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# Side effects of mirabegron studied on ants as models

## Abstract

The overactive bladder syndrome (OAB) affects the quality of life. It is most often treated with anticholinergic drugs, but at the cost of many unwanted effects. In the last decade to date, mirabegron, an adrenergic receptor agonist drug used to relieve OAB has appeared in the pharmacopoeia. It works through a different mechanism than antimuscarinic drugs, with apparently fewer side effects. In the same way that we have examined the adverse effects of the anticholinergic oxybutynin on the ant *Myrmica sabuleti* used as a biological model, here we examine the side effects of mirabegron. Unlike oxybutynin, mirabegron had no adverse effect on ants, except that it increased their meat consumption, reduced their sugar water intake and decreased their general activity. No adaptation was observed for this last-mentioned effect, which may correspond to the tiredness reported in humans. The ants did not become dependent on the consumption of mirabegron, whose effect on general activity decreased very slowly and disappeared about 52 hours after weaning. Patients are advised to take mirabegron daily though its elimination half-life is 50 hours, and fatigue is one of the side effects of the treatment. In the event that patients under mirabegron medication show a decrease in activity over their treatment, it would be careful to space out the medication or to add an appropriate stimulant or to alternate mirabegron with an anticholinergic drug that does not affect the patient's level of activity. The kind of antimuscarinic drug that could be used together with mirabegron, the respective dosage and the timing of each drug should be defined depending on their combined efficacy and side effects, and taking into account their elimination time.

**Keywords:** Adrenergic receptor agonist, antimuscarinics, combination therapy, general activity, *Myrmica sabuleti*, overactive bladder.

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## Abbreviations:

ang.deg., angular degrees; ang.deg./cm, angular degrees per cm; mm/s, millimeter per second; $\chi^2$, chi-square; vs, versus; n°, number; cm, centimeter; mm, millimeter; ml, milliliter; mg, milligram; kg, kilogram; s, second; min, minute; h, hour; t, time; %, percentage

## Introduction

The crippling overactive bladder (OAB) syndrome is most usually treated thanks to anticholinergic drugs which block the acetylcholine neurotransmission to the muscarinic M3 receptors responsible for the involuntary contraction of the detrusor bladder muscle during the filling phase.[1,2] For many years, antimuscarinic drugs were considered as being the only treatment with undisputed effectiveness.[3] However, they present unwanted effects because acetylcholine neurotransmitters are present in many organs. Among seven antimuscarinic drugs used to treat overactive bladder (oxybutynin, tolterodine, darifenacin, fesoterodine, solifenacin, propiverine and trospium chloride), solifenacin and trospium lead to the less adverse effects,[4] trospium thanks to the fact that it barely crosses the blood-brain barrier.[3] Oxybutynin, the most commonly used of these anticholinergic drugs, appears to produce a high benefit against OAB symptoms, but it has the worst adverse effects, among which dry mouth and constipation.[4,5]

Drugs with a different mechanism of action than anticholinergists were thus wanted for treating OAB symptoms. The first of them to have entered clinical practice is mirabegron, a selective β3-adrenergic receptor agonist which relaxes the detrusor muscle, improving the filing capacity of the bladder, reducing the urgency to empty it, without weakening bladder contraction during voiding.[6] Approved in USA and in the European Union in 2012, it is nowadays the 225th most prescribed medication in the United States, i.e. the second most prescribed drug for OAB after oxybutynin, which is the 99th in the top 300 of prescribed drugs.[7] As mirabegron diminishes more the bladder pressure and number of microcontractions than oxybutynin[8] and has less adverse effects than it, especially less dry mouth and constipation occurrences,[9] it may be preferred to oxybutynin in the future.

Using ants as a biological model, we showed that the consumption of oxybutynin had several adverse effects partly agreeing with some observed in humans treated with this drug such as restlessness, anxiousness, anorexia, as well as cognition and memory impairment. Social relationship impairment was also observed in ants, what may correspond to psychotic disorders sometimes reported in humans treated with this drug.[10] Among the adverse effects of mirabegron reported in the Medscape Drugs Database[11] are dry mouth, urinary tract infection, headache, dizziness, tachycardia, tiredness and gastrointestinal disorders. However, no information can be found about the potential impact of the drug on the sensory perception, social relationship, cognition, and memory, as well as about adaptation, habituation and dependence. We here intend to examine the potential adverse effects of mirabegron by using the ant *Myrmica sabuleti* Meinert 1861 as a biological model just like we examined oxybutynin as for its adverse effects.[10]

Before relating then commenting our results and comparing them with the available information on it, we explain here below why we used ants as models, and then, we relate our experimental processes. Since, for comparative purpose, the present work had to be done in the same way as our previous one on oxybutynin, the experimental protocols and statistical analysis were identical. They are thus here briefly related, and readers are invited to find details in our previous study.[10]

### Why we used ants as models

Most biological processes are identical in every animal species

including humans (e.g. genetics, embryology, molecular biology such as respiration, anabolism and catabolism, nervous cell functioning, etc.). Invertebrates and vertebrates are thus used as models for studying a lot of biological traits.[12] Invertebrates are more and more used because they are small and easily maintained in a laboratory, have a short life cycle and a rather simple anatomy.[13] Some species are widely used as models: for example, the flatworm *Dendrocoelum lacteum*, the nematode worm *Caenorhabdotes elegans*, the mollusk *Aplysia californica*, the beetle *Tribolium castaneum*, the fruit fly *Drosophila melanogaster*, and the honeybee *Apis mellifera*. Insects and among them social hymenoptera are largely used since they present experimental and biological advantages.[14] Therefore, ants can be used as models, the more so because they possess numerous sophisticated biological traits. These eu-social insects among other particularities navigate using learned cues, recruit nestmates, differently mark parts of their territory, take care of their brood, build a complex nest, clean it and manage cemeteries at the frontiers of their territory.[15] We have often used ants as models and, each time we examined on them the side effects of products or situations employed by humans, we found impacted ethological and physiological traits valuable for any animal species, humans included. In the present work, we used again the species *Myrmica sabuleti*.

## What we know on the used ant species' cognition

The workers of *M. sabuleti* adapt their nesting site and their navigation to their visual and olfactory perception, adequately recruit nestmates, recognize themselves in a mirror, are imprinted to the appearance of the front of their congeners at their emergence as an imago, learn several social behaviors in the presence of older congeners,[16–18] have natively a number line, acquire the notion of zero through experiences, and can acquire numerical symbolisms.[19,20] Such as for vertebrates, including humans, their perception respond to the distance and the size effects as well as to Weber's law.[21,22]

## Which traits we examined

As we did for previously examined products used by humans,[23–26] we intended to examine the impact of mirabegron on the workers' food consumption, general activity, locomotion, orientation ability, audacity, tactile (pain) perception, social relationships, cognition, stress, learning ability and memory, as well as their adaptation to the side effects of the drug, their dependence on its consumption, and the decrease of the effect of the drug after its consumption was stopped. Our experimental protocols were identical to those previously used, e.g.[10,27,28] Consequently, we only briefly relate them, however without avoiding inevitable plagiarism. We affirm having performed our experimental work being blind to the known side effects of the examined drug.

