11 12 12 12 12 11
	B	2 2 2 2 1 1	2 1 2 1 2 2	8 9 9 9 9 8	0 0 1 0 0 0	2 2 1 1 1 1	8 8 9 9 9 8
IV	A	2 2 1 1 2 2	4 4 3 5 5 4	11 12 11 13 12 12	0 1 0 1 0 0	3 3 3 3 4 3	10 9 9 9 10 10
	B	2 1 2 2 2 3	1 2 2 2 2 1	9 9 8 9 9 8	1 0 0 0 0 1	1 2 2 2 2 1	8 7 8 7 8 8
V	A	1 1 0 1 1 0	5 5 4 5 5 6	12 11 12 12 12 11	1 1 0 0 0 1	2 2 1 1 2 2	11 11 12 11 12 12
	B	0 0 1 1 1 1	1 1 2 2 2 1	10 9 9 9 9 10	0 0 0 0 1 0	4 4 3 4 4 3	7 8 8 8 8 9
VI	A	1 1 1 1 1 1	6 6 5 6 6 6	12 11 11 11 12 12	0 0 1 1 0 0	2 2 2 2 1 2	12 13 12 13 13 12
	B	1 2 1 2 1 1	2 2 1 2 2 2	9 9 10 10 10 9	1 1 1 1 0 0	2 2 3 2 3 2	8 9 9 8 8 8
Daily means							
I	A + B	1.08	2.00	10.75	0.50	2.58	10.18
II	A + B	1.17	2.33	9.08	0.50	2.50	10.17
III	A + B	1.17	2.00	9.50	0.50	2.08	10.08
IV	A + B	1.83	2.92	10.25	0.33	2.42	8.58
V	A + B	0.67	3.25	10.50	0.33	2.67	9.75
VI	A + B	1.17	3.83	12.17	0.50	2.08	10.42
Average of daily means							
		1.57	2.74	11.28	0.44	2.39	9.86

Table 1: Effect of sildenafil citrate on the ants' food consumption and activity. Experimental details and statistics are given in the text. In brief, the drug decreased the meat food consumption but did not statistically impacted the sugar water consumption and the general activity.

Linear and angular speed

The ants' locomotion was impacted by sildenafil consumption (Table 2, lines 1, 2): the ants consuming this drug moved slower and more sinuously than ants under normal diet and this was statistically significant (linear speed: $\chi^2 = 20.18$, $df = 2$, $P < 0.001$; angular speed: $\chi^2 = 43.62$, $df = 2$, $P < 0.001$). In fact, the ants consuming this drug walked somewhat slowly and sinuously essentially when approaching the entrance of their nest. They moved in front of this entrance but did not immediately enter the nest, moved away, came back, and also did so when approaching their food sites. All happened as if they not well perceived these entrance and sites. An experiment related below tempted to examine the accuracy of the ants' sensitive perception.

Orientation towards an alarm signal

Under normal diet, the ants very well reached a tied nestmate. It was not exactly the case for ants consuming sildenafil citrate. At the beginning of the presentation, such ants oriented themselves rather well, but after a few ants' displacement around the tied worker, the gradient of attractive pheromone becoming less pronounced, they failed in coming close to the tied worker (Figure 3A), moved aside and even over it as if they poorly perceived it. Their orientation statistically differed from the control one: $\chi^2 = 21.80$, $df = 3$, $P < 0.001$ (Table 2, line 3). This was in agreement with the previous result (see above) and was subsequently experimentally examined in the present work (see below: 'Sensitive perception').

Trail following

Ants under normal diet easily followed a circular trail along meanly 12 arcs of 10 angular degrees (Table 2, line 4). While consuming sildenafil citrate, the ants seemed to have some difficulties in promptly perceiving the trail and in going on moving along it. They followed the trail along meanly 5.5 arcs of 10 angular degrees (Table 2, line 4; Figure 3B). The difference of trail behavior between ants consuming sildenafil and ants under normal diet was significant: $\chi^2 = 25.47$, $df = 3$, $P < 0.001$. The ants' olfactory perception might be affected by the drug, a presumption which was later on experimentally examined (see below: 'Sensitive perception').

Audacity

This trait was somewhat affected by sildenafil citrate consumption (Table 2, line 5; Figure 3C). While living under normal diet, the ants were not inclined in coming onto the unknown presented apparatus, although a few ones came on it and stay on it a short time. The ants consuming sildenafil citrate often stopped in front of the apparatus looking for a time to it; they hesitated and seldom climbed on it. All happened as if they had some difficulties in precisely seeing the apparatus. The difference of behavior in front of the unknown apparatus between ants under one and the other kinds of diet was significant: $N = 5$, $T = -15$, $P = 0.031$ (Table 2, line 5). The presumption of an impact of sildenafil on the ants' visual perception was examined in a following experiment (see below: 'Sensitive perception').

