Conditioned food aversion mediated by odour cue and microencapsulated levamisole to avoid predation by canids

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Abstract

Worldwide, predators and humans are in conflict for resources such as game species or livestock, especially in the case of medium-large wild canids. One non-lethal method to reduce predation is conditioned food aversion (CFA), in which animals learn to avoid a food due to the illness after its ingestion, caused by the addition of an undetected chemical compound. Food aversion can be enhanced by adding an artificial odour cue, in a process known as taste-potentiated odour aversion (TPOA). We carried out an experiment on penned dogs with three experimental groups to test CFA and TPOA. We offered the food mixed with a combination of microencapsulated levamisole + vanilla odour (ODO), microencapsulated levamisole (LEV), and plain food as a control. The aims were: a) to test whether dogs are able to detect the microencapsulated levamisole; b) to analyse the strength and extinction of CFA induced by microencapsulated levamisole; c) to analyse the strength and extinction of TPOA. Two-choice tests were carried out during 11 months in the post-conditioning phase, and two reinforcements with microencapsulated levamisole were done during the first month. In the first post-conditioning test, ODO and LEV groups ate significantly less untreated food than control group. After the reinforcement, suddenly the dogs in LEV group started to eat the food. Three of four dogs in ODO group showed long-lasting CFA until the 11th month. These results show that TPOA could be used to induce odour aversion on canids and that the odour cue overshadows the slight bitter taste of microencapsulated levamisole. These results open new possibilities to develop TPOA as tool to reduce predation by wild canids.

Keywords: Learned aversion; taste-potentiated odour aversion; dog; predation conflict; non-lethal predation control; wildlife management.

1. Introduction

One of the main conservancy problems of large carnivores is its historical persecution as consequence of livestock losses due to their predation (Treves and Karanth 2003; Ray *et al.* 2005; Ripple *et al.* 2014). This situation is nowadays changing as the public demands for animal welfare increases in the society (Van Eeden *et al.* 2017; Bergstrom 2017). The predation conflict has led to a sharp controversy between ranchers who wish to reduce livestock losses and conservationists who wish the survival of carnivores in the wild. This conflict is especially pronounced in the case of medium-large wild canids such as red foxes (*Vulpes vulpes*), culpeo foxes (*Pseudalopex culpaeus*), Ethiopian wolves (*Canis simensis*), coyotes (*Canis latrans*) or grey wolves (*Canis lupus*), almost everywhere where these species coexist with livestock or game species (Macdonald and Sillero-Zubiri, 2004; Din *et al.* 2017). The main method employed so far to reduce predation has been the lethal control by shooting, trapping or poisoning (Reynolds and Tapper, 1996; Sánchez-Barbudo *et al.* 2012). These methods can affect non-target species, produce secondary poisoning, and it is not selective for problematic individuals". Consequently, the predator control paradigm should be replaced by a new "predation reduction paradigm", by using non-lethal methods for preventing predation of livestock or wild prey, such as modifying predator behaviour (Bergstrom 2017; Van Eeden *et al.* 2018; Smith and Appleby 2018).

Conditioned food aversion (CFA) has been explored to reduce predation by medium and large carnivores (Gustavson *et al.* 1976; Nicolaus *et al.* 1989; Massei *et al.* 2003a, b). CFA occurs when an animal associates the taste and other characteristics to a food that causes an illness or adverse effect, eliciting a rejection of that food in following encounters (Garcia *et al.* 1974; Gustavson *et al.* 1974). Experimentally, we can induce CFA by adding a chemical substance into the food or prey that we want to protect from predation (Gustavson *et al.* 1974; Cowan *et al.* 2000). CFA has been widely tested in laboratory studies and also used in the field (Riley and Tuck, 1985; Smith *et al.* 2000), but studies with wild canids are still scarce (Gustavson *et al.* 1976; Ellins *et al.* 1977; Jelinski *et al.* 1983; Gentle *et al.* 2004). In order to create CFA, especially in wild animals, the correct selection of the aversive compound is the key, which must comply with several characteristics: (1) to induce slight adverse effects, mainly gastrointestinal, as vomit or diarrhoea; (2) to have a wide (or great) margin of safety, which means a high toxic dose together with a low effective dose; (3) to have a short period of latency, between 30 min and two hours to improve the learning of CFA (Garcia and Kimeldorf 1957); and (4) to be undetectable by

predators, i.e. odourless, colourless and tasteless. However, safe and undetectable compounds to be used as aversive for wildlife need to be identified.

