Conditioned placebo effect in dogs decreases separation related behaviours.

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ABSTRACT

In humans, placebo effect can be produced by giving verbal information and also by conditioning when, after repeated administration of an active substance, an inactive compound that just looks like the drug administered before, can produce the effect of the active substance. Conditioned placebo effect has been reported in rodents, however, the dog (*Canis familiaris*) may also provide a promising model species. In our study dogs' behaviour was observed while they were repeatedly separated from their owners in the same unfamiliar

room. First, subjects did not receive any pre-treatment (Baseline trial), then they participated in either of two different conditioning contexts: after having received either sedative drug (Conditioned group) or non-sedating vitamin (Control group) treatment, subjects participated in 3 conditioning trials on consecutive days. Finally, in the 'Test trial', both groups were separated from their owners after receiving placebo (non-sedating vitamin). Results show significant effect of the sedative drug conditioning; when comparing the change from Baseline to Test trials in the *Conditioned* and the *Control* group, conditioned subjects showed less active signs of distress ($U_{(26)}$=48, p=0.021) and more passive behaviours ($U_{(26)}$=50, p=0.027). We also investigated the association between dogs' susceptibility to conditioned placebo effect and their expectancy bias towards positive outcomes and found a positive correlation ($r_{(12)}$= 0.697 p = 0.008), suggesting that dogs with more positive expectations are more responsive to placebo treatment. Considering previous human findings about stronger responsiveness to placebo in optimistic people, our results support the validity of the application of a dog model towards a better understanding of some aspects of the placebo phenomena in humans.

Keywords: placebo-effect, conditioning, dog, expectancy, cognitive bias

Highlights:

Dogs can be a useful model species for studying the placebo phenomenon.

Results showed evidence of the conditioned placebo-effect in dogs.

Sedative drug conditioning affects later behaviour in the Strange Situation Test.

There is a relationship between individual placebo response and positive expectancy.

1. INTRODUCTION

Investigation of the mechanisms as well as the behavioural and psychological dimensions of the placebo effect has become a burgeoning field of life sciences in the last few decades. According to the widely accepted definition, placebo is a substance or procedure that has no inherent power to produce an effect that is sought or expected (Stewart-Wiliams and Podd, 2004). The effect that placebos have can be highly variable involving both psychological and physiological changes (e.g. endogenous opiate release Petrovic et al., 2002, Wager et al., 2004).

Nevertheless, placebo effect is often conceptualized as a psychosocial context effect (Benedetti et al., 2004) involving the formation of cognitive expectancies, a process driven by verbal information from a trustworthy, certified person (Benedetti et al., 1999; 2003). Although this view would strongly suggest that placebo effects are limited to humans, experimental evidence indicates that this complex phenomenon stems from both higher mental functions and lower conditioning effects, and thus can also be studied in nonhuman subjects (see Price et al., 2008 for a review).

Increasing evidence suggests that placebo responses can be formed by classical conditioning in both humans (Voudouris et al., 1990) and different species of animals (McMillan 1999). This process is based on the association between an active substance (unconditioned stimulus) and some characteristic property of the substance (smell, taste, colour) and/or some environmental cues (places, persons, procedures, rituals) surrounding the treatment (conditioned stimuli). After repeated experience of the specific effects of the treatment, a procedure with the same features but without the active substance can produce the very same physiological and/or behavioural effects evoking a conditioned response. The induction of a placebo effect via conditioning is possible even when the effect of the treatment

is unconscious and imperceptible to the subject (e.g. change in hormone level - Benedetti et al., 2003 or immune response - Goebel et al., 2002).

In addition to rats and other laboratory rodents that are often used to demonstrate the conditioned placebo effect (see Stewart-Williams and Podd, 2004 for a review), some evidence suggests a placebo-like effect in pet dogs that have undergone veterinary treatment. However, it is important to note that in all placebo studies on dogs, assessment of the magnitude of placebo responses has been based solely on the owners' subjective evaluation; therefore, the results could be strongly influenced by the owners' expectations (Muñana et al., 2010; Jaeger et al., 2005). Although the mechanism mediating the effects of placebo treatment in dogs is still unclear, Cracknell and Mills (2008) investigated the role placebo treatment plays in overcoming fear and anxiety. They found a significant anxiolytic effect in dogs that showed excessive fear response to fireworks. This result was also based on owners' reports, so further confirmation of conclusions about the role of placebo in alleviating fear or relieving pain would require the collection of behavioural data through direct observations.