Tactile (pain) perception

Under a sildenafil citrate diet, the ants seemed to less perceive the rough character of the substrate than while living under normal diet (Table 2, the two last lines; Figure 3D). Indeed, while walking on such a substrate, the ants under sildenafil citrate diet presented a higher linear speed and a lower sinuosity than ants living under normal diet, and this was statistically significant (linear speed: $\chi^2 = 25.31$, $df = 2$, $P < 0.001$; angular speed: $\chi^2 = 23.99$, $df = 2$, $P < 0.001$). This was the fourth experiment in the course of which all happened as if the ants' sensitive perception was reduced by the examined drug consumption. An experiment was thus conducted to check the validity of this presumption (see below: 'Sensitive perception').

Traits	Sugar water diet	Sugar water+ sildenafil citrate diet
Linear speed (mm/s)	12.2 (10.9- 13.6)	10.8 (9.6 - 11.4)
Angular speed (ang.deg./cm)	121 (103 - 139)	188 (168 - 208)
Orientation (ang. deg.)	35.4 (25.2 - 46.7)	62.7 (45.9 - 72.3)
Trail following (n° arcs)	12.0 (8.0 - 16.0)	5.5 (4.0 - 8.0)
Audacity (n° ants)	1.70 [0 - 3]	0.95 [0 - 2]
Tactile (pain) perception:		
Linear speed (mm/s)	4.3 (3.8 - 4.9)	8.0 (6.9 - 8.7)
Angular speed (ang.deg./cm)	265 (248 - 321)	196 (182 - 219)

Table 2: Effects of sildenafil citrate on six ethological and physiological traits.

Experimental details and statistics are given in the text. Sildenafil citrate appeared to increase the ants' sinuosity of movement, and to decrease their linear speed, orientation ability, trail following, audacity and tactile perception. mm/s: Millimeter Per Second; ang.deg./cm: Angular Degrees Per Centimeter; ang.deg.: Angular Degree; n°: Number.

Brood caring

The ants consuming sildenafil citrate took care of the larvae experimentally removed from the nest just like the ants living under normal diet (Figure 3E). They hold the larvae and tried to replace them inside the nest, but were seldom able to quickly find the nest entrance. They generally moved in the entrance vicinity for a rather long time (5 to 15 minutes) before succeeding in entering with the larva. Consequently, after the 10 experimental minutes, three larvae were still not re-entered and transported all around the foraging area (Table 3, line 1). However, the difference of brood caring behavior between ants under normal diet and under sildenafil diet was not significant (N = 3, NS). The observation of the difficulties of the ants under sildenafil diet in re-entering their nest was in agreement with that made while analyzing such ants' locomotion (see 'Food consumption, general activity'), and may result from a decrease of visual or olfactory perception in ants consuming the drug, a presumption investigated below (see: 'Sensitive perception').

Aggressiveness against nestmates

This trait was not affected by sildenafil citrate consumption (Table 3, line 2; Figure 3F). The ants under that diet did not aggress their nestmates. Ants under normal diet also did not. The variable assessing the aggressiveness equaled 0.11 for the two kinds of ants, and the difference of behavior between them was not significant ($\chi^2 = 0.38$, $df = 2$, $0.38 < P < 0.90$). The odor of a nestmate is already known by callow ants, being 'learned' by habituation during the larval life, and the appearance of a nestmate is 'learned' by the callow ant at its emergence, through imprinting [22]. No new olfactory and visual elements for the ants were present in this experiment. Sildenafil citrate might thus not impact the ants' visual and olfactory memory, a presumption checked by a following experiment (see below: 'Visual and olfactory conditioning and memory').

Aggressiveness against aliens

This trait appeared to be affected by sildenafil citrate (Table 3, line 3; Figure 3G). Ants under normal diet soon aggressed an alien ant, gripping it and, generally after a few seconds or minutes, stinging it or trying to do so. Ants consuming sildenafil citrate delayed in identifying the opponent as an alien, and consequently in attacking it. Finally, the ants consuming sildenafil citrate gripped the alien, but were often stung by it instead of succeeding in stinging it. The difference of behavior between ants under normal diet and under sildenafil citrate diet was significant ($\chi^2 = 24.56$, $df = 3$, $P < 0.001$). This result, as previous ones (see above), may be due to a decrease of the sensitive perception of ants consuming sildenafil citrate, a presumption examined below (see: 'Sensitive perception').

Cognition

While living under sildenafil citrate diet, as while living under normal diet, the ants soon left the small area and walked across the twists and turns way. In the course of the 12 experimental minutes, a few ones (5 for ants under normal diet, 4 for ants under sildenafil diet) reached the large area beyond the difficult way (Table 3, line 4). There was no statistical difference as for the ants' cognitive ability in performing this task between the ants living under one or the other kind of diet (small area: N = 5, T = -6, +9, P = 0.406; large area: N = 4, T = -5, +5, P = 0.563).