Several compounds have been tested to induce CFA on carnivores (Conover 1989; Gill *et al.* 2000; Massei and Cowan, 2002; Norbury *et al.* 2005). One compound tested with high potential as aversive for carnivores is levamisole hydrochloride, which induced CFA in grey foxes (*Pseudalopex griseus*) (Nielsen *et al.* 2015), but failed in ferrets (*Mustela furo*) (Massei *et al.* 2003b; Norbury *et al.* 2005) and badgers (*Meles meles*) (Cagnacci *et al.* 2005). Levamisole also produced contrasting results in red foxes (Massei *et al.* 2003a; Gentle *et al.* 2004) and domestic dogs (*Canis lupus familiaris*) (Tobajas *et al.* submitted). The main problem of levamisole as a CFA agent for carnivores is its detectability by odour and taste (Gentle *et al.* 2004; Cagnacci *et al.* 2005; Nielsen *et al.* 2015). Microencapsulation is a technique to coating the chemical compound with hydrophobic binder to reduce its solubility, and can be used to mask its taste and smell. However, results so far are limited (Tobajas *et al.* submitted) and the microencapsulation technique has to be improved.

An alternative method of CFA that could be used as a non-lethal method for reducing predation is taste-potentiated odour aversion (TPOA). In this case, the aversion is created to an artificial odour cue rather than to the food taste, with the added advantage of getting avoidance at a distance (Rusiniak *et al.* 1979; Nicolaus and Nellis 1987; Baker *et al.* 2007). The TPOA occurs when the strength of the odour aversion is enhanced following taste + odour compound conditioning (Rusiniak *et al.* 1979). Experimentally, it has been observed that the salience ratio or relative concentration of the taste and odour cues is crucial to establish an odour potentiation (Bouton *et al.* 1986), where the aversion of the weak cue is enhanced. In this sense, the strong taste of levamisole could act as strong salience cue in a compound conditioning with a weak odour cue, being the odour aversion potentiated. Although widely developed in laboratory conditions (Durlach and Rescorla 1980; Bouton *et al.* 1986; Batsell and Paschall 2009), few attempts with TPOA have been done in wild conditions (Nicolaus and Nellis 1987; Baker *et al.* 2007, 2008). The results obtained by Baker *et al.* (2007, 2008) using the food aversion + odour cue to protect crops and baits from wild badgers, have opened new opportunities to develop this technique in the predation control of wild animals. In this sense, the use of odour aversion could be a tool to avoid the livestock predation by large carnivore, as grey wolf (*Canis lupus*). It could be used to protect areas by the creation a buffer with the odour, creating a disruptive effect caused by the odour aversion to stop predation during the predatory behaviour.

The aims of this paper are: a) to test whether dogs are able to detect a microencapsulated levamisole; b) to analyse the strength and extinction of CFA induced by this microencapsulation; c) to analyse the strength and extinction of the TPOA to generate an enhanced aversion in dogs.

2. Material and methods

2.1. Animals

A total of twelve adult English foxhound dogs (*Canis lupus familiaris*, six males and six females), ranging from 16.6-25 kg body weight, were used in the experiment. The dogs were born in the Laboratory Animal Section (Research Support Service, University of Murcia, Spain), where all the experiments were performed following the appropriate European regulations. The project has been evaluated by the Ethics Committee of the University of Murcia, and approved subsequently by the Government of the Region of Murcia (Spain) with the permit Nº A13170703. During the experiment, dogs were fed every morning with the habitual diet formed by dry food (Gosbi® Premium Performance). Tap water was available ad libitum and the dogs were released for exercise and physical contact with the roommates for 30 minutes every day. Each dog was housed individually in a separate pen (size: 1.6 × 4.3 × 3 m) within animal room facilities, following the "Guidelines for accommodation and care of animals of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (European Commission, 2007) conform to Directive 2010//63/EU: room temperature: 20-22°C; relative humidity: 55 %; air exchange: 15-20 times/h; 12h light/darkness cycle. A digital video camera (Spartan, HCO Outdoor Products, Norcross, GA, USA) was placed at each pen door to record dog behaviour during feeding with as little disturbance as possible.