## Material and methods

### Collection and maintenance of ants

The experiments were made on two colonies of *M. sabuleti* collected in autumn 2019 in an abandoned quarry located at Olloy/Viroin (Ardenne, Belgium). The colonies contained about 500 workers, a queen and brood, and were maintained in the laboratory in one to three glass tubes, being separated from a bottom water-filled compartment by a cotton plug. The nest tubes of each colony were set in a tray (34cm x 23cm x 4cm) into which a cotton-plugged tube containing a 30% aqueous solution of sugar was permanently provided, as well as pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) which were deposited three times per week. The lighting of the laboratory was *ca* 330 lux, the ambient temperature *ca* 20°C, the

humidity *ca* 80%, and the electromagnetism *ca* 2 µWm². The ants are here often named workers or nestmates as do researchers on social insects.

## Solution of mirabegron given to the ants

A package of Betmiga® in extended-release formulation, produced by Astellas Pharma Europe B.V. Branch (Belgium), was furnished by the pharmacist Wera (Brussels, Belgium). It contains tablets with mirabegron as the active substance. Humans treated with this drug are advised to consume each day two tablets of 25mg (or one tablet of 50mg). They also daily consume about one liter of water. Insects drink about ten less water than mammals due to their physiology and anatomy. Therefore, to maintain ants under a drug diet similar to that of humans, they have to be provided with a solution of 50mg mirabegron in 100ml of their usual sugar water, or of 25mg in 50ml of this liquid. To get this drug as an immediate-release product, a tablet of Betmiga® 25mg was ground then dissolved into 50ml sugar water. This solution was delivered to the ants in their usual cotton-plugged tubes (Figure 1). The plug of these tubes was refreshed every 2-3 days, and the entire solution was renewed every 7 days. Each day, it was checked if the ants drunk the provided solution, what they did. All control experiments were firstly made on the colonies maintained under normal diet. Then, these colonies were provided with the drug solution, and different tests were conducted starting the day after the drug delivery.
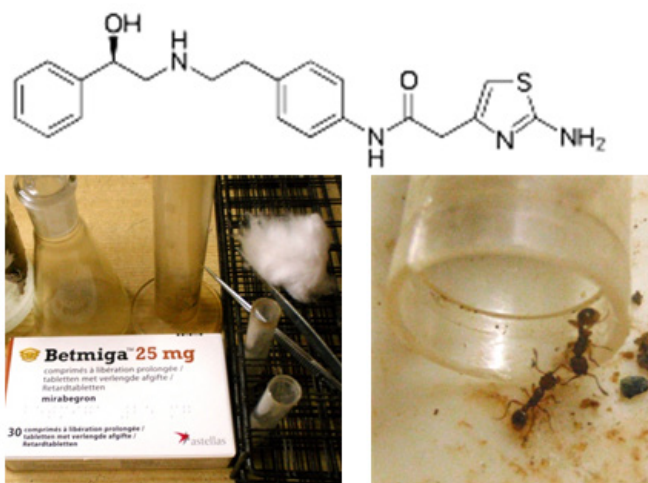


**Figure 1** Chemical structure of mirabegron, the side effects of which are here examined on ants as models; preparation of this drug solution given to the ants, and two ants drinking this solution.

## Meat and sugar water consumption, general activity

For each kind of diet (normal and with mirabegron), we counted the ants present on the meat food, at the entrance of the sugar water tube, as well as, for assessing the ants general activity, those being active anywhere (on their foraging area, at their nest entrance and inside the nest). A single punctual count was made four times during the day and two times during the night, during 6 days, each day at the same time o'clock. For each kind of count and diet, the daily mean of the 6 times x 2 colonies = 12 counts was established (Table 1, lines 1 - 6), as well as the mean of the six daily means (Table 1, last line). For each three kinds of count, the six daily means obtained for ants under mirabegron diet were compared to the six daily means obtained for ants under normal diet using the non-parametric test of Wilcoxon,[29] the level of significance being set at P = 0.05.
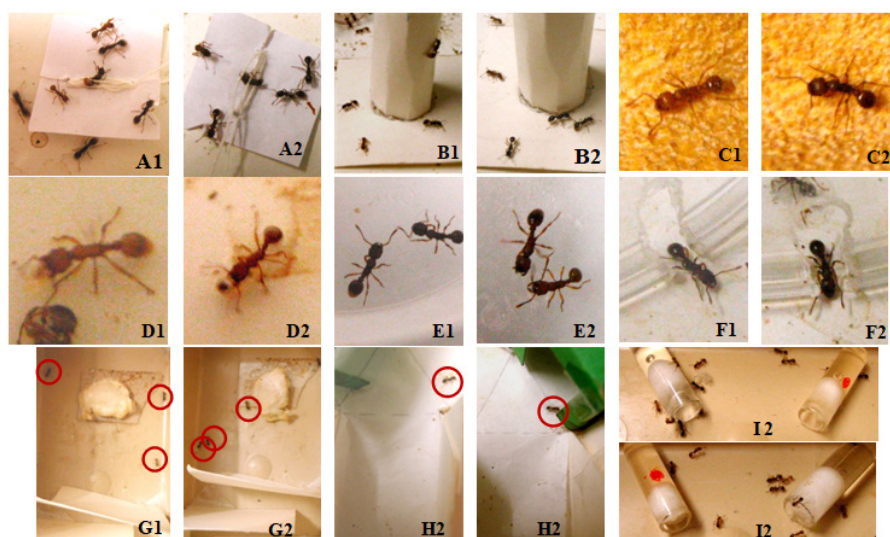
**Table 1** Effect of mirabegron on ants' food consumption and general activity

| Days | Normal diet | | | Diet with mirabegron | | |
|---|---|---|---|---|---|---|
| | Meat | Sugar water | Activity | Meat | Sugar water | Activity |
| I | 1.50 | 2.00 | 9.00 | 1.25 | 1.50 | 8.16 |
| II | 2.25 | 1.75 | 12.50 | 2.75 | 1.50 | 7.75 |
| III | 1.25 | 2.25 | 10.75 | 2.25 | 1.00 | 9.50 |
| IV | 1.50 | 2.25 | 10.00 | 2.00 | 1.50 | 9.25 |
| V | 2.75 | 3.75 | 12.25 | 3.00 | 1.00 | 9.25 |
| VI | 2.50 | 2.00 | 9.50 | 3.50 | 1.25 | 8.00 |
| mean | 1.96 | 2.33 | 10.67 | 2.46 | 1.29 | 8.65 |

The table gives the daily mean number of ants eating meat, drinking sugar water, and being active anywhere in their environment, and the overall mean. Mirabegron increased the ants' meat consumption, and decreased their sugar water consumption and their general activity. Details on the methods and statistics are given in the text

## Linear and angular speeds, orientation

These traits were assessed on ants walking in their foraging area, the speeds without stimulating them, the orientation while stimulating them with a nestmate tied to a piece of paper (Figure 2, A). Such a nestmate emits its attractive mandibular glands alarm pheromone. To assess the ants' speeds as well as their orientation, 40 trajectories were recorded and analyzed using appropriate software[30] and following definitions. The linear speed (in mm/s) is the length of a trajectory divided by the time spent to travel it; the angular speed (in ang.deg./cm) is the sum of the angles made by successive adjacent segments, divided by the length of the trajectory; the orientation (in ang. deg.) to a location is the sum of successive angles made by the direction to the location and the direction of the trajectory, divided by the number of measured angles. A value lower than 90° indicates that the animal tends to orient itself to the location; a value higher than 90° indicates that it tends to avoid the location. For the linear speed, the angular speed, and the orientation, the median and quartiles of the 40 obtained values were established (Table 2, lines 1, 2, 3). The distributions obtained for ants consuming mirabegron were compared to those obtained for ants under normal diet by using the non-parametric $\chi^2$ test.[29]