Escaping behavior

Contrary to ants living under normal diet, those consuming sildenafil citrate seemed to have difficulties in perceiving with precision the enclosure exit, and consequently in going out of the enclosure. The ants consuming sildenafil citrate did not stress; they duly walked rather calmly all along the rim of the enclosure and found the exit, but at that time they often stopped, tried to go out, could not succeed, tried again just as if they did not very well perceive the limit, the borders of the exit. However, a few ones could escape (Figure 3H), but there was a statistical difference between ants under one or the other kind of diet (Table 3, line 5; N = 6, T = +21 for ants still enclosed, -21 for ants escaped, P = 0.016). The fact that, obviously, the ants identically wanted and tried to escape whatever their diet was in agreement with the result of the previous experiment on cognition. A following experiment will again examine the ants' cognition while consuming sildenafil citrate (see: 'Visual and olfactory conditioning and memory'). The fact that the ants under sildenafil diet did not precisely perceive the exit of the enclosure was in agreement with observations previously made in the present study (see the results concerning the ants' orientation, trail following, audacity, tactile perception, brood caring, aggressiveness against aliens). A subsequent experiment tempted to examine the impact of sildenafil citrate on the ants' sensitive perception (see: 'Sensitive perception').

Traits	Sugar water diet	Sugar water+ sildenafil diet
Brood caring: numbers of larvae not re-entered in the nest over 10 minutes	Time: 30" 2 4 6 8 10 n°: 10 8 6 4 2 0	Time: 30" 2 4 6 8 10 n°: 10 8 8 6 6 4
Aggressiveness against nestmates	Levels 0 1 2 3 4 var 'a' n°: 74 57 15 0 0 0.11	Levels 0 1 2 3 4 var 'a' n°: 57 37 11 0 0 0.11
Aggressiveness against aliens	Levels 0 1 2 3 4 var 'a' n°: 7 38 66 89 45 4.44	Levels 0 1 2 3 4 var 'a' n°: 21 41 66 41 9 1.87
Cognition: ants in front of and beyond twists and turns in the course of 12 minutes	t n° in front n° beyond 30s 30 0 2 25 0 4 19 0 6 19 2 8 18 4 10 15 4 12 14 5	t n° in front n° beyond 30s 28 0 2 26 0 4 22 1 6 20 1 8 19 3 10 15 4 12 14 4
Escaping from an enclosure: ants in and out of the enclosure in the course of 12 minutes	t: 30s 2 4 6 8 10 12 n° in: 12 11 9 7 5 4 3 n° out: 0 1 3 5 7 8 9 Variable = 9/12 = 0.75	t: 30s 2 4 6 8 10 12 n° in: 12 12 10 9 7 7 6 n° out: 0 0 2 3 5 5 6 Variable = 6/12 = 0.50

Table 3: Effect of sildenafil citrate on five ethological and physiological traits.

Statistics and details can be found in the text. Sildenafil citrate did not affect the ants' social relationship and cognition, but it decreased their aggressiveness against aliens and their escaping behavior, this two effects resulting probably from a decrease of sensitive perception. s: second; n°: number; level 0: doing nothing, 1: contacting the opponent with the antennae, 2: opening the mandibles, 3: gripping the opponent, 4: stinging the opponent or trying to do so.

Visual and olfactory conditioning and memory

These traits were not impacted by sildenafil citrate consumption (Table 4; Figure 3I, 3J).

Concerning the visual conditioning, the ants consuming sildenafil citrate reached the same conditioning score (80%) as those living under normal diet, though a little slower, but this difference was not statistically significant (N = 3, NS). After removal of the visual cue, the ants consuming sildenafil citrate retained some more of their visual conditioning than ants living under normal diet, and this difference in visual memory between ants under the two kinds of diet was significant (Table 4, visual memory; N = 5, T = 15, P = 0.031). This could be explained by the somewhat larger sugar consumption of ants under sildenafil citrate diet (learning and memorization require glucose), and by the lower rapidity in acquiring conditioning (what is more quickly learned is generally less well memorized). This may also be due to a small increase of cAMP caused by sildenafil citrate (see below, the subsection devoted to a proposed physiological explanation for the effects of this drug), what should finally be identical to an effect caused by caffeine and similar substances [23].