2.2. Drugs and dose

In order to reduce its bitter taste and solubility, levamisole hydrochloride (levamisole hereafter) was microencapsulated with Precirol® Ato 5 (glyceryl palmitostearate) as hydrophobic binder in a melt-granulation technique (Hamdani *et al.* 2003; Mašić *et al.* 2012). Presented as a fine white powder, Precirol® Ato 5 is a high melting point lipid for use in modified release oral solid dosage forms (lipid

matrix for sustained release, delayed release), used in coating techniques to provide taste masking (Amrutkar *et al.* 2010; Mašić *et al.* 2012). The microencapsulation of levamisole has been carried out by the Service of Development of Medicines (Pharmacy Faculty, University of Barcelona, Spain).

The dose of levamisole was selected based on previous toxicity studies, as it would be able to cause digestive symptoms (vomiting, nausea and/or diarrhoea) without causing severe adverse health effects. Tobajas *et al.* (submitted) used a dose of 50 mg/kg in penned dogs creating aversion without negative health effect, but we tried to find a lower effective dose. Therefore, an initial dose of 20 mg/kg was chosen to be tested in a male and a female dog in a preliminary trial. The dogs were monitored for 8 h but no digestive symptoms were observed. Hence, in a second preliminary trial, a dose of 30 mg/kg was chosen and administered to another couple of dogs. As nausea and vomits were found one hour after the administration, 30 mg/kg was the chosen as the dose for the conditioning study.

2.3. Experimental design

The animals were assigned to two experimental rooms to avoid odour interferences among treatments. Room A housed two males and two females which were treated with levamisole (LEV group), and a control pair (male and female not treated with levamisole, CONTROL group). Room B housed two males and two females which were treated with levamisole and vanilla essence (Dr Oetcker™) as an odour cue (ODO group), and another control pair (male and female not treated with levamisole, CONTROL group). Therefore, treatment groups were LEV (n=4), ODO (n=4) and CONTROL (n=4). The experiment was performed in three phases used in CFA experiments: pre-conditioning (untreated food); conditioning (food + aversive agent); and post-conditioning (untreated food) phases. Additionally, two reinforcements (food + aversive agent) were done to induce aversion on unconditioned dogs after the conditioning. We compared pre- and post-conditioning food intake of untreated food by dogs as a measure of CFA response.

The dogs were enrolled in the experiment 30 days before the conditioning trial (pre-conditioning phase). During pre-conditioning, the dogs were fed with the habitual diet of dry food *ad libitum* (1500 g), and in alternate days the excess of dry food was retired and the dogs were fed with wet food (735 g of Gosbi® Fresko Chicken). On day 0, the dogs on LEV and ODO treatments were conditioned with levamisole. To achieve this, the amount of the substance corresponding to each dog's weight according to

the selected dose (30 mg/kg) was homogenously mixed with 735 g of wet food and offered to each dog. The dogs fasted 24 hours prior the conditioning trial. In the ODO treatment, the vanilla (four drops) was applied on the outer surface of the dog bowls without contacting the wet food. The bowl was assigned to the same dog and after its use was cleaned. CONTROL dogs received the same amount of wet food and were studied under the same conditions. The dogs were evaluated by a veterinary practitioner for 8 hours after exposure, checking every 2 h for signs of illness such as nausea, vomiting, diarrhoea, and 24 h later to observe for the normal consumption of their usual diet.

