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Research Article

Some Physiological and Ethological Effects of Aluminum Hydroxide: A Study Using Ants as Models

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Abstract

Aluminum is largely present on the earth but has no biological function. Aluminum hydroxide is nowadays used in drugs for caring of persons suffering from stomach acidity, as an adjuvant in vaccines, in cosmetics and other products of common use, but has been suspected to have severe adverse effects. We thus examined its impact on 22 physiological and ethological traits, by using ants as models. We found that aluminum hydroxide decreased their meat consumption, general activity, linear speed, orientation ability, trail following, audacity, tactile perception, cognition, escaping ability and conditioning capability, thus their memory. It increased their sinuosity of movement and impacted their locomotion and movement coordination. It did not affect their relationship with their brood, nestmates and aliens. Ants did not adapt themselves to the adverse effects of aluminum hydroxide and developed no dependence on this compound. After weaning, the effects of aluminum hydroxide decreased linearly and fully vanished in a total of 12 hours. On basis of these results and of literature reports on motor and cognitive impairments in animals and humans we recommend limiting the use of aluminum hydroxide as much as possible, and to search for natural alternatives.

Keywords: Cognition; Locomotion; Memory; Straightforwardness; Tactile Perception

Abbreviations

Al: Aluminum; ang.deg.: Angular Degrees; ang. deg./cm: Angular Degrees per cm; mm/s: Millimeter Per Second; χ^2: Chi Square; M-W: Mann-Whitney Test; vs: Versus; n°: Number; cm: Centimeter; mm: Millimeter; ml: Milliliter; µl: Micro Liter; g: Gram; mg: Milligram; s: Second; min: Minute; h: Hours; t: Time; %: Percentage

Introduction

Aluminum is the third most abundant element in the lithosphere [1], while having no known beneficial role in any biological systems and being normally not present in them [2]. When present in the body, aluminum is essentially toxic [3]. However, aluminum salts, oxides, hydroxide, sucralfate, and aminoacetate are largely used by humans, and can be present in treated tap water, in numerous food additives with diverse usages [4], as metal leakage from cookware and food packaging, in pharmaceuticals and in cosmetics (deodorants and anti-perspirants). Moreover, aluminum hydroxide, aluminum phosphate and aluminum potassium sulfate are used as vaccine adjuvants, these vaccines being directly injected into muscles [2]. Humans are thus largely exposed to aluminum contamination. The ascertained, suspected or debated adverse effects of aluminum intake are numerous, among them, bone disease (osteomalacia), breast cancer, developmental retardation, cognitive and motor impairments as well as autism and diverse neurodegenerative disorders [5-8].

Using ants as biological models, we previously studied the effects of aluminum foil leaching into the sugar water given as food to ants and showed that this metal increased their general activity, sugar water consumption, sinuosity of locomotion, and decreased their meat consumption, audacity, tactile perception, cognition, ability in escaping from an enclosure, as well as their ability in acquiring conditioning, affecting thus their short and middle term memory. To summarize, aluminum (Al) essentially adversely affected the locomotion of the ants and the functioning of their nervous system [9].

Aluminum hydroxide, $Al(OH)_3$, is largely used as an efficient adjuvant of intramuscular injected Tetanus, Diphtheria, Pertussis, Hepatitis A and B and Anthrax vaccines, because it increases the immunological reaction towards the antigen adsorbed on its surface. It is designed to provide a long-lasting cellular exposure [7], but may lead to two health problems. It may induce an inflammatory response even in absence of antigen, and it can make its way into the central nervous system and impact it [8]. Injected subcutaneously in mice, aluminum hydroxide is reported to decrease their linear speed, to increase their angular speed, to decrease their muscular strength and endurance and to impair their learning and spatial memory as well as to promote motor neuron death [10,11]. Moreover, intramuscular injection of this adjuvant in humans can lead to diffuse arthromyalgia, chronic fatigue and cognitive dysfunction, suggesting demyelination [12]. Cognitive deficits not correlated with pain, fatigue or depression in patients have also been observed, as well as deficiency in visual and verbal memory, attention, working memory and planning [13]. The injection site may be characterized by muscular infiltration and abnormal long-term persistence of Al hydroxide-loaded macrophages what forms a granulomatous lesion called macrophagic myofasciitis [12,14-17].

Aluminum hydroxide is also used for caring of humans. Numerous oral pharmaceuticals containing aluminum hydroxide as an active ingredient act as gastric antacids, buffered analgesics or phosphate binders against hyperphosphatemia [18,19]. These drugs can be bought in any drugstore without a medical prescription.

The most common way of exposure to aluminum (Al) is the gastrointestinal tract (0.2% absorption rate: [20]). Oral ingestion of antacids is known to produce osteomalacia by phosphate depletion in growing infants [21]. Moreover, by entering the vascular system, Al salts are bound to transferrin, and ca 0.005% of these aluminum-protein complexes enter the brain [20], the degree of incidence of neurological disease being proportional to the Al intake [22]. However, the uptake of Al from food can vary 10-fold depending on its chemical form [20]. Initially, oral administration of Al hydroxide has been stated to not impair neuropsychological functions in patients. This statement was based on tests examining general functions (intelligence, reasoning, memory) and specific abilities (visual memory, verbal and reading fluency, manual dexterity) [23]. However, there is a growing evidence for a link between neurodegenerative diseases, such as Alzheimer's disease or Parkinson disease, and the amount of ingested Al via drinking water and antacids [22].

We here aimed to examine, on ants as models, the physiological and ethological effects of ingested Al hydroxide, in the same manner we previously examined the effect of aluminum foil [9]. Here below, we explain why we use ants as models, which species was used in the present paper, what we know on it, and which traits we intended to examine.

Why ants can be used as models

Physiological mechanisms, nervous system functioning, muscle functioning, genetics, some kinds of behavior, and nearly all the vital functions are similar for all animals, including humans [24,25]. It is the reason why most biological processes have been and are still studied on suitable animals used as models such as monkeys, rats, mice, parrots, as well as bees, fruit flies, crickets, cockroaches, mealworms and some other invertebrates [26]. Invertebrates are preferentially used when it is possible due to their small size, rapid development, and easy maintenance, in large samples, in a laboratory [27]. Insects are often used, above all the hymenoptera such as bees [28]. Ants too can be used. They are highly evolved social insects. They have numerous glands producing efficient pheromones [29]. Their colonies present labor division, polyethism and social regulation [30,31]. They build complex nests, take care of their brood, differently mark the different parts of their habitat [30], and communicate thanks to informative chemical and tactile signals [31]. They navigate using memorized cues, adequately recruit nestmates, clean their nest and even manage cemeteries [30,31]. All these characters and abilities allow using them to examine the impact of situations, products and environmental elements [32]. Moreover, ant colonies, containing large numbers of individuals, can be maintained during many years, at low cost, in ordinary rooms.

Which species we used

Our knowledge of the biology of ants is largely based on our study of ants belonging to the genus *Myrmica*. We made research on their ecology, eyes, angle of vision, visual perception, recruitment, navigation, learning [33], as well as on the ontogenesis of some of their abilities [34]. Studying the effect of manmade electromagnetism on their learning ability and their response to pheromones showed that they can be good biological models [35,36]. They were effectively good models when we examined, on them, the effects of alkaloids, nicotine, morphine and quinine, buprenorphine and methadone, fluoxetine, anafranil and efexor [32], paroxetine [37], carbamazepine [38], alprazolam, sweeteners, food complements and analgesics [39,40]. Each time, the effects presented by humans were observed, novel information was brought on them, and other effects from which humans may suffer were revealed. In the present work, as in the previous submitted one, we used *M. sabuleti* Meinert 1861 for studying the potential harmful impacts of aluminum hydroxide.