Traits Time (hrs)	Normal diet*		Sildenafil citrate diet		
	Colony C	%	Colony A	Colony B	%
Visual conditioning					
7	7	70	6	6	60
24	8	80	8	6	70
31	8	80	8	8	80
48	8	80	9	8	85
55	8	80	8	8	80
72	8	80	9	7	80
Visual memory					
7	8	80	10	9	95
24	7	70	8	9	85
31	8	80	9	7	80
48	7	70	8	9	85
55	7	70	8	8	80
72	7	70	9	7	80
Olfactory conditioning					
7	7	70	7	7	70
24	8	80	7	8	75
31	8	80	8	8	80
48	9	90	8	8	85
55	9	90	9	9	90
72	9	90	9	9	95
Olfactory memory					
7	9	90	9	10	95
24	8	80	9	9	90
31	8	80	8	8	80
48	7	70	9	7	80
55	8	80	7	9	80
72	8	80	9	8	85

Table 4: Impact of sildenafil citrate on the ants’ visual and olfactory conditioning and memory. Details are given in the text. Sildenafil citrate did not impact the ants’ conditioning ability and somewhat improved their memory. hrs = Hours; % = Percentage; * = Results Previously Obtained [20].

Concerning the olfactory conditioning, the ants consuming sildenafil citrate acquired it a little slower than ants under normal diet but reached a similar score (about 90%). The difference of olfactory conditioning ability between ants under the two kinds of diet was not significant ($N = 3$, NS). After removal of the olfactory cue, ants consuming sildenafil citrate retained about 5% more of their conditioning than ants under normal diet, this difference of olfactory memory between ants under the two kinds of diet being at the limit of significance ($N = 4$, $T = +10$, $P = 0.063$). The same explanation as that given for the improved visual memory could be here again proposed (see here above).

Sensitive perception

After having had, for one day, their nest entrances marked, one with blue cues, the other with carrot odor, the ants under sildenafil citrate diet did not preferentially go towards such marked entrances when tested in front of marked and unmarked entrances (Table 5, line 1). They were even less numerous in front of and inside the blue marked entrance, this being at the limit of significance ($\chi^2 = 7.90$, $df = 3$, $P \sim 0.05$). After having lived during two days in a nest, the entrances of which being marked, the one with blue cues the other with carrot odor, the tested ants did not go more often towards the entrances marked with blue cues or carrot odor than towards the unmarked entrances (Table 5, line 2). On the contrary, they were statistically more numerous in front of and inside the unmarked entrances located on the left of the blue marked entrance ($\chi^2 = 21.06$, $df = 3$, $P < 0.001$). The number of ants going to the two unmarked entrances equaled $43 + 12 = 55$, and that of ants going to the marked entrances equaled $28 + 19 = 47$. These two numbers (55, 47) did not statistically differ from those expected (51, 51) if ants randomly went towards the marked and the unmarked entrances ($\chi^2 = 0.62$, $df = 1$, $0.30 < P < 0.50$).

Tests in front of marked and unmarked entrances	N° of ants (under sildenafil diet) in front of and in entrances			
	unmarked	marked with blue cues	marked with carrot odor	unmarked
after one day of habituation	23	10	27	20
<i>random numbers</i>	20	20	20	20
after two days of habituation	43	28	19	12
<i>random numbers</i>	25.5	25.5	25.5	25.5

Table 5: Assessment of the accurate sensitive perception of ants consuming sildenafil citrate. The nest entrances of two colonies provided with sildenafil citrate were marked, the one with blue cues, the other with carrot odor, and the ants were tested, one and two days later, in front of such marked and of unmarked entrances. They did not preferentially go to the marked entrances. Their sensitive perception was thus somewhat impacted by the drug. Statistics are given in the text.

The sum of the results of the two experiments (i.e. 66, 38, 46, 32) also revealed that more ants went towards the unmarked entrance located on the left of the blue marked one ($\chi^2 = 11.50$, $df = 3$, $0.01 < P < 0.02$), and that the number of ants going to the marked entrances ($38 + 46 = 84$) did not statistically differ from that of ants going to the unmarked entrances ($66 + 32 = 98$) ($84, 98$ vs $91, 91$: $\chi^2 = 1.078$, $df = 1$, $P \sim 0.30$) (Figure 3M). Consequently, ants did not memorize (though having an intact memory) the small visual and olfactory cues set for two days at their nest entrances. Their visual and olfactory perceptions were thus slightly impacted by sildenafil citrate consumption. Note that a few ants were seen stopping for a few minutes in front of the blue marked entrances, looking attentively to the blue cue as if having difficulty in perceiving it (Figure 3L). Such a behavior, seldom observed in ants, has also been seen in this work while testing trained ants in their y-apparatus provided with a yellow hollow cube (see above, the subsection on visual conditioning).

The presence of more ants in front of and inside the unmarked entrance located on the left of the experimental design (Figure 2, test) was not due to the lighting of the laboratory, located on the left, since ants prefer poorly lighted places. It might be due to the setting of the ants in the experimental tray, which might have been made not just in front of the two marked entrances but slightly to their left (Figure 2, test).