These findings are in line with the increasing evidence of dogs' human-tuned social cognitive skills (Kaminski, 2008) and support the idea that the fear/anxiety-alleviating effect of placebo treatment in dogs is a phenomenon worth investigating within the context of the dog-human social bond. It has been suggested that dogs possess a specific behaviour organising mechanism (called interspecific attachment), which evokes specific responses in stress situations related to separation from the attachment figure (see Topál and Gácsi, 2012 for a review). Separation related behaviours, the fear or dislike of isolation from the owner even in familiar environments, are frequently reported problems in pet dogs (Wright and Nesselrote, 1987). Behaviour symptoms associated with physiological changes (Palestrini et al., 2010) can be reduced by medication or behaviour therapy (Butler et al., 2011; Appleby and Plujimakers, 2004). Concerning the medication to treat anxiety disorders in dogs, Sedalin

100 is one of the widely used psychoactive drugs. Its active substance is acetylpromazine, which
101 has a tranquilizing effect (Booth, 1991) as it causes a general depression of the nervous
102 system characterised by both neuronal and behavioural changes (Tontodonati et al., 2007).

103 The most widely used experimental paradigm to study dog-human attachment and
104 separation anxiety is the Strange Situation Test (SST), which capitalizes on the tendency of
105 dogs to show specific behaviours when separated from the owner in an unfamiliar room
106 (Topál et al., 1998). In this context, efforts to re-establish the proximity (scratching the door,
107 orientation to the door, vocalisation) are typical characteristics of dogs' behaviour (e.g. Prato-
108 Previde et al., 2003; Palmer and Custance, 2008).

109 Although behavioural manifestations of separation anxiety in dogs are easy to observe
110 and behavioural symptoms of anxiety can be reduced by tranquilizers, placebo conditioning
111 studies are missing. Thus, in the first experiment of the present study we aimed to investigate
112 the role of placebo in reducing dogs' separation related distress behaviours and to determine
113 whether it is possible to produce a conditioned placebo-effect after repeated experiences of
114 the anxiolytic effects of psychoactive drug (Sedalin) treatment in the experimental situation.

115 Moreover, since responsiveness to expectancy based placebo treatment in humans is
116 positively affected by subjects' dispositional optimism (Geers et al., 2005; 2007; 2010;
117 Morton et al., 2009), in a follow up study (Experiment 2) we aimed to test whether individual
118 differences in dogs' susceptibility to the placebo effect are linked to the subjects' tendency to
119 form positive expectations about upcoming events.

120 Discrimination learning tasks are standardly used to assess positive expectation bias in
121 non-human animals (Harding et al., 2004) including rats (Burman et al., 2009), sheep (Doyle
122 et al., 2010), starlings (Bateson and Matheson, 2007), and honeybees (Bateson et al., 2011).
123 After the subjects have learned that one stimulus (sound, colour, location, etc.) is negative
124 (non-reinforced), while another one is positive (reinforced) they typically respond with higher

latency to the negative stimulus. When subjects are presented with an ambivalent stimulus (transition between negative and positive stimuli), "optimistic" subjects respond as if they were presented with the positive stimulus (Mendl et al., 2009). This method was successfully applied for dogs with location cues (Mendl et al., 2010; Müller et al., 2012) and in colour discrimination contexts (Burman et al., 2011).

In the present study we hypothesised that there would be a significant positive correlation between dogs' susceptibility to placebo conditioning (measured by the relative change in behaviour signs of distress - Experiment 1), and their positive expectation bias scores (measured by Mendl et al.'s 2010 discrimination learning task - Experiment 2).