Which traits we examined

We aimed to examine the effect of aluminum hydroxide on 18 physiological and/or ethological traits, first on ants under normal diet, then on the same ants consuming aluminum hydroxide, and thereafter, on 4 more traits only on ants consuming or having consumed aluminum hydroxide. The 18 traits were: meat consumption, sugar water consumption, general activity, linear speed, angular speed, orientation towards an alarm signal, trail following, tendency in moving on an unknown apparatus, tactile ("pain") perception, caring of brood, cognitive ability, aggressive behavior against nestmates, aggressiveness against aliens, ability in escaping from an enclosure, visual conditioning ability, visual memory, olfactory conditioning ability, and olfactory memory. The four other traits were: adaptation to the adverse effects of aluminum hydroxide, habituation to its beneficial effects, dependence on its use, and the vanishing of its effects after its consumption was stopped.

Adaptation exists when the adverse effects decrease over time. Habituation occurs when the beneficial effects decrease over time. Dependence appears when individuals consuming the product prefer food containing it than food free of it.

Material and Methods

Most of the material and the methods are similar to those we previously employed and are thus only briefly related here, inviting readers to find details in, among others, [39-41].

Collection and maintenance of ants

All the experiments were made on two colonies of *M. sabuleti* collected in the Aise Valley (Ardenne, Belgium) in June 2016, and on a third colony, collected the same day on the same site, which furnished aliens and some controls. Each colony was kept in glass tubes half filled with water, these nest tubes being deposited in a tray (34 cm x 23 cm x 4 cm) [39-41]. The ants were fed with an aqueous solution of sugar given *ad libitum* in small cotton plugged tubes and with cut *Tenebrio molitor* larvae (Linnaeus, 1758) given three times per week. Laboratory parameters were optimum for the species [40,41]. The ants are here often named 'nestmates' as commonly do researchers on social insects.

Aluminum hydroxide given to the ants in their sugar water

Aluminum hydroxide is scarcely soluble in water at pH 7 (its less solubility occurring at pH 6). Being amphoteric, aluminum solubility is substantial at a slightly lower or higher pH [42]. We thus made a solution of 0.6 g of baking soda ($NaHCO_3$) into 250 ml tap water, the pH of which equaled 8.0. This value was assessed using a water test (sera GmbH, Heinsberg, Germany). Higher values of pH would have increased the solubility of aluminum hydroxide but may have affected the ants' health and the aluminum hydroxide solution consumption. This aqueous solution of baking soda was then sugared exactly as we sugared the water given *ad libitum* to the ants (see previous paragraph). Aluminum hydroxide was furnished by the manufacturer 'Alumine Durmax SARL' (Cenon sur Vienne, France). The product had the following characteristics: SG-6, particles size: 0.3 microns, Na_2O: 0.5%, Fe_2O_3: 0.03%, SiO_2: 0.02%; density = 240 g/liter. Humans obliged to consume aluminum hydroxide for caring of themselves (e.g. from an excess of digestive acidity) are advised to ingest about 4.8 g of aluminum hydroxide per day, whatever the kind of drug they use. Humans drink about one liter of water per day. While consuming aluminum hydroxide, they thus ingest 4.8 g of that compound together with one liter of water. Insects and so ants consume proportionally about ten times less water than mammals. Consequently, for setting ants under an aluminum hydroxide diet similar to that of humans using this product, we must provide the ants with a solution of 4.8 g of aluminum hydroxide into 100 ml of water, or more exactly of sugar water containing some baking soda for having a pH value slightly higher than 7. This sugar + baking soda + aluminum hydroxide aqueous solution constituted a stock solution, kept at – 25°C when not used. The solution was delivered to the ants in their usual feeder tubes (volume = 5 ml). Immediately after these tubes deposit on the ants' foraging area, a few workers already came drinking the provided liquid. Thereafter, we checked each day if ants effectively went on drinking the provided solution, and they did. The cotton plug shutting the tubes was refreshed every 2 - 3 days, and the entire solution was renewed every 7 days. The test experiments started after the ants had consumed aluminum hydroxide during one day.

Let us recall that the first following 18 traits were assessed once while the ants were under a sugar diet, and then while they consumed aluminum hydroxide, and that the last 4 traits were assessed only on ants consuming or having consumed that product.

Sugar water and meat consumption, general activity

During six days, the ants eating the *T. molitor* larvae, drinking the sugar water, and being active anywhere in their habitat were counted six times per day for colony A as well as for colony B, what provided 12 counted numbers of ants for those eating meat, those drinking sugar water and those being active. These counting were made at the same times o'clock each day (Table 1, Daily counts), as done for previous works [40,41]. The mean of these daily counts were established (Table 1, Daily means) and the six daily means obtained for ants consuming sugar water containing Al hydroxide were compared to those obtained for ants under normal diet using the non-parametric Wilcoxon test [43]. The mean of the daily means were also calculated (Table 1, Average of daily means).

Speeds of locomotion and orientation ability

As previously [40,41], the experiments were made on ants freely moving in their tray. The linear and angular speeds were quantified giving no stimulus to the ants; the orientation to an alarm signal was quantified presenting them with a nestmate tied to a piece of white paper (Figure 1B). Such a tied worker emits its alarm pheromone produced by the mandibular glands. As previously [40,41], for each variable, the trajectory of 20 ants of each colony (n = 20 ants x 2 colonies = 40 trajectories) was recorded on glass and then copied on a transparent foil which remained affixed to a PC monitor screen due to its static electricity charge. The recorded trajectories were analyzed using specifically designed software [44]. The use of this software is explained in [44], and summarized in previous works [40,41]. The linear speed (in mm/s) is the length of a trajectory divided by the time spent for travelling it. The angular speed (in ang. deg./cm) is the sum of the angles, measured at several successive points of the trajectory, between the segment 'point i to point i – 1' and the following segment 'point i to point i + 1', divided by the length of the trajectory. The orientation to a given point (in ang. deg.) is the sum of the angles, measured at several successive points of the trajectory, between the segment 'point i of the trajectory - given point' and each segment 'point i - point i + 1', divided by the number of measured angles. If the obtained value is lower than 90°, the animal has a tendency to orient itself towards the point; if it is larger than 90°, the animal has a tendency to avoid the given point. Each distribution of 40 values was characterized by its median and quartiles (Table 2, lines 1, 2, 3) and the values obtained for ants consuming Al hydroxide were compared to those obtained for ants under normal diet, using the non-parametric Mann-Whitney test [43].

Trail following

The method is described in many previous works [40,41]. The trail pheromone of *Myrmica* ants is produced by the workers' poison gland. A solution of 10 of these glands in 500 µl of hexane was made and set for 15 min at -25°C. To make an experiment, 50 µl of the solution was deposited, thanks to a metallic normograph pen, on a circle (radius = 5 cm) pencil drawn on white paper and divided into 10 angular degrees arcs. One minute later, this artificial trail was deposited in the ants' tray, and the response of 20 ants of each colony to that trail was assessed by the number of arcs of 10 angular degrees the ants walked along the trail without departing from it (Figure 1C). The distribution of the 40 values was characterized by its median and quartiles (Table 2, line 4), and the distribution obtained for ants consuming Al hydroxide was compared to that obtained for ants under normal diet using the non-parametric χ^2 test [43].