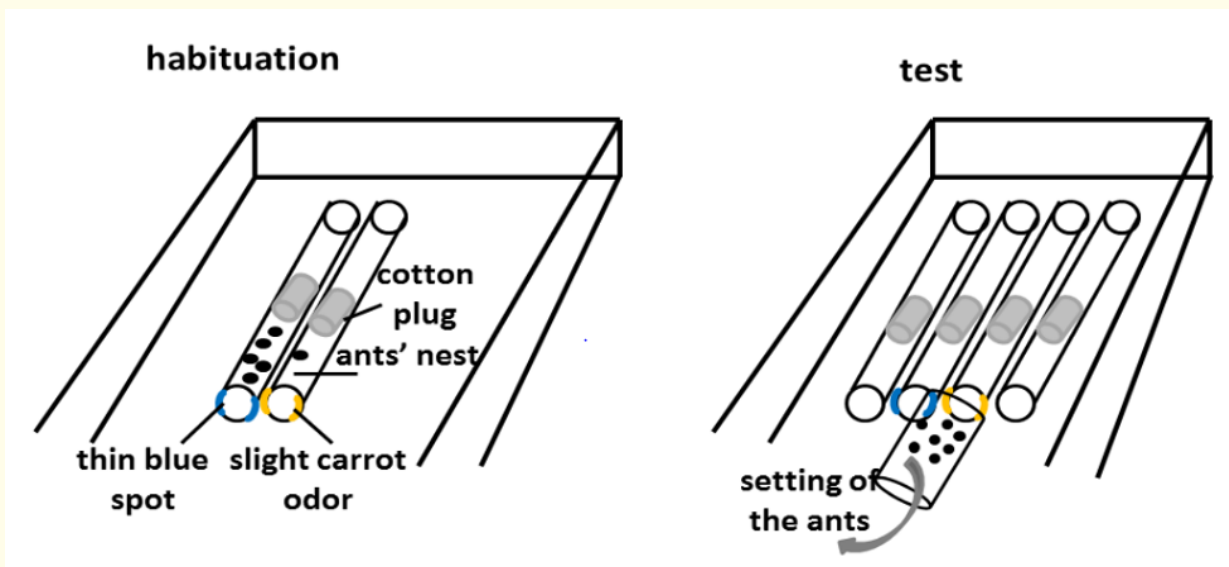


Figure 2: Experimental design allowing examining the ants’ visual and olfactory sensitivities. The ants’ nest entrances were slightly marked with blue spots (one nest tube) and carrot odor (the other nest tube), and after one and two days, the ants were tested, in a separate tray, in front of identically marked as well as of not marked tubes (right schema). Ants perceiving very well the blue spot and the carrot odor will become habituated to them, and when tested, should choose the tubes marked by them.

Adaptation to the impact of sildenafil citrate on the locomotion

Such a potential adaptation was examined after the ants consumed sildenafil citrate for 8 days, and without remembering or looking to the results obtained after one day of that drug consumption. As inferred from their locomotion (Table 6) and from their health (personal observation), ants appeared to not adapt themselves to the impact of sildenafil citrate. Indeed, the ants went on presenting some difficulties in finding their nest entrance and food sites, and went on moving slower and more sinuously than usually. Their linear and angular speeds still differed from the control ones (linear speed: $\chi^2 = 18.29$, $df = 2$, $P < 0.001$; angular speed: $\chi^2 = 52.69$, $df = 3$, $P < 0.001$). Moreover, their linear speed was even somewhat lower than that presented after one day of consumption and this was statistically significant ($\chi^2 = 11.86$, $df = 2$, $0.001 < P < 0.01$), and their angular speed somewhat higher, but this last result was not statistically significant ($\chi^2 = 1.67$, $df = 2$, $0.30 < P < 0.50$). Such a lack of adaptation to the adverse effect of sildenafil citrate is not in favor of its use.

Traits	Normal diet	Sildenafil citrate since one day	Sildenafil citrate since 8 days
Linear speed (mm/sec)	12.2 (10.9 - 13.6)	10.8 (9.6 - 11.4)	9.8 (8.7 - 11.4)
Angular speed (ang.deg./cm)	121 (103 - 139)	188 (168 - 208)	196 (175 - 217)

Table 6: Ants’ adaptation to the effect of sildenafil citrate, as inferred from their locomotion. The ants did not adapt themselves to such an impact, their locomotion being affected over the drug consumption. Statistics are given in the text. mm/sec = Millimeter Per Second; ang. deg./cm = Angular Degrees Per Centimeter.

Habituation to beneficial effects of sildenafil citrate

Having found no beneficial effect of the examined drug, this trait could not be studied.

Dependence on sildenafil consumption

Ants developed no dependence on sildenafil citrate consumption (Figure 3N). Confronted to sugar water containing this drug and to sugar water free of it, 11 ants of colony A went onto the former liquid and 20 ones on the latter liquid, while 15 ants of colony B went onto the former liquid and 14 ones on the latter liquid. Thus, in total, 26 ants preferred the sugar water containing sildenafil citrate, and 35 ants preferred the sugar water free of that drug. These numbers (26, 35) did not statistically differ from those expected if ants went randomly on each of these two liquids (30, 30) ($\chi^2 = 0.30$, $df = 1$, $0.50 < P < 0.70$). In conclusion, sildenafil citrate did not lead to physical dependence, what is in favor of its use.