2. MATERIALS AND METHODS

2.1. Experiment 1: Conditioned placebo effect

2.1.1. Subjects

Participants were recruited on a voluntary basis. Owners completed a brief questionnaire about their dog's behaviour during different separation situations, and those dogs that were affected in at least 3 out of the 7 contexts, and were reported to show behavioural problems (e.g. excessive barking, salivating, destructive behaviour) when left alone in an unfamiliar place were selected. An additional criterion for selection was that the dog was not taking any medication and had no known health problem. All owners were provided with adequate information about the effects of Sedalin and they signed the informed consent form to participate. However, owners were not informed of the specific aims and design of the study, and they did not know if their dogs had been given Sedalin or vitamin before the trials. The procedure was approved by the Ethical Committee for Animal

Experimentation of Eötvös University (No. XIV-I-001/521-4/2012), and conducted in accordance with the national laws regulating animal research.

Thirty-one adult (> 1 year) pet dogs were included in the experiment, but 3 owners and their dogs did not come back to all trials. The remaining 28 dogs (mean age±SD: 1.8±3.09 years, 15 males and 13 females from 13 different breeds and 13 mongrels) were tested and included in the data analysis. Subjects were randomly assigned to either the *Conditioned* or the *Control* group (N= 14-14). The two groups did not differ in their mean age ($t_{(26)}$=0.905, p=0.374), sex ratio ($\chi 2_{(1)}$=0.144, p=0.705), breed distribution ($\chi 2_{(7)}$=3.0, p=0.885), body weight ($t_{(26)}$=0.786, p=0.439), separation anxiety questionnaire score ($U_{(26)}$=84, p=0.541) and in terms of duration from baseline to test trial ($t_{(26)}$=1.047, p=0.305), and duration from the last conditioning event to test trial ($t_{(26)}$=0.0, p>0.999).

2.1.2. Experimental arrangement

The experiment took place in a room (3.9 m x 4.1 m) at the Family Dog Project lab, at Eötvös University, Budapest. Only a chair and some toys for the dog were placed in the room. Two different doors were used by the two human participants, the owner and the stranger (Figure 1). The stranger was always a woman who was unfamiliar to the dogs.

2.1.3. Procedure

Dogs participated in five trials, taking 1-4 day breaks (at least 24 hours) between them.

Baseline trial

The procedure was identical for both groups. Subjects participated in a modified and shortened version of Strange Situation Test (Topál et al., 1998). It consisted of 3 episodes, each lasting for 2 minutes. Human participants (owner and stranger) followed detailed

instructions that determined their behaviour during the test. The three episodes were preceded by a short introductory phase during which the experimenter introduced the dog and the owner to the experimental room through Door 2, and the dog was allowed to explore the room for 30 s. Then, the experimenter left the room with the owner through Door 2.

The episodes followed each other in a fixed order: the dog was 1) alone, 2) with a stranger, 3) with the owner in the experimental room.

Episode 1: Dog alone

The dog was left alone, and observed by the owner and experimenter on the monitor in the adjacent room (without speaking, thus the dog could not hear people in the adjacent room).

Episode 2: Dog & Stranger

The stranger entered the room (through Door 1), stepped up to a predetermined point (SP) and stood there for 1 minute. She adjusted her behaviour to that of the dog (petted its head and back if the dog initiated contact) and tried to keep the dog away from the doorway by playing or petting (depending on the preference of the dog). After 1 minute, she sat on the chair and stopped playing. During the second minute she was allowed to pet the dog if it initiated contact.

Episode 3: Dog & Owner

The owner entered the room through Door 2 and stepped up to a predetermined point. Meanwhile, the stranger left through Door 1. The owner then greeted and comforted the dog (petting and playing – depending on the dog's reaction). The owner stood at the predetermined point (OP) until the end of the episode, playing with and/or petting the dog if it initiated.