Audacity

The method has already been used in several previous works [40,41]. A tower standing on a platform, both being made of strong white paper (Steinbach®, height = 4 cm; diameter = 1.5 cm), was set in the ants' tray, and the ants present at any place of this unknown apparatus were counted 12 times over 12 min (Figure 1D). The mean and the extremes of the obtained values were established (Table 2, line 5). The values obtained for the two colonies as well as those obtained during each successive time period of two minutes were pulled, as done in [40,41], and these values for ants under Al hydroxide and normal diet were compared using the non-parametric Wilcoxon test [43].

Tactile ('pain') perception

An ingested product may adversely impact the central and the sensitive nervous system. This is why we assessed the ants' locomotion on a rough substrate (and later on several cognitive abilities), doing so after the ants consumed Al hydroxide for 5 days. Ants correctly perceiving the uncomfortable character of the substrate walk with difficulties, slowly, sinuously, while ants poorly perceiving such a character walk more confidently, more quickly and less sinuously. As previously [40,41], a folded piece (length: 3 cm, width: 2 + 7 + 2 = 11 cm) of rough emery n° 280 paper was tied to the bottom and the borders of a tray (15 cm x 7 cm x 4.5 cm), the tray becoming so divided into a small zone 3 cm long, a zone 3 cm long where the ants' moving was difficult (Figure 1E), and a 9 cm long smooth zone. Each colony had its own apparatus (3 + 3 + 9 cm = 15 cm, the length of the tray). For each of them, 12 ants were deposited, at a time, in the small zone. When moving away from that zone, the ants walked for a time on the rough paper. During that time, their speed of locomotion and their sinuosity were assessed (n = 12 trajectories x 2 colonies = 24; Table 2, line 6). The values obtained for ants consuming Al hydroxide were compared to those obtained for ants under normal diet using the non-parametric Mann-Whitney test [43].

Brood caring

A few larvae were removed from the inside of the nest of the two used colonies and deposited in front of the nest entrance. For each colony, five of these larvae were observed, as well as the ants' behavior in front of a larva (Figure 2F). The larvae among the five observed ones of each colony still remaining out of the nest after 5 seconds, 2, 4, 6, 8, and 10 minutes were counted, the numbers recorded for each colony being added (Table 3, line 1). The six numbers obtained for ants under normal diet were compared to the six ones obtained later on for ants consuming Al hydroxide using the non-parametric Wilcoxon test [43].

Cognition

This protocol was set up when studying the effects of nicotine [45]. Two folded pieces of white strong paper (Steinbach®, 12 cm x 4.5 cm) were inserted in a tray (15 cm x 7 cm x 4.5 cm) for creating a path with twists and turns between an initial small loggia and a large one. Each colony had its own apparatus. For each of them, 15 ants were set all together in the initial loggia, and just after, the ants present in this initial loggia and in the large one were counted after 30s, 2, 4, 6, 8, 10 and 12 minutes. The numbers obtained during each count for the two colonies were added (Table 3, line 2), and the sums obtained for ants consuming Al hydroxide were compared to those obtained for ants under normal diet using the non-parametric Wilcoxon test [43].

Aggressive behavior against nestmates and aliens

This treat was assessed as previously [40,41]. Five dyadic encounters with a nestmate and with an alien were realized for each colony. Each encountering was conducted in a small cylindrical cup (diameter = 2 cm, height = 1.6 cm), the borders of which being slightly covered with talc. Each time (5 x 2 = 10 encounters with nestmates, 5 x 2 = 10 encounters with aliens), one ant of colony A or B was observed during 5 min and its encounter with the opponent was characterized, as previously, by the number of times it did nothing (level 0 of aggressiveness), touched the other ant with its antennae (level 1), opened its mandibles (level 2), gripped and/or pulled the other ant (level 3), tried to sting or stung the other ant (level 4) (Figure 1G, H). The numbers recorded for the two colonies were added (Table 3, lines 3, 4), and the results obtained for ants under Al hydroxide diet were compared to those obtained for ants under normal diet using the non-parametric χ^2 test [43]. As in previous works [40,41], the ants' aggressiveness was also quantified by the variable 'a', equaling the number of recorded aggressiveness levels 2 + 3 + 4 divided by the number of recorded levels 0 + 1.

Ability in escaping from an enclosure

As in previous works [40, 41], for each colony, six ants were enclosed in a reversed polyacetate glass (h = 8 cm, bottom diameter = 7 cm, ceiling diameter = 5 cm) set in the ants' foraging area. The rim of the bottom of the glass was provided with a small notch (3 mm height, 2 mm broad) for giving to the ants the opportunity of escaping from the enclosure (Figure 1I, J). To quantify such ability, the ants still under the glass and those escaped after 30s, 2, 4, 6, 8, 10 and 12 minutes were counted. The results obtained during each count for the two colonies were added (Table 3, line 5), and the sums obtained for ants consuming Al hydroxide were compared to those obtained for ants under normal diet using the non-parametric Wilcoxon test [43]. As previously [40,41], we also evaluated the ants' ability in escaping by the variable "n° of ants escaped after 12 min/12".

Visual and olfactory conditioning and memory

The protocol of these experiments has been set up many years ago, and has been used several times [40,41]. Here, we used it again for studying the effect of Al hydroxide on learning and memorizing capabilities, experimenting on colonies A and B after they had consumed aluminum for 7 days, and using results previously obtained on a colony never provided with that compound, here named colony C, a colony previously used while studying the effects of statins [46]. At a given time, a yellow hollow cube under which the ants could go was set above the entrance of the sugar water tube, the ants undergoing so visual operant conditioning. One week later, after the end of that visual conditioning experiment, pieces of basilica were deposited all around the entrance of the sugar water tube, the ants undergoing then olfactory operant conditioning. Tests were performed over time, while ants were expected to acquire conditioning and after removal of the cue while they were expected to lose it. The ants were individually tested in a Y-apparatus constructed of strong white paper, and set in a small tray (30 cm x 15 cm x 4 cm), as explained in previous studies [same references as above]. The Y-apparatus was provided with a yellow hollow cube (Figure 1K) or pieces of *basilica* in one branch. Half of the tests were conducted with the cue in the left branch and the other half with the cue in the right branch. Moving into the branch containing the cue was considered as giving the correct response. For each test, 10 ants of each colony were tested, the numbers of ants under Al hydroxide diet being thus 10 ants x 2 colonies = 20 ants, and of ants under normal diet, n = 10. The percentage of correct responses was established for each test (Table 4). The numerical results obtained for ants under one and the other diet were compared thanks to the non-parametric Wilcoxon test [43]. Note that not conditioned ants present the control score of 50%, and that only higher scores reveal some conditioning acquisition.

Adaptation to aluminum hydroxide consumption

After the ants lived under a diet with Al hydroxide during 8 days, their linear and angular speeds were again assessed (Table 5, upper part) in the manner they had been before the ants consumed this product and after they had consumed it for one day, in order to examine if ants went on walking slowly and sinuously or if they became adapted to the effect of Al hydroxide on their locomotion. The values obtained after 10 days of Al hydroxide consumption were compared to the control ones and to those obtained after one day of that product consumption using the non-parametric Mann-Whitney test [43].