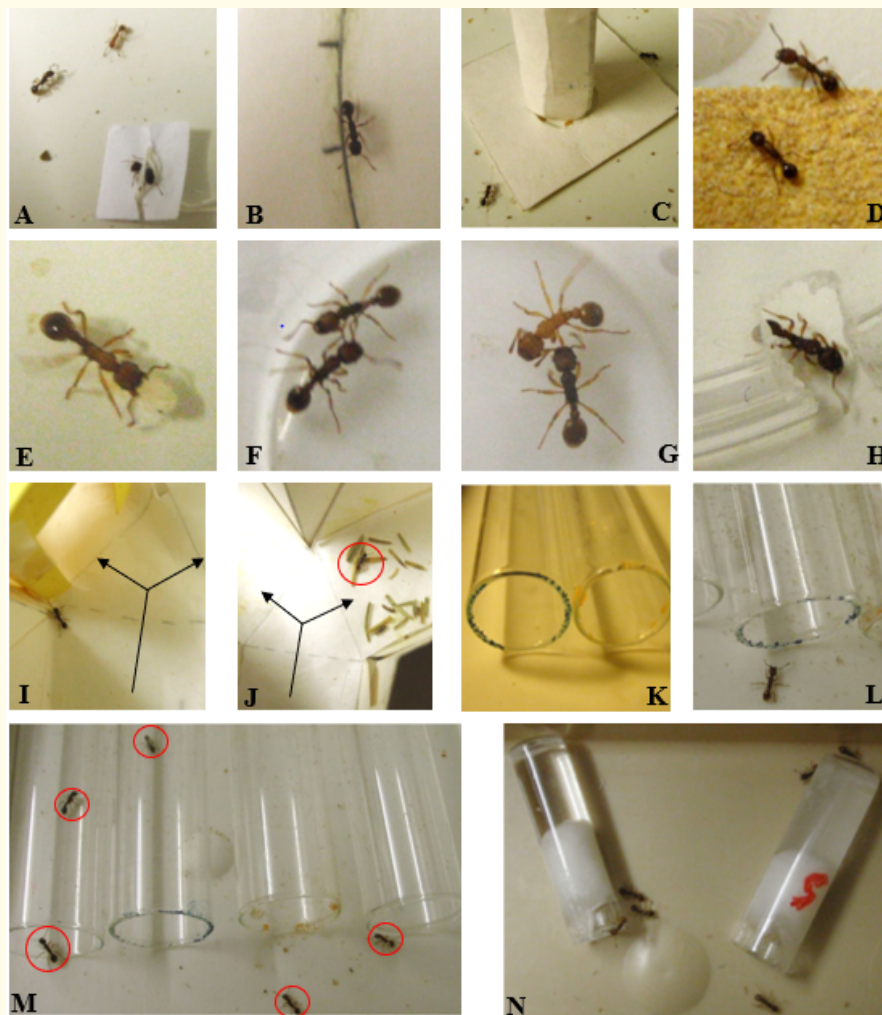


Figure 3: A few photos of the experiments. All the photographed ants were under sildenafil citrate diet. A: Two ants having some difficulties in reaching a tied nestmate which emitted its alarm pheromone. B: An ant following for a time a circular trail. C: Two ants looking to an unknown apparatus and not yet coming onto it. D: An ant having walked and an ant walking on a rough substrate, apparently slightly perceiving the uncomfortable character of the substrate. E: An ant holding a young nymph experimentally removed from the nest, and in the process of re-enter it. F: Two nestmates staying side by side without aggressiveness. G: An ant (the darker one) gripping (thus attacking) an alien ant. H: An ant escaping from an enclosure through a notch, and moving as if it did not precisely see the limit of the notch. I: An ant, trained to a yellow cube, giving the correct response when tested in a Y-apparatus, but coming very near the yellow cube before going on responding. J: An ant (red circle), trained to rosemary, having given the correct response when tested in a Y-apparatus, but coming very near, even on, the rosemary. K: Two nest entrances marked, one (on the left) with thin blue spots, the other (on the right) with carrot odor, an experimental design used for examining the ants' precise sensitive (visual, olfactory) perception. L: An ant, habituated to a nest entrance slightly marked in blue, looking motionless, for a few minutes, to this marking. M: Ants (red circles), habituated to slightly marked nest entrances, tested in front of such marked entrances and unmarked ones, not preferentially going to the marked entrances. N: Ants presented with sugar water containing sildenafil citrate (red 'S') and sugar water free of that drug, not preferring the former solution, having thus developed no dependence on sildenafil citrate.