Conditioning trials (2-4)

In case of the three conditioning trials, 25 minutes before each trial, dogs received either a sedative drug (Sedalin Gel Oraldoser A.U.V. manufactured by Vetoquinol Biowet Sp.z.o.o., dose: 1 ml/35 kg body weight) in a piece of liverwurst (approx. 10 g, manufactured by Szegedi Paprika Zrt.) or a non-sedating vitamin formulation (dose: 1 ml/35 kg body weight, Canigest Paste manufactured by TRM Pet Products) in a piece of liverwurst. Sedalin is widely applied by veterinarians as tranquilizer and anesthetic premedicant; it shows effects in 20 minutes and lasts 6-12 hours. The vitamin did not have any effect during the experiments. Dogs received the treatment in the kitchen of the department and spent the 25 minutes there resting next to the owner.

In order to increase the saliency of 'treatment' and to facilitate the formation of associations between the physiological effects of pre-trial treatment and the unfamiliar test environment, we introduced an additional salient treatment right before the conditioning trials in both groups. The experimenter sprayed the dogs' muzzle and paws with clear water (using a hand pump spray bottle) and during the spraying she gave one more piece of liverwurst to the dog.

Conditioning trials included three episodes similar to the Baseline, however, the owner was present with the dog in all three episodes in order to avoid any possibility of separation from the owner being directly associated with the anxiolytic effects of Sedalin. Episodes 1 and 3 were identical to episode 3 in the Baseline trial. In episode 2, in contrast to the Baseline trial, the owner did not leave but was standing at the predetermined point and was allowed to interact with the dog while the stranger was in the room.

Test trial

In the test trial, all dogs were treated similarly. Both groups received placebo (vitamin treatment) in a piece of liverwurst 25 minutes before the trial. Their muzzles/paws were

sprayed with water and they received one more piece of liverwurst right before the trial (Table 1). The procedure of this trial was identical to that described in the Baseline.

After the conditioning trials and test trial the owners' opinion about the type of treatment (Sedalin or placebo) their dogs received was asked.

2.1.4. Behaviour coding

As behaviours related to separation anxiety are typically displayed close to the exit/entry door (see e.g. Prato-Previde et al. 2003, Palmer and Custance 2008, Palestrini et al. 2005), we recorded the durations of anxiety-related behaviours while staying close (< 1 m) to the doors. The two doors were not differentiated because both could be considered as a potential exit by the dogs. On the other hand to examine the sedative effect of the drug the time spent passively was also measured. Relative durations were recorded for both variables.

Definitions of the behaviour categories:

Passive behaviours: standing, sitting or lying down anywhere but at the door while alone (PASS-A), in the presence of the stranger (PASS-S), or in the presence of the owner (PASS-O).

Door-distress: displaying behavioural signs of distress while staying close to the door; active behaviours resulting in physical contact with the door (scratching, jumping at etc.) and/or vocalising (i.e. barking, growling, howling, whining) in the close proximity (< 1 m) of the door while alone (D/DISTR-A), in the presence of the stranger (D/DISTR-S), or in the presence of the owner (D/DISTR-O).

Door-passive: staying (standing, sitting, or lying down) in the close proximity of the door (< 1 m) without physical contact with it, and/or vocalisation while alone (D/PASS-A), in the presence of the stranger (D/PASS-S), or in the presence of the owner (D/PASS-O).

Inter-observer agreement was assessed by parallel evaluation of the behaviour of 20% of the total sample by two independent coders who were blind to the conditions. The analysis of inter-observer agreement yielded a very good inter-observer reliability (Cohen's kappa values; PASS: 0.92, D/DISTR: 0.87, D/PASS: 0.91).