On day 8, a two-choice test between the dry and wet food was performed, followed by a reinforcement on day 9 to try to induce aversion in the not conditioned dogs. Reinforcements were performed following the same protocol than conditioning. A new two-choice test was then performed on day 11. A second reinforcement was made on day 16, followed by a two-choice test on day 18. Since then, until day 60, two-choice tests were run every 7 days. Between day 60 and 120, two-choice tests were run every 15 days. After day 120, the dogs were grouped with other dogs and allocated in bigger pens until day 241 (8th month) and 334 (11th month), when they were separated for two-choice tests.

The two-choice feeding tests were run as follows: The day before of the two-choice test, all dogs were fed in the morning with the dry food, afterwards they fasted until the two-choice test (24 hours approximately). In the two-choice test the food (dry and wet) was weighted (± 1 g) in separate stainless-steel dog bowls with a balance (Mettler® PJ15, Mettler Instrumente®, Greifensee-Zurich, Switzerland) and was offered to each dog during 30 minutes. Afterwards, the plates were retrieved and weighted to calculate the amount of food eaten. Wet food in these tests was not treated with levamisole, but vanilla essence was always applied on the bowls containing the wet food of the ODO treatment group. Dog behaviour performed after all the procedures was recorded with a video camera to analyse the effects of conditioning on dog health and possible modifications of the feeding behaviour.

2.4. Haematology and serum biochemistry

To evaluate the possibility of detrimental effects on dogs' health, haematology and serum biochemistry were studied after three exposures to levamisole. Thus, after the second reinforcement on day 30 blood samples were obtained in all the dogs, including controls. Blood samples (4-5 mL) were obtained by puncturing the brachial vein, using a 5 mL syringe and a 21 G needle. All the analyses were

made at the Interdisciplinary Laboratory of Clinical Pathology, Interlab-UMU, Campus of Excellence Mare Nostrum, University of Murcia, Spain

It should be clear; it is not, that these samples were taken sufficiently after having received the aversive, when this had the opportunity to affect the health of the dogs.

To evaluate the effect of levamisole on the parameters considered, it would have been convenient to take a blood sample, on the same dogs, before and after the treatment with the aversive. The comparison between treated and untreated dogs is not adequate, especially with such a small sample size, in which individual variability could mask any effect.
Instead of this extensive list of biochemical parameters, it would be convenient to identify (in a well-founded manner) those that a priori are considered good indicators of possible toxic or harmful effects of levamisole, predict their effects and put them to the test with these evaluations.

2.5. Statistical analyses

To examine if levamisole induced CFA on dogs, we used a general linear mixed model (GLMM, Lindstrom and Bates 1988) to analyse the effect of treatment on the proportion of food consumed during the pre-conditioning phase, the conditioning trial, and the post conditioning phase among LEV and ODO treatments and CONTROL group. The model included treatment as fixed effect and the individual as random effect. The strength of the CFA generated by each treatment was tested using a GLMM and comparing food consumption between treatment groups at first post-conditioning test (Massei and Cowan 2002). To test the CFA extinction, the data of post-conditioning tests were grouped monthly, and were compared between groups using a GLMM. The long-lasting CFA was tested using a GLMM comparing food consumption among groups in one test at 8 and 11 months after conditioning. Normality of residuals was checked and non-normal data of food ingestion were logit transformed. Student's t tests were used to compare haematology and serum biochemical parameters between control and levamisole groups. All the statistical analyses were carried out with the R software version 3.4.0 (R Core Team, 2017).

3. Results

I should start with what is central to the article. Reverse the order of the sections.