**Figure 2** Some views of the experiments made for examining the side effects of mirabegron. 1: ants under normal diet; 2: ants under mirabegron diet. A: ants coming to a tied nestmate; B: ants coming onto a unknown apparatus; C: ants walking with difficulty on a rough substrate; D: ants holding a larva removed from the nest; E: two nestmates behaving peacefully towards one another; F: ants escaping from an enclosure; G: ants having successfully crossed a twists and turns path; H: ants, trained to a green hollow cube, tested in a Y-apparatus and giving the wrong (left photo) as well as the right response (right photo); I: ants consuming mirabegron choosing the tube filled with drug-free sugar water, neglecting the tube filled with a sugared solution of the drug (red dot). Mirabegron affected none of the traits here illustrated. Details are given in the text and in Tables 2, 3 and 4.

**Table 2** Effect of mirabegron on five ants' physiological and ethological traits.

| Traits | Normal diet | Diet with mirabegron |
|---|---|---|
| Linear seed (mm/s) | 9.9 (8.6 – 11.0) | 9.0 (8.3 – 10.0) |
| Angular speed (ang.deg./cm) | 126 (108 – 147) | 129 (115 – 141) |
| Orientation (ang.deg.) | 30.6 (25.2 – 47.9) | 31.1 (22.6 – 44.8) |
| Audacity (n°) | 2.65 [2 – 5] | 2.40 [1 – 4] |
| Tactile (pain) perception | | |
| linear speed (mm/s) on a rough substrate | 4.6 (4.2 – 5.3) | 4.7 (4.3 – 5.2) |
| angular speed (ang.deg.cm) on a rough substrate | 260 (234 – 285) | 268 (222 – 284) |

The table gives the median (and quartiles) or the mean [and extremes] of the recorded experimental values. Mirabegron did not impact any of these five traits. Details and statistics can be found in the text, and some photos are shown in Figure 2.

## Audacity

A cylindrical tower (height = 4cm; diameter = 1.5cm) tied to a squared platform (9 cm²), both in Steinbach® white paper, was deposited in the ants' foraging area,[10,27,28] and the ants present on this apparatus were counted 10 times over 10 min (Figure 2, B). The mean and extremes of the recorded numbers were established (Table 2, line 4). The numbers obtained for the two colonies were pooled and those obtained during every two successive minutes were added. The five sums obtained for ants under mirabegron diet were compared to those obtained for ants under normal diet by using the non-parametric test of Wilcoxon.[29]

## Tactile (pain) perception

The ants perceiving the uncomfortable character of a rough substrate walk on it slowly, sinuously, often touching the substrate with their antennae (Figure 2, C1). Ants not perceiving this uncomfortable character walk more easily, more quickly and less sinuously. Therefore, to assess the ants' tactile perception, we assessed their locomotion on a rough substrate. A folded piece (3cm x 2 + 7 + 2 = 11cm) of n° 280 emery paper was deposited in a tray (15cm x 7cm x 4.5cm) dividing it into a first 3cm long zone, a second 3cm long zone covered with the emery paper, and a last 9cm long zone. To make an experiment on a colony, 12 ants were deposited in the first zone of the apparatus, and their trajectories on the emery paper were recorded. Two colonies being used, 24 trajectories were recorded, and the ants' linear and angular speeds on the rough substrate were assessed as usually (see the subsection relative to linear and angular speeds). For each speed and each kind of diet, the median and quartiles of the 24 obtained values were established (Table 2, lines 5, 6). The distributions corresponding to ants consuming mirabegron were compared to those corresponding to ants under normal diet by using the non-parametric $\chi^2$ test.[29]

## Brood caring

For each colony, a few larvae were removed from their nest and set in front of its entrance, and five of them were observed during five minutes (Figure 2, D). Only five larvae were observed in order to be able to keep track of them all together, simultaneously. The experiment was not repeated because it caused an important trauma for the colony. The larvae among the five observed which were not re-entered in the nest after 30 seconds, 1, 2, 3, 4 and 5 minutes were counted, and the numbers obtained for the two colonies were added (Table 3, line 1). The six numbers obtained for ants consuming mirabegron were compared to the six numbers obtained for ants under normal diet using the non-parametric test of Wilcoxon.[29]

**Table 3** Effect of mirabegron on four biological and ethological traits

| Traits | Normal diet | | | | | | Diet with mirabegron | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N° of larvae among 10 not re-entered over time | time: 30s | 1 | 2 | 3 | 4 | 5min | time: 30s | 1 | 2 | 3 | 4 | 5min |
| | n°: 8 | 7 | 5 | 5 | 3 | 0 | n°: 10 | 8 | 6 | 4 | 2 | 0 |
| Level of aggressiveness against nestmates and n° of cases | level: 0 | 1 | 2 | 3 | 4 | 'a' | level: 0 | 1 | 2 | 3 | 4 | 'a' |
| | n°: 71 | 36 | 13 | 0 | 0 | 0.12 | n°: 70 | 43 | 14 | 0 | 0 | 0.12 |
| N° of ants in front and beyond a twist and turns path over time | time: | 2 4 | 6 | 8 | 10 | 12min | time: | 2 4 | 6 | 8 | 10 | 12min |
| | n° in front: 26 | 21 | 18 | 16 | 16 | 14 | n° in front: 21 | 20 | 18 | 17 | 17 | 17 |
| | n° beyond: 0 | 0 | 1 | 3 | 5 | 6 | n° beyond: 0 | 0 | 1 | 1 | 2 | 4 |
| N° of ants among 12 escaped over time | time: | 2 4 | 6 | 8 | 10 | 12min | time: | 2 4 | 6 | 8 | 10 | 12min |
| | n° ants: 2 | 4 | 6 | 8 | 10 | 12 | n° ants: 2 | 4 | 5 | 7 | 9 | 11 |

Even if the recorded numbers were not identical for ants under the one and the other kinds of diet, mirabegron did not substantially impact the four examined traits. The slight observed effect on brood caring, cognition and escaping may result from an effect of the drug on the ants' general activity. Explanation as well as statistics and details on the methods are given in the text. Photos are shown in Figure 2.

## Social relationships

Drugs may affect the usual peaceful social relationship existing between members of a colony. To examine the potential impact of mirabegron on social relationship, five dyadic encounters were performed on ants of each two colonies living under normal then under the drug diet (10 encounters in total). The encounters occurred in a cup (diameter = 2cm, height = 1.6cm) the borders of which had been lightly covered with talc. Each time, one ant of each pair was observed during 5 min and its behavior described by the numbers of times it did nothing (level 0 of aggressiveness), touched the other ant with its antennae (level 1), opened its mandibles (level 2), gripped and/or pulled the other ant (level 3), tried to sting or stung the other ant (level 4) (Figure 2, E). The numbers obtained for the two colonies were added (Table 3, line 2). The five sums obtained for ants consuming mirabegron were compared to those obtained for ants under the normal diet by using the non-parametric $\chi^2$ test.[29] Also, the ants' behavior was characterized by the variable 'a' which equaled to the number of aggressiveness levels 2 + 3 + 4 divided by the number of levels 0 + 1.