Habituation to aluminum hydroxide consumption

This trait was not examined, a decision taken after having examined all the here above potential effects of aluminum.

Dependence on aluminum hydroxide consumption

After the ants lived under an Al hydroxide diet during 12 days, we conducted an experiment in order to reveal if they became dependent on that product. The protocol of this experiment was identical to that used for examining potential ants' addiction to different substances [40,41]. For colony A and for colony B, 15 ants were removed and set in a small tray (15 cm × 7 cm × 5 cm) in which two tubes (h = 2.5 cm, diam. = 0.5 cm) had been deposited, one containing sugar water, the other containing a sugar solution of Al hydroxide i.e. the solution used throughout the hole study (Figure 1L). In one tray, the tube containing Al hydroxide was deposited on the right, and in the other tray, it was deposited on the left. After that, the ants seen drinking each liquid were counted 15 times over 15 minutes. The two sums of these two different counts were compared to those which should have been obtained if ants randomly went drinking each kind of provided liquid, using the non-parametric goodness of fit χ^2 test [43] (Table 5, lower part). On the basis of these two sums, we also established the proportion of ants which have chosen each kind of liquid.

Loss of the effects of aluminum hydroxide after its consumption was stopped

The protocol of such a study is detailed in previous works, for instance [40,41]. The solution of Al hydroxide was renewed 12 hours before the start of the present experiment. Weaning began at t = 0 when the solution of Al hydroxide provided to the ants was replaced by an aqueous solution of sugar, free of the Al compound. Since that time, the ants' angular speed (= sinuosity) was assessed each two hours just like it had been before the ants consumed Al hydroxide and after they had consumed it for one day or for 8 days, except that 20 instead of 40 ant's trajectories were recorded for being able to make the assessments in the course of the experimentation. The obtained values were compared to those of the control (diet without Al hydroxide) and to those obtained at t = 0, taking these two latter groups of values as control groups, in a non-parametric Kruskal-Wallis ANOVA test for multiple comparisons [43], using Statistica® v.10 software. The results of the present experiment are given in table 6, and graphically presented in figure 2. The experiment ended when the ants' angular speed became similar to that presented under normal diet.

Results and Discussion

Sugar water and meat consumption, general activity

While consuming aluminum hydroxide, ants eat somewhat less meat, drank a little more sugar water, and were less active than when living under normal diet (Table 1). On average, less ants consuming aluminum hydroxide were seen eating the provided *T. molitor* larvae than ants not receiving this compound, a result at the limit of significance (N = 5, T = -13, P = 0.09). Meanly more ants under aluminum diet than ants under normal diet were observed drinking sugar water, but this results was not significant (N = 6, T = +17, P = 0.109) what may be due to the few number of days (6) during which the counting was performed. Ants consuming aluminum hydroxide were less active than ants living under normal diet, a difference statistically significant (N = 6, T = -21, P = 0.016). This may be explained by the ants' difficulties in moving when they had consumed aluminum hydroxide (see the following paragraph).

	Sugar water diet			Sugar water + $Al(OH)_3$ diet		
Days	Meat	Sugar water	Activity	Meat	Sugar water	Activity
Daily counts						
I A	1 0 0 2 1 2	4 4 5 2 2 2	17 15 16 13 16 15	0 0 1 1 0 0	3 3 3 7 7 6	7 7 8 8 8 7
B	1 1 1 1 0 0	5 5 5 1 0 0	13 14 15 15 16 15	1 0 0 1 0 0	4 4 4 5 5 5	7 7 7 7 7 7
II A	1 1 2 2 2 2	5 4 5 7 6 7	9 8 9 10 11 10	0 0 1 1 1 1	5 4 5 2 3 4	7 7 8 9 9 10
B	1 1 0 1 1 1	5 5 4 6 7 7	8 8 9 12 11 12	1 0 0 0 0 1	5 6 5 3 4 4	5 6 5 10 11 11
III A	0 0 1 1 1 0	5 5 4 2 2 2	9 10 11 12 13 12	1 1 0 0 0 0	5 5 5 5 5 5	7 6 7 9 9 10
B	1 0 0 1 1 1	4 4 5 1 1 0	9 9 10 13 12 12	1 1 1 0 1 1	8 7 9 6 6 7	10 10 11 7 8 9
IV A	1 1 0 1 1 0	6 6 5 5 6 6	8 8 9 11 11 10	0 0 1 1 1 0	6 6 7 3 3 4	9 10 9 8 7 8
B	1 1 1 0 0 1	5 6 6 5 5 5	9 9 10 12 13 12	0 1 0 1 1 0	5 6 6 10 10 9	9 9 10 6 7 6
V A	0 0 1 1 0 0	4 4 5 5 5 6	7 8 8 12 11 12	1 1 0 2 2 1	6 6 7 4 4 4	4 5 4 9 9 10
B	1 0 0 0 0 1	5 4 4 6 6 7	8 8 7 11 12 12	0 0 1 0 0 1	9 9 10 4 4 5	7 8 7 8 9 9
VI A	1 1 0 1 1 0	5 5 6 4 4 4	9 10 10 14 15 15	0 0 1 1 1 0	7 7 6 4 5 5	4 5 5 10 10 9
B	1 1 1 1 1 1	5 5 4 4 4 4	6 7 7 17 18 18	0 0 0 1 0 0	7 5 7 5 5 6	4 4 5 10 9 10
Daily means						
I	0.83	2.91	15.00	0.33	4.67	7.25
II	1.25	5.67	9.75	0.50	4.17	8.17
III	0.58	2.91	11.00	0.58	6.08	8.58
IV	0.67	5.50	10.17	0.50	6.25	8.17
V	0.33	5.08	9.67	0.67	6.25	7.42
VI	0.83	4.50	12.17	0.33	5.83	7.08
Average of daily means						
	0.75	4.43	11.29	0.48	5.54	7.78

Table 1: Impact of aluminum hydroxide on the ants' food consumption and general activity.
During six days, the ants of colonies A and B eating meat, drinking sugar water and being active were counted six times (Daily counts). The mean of these 12 counts was established each day (Daily means), and finally the average of the six daily means was calculated for each ants' trait. Aluminum hydroxide decreased the ants' meat consumption, somewhat increased their sugar water consumption, and decreased their general activity, this latter result being probably due to the ants' difficulties in moving (see Figure 1A).

Mice having been injected with Al hydroxide moved along shorter distances and with slower movement [11], a result similar to that on ants, while rats intubated with AlCl3 traveled greater distances according to the dose [47]. Motor deficits and neuron apoptosis (expressed through decrease in muscular strength and endurance) were observed in Al hydroxide subcutaneously injected mice [8,10,11]. More severe motor deficits including limb paralysis and developmental retardation appeared in Al lactate fed gestating mice and their offspring [48].

Linear and angular speed

After 1 day of consuming aluminum hydroxide, the ants walked at a lower linear speed (M-W: U = 56, Z(adjusted) = 7.1516, $P < 0.001$) and at a higher angular speed (M-W: U = 87, Z (adj.)= -6.857, $P < 0.001$) than when under a normal diet, what was obvious to the observer (Table 2, lines 1, 2). Sometimes, ants had difficulties in walking, and even in staying on their legs (Figure 1A). Their muscular functioning, movement coordination and nervous control of locomotion may be affected by aluminum hydroxide.