3.1 Strength and extinction of CFA

No significant differences in the proportion of food intake were found between treatment and control groups during the pre-conditioning phase (LEV: $t = 1.06$, $P = 0.31$; ODO: $t = 0.05$, $P = 0.9$; Fig. 1) neither in the conditioning trial (LEV: $t = 0$, $P = 1$; ODO: $t = -1.22$, $P = 0.25$; Fig. 1). At the first post-conditioning test, only one dog of LEV group but none of the ODO group ate all wet food. LEV and ODO dogs ate significantly less food than CONTROL dogs (LEV: $t = -2.43$, $P = 0.03$; ODO: $t = -2.27$, $P = 0.04$; Fig. 1) at the first post-conditioning test. After the reinforcements, three out of four dogs in the LEV group started to eat all the wet food (Fig. 1), suggesting that they learned to detect when levamisole was absent. In the case of the ODO group, all the dogs showed CFA after the reinforcements, suggesting they did not associate the adverse signs with levamisole presence (Fig. 1). During the following 4 months after conditioning, the food consumption was significant lower in the ODO group than in the CONTROL group ($P < 0.05$; Fig. 2), but this difference was not found for the LEV group ($P > 0.05$; Fig. 2). In the long-lasting CFA tests, the dogs showed aversion until 8 months after conditioning in the ODO group ($t = -2.81$, $P = 0.02$; Fig. 2) but not in the LEV group ($t = -1.78$, $P = 0.10$; Fig. 2). However, at 11 months, 75% of dogs from the ODO group continued to manifest CFA and differences among the groups were not significant for both treatment groups compared to control group (LEV: $t = -1.82$, $P = 0.10$; ODO: $t = -2.06$, $P = 0.06$; Fig. 2).

3.2. Adverse effects by the conditioning

During conditioning, salivation and vomit were the main observed signs in six of the eight dogs which ingested the microencapsulated levamisole. Vomit appeared between 3 h 15 and 5 h 30 min after ingestion; and salivation was observed between 2 h and 8h 10 min after ingestion. Only two females, one from LEV and one ODO groups, respectively, showed no clinical signs related with levamisole ingestion.

In the first reinforcement, vomit and salivation were also the main signs. Despite four dogs did not ingest the whole portion of food, they vomited and salivated 1 h 20 min and 5 h 30 min after reinforcement, respectively. It should be noted that vomit appeared earlier than in the conditioning phase (1 h 20 min vs. 3 h 15 min), while salivation appeared later (about 5 h 30 min). Diarrhoea was found in one dog between 1 h 15 min and 3 h 15 min after ingestion. Two dogs did not show any adverse clinical signs, although the estimated doses of levamisole ingested was very different between them (6 mg/kg and 24 mg/kg). On the contrary, during the second reinforcement only two dogs showed signs (vomit at 3 h

and diarrhoea at 1 h 45 min after ingestion), which we estimated that ingested respectively 30 and 26.1 mg/kg of levamisole. The rest of dogs ingested a dose of levamisole between 12.5 and 26 mg/kg. No significant differences were found between the control and treated groups in haematology and serum biochemistry as a whole (Supplementary material), nor comparing males and females separately. However, there were some individuals (both treated and controls) with CK and ALP values higher than those considered normal for dogs and could be related to the stress.

These results are meaningless insofar as changes in haematology and serum biochemistry have not been predicted based on the potential effects of levamisole. Even so, to evaluate its effect on the treated animals, the evaluations had to be done on the same animals, before and after having consumed levamisole.

4. Discussion

According to our results, microencapsulated levamisole can generate CFA in penned dogs, in agreement with previous studies on other canids using pure levamisole (Massei *et al.* 2003a; Gentle *et al.* 2004; Nielsen *et al.* 2015). Strong CFA caused by levamisole, with and without odour, was found at the first post-conditioning test, but dogs could detect the microencapsulated levamisole. The modification of the original food characteristics has been previously described as the main problem for the application of levamisole as a CFA agent (Gentle *et al.* 2004; Tobajas *et al.* submitted). In this sense, the reinforcement with levamisole, when three out of four dogs in LEV group showed strong aversion (Fig. 1), apparently expedited the CFA extinction according to the post-conditioning test after reinforcement (Fig 1). After the reinforcement, three out of four dogs of LEV group learned to discriminate when the levamisole was absent, showing no CFA during the rest of the study (Fig. 2). On the contrary, the four dogs in the ODO group still showed CFA after the reinforcement (Fig. 1). The fact that dogs in ODO group ate most of the wet food during the reinforcement, and they continued rejecting the food during the post-conditioning tests, could be explained by a competition between cues, where the vanilla odour acted as weak cue and could be potentiated by the strong flavour of levamisole following a TPOA process (Rusiniak *et al.* 1979; Bouton *et al.* 1986). In this situation, the dogs did not recognize the levamisole in the food during the reinforcement. This was probably because the levamisole flavour could be overshadowed or blocked in a cue competition, being the aversion to vanilla odour stronger (Rescorla and Wagner 1972; Wesbrook *et al.* 1983). In any case, the CFA was maintained during the next eight months after conditioning in all