## Cognition

This trait was assessed by depositing 15 ants of each colony in an own apparatus made of a tray (15cm x 7cm x 4.5cm) into which two duly folded pieces of paper (Steinbach®, 12cm x 4.5cm) had been set in order to create a twists and turns path between a 2cm long area (where the 15 ants were deposited) and a 8cm long one beyond the difficult path (Figure 2, G). The ants still in front of the difficult path and those present beyond it were counted after 2, 4, 6, 8, 10 and 12 min. The numbers obtained for the two colonies were added (Table 3, line 3), and the numbers obtained for ants - in front as well as beyond the difficult path - under mirabegron diet were compared to those obtained for ants under normal diet using the non-parametric Wilcoxon test.[29]

## Escaping behavior

Six ants of each colony were enclosed under a reversed polyacetate cup (height = 8cm, bottom diameter = 7cm, ceiling diameter = 5cm) set on their foraging area. The rim of the bottom of the cup presented a notch (3mm height, 2mm broad) so that the ants could escape (Figure

2, F). The numbers of ants of the two colonies which could escape after 2, 4, 6, 8, 10 and 12 minutes were counted and added (Table 3, line 4). The six sums obtained for ants consuming mirabegron were compared to those obtained for ants living under normal diet by using the non-parametric Wilcoxon test.[29]

## Conditioning ability, memory

At a given time, for each two colonies, a green hollow cube build in strong green paper (Canson®) was placed above the entrance of the sugar water tube and pieces of mealworms were relocated just aside that tube. Since that time, the ants underwent operant visual conditioning. The control experiment had been previously made on another colony (results of this control experiment are given in[10]) because after individuals are conditioned to a cue, they are no longer naïve as for this cue, and their conditioning to that cue can no longer be assessed. Since the deposit of the cube, the ants were tested over their conditioning acquisition, then the cues were removed, and the ants were tested over the loss of their conditioning. The tests were performed in a Y-apparatus made of strong white paper, having its sides slightly covered with talc, and set in a tray. Each Y-apparatus was provided with a green hollow cube in one of its branch, half of the tests being made with the cube in the left branch and the other half with the cube in the right branch.

To make a test, 10 ants of each colony were one by one deposited in the area of the Y-apparatus lying in front of the two branches of the apparatus. The ants' first choice of one or the other branch of the Y-apparatus was noted (Figure 2, H). Choosing the branch containing the green cube was considered as giving the correct response. For not testing twice the same ant, after having been tested, each ant was kept into a cup until 10 ones were tested. After the test, the 10 ants were replaced into their foraging area. For each test, the recorded responses of 10 ants of each colony were added (n = 10 x 2 = 20 responses) and the proportions of correct responses obtained over time were calculated (Table 4). The numbers of correct responses obtained over the six tests made during the acquisition then the loss of conditioning for ants consuming mirabegron were compared to those previously obtained for ants living under normal diet by using the non-parametric Wilcoxon test.[29]

## Adaptation to side effects of mirabegron

Adaptation to a drug or a situation occurs when an individual becomes less and less affected by them over their use. To study adaptation to mirabegron, a trait impacted by this drug has to be examined after one or two days of use, then again after several days of use and the results compared. In the present work, the only trait largely impacted by mirabegron consumption and not appearing to decrease over time was the ants' general activity, which was moreover easy to assess. Consequently, for each colony, this trait was assessed six times per day, during six days of mirabegron consumption (as it is explained in the subsection 'Meat and sugar water consumption, general activity'), and for each of these six days, a mean of 12 counts (6 counts x 2 colonies) was calculated. The ants continuing to be under mirabegron diet, at the tenth day, six counts were made in the course of 12 hours, i.e. every two hours and the means of these 12 counts (6 counts x 2 colonies) was calculated. The overall mean for each kind of diet, and period of activity is also reported (Table 5). The six means obtained at the tenth day of mirabegron consumption were compared to the six means obtained for ants consuming that drug during the days 1 to 6, as well as to the six means previously obtained for ants maintained under normal diet, by using the non-parametric Wilcoxon test.[29]

**Table 4** Impact of mirabegron on learning and memory

| Time (hours) | Under normal diet; score | Under a diet with mirabegron; correct vs wrong responses; score | | |
|---|---|---|---|---|
| 7h | 50% | colony A: 4 vs 6 | colony B: 6 vs 4 | score = 50% |
| 24h | 65% | colony A: 6 vs 4 | colony B: 6 vs 4 | score = 60% |
| 31h | 70% | colony A: 7 vs 3 | colony B: 6 vs 4 | score = 65% |
| 48h | 70% | colony A: 8 vs 2 | colony B: 7 vs 3 | score = 75% |
| 55h | 75% | colony A: 8 vs 2 | colony B: 7 vs 3 | score = 75% |
| 72h | 80% | colony A: 8 vs 2 | colony B: 8 vs 2 | score = 80% |
| cue removal | | | | |
| 7h | 70% | colony A: 8 vs 2 | colony B: 8 vs 2 | score = 80% |
| 24h | 70% | colony A: 8 vs 2 | colony B: 9 vs 1 | score = 85% |
| 31h | 70% | colony A: 8 vs 2 | colony B: 7 vs 3 | score = 75% |
| 48h | 70% | colony A: 7 vs 3 | colony B: 7 vs 3 | score = 70% |
| 55h | 70% | colony A: 8 vs 2 | colony B: 6 vs 4 | score = 70% |
| 72h | 70% | colony A: 7 vs 3 | colony B: 7 vs 3 | score = 70% |

Mirabegron did not impact the ants' conditioning ability or their memory. They acquired their conditioning a little more slowly and remembered better the cue than ants not consuming the drug. This may be due to their lower general activity while consuming mirabegron. Details on the method are given in the text and photos are shown in Figure 2.

**Table 5** Ants 'adaptation to the impact of mirabegron on their food consumption and general activity.

| Traits | 1 to 6 days under normal diet | 1 to 6 days on the drug diet | 10th day on the drug diet |
|---|---|---|---|
| meat intake | 1.50; 2.25; 1.25; 1.50; 2.75; 2.50　mean = 1.96 | 1.25; 2.75; 2.25; 2.00; 3.00; 3.50　mean = 2.46 | 2.75; 3.00; 3.00; 2.50; 2.25; 2.50　mean = 2.67 |
| sugar water intake | 2.00; 1.75; 2.25; 2.25; 3.75; 2.00　mean = 2.33 | 1.50; 1.50; 1.00; 1.50; 1.00; 1.25　mean = 1.29 | 0.50; 1.00; 1.00; 1.00; 0.75; 1.25　mean = 0.92 |
| general activity | 9.00;12.50;10.75;10.00;12.25;9.50　mean = 10.67 | 8.16; 7.75; 9.50; 9.25; 9.25; 8.00　mean = 8.65 | 6.50; 7.25; 8.50; 8.50; 8.50; 7.50　mean = 7.79 |

The ants did not adapt themselves to the impact of mirabegron on their food consumption and general activity. They went on eating more meat, drinking less sugar water, and being less active than while living under normal diet. For each diet, six means of 12 counts (6 counts x 2 colonies) are reported, obtained over six days (columns 2, 3) or over 12 hours (column 4). The overall means for each kind of diet, period and activity are also reported. Statistics and details can be found in the text.