Changes in locomotion behavior such as lower linear speed and higher angular speed were also observed in Al hydroxide injected mice [11].

Orientation towards an alarm signal

Ants consuming aluminum hydroxide could not orient themselves towards an alarm signal as well as ants under normal diet (Table 2, line 3). Instead of several ants surrounding the presented tied worker, there were only few ones (Figure 1B). This was thus obvious while experimenting, and was statistically significant (M-W: U = 156.5, Z (adj.) = -6.1873, P < 0.001). Their central nervous system and/or olfactory perception may thus be affected by aluminum hydroxide.

Trail following

Under normal diet, the median of the numbers of followed angular degrees arcs was 10. While consuming aluminum hydroxide, only a median of 4 ang. deg. arcs was followed (Table 2, line 4). This difference of ants' trail following behavior according to the ants' diet was obvious while experimenting (Figure 1C), and statistically significant (χ^2 = 36.41, df = 3, P < 0.001). Aluminum hydroxide impacted thus the ants' ability in correctly moving along a line (what is in agreement with the result about the ants' locomotion) and/or their olfactory perception (what is in agreement with the result about the ants' orientation towards an alarm signal).

Audacity

In front of an unknown and risky apparatus, the ants have usually some reluctance of walking and climbing on it: under normal diet, meanly 2.10 ants were seen on such an apparatus. Under an aluminum hydroxide diet, the ants were even more reluctant in coming onto the apparatus: they soon moved away from it or stopped in front of it (Figure 1D). Meanly only 0.80 ants under aluminum diet were seen on the apparatus (Table 2, line 5). The difference of audacity between ants under the one and the other kind of diet was significant (N = 5, T = -15, P = 0.031). This result suggests that aluminum hydroxide may impact the ants' nervous system.

Mice injected with Al hydroxide presented a behavior which resembled that of ants under this substance diet and in presence of an unknown apparatus: they spent more time in the corners or near the edges of an arena instead of moving into the center [10], and less and more lately entered the centre of an open field [11]. This change of behavior was interpreted as anxiety or fear-related behavior. $AlCl_3$ fed rats spent more time in the arms of a maze, also suggesting some increase of their anxiety level [49].

Tactile ('pain') perception

This trait was largely impacted by aluminum hydroxide (Table 2, line 6). Under normal diet, the ants walked cautiously, slowly and sinuously on a rough substrate, clearly perceiving its uncomfortable character (Figure 1E). They often touched with their antennae the substrate lying in front of them. While consuming aluminum hydroxide, the ants walked more frankly, more rapidly and less sinuously on such a substrate, just as if they far less perceived its uncomfortable character. Between the ants under normal diet and those under an Al hydroxide diet, the linear and angular speeds on a rough substrate differed significantly (linear speed: χ^2 = 35.78, df = 2, P < 0.001; angular speed: χ^2 = 30.55, df = 2, P < 0.001). Note that, under an aluminum hydroxide diet, the ants walked on a rough substrate nearly exactly as on a usual substrate (Table 2, line 6 vs lines 1, 2). The present result allowed concluding that Al hydroxide impacted the ants' sensitivity.

Traits	Sugar water diet	Sugar water + $Al(OH)_3$ diet
Linear speed (mm/s)	14.9 (13.7 - 16.3)	10.0 (8.9 - 11.3)
Angular speed (ang. deg./cm)	122 (106 - 135)	179 (161 - 195)
Orientation (ang. deg.)	39.4 (27.6 - 50.2)	71.0 (59.4 - 82.8)
Trail following (n° arcs)	10.0 (8.8 - 15.5)	4.0 (2.0 - 7.3)
Audacity (n° ants)	2.10 [1 - 3]	0.80 [0 - 2]
Tactile (pain) perception:		
linear speed (mm/s)	5.3 (4.9 - 6.1)	9.1 (8.6 - 10.0)
angular speed (ang. deg./cm)	271 (243 - 302)	173 (153 - 190)

Table 2: Impact of aluminum hydroxide on six physiological and ethological traits.
Details and statistics can be found in the text. After one day of consumption, aluminum hydroxide appeared to decrease the ants' linear speed, to increase their sinuosity of movement, to decrease their orientation and trail following ability, to decrease their audacity and their tactile perception (or sensitivity). The table gives the median (and the first and third quartiles) or the mean [and the extremes] of the obtained values.

A seemingly opposite response was obtained in humans as well as in rats that received $AlCl_3$ or $AL(OH)_3$, as their sensitivity to flicker increased with the level of Al in serum (humans) or in diet (rats) [47].

Brood caring

Ants consuming aluminum hydroxide replaced the larvae that were experimentally removed from the nest a little more rapidly than ants living under normal diet (Table 3, line 1, Figure 1F). However, this was not particularly obvious while experimenting and on basis of the numbers of counted larvae, the result was only at the limit of significance (N = 4, T = 10, P = 0.063). As a matter of fact, aluminum hydroxide appeared not to impact the ants' relation with the brood.

Cognition

This trait was impacted by aluminum hydroxide (Table 3, line 2). It was obvious while experimenting: ants under aluminum hydroxide diet hesitated during 2 to 6 minutes before moving into the twists and turns path, or never did so, and those doing so often came back on their way after one or two minutes. No ant could reach the large area lying beyond the difficult path, while 7 ants among 30 that were under normal diet could do so during the same time period. Statistically, the difference in the numbers of ants remaining in the area in front of the difficult path was at the limit of significance (N = 7, T b= +23, P = 0.078), while the difference in the numbers of ants reaching the area lying beyond that path was significant (N = 5, T = -15, P = 0.031). Aluminum hydroxide impacted thus the ants' audacity and cognition, so their nervous system, what was in agreement with results relative to orientation, audacity and tactile perception (see above).

Mice injected with Al hydroxide presented cognitive deterioration when tested in water-mazes. This cognitive impairment was related to Al-induced apoptosis of motor neurons [10,11]. Cognitive damage was also demonstrated in rats fed with amounts of $AlCl_3$ equivalent to those of the urban American dietary. The performance of these rats in T-mazes decreased inversely with the percentage of their Al-loaded entorhinal cortex cells, and they displayed concentration inability, confusion and repetitive behavior. These behaviors resembled those of dementia patients, and were associated with elevated amyloid precursor protein (APP) gene expression in hippocampal and cortical tissue [51,52].

It has to be noticed that a daily ingestion of 2 - 5 g Al hydroxide by dialysis patients has been reported to have not lead to a significant difference in intelligence, reasoning, memory, visual memory, verbal and reading fluency and in manual dexterity [23]. Nevertheless, an accidental exposition to Al sulfate concentrations 500 - 3000 times the acceptable limit had brought a middle-aged woman to have visual hallucinations, to present difficulties in vocabulary, calculation and carrying out any commands, and finally to die [52].

Aggressiveness against nestmates

Ants consuming aluminum hydroxide were never aggressive towards their nestmates. They behaved just like while living under normal diet (Table 3, line 3; $\chi^2 = 1.71$, df = 2, $0.30 < P < 0.50$; Figure 1G). This observation was in agreement with that made on the ants' brood caring behavior while consuming aluminum hydroxide (see above).