dogs in the ODO group, or even longer (11 months) in three dogs. The TPOA seemed to be a good tool to create CFA on penned dogs, but contrary to our expectations, three out of four dogs did not avoid the food and ate a small amount of food in many occasions during the CFA tests. This could be partly due to the high individual variability of the aversion response, or to the experimental design. In this sense, our experimental subjects were domestic animals fed by humans during all their life and they associated the offered food as "learned safety" food, thus reducing the CFA (Kalat and Rozin 1973). Also, the long pre-exposure (pre-conditioning phase) to the food reduces the strength of the aversion (Revusky and Bedarf 1967; Mikulka and Klein 1977). In the same way, Mikulka and Klein (1977) observed that leaving the food available for long periods of time in the aversion tests can mask weak aversion. Finally, the captive conditions of domestic dogs in a pen enclosure during the aversion tests differ from the conditions of other canids in the wild, where animals can avoid the food at a distance and search for alternative food (Nicolaus and Nellis 1987).

In order to use the levamisole in more safety conditions, we decreased the dose to create aversion in this experiment compared to previous studies. Here we used 30 mg/kg of levamisole that is less than the 50 mg/kg used also in penned dogs (Tobajas *et al.* submitted) and far below that dose used with foxes (70 mg/kg) (Massei *et al.* 2003a; Gentle *et al.* 2004; Nielsen *et al.* 2015). The haematology and the serum biochemistry analyses have shown no negative health effect after two or three doses of levamisole. Although physiological differences between wild canids and domestic dogs in front of CFA could exist, this lowest dose of 30 mg/kg should be regarded in the field studies, with the aim to minimize the risk of intoxication on the non-target species, but with the enough doses to ensure the levels of aversion necessary for the method to work.

In the LEV group, because the dogs suddenly started to eat after the reinforcement, we could not evaluate the length of the extinction period, but showed that the tested microencapsulation of levamisole did not avoid its detection by dogs. In addition, the microencapsulation used seemed to produce a delay in the signs after the food ingestion in the conditioning, exceeding the appearance of the signs beyond two hours after ingestion. Although it is possible to induce CFA with longer delays than 2 hours, longer delay times produce weaker aversions (Garcia *et al.* 1972). In order to decrease the flavour of the microencapsulated levamisole, new microencapsulation techniques should be essayed (Shukla *et al.* 2011). However, microencapsulation could have the double effect of delaying and diluting the release of levamisole, with which the effective dose would be even lower. The development of an undetectable and

quick release microencapsulated presentation or an alternative undetectable compound as safe and effective CFA agent, must be on the focus of future research in the CFA development as a method to control predation by wild canids.

The utilization of an odour cue has been demonstrated as an effective tool to induce CFA in wild predators (Nicolaus and Nellis 1987; Baker *et al.* 2007, 2008). Our results showed that the TPOA can induce long-lasting CFA on penned dogs, opening new possibilities to use levamisole plus odour cue as a tool for reducing predation by wild canids. In this sense, two research lines need to be developed. Firstly, to use the odour cue in a conditioning treatment with a combination of non-lethal methods (fences, traditional husbandry, guardian dogs, fladry) such as a protection barrier to avoid the use of the space to be protected (livestock fences, breeding areas) as "living buffer zones" (Smith and Appleby 2018). Secondly, methods to protect the livestock individually using a device (e.g. collar) that emits the odour cue after conditioning. Similarly, these new methodologies could be used as conservation tools to avoid predation of endangered species in nesting or reintroduction areas. At the same time, non-lethal methods for reducing predation such as CFA are crucial to reduce conflicts between humans and wild mesocarnivores and could attenuate the conservation problems derived from the extirpation of large carnivores in human-dominated landscapes (Beschta and Ripple 2009; Ripple *et al.* 2014).