## Habituation to wanted effects of mirabegron

Habituation to a drug or a situation occurs when an individual becomes less and less sensitive to the wanted effect of the drug or situation over time. To study habituation to a drug or a situation, a trait favorably influenced by them must be assessed one or two days after their use as well as after several days of use, and the results of these two assessments compared. In this work on mirabegron, we could not point out any improvement due to the drug. Therefore, habituation to mirabegron consumption could not be examined.

## Dependence on mirabegron consumption

Dependence on a drug or a situation occurs when an individual wants to continue using them, whatever the consequences, enjoys doing so, and cannot do without their use. The occurrence of dependence on mirabegron was studied after the ants consumed this drug during 11 days. To do so, 15 ants of each two colonies were transferred in an own tray (15 cm × 7 cm × 5 cm) which contained two cotton-plugged tubes (h = 2.5 cm, diam. = 0.5 cm), one filled with sugar water, the other filled with the sugar solution of mirabegron provided to the ants over the experimental work. The tube containing the drug was located on the right in the tray of one colony, and on the left in the tray of the other colony (Figure 2, I). During 15 minutes, the ants coming onto the entrance of each tube were counted, and the 15 corresponding counts obtained for each two colonies were added. These sums allowed calculating the proportions of ants having approached the drug and the drug-free solution. The results were analyzed by comparing the two obtained sums with those expected if the ants randomly visited the two tubes, by using the non-parametric $\chi^2$ goodness-of-fit test.[29]
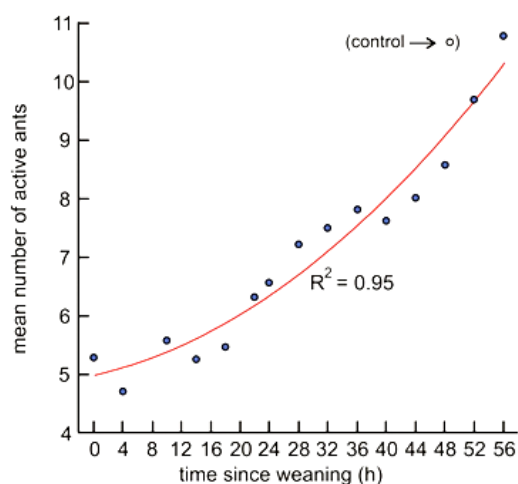
## Decrease of the effect after mirabegron consumption was stopped

This decrease was studied after the ants had consumed mirabegron during 14 days. The experimental protocol resembled that used in previous studies (e.g.[10,27,28]), although here, we assessed the ants' general activity, one of the few traits affected by the drug. The ants received a fresh solution of the drug 12 hours before weaning, and at the end of these 12 hours, their activity was assessed over 12 minutes, during 6 successive counting periods of 2 minutes. For both colonies and during each of these 2-minute periods, 6 counts were made of the number of active ants on their foraging area, at the entrance of their nest and in their nest. The mean of the 12 values thus obtained for each 2-minute period, as well as the mean of the 6 means so obtained during the 12 minutes were established. Just after this assessment done at t = 0h, weaning started: the sugared solution of the drug was replaced by the usual drug-free sugar water, and from that time, the ants' activity was assessed in the same way, every four hours until the mean of the 6 mean counts became similar to that observed for ants under normal diet. The results are given in Table 6 and illustrated in Figure 3.

**Table 6** Decrease of the effect of mirabegron on the ants' general activity after its consumption was stopped

| Time (h) | Six successive means each of 12 counts of active ants, and their mean | | | | | | | vs t = 0 | | statistics | vs control | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | N | T | P | N | T | P |
| t = 0 | 5.50 | 5.00 | 5.50 | 5.25 | 5.25 | 5.25 | 5.29 | | | | | | |
| 4 | 5.00 | 4.75 | 4.75 | 4.50 | 4.50 | 4.71 | 4.71 | 6 | -21 | 0.020 | 6 | -21 | 0.018 |
| 10 | 5.00 | 5.75 | 5.50 | 5.75 | 5.75 | 5.75 | 5.58 | 5 | -14 | 0.033 | 6 | -21 | 0.018 |
| 14 | 5.33 | 5.25 | 5.42 | 5.33 | 5.17 | 5.17 | 5.28 | 6 | -11.5 | 0.187 | 6 | -21 | 0.018 |
| 18 | 5.67 | 5.33 | 6.03 | 5.33 | 5.50 | 5.50 | 5.56 | 6 | +21 | 0.020 | 6 | -21 | 0.018 |
| 22 | 5.50 | 6.25 | 6.25 | 6.92 | 7.00 | 6.00 | 6.32 | 5 | +15 | 0.033 | 6 | -21 | 0.018 |
| 24 | 6.25 | 6.25 | 6.75 | 6.67 | 6.97 | 6.58 | 6.58 | 6 | +21 | 0.020 | 6 | -21 | 0.018 |
| 28 | 7.25 | 7.33 | 7.17 | 7.00 | 7.50 | 7.08 | 7.22 | 6 | +21 | 0.020 | 6 | -21 | 0.018 |
| 32 | 7.25 | 7.75 | 7.75 | 7.75 | 7.25 | 7.25 | 7.50 | 6 | +21 | 0.020 | 6 | -21 | 0.018 |
| 36 | 7.67 | 7.83 | 7.67 | 7.75 | 7.92 | 8.08 | 7.82 | 6 | +21 | 0.020 | 6 | -21 | 0.018 |
| 40 | 7.75 | 7.75 | 7.58 | 7.58 | 7.50 | 7.58 | 7.62 | 6 | +21 | 0.020 | 6 | -21 | 0.018 |
| 44 | 8.17 | 8.00 | 8.08 | 7.75 | 8.08 | 7.92 | 8.00 | 6 | +21 | 0.020 | 6 | -21 | 0.018 |
| 48 | 8.50 | 8.25 | 8.75 | 8.50 | 8.75 | 8.75 | 8.58 | 6 | +21 | 0.020 | 6 | -21 | 0.018 |
| 52 | 9.33 | 9.75 | 9.42 | 9.75 | 9.92 | 10.00 | 9.70 | 6 | +21 | 0.020 | 6 | -16 | 0.167 |
| 56 | 10.0 | 10.75 | 10.50 | 11.08 | 11.17 | 11.17 | 10.78 | 6 | +21 | 0.020 | 6 | +10.5 | >0.50 |
| control | 9.00 | 12.50 | 10.75 | 10.00 | 12.25 | 9.50 | 10.67 | | | | | | |

On the basis of the impact of mirabegron on the ants' general activity, the effect of this drug vanished in a total of about 56 hours. These results are illustrated in Figure 3. Benjamini-Hochberg adjusted P values.