Aggressiveness against aliens

Ants consuming aluminum hydroxide aggressed aliens (Figure 1H) as did the ants living under normal diet. Their relations with others (nestmates and aliens) were thus intact (Table 3, line 4). However, the ants under aluminum hydroxide diet more often opened their mandibles than the ants under normal diet ($\chi^2 = 12.76$, df = 3, $0.001 < P < 0.01$). Such a significant result may be interpreted as an attempt of the ants consuming aluminum hydroxide to avoid a conflict with opponents. The alarm pheromone emitted by the mandibular glands when ants largely opened their mandibles indeed decreases *Myrmica* ants' aggressiveness [53]. After having opened the mandibles many times (emitting so the alarm pheromone) and perceived that the encounter stayed aggressive, each tested ant under aluminum hydroxide diet duly attacked its encounter. This delay in attacking of ants' under Al hydroxide diet reflected some audacity or straightforward decrease. Such a decrease, detected through the present experiment on ants' behavior towards aliens, had already been found when examining audacity and cognition.

Ability in escaping from an enclosure

This trait was largely impacted by Al hydroxide consumption (Table 3, last line). Under normal diet, the enclosed ants walked for a time all around their enclosure, then walked more calmly along the rim of the reversed glass. When perceiving the notch in the rim, the ants escaped from the enclosure (Figure 1I). Under Al hydroxide diet, the ants walked firstly all around, then more calmly along the rim of the enclosure but, when coming near the notch, they did not escape (Figure 1J), and went on moving under the reversed glass. This result was significant for the ants still enclosed as well as for those which escaped: N = 6, T = +21, -21 respectively, P = 0.031. Such a behavior of ants consuming aluminum hydroxide revealed that this compound had an impact on the ants' cognition and straightforwardness, two deductions already made by when examining audacity and cognition.

Traits	Normal Diet	Diet with Aluminum
Brood caring: n° among 10 larvae removed from the nest and not re-entered in the course of 10 minutes	time: 30” 2 4 6 8 12 min n° : 10 10 8 5 3 1	time: 30” 2 4 6 8 12 min n° : 10 8 6 4 2 1
Cognition: ants in front of and beyond twists and turns in the course of 12 min	t n° in front n° beyond 30s 29 0 2 26 0 4 24 1 6 23 1 8 20 3 10 13 7 12 13 7	t n° in front n° beyond 30s 30 0 2 28 0 4 25 0 6 20 0 8 21 0 10 18 0 12 18 0
Aggressiveness against nestmates	levels 0 1 2 3 4 var ‘a’ n° 71 51 8 0 0 0.07	levels 0 1 2 3 4 var ‘a’ n° 63 60 12 0 0 0.09
Aggressiveness against aliens	levels 0 1 2 3 4 var ‘a’ n° 7 41 59 81 24 3.41	levels 0 1 2 3 4 var ‘a’ n° 7 24 82 52 26 5.16
Escaping from an enclosure: ants in and out of the enclosure in the course of 12 min	t: 30s 2 4 6 8 10 12 n° in 12 10 8 7 5 3 2 n° out: 0 2 4 5 7 9 10 variable = 10/12 = 0.83	t: 30s 2 4 6 8 10 12 n° in: 12 12 12 12 12 12 11 n° out: 0 0 0 0 0 0 1 variable = 2/12 = 0.08

Table 3: Impact of aluminum hydroxide on five ethological and physiological traits.
Aluminum hydroxide did not impact the ants' brood caring, and their relationship towards nestmates and aliens. It largely decreased the ants' cognition and their escaping ability (so their straightforwardness).

An increase of escaping an electric shock or more exactly an increase of avoidance of an electric shock was observed in $AlCl_3$ fed rats and interpreted as a cognitive impairment [49]. This could be more exactly interpreted as a higher sensitivity to electricity due to the ingested aluminum.

Visual and olfactory conditioning and memory

Aluminum hydroxide largely affected the ants' ability in acquiring conditioning as well as their short- and middle-term memory (Table 4, Figure 1K).

Indeed, while ants under normal diet acquired a conditioning score of 70% after 7 hours of training and 80% as soon as after 24 hours, ants consuming aluminum hydroxide never acquired such a conditioning, and presented the null score of 45-55% during the entire training time period (72 hours). This result was statistically significant: N = 6, T = -21, P = 0.016. The ants have thus never memorized the presented visual cue.

Traits Time (h)	Sugar water diet *		Sugar water + aluminum diet		
	Colony C	%	Colony A	Colony B	%
Visual conditioning					
7	7	70	3	6	45
24	8	80	5	4	45
31	8	80	6	4	50
48	8	80	5	6	55
55	8	80	6	5	55
72	8	80	6	4	50
Visual memory			Testing pointless: short and middle term memory largely impacted		
7	8	80			
24	7	70			
31	8	80			
48	7	70			
55	7	70			
72	7	70			
Olfactory conditioning					
7	7	70	4	5	45
24	8	80	5	4	45
31	8	80	4	5	45
48	9	90	5	4	45
55	9	90	4	5	45
72	9	90	5	5	50
Olfactory memory			Testing pointless: short and middle term memory largely impacted		
7	9	90			
24	8	80			
31	8	80			
48	7	70			
55	8	80			
72	8	80			

Table 4: Impact of aluminum hydroxide on the ants' ability in acquiring conditioning and on their memory.
Aluminum hydroxide largely affected the ants' ability in acquiring conditioning, and thus their short- and middle-term visual and olfactory memory. * = results previously obtained [46].

In the same way, while ants under normal diet reached an olfactory conditioning score of 70% after 7 hours of training and of 90% as soon as after 48 hours, ants consuming aluminum hydroxide presented the low score of 45-50% all along the 72 hours training period. This result was statistically significant: N = 6, T = -21, P = 0.016. The ants' short- and middle-term olfactory memory was thus largely impacted.

Diminished spatial learning and memory was shown, via water-maze tests, in Al hydroxide subcutaneously injected mice [10,11], and in $AlCl_3$ fed rats [54]. This cognitive impairment was related to Al-induced apoptosis of motor neurons [10,11] and oxidative stress [54]. Impairment of memory occurred in $AlCl_3$ fed rats through impact on Ca^{2+} metabolism in hippocampal cells having accumulated Al [55].

Adaptation to aluminum hydroxide consumption

Ants presented no adaptation to the impact of aluminum hydroxide on the ants' locomotion (Table 5, line 1). After having consumed that compound for 8 days, their linear and angular speeds were still affected, the former being decreased and the latter increased. There was no statistical difference between these speed values and those observed after 1 day of aluminum hydroxide consumption (linear speed: M-W U = 745, Z (adj.) = 0.5247, P = 0.60; angular speed: M-W U = 649.5, Z (adj.) = -1.4437, P = 0.15), even it the ants' speeds were slightly more impacted after 8 days than after 1 day of aluminum hydroxide consumption (Table 5, line 1).

Dependence on aluminum hydroxide consumption

Confronted to sugar water free of aluminum hydroxide and to sugar water containing this compound, 17 ants of colony A and 10 ants of colony B chose the former liquid while 20 ants of colony A and 19 ones of colony B preferred the latter (Table 5, line 2; Figure 1L). In total, 27 ants went drinking the water free of aluminum hydroxide and 39 ants went drinking the liquid containing that compound, what corresponded to 41% of 'aluminum hydroxide avoidance' and 59% of 'aluminum hydroxide choice'. Such a numerical result (27 vs 39) did not statistically differ from that expected if ants went randomly drinking each presented liquids (i.e. 33 vs 33) (χ^2 = 0.76, df = 1, 0.30 < P < 0.50). Consequently, even if ants stayed somewhat longer time periods on the liquid containing aluminum hydroxide (39 > 27), they developed no dependence on that compound consumption.