Decrease of the effect of sildenafil after its consumption was stopped

The result of this investigation is numerically given in table 7 and graphically presented in figure 4. During the first four hours after weaning, the effect of sildenafil citrate only weakly decreased (from 208 to 188 ang.deg./cm). Then, after a total of six hours, its effect, though somewhat lower, was still statistically similar to its initial one (182 vs 208 ang.deg./cm; $\chi^2 = 3.81$, df = 2, $0.10 < P < 0.20$). Eight hours after weaning, the effect of sildenafil citrate became statistically different from its initial one (157 vs 208 ang.deg./cm; $\chi^2 = 18.02$, df = 2, $P < 0.001$), but it still differed from the control value (157 vs 122 ang.deg./cm; $\chi^2 = 17.40$, df = 3, $P < 0.001$). Ten hours after weaning, the effect of sildenafil citrate became very weak, with the ants' sinuosity slightly different from the control value (147 vs 122 ang.deg./cm; $\chi^2 = 5.61$, df = 2, $P \sim 0.05$). Twelve hours after weaning, the effect of sildenafil citrate was statistically no longer detectable (139 vs 122 ang.deg./cm; $\chi^2 = 5.35$, df = 2, $0.05 < P < 0.10$). Fourteen hours after weaning, the ants' sinuosity (123 ang.deg./cm) became identical to the control one (122 ang.deg./cm). Consequently, after weaning, the effect of sildenafil citrate slowly vanished over time, the decrease corresponding better to a linear than to a non-linear (cubic or quadratic) function. This linear function can be expressed by:

$E_t = 212.125 - 6.2768 t$ using the medians of the distributions (Figure 4, left graph)

or $E_t = 211.433 - 5.8789 t$ using the means of the distributions (Figure 4, right graph)

with E = effect of the drug and t = time after weaning (in hours).

Experiments and times	Ants' sinuosity ang.deg./cm	Statistics			
		vs control		vs t = 0h	
		χ^2	K-W	χ^2	K-W
Control (normal diet)	122 (103 - 139)				
After 1 day of consumption	188(168 - 208)				
After 8 days of consumption	196 (175 - 217)				
After 24 days of consumption = just before weaning, t = 0h	208 (192 - 227)				
Weaning,					
Time after weaning: t = 2h	202 (181 - 218)	P < 0.001	P < 0.001	0.80 < P < 0.90	P = 1
t = 4h	188 (161 - 219)	P < 0.001	P < 0.001	0.20 < P < 0.30	P = 0.77
t = 6h	182 (158 - 196)	P < 0.001	P < 0.001	0.10 < P < 0.20	P = 0.26
t = 8h	157 (144 - 163)	P < 0.001	P = 0.03	P < 0.001	P = 0.008
t = 10h	147(129 - 176)	P ~ 0.05	P = 0.19	P < 0.001	P = 0.007
t = 12h	139 (127 - 159)	0.05 < P < 0.10	P = 0.87	P < 0.001	P < 0.001
t = 14h	124 (111 - 139)	0.80 < P < 0.90	P = 1	P < 0.001	P < 0.001

Table 7: Decrease of the effect of sildenafil citrate after its consumption was stopped. The trait examined was the ants' sinuosity of movement. The effect of the drug became at the limit of significance some 10 hours after its consumption was stopped, and persisted thus less than 12 hours. ang.deg./cm = Angular Degree per Centimeter; h = Hours; χ^2 = Chi-square; K-W = One-Tailed Non-Parametric Kruskal-Wallis ANOVA for Multiple Comparisons, taking either 'control' or 't = 0h' as control groups These numerical values are graphically presented over time in figure 4.