**Figure 3** Decrease of the effect of mirabegron on the ants' general activity after its consumption was stopped. Although slightly accelerating over time, on the whole, the decrease was very slow. Few changes occurred during the first 18 hours after weaning, and the effect of the drug totally vanished in 56 hours. This slow loss of effect was in agreement with the absence of dependence on the drug consumption. Numerical and statistical results are given in Table 6.

The six means (each of 12 counts) obtained during each assessment made every four hours after weaning were compared to those obtained at t = 0 and to those obtained while ants were under normal diet by using the non-parametric Wilcoxon test.[29] We did so because comparing six pairs of mean values of a collective behavior is more appropriate than comparing 72 individual values which could not make up real pairs of individuals. The resulting P values were adjusted for multiple comparisons by using the Benjamini-Hochberg procedure[31] with a false discovery rate of 0.05. The function describing the regression of the mean number of active ants after weaning was established using Statistica® v.10 software.

## Results

### Meat and sugar water consumption, general activity

Mirabegron consumption affected these three physiological traits (Table 1). Ants consuming this drug eat somewhat more meat than ants living under normal diet, and this was statistically significant (N = 6, T = 20, P = 0.031). On the contrary, ants consuming mirabegron in sugar water drunk less than ants not consuming this drug, a difference also significant (N = 6, T = -21, P = 0.016). In the same way, and maybe due to their lower consumption of sugar, when living under mirabegron diet, the ants were somewhat less active than while living under normal diet, a significant change (N = 6, T = -21, P = 0.016). We checked if this lower activity persisted over the drug consumption by assessing again the ants' activity after they had consumed mirabegron during nine days (see the subsection relative to adaptation to side effects of mirabegron).

### Linear and angular speeds

These traits were not significantly affected by mirabegron consumption (Table 2, lines 1, 2). Ants consuming this drug walked at a linear speed slightly lower than that of ants living under normal diet, but this difference was not significant ($\chi^2$ = 5.71, df = 2, 0.50 < P < 0.10). The angular speed (sinuosity) of ants consuming mirabegron was similar to that of ants maintained under normal diet ($\chi^2$ = 0.24, df = 2, 0.80 < P < 0.90). These results are in agreement with those

previously obtained on the ants' general activity (see the here above subsection).

### Orientation

As it was obvious to the observer, mirabegron did not affect the ants' orientation to a tied nestmate. This was confirmed by the numerical results (Table 2, line 3; Figure 2, A) and their statistical analysis, as no significant difference existed between the orientation of ants maintained under one or the other kinds of diet ($\chi^2$ = 0.22, df = 1, 0.50 < P < 0.70). This allowed presuming that mirabegron did not affect the ants' olfactory perception since they duly perceived the alarm pheromone emitted by a tied nestmate.

### Audacity

This trait was not impacted by mirabegron consumption (Table 2, line 4; Figure 2, B). The ants consuming the drug went onto the risky apparatus with the same audacity than those not consuming the drug. There was no statistical difference between the numbers of ants under one and the other kinds of diet counted on the apparatus (N = 4, T = +3.5, -6.5, P = 0.375). Even if ants consuming mirabegron were somewhat less active, walked at a slightly lower speed, they still presented the same audacity and curiosity.

### Tactile (pain) perception

This trait was not affected by mirabegron consumption (Table 2, the two last lines; Figure 2, C). Ants consuming this drug walked on a rough substrate at a small linear speed and a large angular speed, similar to those of ants living under normal diet. This was obvious to observers and confirmed by statistical analysis: linear speed: $\chi^2$ = 2.06, df = 2, 0.30 < P < 0.50; angular speed: $\chi^2$ = 0.25, df = 2, 0.80 < P < 0.90. There was thus no difference between the ants maintained under one and the other kinds of diet as for their tactile (and probably pain) perception, a result in agreement with that obtained for the ants' orientation ability (see the above subsection relative to orientation).

### Brood caring

Mirabegron diet did not affect the ants' brood caring behavior (Table 3, line 1; Figure 2, D). Ants under normal diet very quickly took and re-entered the larvae removed from their nest. Ants consuming mirabegron did so with some delay or some slowness, but finally re-entered in time all the larvae removed from the nest. The difference over time between ants under the two kinds of diet, probably due to the lower activity of those consuming mirabegron, was on the whole not significant (N = 5, T = 10, P = 0.313).

### Social relationships

Mirabegron did not at all affect the ants' social relationships (Table 3, line 2, Figure 2, E). During the experimental dyadic encounters as well as when being in their colony, the ants consuming the drug behaved as those not consuming it. They essentially stayed near nestmates, doing nothing or entering in contact by their antennae, and seldom slightly opened their mandibles. There was no statistical difference of behavior between ants living under the one or the other kind of diet ($\chi^2$ = 0.46, df = 2, P ~ 0.80). The variable 'a' assessing the ants' aggressiveness equaled 0.12 for ants under each two kinds of diet. This result was in agreement with that concerning the ants' brood caring (see the here above subsection).

### Cognition

This trait was *a priori* not affected by mirabegron consumption (Table 3, line3; Figure 2, G). Ants under normal as well as under

the drug diet moved into the twists and turns path and tried to cross it, but those consuming the drug did so more slowly that those not consuming it. This was obvious to the observer and was pointed out by the numerical results, e.g. only the last recorded numbers differed, revealing some slowness in the travelling of ants under the drug diet. The difference between the ants under one and the other kind of diet was on the whole not significant: n° of ants in front of the difficult path: N = 5, T = 8, P = 0.50; n° of ants beyond this path: N = 3, T = 6, P = 0.125. However, the observed small, and not significant, difference may be due to some slight difference in cognitive ability between the ants under the two kinds of diet, a presumption checked in the two following experiments.

## Escaping behavior

This trait was very slightly affected by mirabegron consumption (Table 3, last line, Figure 2, F). Enclosed ants under normal diet walked firstly erratically all around the enclosure, then, after a few seconds, more calmly, essentially along the rim of the enclosure. They so found the exit and went out. When consuming mirabegron, the ants similarly behaved but they were simply a little less active and went out the enclosure with some delay comparatively with ants under normal diet. The difference of behavior between the ants under one and the other kinds of diet was just under the level of significance (N = 4, T = -10, P = 0.063). This probably resulted from the somewhat lower activity of ants consuming the drug (as we pointed out for the ants' general activity: see the subsection relative to this trait), but might also reveal some slight decrease in cognitive ability, a presumption examined in the following experiment.

## Conditioning ability, memory

The ants' conditioning ability was not impacted by their consumption of mirabegron (Table 4, Figure 2, H). Even if ants consuming the drug acquired their conditioning a little more slowly than those not consuming it (during the first 31 hours of training, their scores were somewhat lower than those of ants under normal diet), the ants consuming mirabegron finally reached a conditioning score similar to that of ants not consuming this drug. There was no statistical difference between the scores presented by ants under the one or the other kinds of diet (N = 3, T = -4, P = 0.375). Concerning the ants' memory (i.e. the scores they presented after removal of the cue), the ants consuming the drug presented a score of 80%, 85%, and 75%, 7, 24, and 31 hours after the cue removal while ants not consuming it presented a score of 70%, 70% and 70% after the same time periods. The ants' memory was thus stronger under mirabegron diet. This could be explained by the longer time required by ants consuming the drug for acquiring their conditioning (what is learned quickly is quickly forgotten, and what is learned slowly is slowly forgotten),[32] as well as by the lower activity of ants under mirabegron diet (the more the ants are active, the less good is their conditioning acquisition).[33] Finally, 48, 55 and 72 hours after the cue removal, the conditioning scores of ants under mirabegron diet became identical to those of ants under normal diet (70%). Consequently, in the course of our experiment, there was no statistical difference as for middle and long term memory between ants under normal and under mirabegron diets (N = 3, T = 6, P = 0.125).