The microencapsulation used in this work delays the manifestation of the toxic effects of levamisole, something that could be due to the fact that the formulation used to encapsulate it is dissolved only in places furthest from the mouth, somewhere in the digestive tract. This is in some way contradicted by the ability of dogs to detect it in food. Something else could have had an influence on this, and that is that the concentration of the aversive in the food was different according to the body weight of the dog.

Acknowledgments

This study is a result of CGL2013–40975-R project, from I+D+I National Plan funded by the Spanish Ministry of Economy and Competitiveness. Jorge Tobajas benefitted from a FPI PhD scholarship (BES-2014-068987) funded by the Spanish Ministry of Economy and Competitiveness. We thank Nuria García and José J. Cerón for welfare and analytical support, respectively. Thanks to Josep Ramon Ticó from the Service of Development of Medicines (Pharmacy Faculty, University of Barcelona) to help in the levamisole microencapsulation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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Fig. 1 Mean (± 1 S.E.) of proportion of food intake by four dogs in each treatment group during all pre-conditioning phase (11 trials), conditioning and reinforcement trials, and the first two post-conditioning tests. Data are expressed as the percentage of food consumed from the total food offered. The conditioning and reinforcement were did in a one-choice trial during two hours and the two-choice test across 30-min. LEV: levamisole groups; ODO: levamisole + odour group. * indicate significant differences with control group ($p < 0.05$)

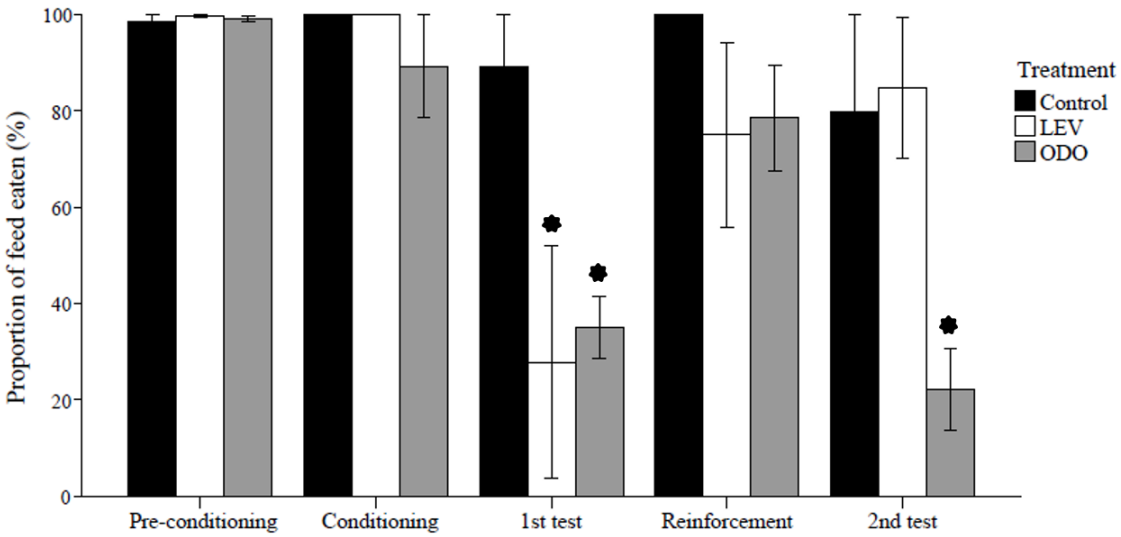

Fig. 2 Mean (± 1 S.E.) of proportion of food intake by four dogs in each treatment group during the post-conditioning phase, expressed as the percentage of food consumed from the total food offered across 30-min two-choice test. In brackets the number of taste aversion tests. LEV: levamisole groups; ODO: levamisole + odour group. * indicate significant differences with control group ($p < 0.05$).