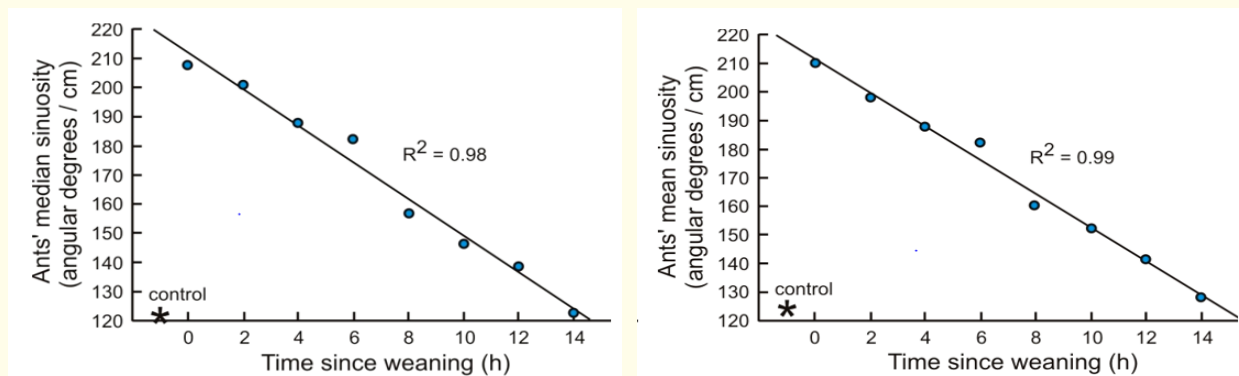


Figure 4: Decrease of the effect of sildenafil after its consumption was stopped. The numerical results and statistics are given in table 7 and in the text. The left graph uses the medians (given in table 7) and the right graph the means of the distributions of the obtained values. The drug was efficient during nearly 10 hours after weaning, and became inefficient just after. The decrease after weaning was slow, what allowed avoiding dependence.

Such a slow decrease was in agreement with the absence of dependence on sildenafil citrate consumption. On ants, the drug appeared thus to be active during ten hours (or slightly a little more) when taking ants consuming sildenafil for 24 days as a start. Using the effect of the drug after one day of consumption as a start (188 (168 - 208) ang.deg./cm), the drug appeared to be active during eight hours (157 ang.deg./cm) (statistics not in table 7: 6h: 188 vs 182; $\chi^2 = 1.11$, $df = 3$, $0.70 < P < 0.80$; 8h: 188 vs 157; $\chi^2 = 10.47$, $df = 3$, $0.01 < P < 0.02$; 10h: 188 vs 147; $\chi^2 = 23.29$, $df = 2$, $P < 0.001$). Looking for information about the duration of action of sildenafil citrate in humans, we found that a time period of twelve hours is mentioned [24]. This might be a little overestimated.

Proposed physiological explanation for the here observed adverse effects of sildenafil citrate

We found that sildenafil citrate impacted the ethological and physiological traits requiring an intact visual, olfactory and tactile perception, but did not affect cognition, conditioning ability and memory. It even somewhat improved memorization, as did caffeine and similar substances [23]. No adaptation to the harmful effects of sildenafil citrate occurred, and no dependence developed on its consumption.

Sildenafil citrate inhibits type 5 phosphodiesterase. For mammals, this enzyme allows hydrolyzing cGMP into GMP what leads to the shutting of membrane channels and consequently to the visual perception. In invertebrates, another enzyme acts, but it also makes hydrolysis, what also leads to the closure of ion channels and triggers visual perception. So, sildenafil citrate might inhibit some enzymes able to hydrolyze, and might therefore have other harmful effects than those actually known in humans.

Also, under a sildenafil citrate diet, cGMP as well as (to a lesser extend) cAMP amounts increase since they are no longer hydrolyzed, and this may have several physiological effects because these two co-enzymes have a lot of physiological functions in the organism. Cyclic GMP acts, among others, on kinases, some phosphodiesterases, some ionic channels, as well as smooth muscles (https://fr.wikipedia.org/wiki/Guanosine_monophosphate_cyclique). Cyclic AMP acts, among others, on kinases, proteins located at ion channels, striated muscles, hormonal activities, neurotransmitter activities, and glucose release (https://fr.wikipedia.org/wiki/Adénosine_monophosphate_cyclique).

Other bibliographical information useful for concluding

Looking to information available about the effects of sildenafil citrate on humans, anyone essentially finds that this product is very efficient as for its beneficial effect (maintaining an erection) and has only weak adverse effects [25-27]. Moschos and Nitoda [28] describe

the adverse effect of sildenafil citrate on visual perception and state that this effect is reversible. However, for a time, persons consuming sildenafil citrate have their vision more or less affected. We could not but estimate that the adverse effects of sildenafil citrate observed in humans (here also observed on ants) are not 'put forward' by practitioners and pharmacists, contrary to its beneficial effect.

Conclusion

Working on ants as biological models, we found that sildenafil has several ethological and physiological adverse effects, in agreement with effects observed in humans. On basis of bibliographical research, we estimate that practitioners and pharmaceutics attach not enough importance to the adverse effects, putting forward the beneficial effect of sildenafil citrate. We also presume, on basis of our results, that this drug may also have still unknown harmful effects. Indeed, it inhibits not only type 5 phosphodiesterase but also other hydrolyzing enzymes, and increases the amounts of cGMP as well as to a lesser extend cAMP, these different affected enzymes and co-enzymes having important functions in any living organism. Our advice, based on our observations and bibliographical researches, is to use parsimoniously the drug and no longer if adverse effects appear. Other remedies, natural if possible, should be researched for solving erection problems, and such ones, poorly lucrative for the pharmaceutical industry, are already known [29].

Conflict of Interest

We affirm having no conflict of interest as for the use of sildenafil citrate; we work only on ants, and receive no money for making our research.

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Volume 6 Issue 8 August 2018

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