## Adaptation to the impact of mirabegron on the ants' food consumption and general activity

The ants did not significantly adapt themselves to the impact of mirabegron on their food consumption and general activity, at least after 9 days on the drug diet (Table 5). After that time period, they

still eat more meat than when living under normal diet, but this was at the limit of significance (mean numbers of ants eating meat: 2.67 *vs* 1.96: N = 5, T = 14, P = 0.063). However, after 9 days of mirabegron consumption, the ants did not eat more meat than during their first 6 days of drug consumption (mean numbers of ants eating meat: 2.67 *vs* 2.46: N = 6, T = 16, P = 0.156). At their 10th day on mirabegron diet, the ants still drunk less sugar water than when living under normal diet and this was statistically significant (mean numbers of ants drinking sugar water: 0.92 *vs* 2.33: N = 6, T = 21, P = 0.016). However, their sugar water consumption was then slightly lower than that they presented during their first 6 days on that drug diet, albeit this was at the limit of significance (mean numbers of ants drinking sugar water: 0.92 *vs* 1.29: N = 4, T = 10, P = 0.063). At their 10th day on mirabegron diet, the ants were still significantly less active than while living under normal diet (mean numbers of active ants: 7.79 *vs* 10.67: N = 6, T = -21, P = 0.016). Moreover at their 10th day, the ants were somewhat, although significantly, less active than during their first 6 days on that drug diet (mean numbers of active ants: 7.79 *vs* 8.65: N = 6, T = -21, P = 0.016).

To summarize, under mirabegron diet, the ants eat more meat than usually and this impact did not significantly increase over the drug consumption, they drunk less sugar water than usually and this impact only slightly increased over the drug consumption, and they became less active than usually, this last impact appearing to increase over the drug consumption. Consequently, the ants did not adapt themselves to the impact of mirabegron on their food consumption and general activity. On the basis of these results, we chose to study the decrease of the effect of mirabegron by assessing the ants' general activity over time after weaning (see the subsection relative to that decrease).

## Habituation to wanted effects of mirabegron

Having not found any favorable effect of mirabegron on the ants' physiology and behavior, we could not examine this potential occurrence.

## Dependence on mirabegron consumption

Ants did not become dependent on mirabegron consumption. During the experiment, 47 ants of colony A went onto the entrance of the tube containing the drug-free solution and 3 ones went onto that of the tube containing the drug solution. At the same time, 3 ants of colony B visited the drug solution while 29 ones visited the drug-free solution. In total, 76 ants chose the solution free of drug and only 6 ants chose the drug solution. This corresponded to 92.68% of ants choosing the solution free of drug and 7.32% of ants choosing the drug solution. This result statistically differed from what could be expected if ants randomly chose the two kinds of solution ($\chi^2$ = 34.48, df = 1, P < 0.001). Consequently, as the ants largely preferred avoiding consuming mirabegron, they developed no dependence at all on that drug, on the contrary.

## Decrease of the effect after mirabegron consumption was stopped

Until 18 hours after weaning, the ants' general activity was low, with a mean number of active ants under the six. Fourteen hours after weaning, the recorded numbers were even similar to that measured at weaning (*ca* 5.30 ants; P = 0.187). After that, the number of active ants went above six until 52 h after weaning, at which time it became above nine. From then, the number of recorded active ants became similar to that observed for a diet without mirabegron (i.e. the control: *ca* 10.70 ants; P > 0.17). On the whole, after weaning, the decrease of the effect of mirabegron on the ant's general activity was better

described by a second order curve (y = 4.916 + 0.087x + 0.018x$^2$, with R$^2$ = 0.95) than by a linear function (R$^2$ = 0.91). It means that the decay of the effect of the drug, although slow, slightly accelerated from its start to its end. The fact that this acceleration was slight was confirmed by the absence of statistical significance between every two successive counts made over the loss of the effect of mirabegron (this result is not in a table).

## Discussion

We used the ant *Myrmica sabuleti* as a biological model to assess the side effects of mirabegron, a beta3-adrenergic agonist allowing reducing OAB symptoms, in the same way that we assessed the side effects of oxybutynin, an anticholinergic used for the same purpose.[10] Contrary to oxybutynin, mirabegron had nearly no adverse effects. The ants' behavior under mirabegron diet was the same as that under a diet without this drug, with the only exception that the ants eat less meat (the same effect was observed under oxybutynin diet), drunk more (the contrary was observed under oxybutynin diet) and were less active (on the contrary, an enhanced activity was observed under oxybutynin diet). There was no adaptation to this decrease in general activity which even seemed to somewhat develop over time.

Contrary to mirabegron, oxybutynin had far more adverse effects on the ants' health: it decreased their orientation ability, tactile perception, cognition and memory, impacted their social relationships and induced restlessness and stress.[10] Moreover, consuming oxybutynin induced dependence on this drug, what was not the case for mirabegron. This can be explained by the fact that the action of oxybutynin after weaning vanished in only 10 hours (terminal elimination half-life in humans: 2 to 13 h, according to the kind of release formulation),[34,35] while that of mirabegron needed 56 hours to disappear (elimination half-life in humans: 50 h),[11] such a slow decrease of effects not entailing dependency. An impact like that of mirabegron on the ants' food consumption and general activity has not been reported in humans consuming this drug. The only effects on human health that could have any relation with those observed on ants are digestive disturbances (indigestion and gastritis) and fatigue, but these effects are among the less severe ones observed in humans.[11]

Used by humans to care OAB symptoms, mirabegron has many advantages over antimuscarinic drugs. Although in comparison to other antimuscarinic drugs oxybutynin produces a high benefit against OAB symptoms,[5] tested on rats, the advantage of a mirabegron treatment over oxybutynin is that it diminishes more the bladder pressure and the number of microcontractions[8] and increases significantly the interval length between the rhythmic bladder contractions.[36] A comparison of peer-reviewed articles on the effectiveness of mirabegron and of six antimuscarinics used for OAB treatment (oxybutynin, tolterodine, darifenacin, fesoterodine, solifenacin and trospium chloride) showed that mirabegron scored the best after solifenacin and trospium as for the decrease of the number of daily micturitions. Solifenacin, followed by mirabegron, was the most effective concerning the reduction of the number of episodes of incontinence. Solifenacin was also the most effective concerning the number of episodes of urgency incontinence, followed by oxybutynin, fesoterodine and trospium. Mirabegron scored the best as for dry mouth and constipation occurrence.[9] In a randomized, double-blind study of patients aged over 18 years, dissatisfied with their previous antimuscarinic-based treatment, mirabegron was found having no less efficiency and safety than solifenacin, and this with a lower dry mouth incidence.[37]