Examined traits	Assessment of the traits		
Adaptation	Control	1 day of $Al(OH)_3$ diet	8 days of $Al(OH)_3$ diet
Linear Speed (mm/sec)	14.9 (13.7-6.3)	10.0 (8.9 - 11.3)	9.5 (8.7 - 10.9)
Angular Speed (ang.deg./cm)	122 (106-135)	179 (161 - 195)	187 (168 - 208)
Dependence	Random choice	n° on pure water	n° on water + $Al(OH)_3$
ants being under $Al(OH)_3$ diet	33 *vs* 33	17 + 10 = 27	20 + 19 = 39

Table 5: Ants' adaptation to aluminum hydroxide consumption (i.e. to its adverse effects), and dependence on that consumption. Ants never adapted themselves to the adverse effects of aluminum hydroxide; on the contrary, these effects increased in the course of consumption (see also the values obtained after 14 days of consumption in Table 6). Ants developed no dependence on aluminum consumption.

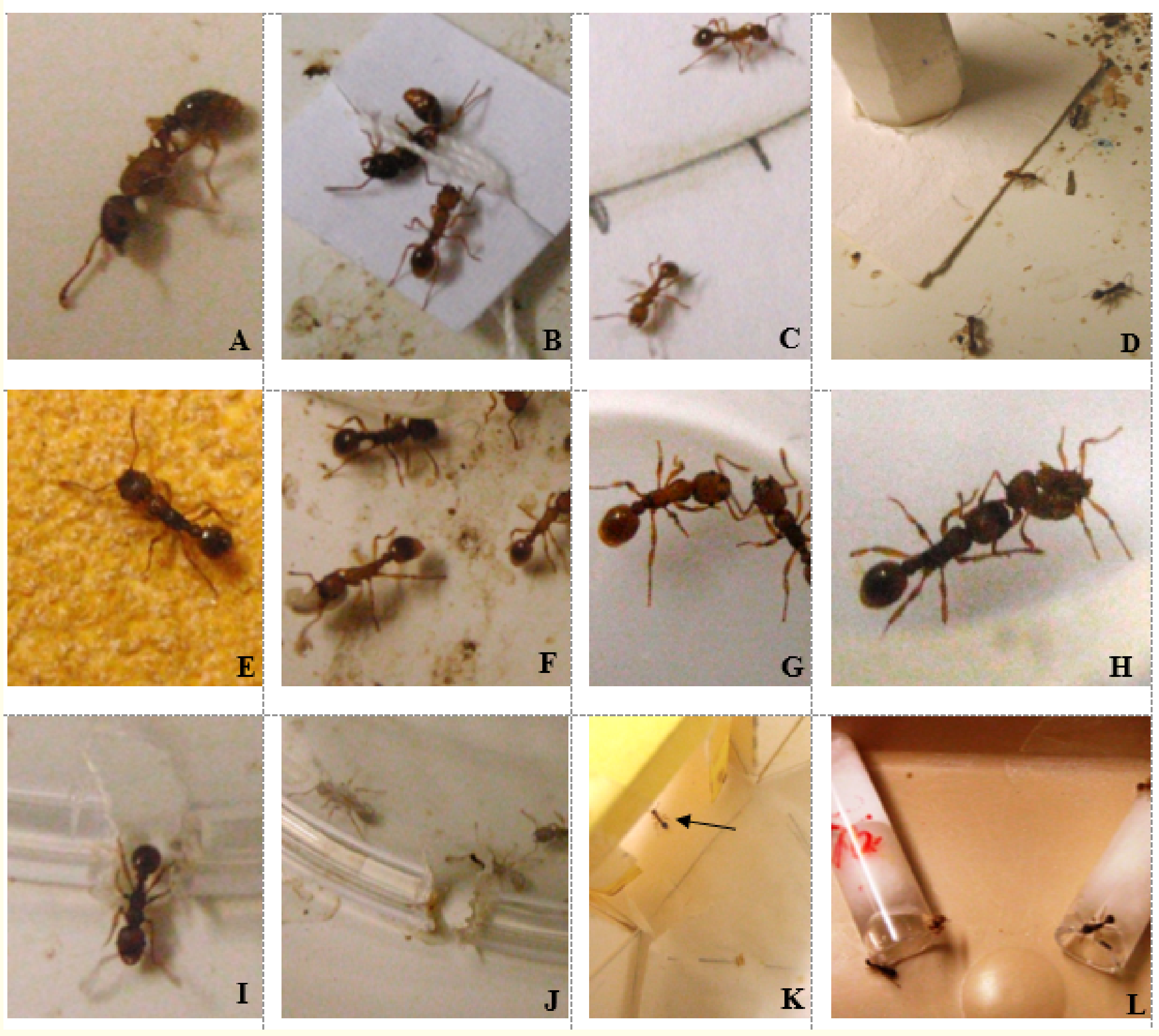

Figure 1: Some views of the experiments.

A: an ant under $Al(OH)_3$ diet presenting moving and perhaps balance difficulties. B: an ant under $Al(OH)_3$ diet having reached a tied worker emitting its alarm signal; it was the only ant reaching the tied nestmate at that time. C: ants under $Al(OH)_3$ diet departing from a circular trail. D: ants under $Al(OH)_3$ diet having not the audacity to come onto an unknown apparatus. E: an ant under normal diet having difficulties in moving on a rough substrate, touching it with its antennae. F: ants under $Al(OH)_3$ diet taking care of their brood. G: two nesmates under $Al(OH)_3$ diet not aggressing one another. H: an ant under $Al(OH)_3$ diet stinging an alien ant. I: an ant under normal diet escaping from an enclosure. J: ants under $Al(OH)_3$ diet moving near the exit of an enclosure, but not escaping through it. K: an ant (arrow) under $Al(OH)_3$ diet, trained to a hollow yellow cube, tested in a Y apparatus provided with that cue in one of its branch, and giving the correct response, i.e. moving towards the yellow cube. L: ants under $Al(OH)_3$ diet, having the choice between pure sugar water and sugar water containing $Al(OH)_3$ (red note); there were no more ants going onto the tube containing $Al(OH)_3$ than onto the other tube; the ants thus developed no dependence on Al hydroxide.

Decrease of the effects of aluminum hydroxide after its consumption was stopped

Results are presented in table 6 and figure 2. Briefly, after its consumption was stopped, the effects of aluminum hydroxide decreased slowly and linearly in 12 hours.

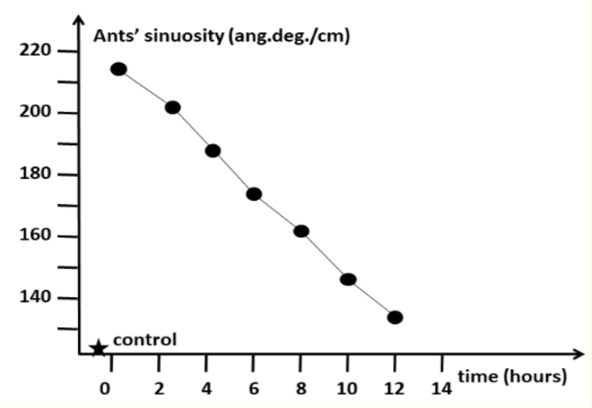

Figure 2: Decrease of the effect of aluminum hydroxide on the ants' angular speed, after its consumption was stopped. The effect of aluminum hydroxide linearly decreased after weaning, without latency, and vanished in 12 hours, the ants moving then statistically as before consuming the drug. $Al(OH)_3$ thus lost 1/12 of its intensity effect each successive hour after weaning.