While no less efficient against OAB than tolterodine, mirabegron led to six times less dry mouth occurrences.[38] Mirabegron presented no more evidence of increased cardiovascular risk than oxybutynin,[39] solifenacin, tolterodine and placebo.[40] In comparison to placebo, it showed to be an efficient drug for OAB elderly patients, the prevalent group suffering from OAB and urgency incontinence, with on the whole no more adverse events than placebo (the incidence on dry mouth and constipation was not investigated).[41] Although improving OAB symptoms with moreover less adverse effects than antimuscarinics, especially without the Central Nervous System and cognitive impairments typical to the anticholinergic drugs, mirabegron is not without side effects. The more noticeable are elevated blood pressure in patients with preexisting hypertension (> 10% of the reported adverse effects), dry mouth (3-9%), urinary tract infection (3-4%), and nasopharyngitis (4%). Headache (2-3%), dizziness (2%), constipation (1.6%), diarrhea (ca 1.5%) and fatigue (ca 1.5%) are among ten other disadvantages with a frequency of at least 1%.[10] However, mirabegron reduces better dry mouth occurrences and constipation inconvenience than the antimuscarinic drugs, the worst drug as for such side effects being oxybutynin.[9] Finally, in the current state of our knowledge, mirabegron appears to be a valuable substitute to anticholinergic drugs, and even to be preferable to the latter kind.

In a clinical trial,[42] the main cause of discontinuing a mirabegron OAB treatment was a lack of efficiency (26% of withdrawals), before that of side effects (10%), while patients who previously used anticholinergics, thus non-naive patients, were significantly more likely to continue mirabegron treatment. A survival analysis has shown that a treatment of OAB with mirabegron lasts during a longer time than a treatment with any of 8 antimuscarinic drugs, including oxybutynin, with less withdrawals due to side effects.[43] Another literature review based on routine clinical practice also concluded to a greater persistence of the treatment for mirabegron compared to antimuscarinics.[44]

For unknown reasons, some patients do not respond to antimuscarinics for OAB treatment and for others the addition of mirabegron leads to an improvement.[45] Treating elderly patients for severe symptoms of OAB with a combination of mirabegron and the antimuscarinic solifenacin appeared to be more efficient than the use of the one or the other drug, but with the side effects of both drugs (dry mouth, high blood pressure, increased heart rate).[46] A randomized, double-blind trial showed that mirabegron and solifenacin in a combined therapy provided consistent improvements in the most relevant OAB symptoms, urgency and urinary incontinence, compared to the respective monotherapies and this as early as by the fourth week of this treatment.[47] A meta-analysis of 64 studies showed that compared to mirabegron used in monotherapy, adding mirabegron to solifenacin improved the number of micturition episodes and of urgency urinary incontinence episodes, lowered the number of zero incontinence episodes, but as an add-up of the adverse effects of the two substances, increased dry mouth and constipation as well as blurred vision although reducing hypertension. Mirabegron alone or in combination with solifenacin on the whole scored better than monotherapy treatments with the antimuscarinics darifenacin, fesoterodine, imidafenacin, oxybutynin, propiverine, solifenacin, tolterodine and trospium chloride.[48] Another meta-analysis of literature based on 51 studies comes to the conclusion that solifenacin reduces incontinence over mirabegron, and that combined they are more effective than monotherapy. The side effects of their combination remain those of the substances taken alone.[49] A same conclusion follows from an analysis of 5 different trials on adults 65year old and over, which shows that mirabegron compared to placebo is efficient to reduce OAB symptoms and more efficient when add to solifenacin, the adverse effects of this combination being those of each substance, thus no higher than with monotherapy.[50] It

is to be noted that combining the usual solifenacin dose (5 mg) with the highest dose of mirabegron (50 mg instead of 25 mg), although superior in efficacy to solifenacin alone, did not demonstrate superior efficacy to mirabegron alone.[45]

An experimental study on rats showed that mirabegron plus fesoterodine had a combined effect on the increase of the bladder capacity exceeding the sum of the effects of the two monotherapies.[51] Similarly, mirabegron used as an add-up therapy to tamsulosin, an alpha blocker, improved OAB symptoms and was well-tolerated.[52,53] In a trial with 50 patients, mirabegron in combination with oxybutynin also had a good effect on lower urinary tract symptoms such as prostate symptom and on quality of life.[54]

Returning to ants, the most obvious adverse effect of mirabegron was a decrease in general activity. As said here above, no severe impact on food consumption and general activity has yet been reported in humans under mirabegron treatment. There is also no difference in locomotor activity in rats placed on a mirabegron diet after being induced into depression following corticosterone treatment.[55] However, if there were to be a decrease in general activity in humans, it would have unfortunate consequences in their daily lives, especially since practitioners recommend consuming mirabegron each day, without interruption, while its elimination half-life lasts 50 hours.[11] In the tested ants, the effect lasts about 50 hours. The possibility of an activity impairment cannot therefore be ruled out. In the event of a serious decrease in general activity in patients treated with mirabegron, we suggest to restore the normal level of activity by spacing out this medication (for example, by taking it every third day) or by adding an appropriate stimulant. Mirabegron may also be alternated with an anticholinergic medication if the latter does not affect the patient's activity level and does not lead to severe side effects. Let us recall that a combination of mirabegron and an anticholinergic, but in simultaneous use, has already been shown to be effective in the OAB treatment (see above). The choice of the anticholinergic should depend on its efficacy and side effects, and on the similarity of its elimination time with that of mirabegron. In ants for example, oxybutynin increases the general activity while mirabegron decreases it, but the former drug has severe adverse effects and its action lasts only 10 hours while that of mirabegron lasts about 50 hours. In humans, delivered as an immediate-effect medication, the half-life of oxybutynin lasts only 2 to 3 hours while that of mirabegron lasts 50 hours.[34,35]

According to the literature, the combination of solifenacin, an antimuscarinic with a high selectivity for the bladder, and of mirabegron appears to be an effective therapy, with no more side effects than either of these substances. In addition, the elimination half-life of these two drugs is similar (50 hours for mirabegron[11] and 45-68 hours for solifenacin).[34,35] Patients treated with mirabegron should also be checked for their habituation to the wanted effect of this drug. If habituation occurs, the treatment would become less and less effective over time and the patient would tend to increase the daily amount of consumed drug. Habituation could not be investigated in the present study nor in that of oxybutynin,[10] as no favorable effect was found on ants.

## Conclusion

Contrary to anticholinergic drugs such as oxybutynin, which increases the ants' activity, leading them to restlessness and among other unwanted effects, impairing their memory, mirabegron has very little adverse effects on the ants, the only noticeable ones being a decrease of their general activity and of their sugar water consumption. Adaptation to the decrease of the ants' general activity did not occur. Potential habituation could not be studied on the ants and has still to be examined in humans. In humans, a decrease of activity along a mirabegron treatment may possibly occur as tiredness has been reported. We thus recommend paying attention to this trait. In the event of a serious decrease in general activity in humans treated with mirabegron, we suggest to restore the normal level of activity by spacing out this medication, or by adding an appropriate stimulant. The use of mirabegron may also be alternated with that of an anticholinergic which does not affect the patient's activity level and does not have severe side effects. The latter drug, the dosage and timing of the two substances, should be chosen according to the efficacy and side effects of their combination, as well as on the similarity of their elimination time.

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