Experiments Time (t, hours)	Ants' angular speed ang.deg./cm	Statistics *vs*	
		Control	t = 0
Control (diet without $Al(OH)_3$)	122 (106 - 135)		
After 1day on $Al(OH)_3$ diet	179 (161 - 195)		
After 8 days on $Al(OH)_3$ diet	187 (168 - 208)		
After 14 days on $Al(OH)_3$ diet, t= 0	216 (187 - 234)		
Time after weaning, t = 2	203 (181 - 220)	P < 0.001	P = 1
t = 4	184 (170 - 216)	P < 0.001	P = 0.74
t = 6	172 (150 - 212)	P < 0.001	P = 0.09
t = 8	166 (145 - 189)	P = 0.002	P = 0.002
t = 10	148 (137 - 162)	P = 0.01	P < 0.001
t = 12	136 (121 - 152)	P = 0.18	P < 0.001

Table 6: Decrease of the effect of aluminum hydroxide after its consumption was stopped.

After weaning, without latency, the effect of aluminum hydroxide linearly decreased and vanished in 12 hours. Column 3 gives one-tailed probabilities from a non-parametric Kruskal-Wallis ANOVA for multiple comparisons using the values under diet without Al hydroxide and those of the start of weaning (t = 0) as control groups. Twelve hours after weaning, the ants' angular speed was statistically similar to the control one and very different from that before weaning. A graphic presentation of the results can be seen in the figure 2.

In details, there was no latency period. Just after weaning, the ants' sinuosity decreased, what was obvious while experimenting. Six hours after weaning, the ants' sinuosity was still different from the control one ($P < 0.001$) and almost similar to that before weaning ($P = 0.09$). Eight hours after weaning, the ants' sinuosity remained still different from that of the control one ($P = 0.002$), but was now different from that before weaning ($P = 0.002$). Ten hours after weaning, the ants' sinuosity remained different from the control one ($P = 0.01$). Twelve hours after weaning, the ants' sinuosity was no longer different from that of the control ($P = 0.18$) and of course remained different from that before weaning ($P < 0.001$). The effect of aluminum hydroxide vanished thus in a total of 12 hours after the end of its consumption.

The decrease of the effect of aluminum hydroxide was linear. The values of sinuosity (E_t) observed in the course of time (t) after weaning, from the initial value (E_i), decreased according to the linear function:

$$E_t = E_i - 6.7\ t$$

(6.7 is the mean angular coefficient obtained in the course of the effect decrease)

The effect of aluminum hydroxide decreased from 216 to 136 ang. deg. (i.e. 80 ang. deg.), losing 6.7 (i.e. 80/6.7 = 1/12) of its value each hour.

Conclusions

Having previously found that aluminum foil (largely used by humans for packaging foods for instance) has several adverse effects [9], we examined, in the present work, using again ants as models, the putative effects of ingested aluminum hydroxide, a compound used by humans for reducing an excess of acidity in the stomach and as phosphate binder. Tested on ants, aluminum hydroxide increased their sinuosity of movement, decreased their linear speed, activity, orientation ability, trail following ability, audacity, tactile perception, cognition, escaping behavior (so their straightforwardness), learning, as well as their visual and olfactory memory. However, it did not affect their social relationship. The ants did not adapt themselves to the adverse effects of aluminum hydroxide and developed no dependence on that compound. After the Al hydroxide consumption was stopped, its effects decreased linearly in 12 hours (at that time, no statistical difference with the control could be detected). All this was in agreement with our previous results on the effects of aluminum foil, as shows the comparison given in table 7. The effects of aluminum hydroxide were somewhat stronger than those of aluminum foil, due to some more amounts or a better solubility of the former product (see the numerical results summarized in table 7). In any way, the use of aluminum and aluminum hydroxide in food, pharmaceuticals and cosmetics should be limited. Other systems of food packaging and cooking should be found. Natural (vegetal) alternatives should be researched for decreasing the excess of acidity in stomach (clay, fennel seeds, cumin seeds, baking powder without Al, fenugreek seeds, and so on).

Ants' examined traits	$Al(OH)_3$	Al foil
Meat consumption	c: 0.75 t: 0.48 ↓	c: 0.61 t: 0.46 ↓
Sugar consumption	c: 4.43 t: 5.54 ↑	c: 1.04 t: 1.43 ↑
Activity	c: 11.29 t: 7.78 ↓moving difficulties	c: 10.35 t: 13.36 ↑
Linear speed	c: 14.9 t: 10.0 ↓	c: 14.1 t: 13.7 ↓
Angular speed	c: 132 t: 179 ↑	c: 126 t: 148 ↑
Orientation	c: 39.4 t: 71.0 ↓	c: 38.3 t: 59.9 ↓
Trail following	c: 10.0 t: 4.0 ↓	c: 12.5 t: 5.0 ↓
Audacity	c: 2.10 t : 0.80 ↓	c: 1.25 t: 0.90 ↓
'Pain': linear speed	c : 5.3 t : 9.1 } ↓	c: 4.7 t: 8.4 } ↓
angular speed	c : 271 t : 173	c: 257 t: 192
Brood caring	c: 1 t: 1 =	c: 0.53 t: 0.53 =
Cognition: initial	c : 13 t : 18 } ↓	c : 10 t : 16 } ↓
final	c : 7 t : 0	c : 6 t : 4
Aggressiveness		
Against nestmates	c : 0.07 t : 0.09 =	c : 0.10 t : 0.16 =
Against aliens	c : 3.41 t : 5.16 =	c : 4.50 t : 3.90 =
Escaping ability	c : 0.83 t : 0.08 ↓	c : 0.91 t : 0.17 ↓
Visual conditioning	c: 80 t: 50 ↓	c : 80 t : 50 ↓
Olfactory conditioning	c: 90 t: 50 ↓	c : 90 t : 45 ↓
Adaptation		
linear speed	1 day: 10.0 8 days: 9.5 : none	
angular speed	1 day: 179 8 days: 187: none	5 days: 148 10 days: 159 :none
Dependence	$AlOH_3$: 39, pure water: 27: none	Al: 25, pure water: 20 : none
Loss of effects after	no latency	0 - 4h: no change = latency
weaning	0 - 12h: slow, linear decrease	4 -16h: slow, linear decrease

Table 7: Comparison of the effects of aluminum hydroxide (present work) and aluminum foil [9].
c: Control Value; t: Test Value Under Consumption; ↓ : Decrease; ↑: Increase; =: No Change. The two kinds of Al had the same adverse effects. The only differences are that Al hydroxide showed somewhat stronger effects, impacting the ants' moving and so reducing their general activity among others, and that its effect decreased without latency after weaning.

Conflict of Interest

We affirm having no competing interest as for the use of aluminum or aluminum hydroxide for manufacturing drugs, vaccines or any other aluminum-based products used by humans. Being independent researchers and working on ants, we received no funds for conducting our research.

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