2.1.5. Data analysis

The relative percentage of the time spent in the above behaviours was calculated for the statistical analyses. Variables did not have Gaussian distribution (Kolmogorov Smirnoff test). At first we analysed the data with Generalized Estimating Equation (GEE) which is an extension of the GLM algorithm to accommodate the modelling of repeated measurement following non-normal distribution (Hardin & Hilbe, 2003). We employed a GEE analysis to examine the effect of the trial (1^{st}, 2^{nd}, 3^{rd} conditioning and test trials) as within-subject factor and the effect of the group (conditioned vs control) as between subject-factor on the owners' opinion about the treatment. GEE analysis was also employed to examine the effect of the repetition (1^{st}, 2^{nd} and 3^{rd} conditioning trials) as within-subject factor and the effect of the pre-treatment (administering Sedalin vs. vitamin) as between subject-factor on passive behaviour of dogs during the Conditioning trials. To analyse the effect of the conditioning we used GEE analysis to examine the effect of the trial (baseline vs. test) as within-subject factor and the effect of the pre-treatment (administering Sedalin vs. vitamin) during the Conditioning trials as between subject-factor on the dogs' behaviour. When GEE analysis revealed significant trial x treatment interaction, we calculated the change in the dogs' behaviours from Baseline to Test trials. We assumed that the difference in the relative durations of separation distress related behaviours expressed by the Sedalin conditioned dogs would be an indicator of subjects' susceptibility to the placebo effect. We subtracted the relative duration (time%) of a given behaviour in Baseline from the relative duration of that behaviour in Test trial. The

273 'difference values' of the *Conditioned* and *Control* groups were compared with Mann-
274 Whitney U tests.
275 SPSS version 18 software was used for statistical analyses.
276
277 **2.2. Experiment 2: Cognitive bias**
278
279 2.2.1. Subjects
280 Twenty-one dogs (mean age±SD: 3.3±2.02 years, 11 males and 10 females, from 9
281 different breeds and 8 mongrels) from the 28 subjects that participated in Experiment 1 were
282 called back for Experiment 2, 1-26 months after the first experiment. (One dog from the
283 Conditioned group and six dogs from the Control group of Experiment 1 were not available
284 any more.)
285
286 2.2.2. Procedure
287 The procedure was based on the study of Mendl et al. (2010). The Cognitive Bias Test
288 was conducted in the same room as Experiment 1, the owner and an experimenter were
289 present with the dog throughout the test. At the start of each trial, the owner led the dog to the
290 starting position while the experimenter, standing behind the dog and the owner, baited (or
291 did not bait, depending on trial type) a plastic pot (11cm high, 14 cm in diameter) with a piece
292 of sausage (see Figure 5).
293
294 Training trials
295 Dogs were first trained that, when the pot was placed at one ('positive'- P) location, it
296 contained food, and when it was placed at another ('negative'- N) location, it was empty. The
297 locations were equidistant from the dog. For 11 dogs, P location was on the right hand side,

and for 10 dogs it was on the left. The training always started with four warm up trials; two P trials (baited pot placed at the P location), when dogs could see the baiting, and two N trials (non-baited pot placed at the N location), in which the experimenter showed the empty container to the dog.

Subsequently, P and N training trials were presented in a pseudorandom order, with no more than two trials of the same type being presented consecutively. Importantly however, in these trials, dogs were prevented from witnessing whether the container was baited or not, since the experimenter baited (or not) the pot behind the dog while the owner gently prevented the dog from looking back. When the experimenter had placed the pot and returned to her position behind the owner, the dog was released and allowed to choose. Owners were allowed to encourage their dog (saying "You can go!"). Training trials continued until the latency for each of the last five N trials was longer than any of the latencies for the last five P trials. After the dog had reached this learning criterion, the test trials began.

Test trials

Testing began once the learning threshold was achieved. Test trials were identical to training trials except that in three cases the empty pot was placed at the 'ambivalent' location (A) equally spaced between the P and N locations (see Figure 5). The ambiguous trials were followed by one P and one N trial (9 trials in total; e.g.: APN, ANP, APN) in random order.

The purpose of the test trials was to investigate how dogs responded to the ambivalent location and whether they tended to approach them with a speed more similar to that at P location (indicating anticipation of a food reward – an 'optimistically' biased judgement of the ambivalent cue) or N location, that is, more slowly (indicating lower anticipation of food – a 'pessimistically' biased judgement).

2.2.3. Data analysis

Considering the wide range of time that elapsed between Experiment 1 and 2, we checked the data for any association with this duration (Pearson correlation test) to determine if the conditioning of the subjects might have had an effect on the expectancy scores.

The latency to reach the pot was defined as the time that elapsed between being released by the owner and the moment the dog put its head into the pot, or touched it with its nose. Latency was recorded for each trial. If the dog did not approach the container within 30 s, the trial was terminated, a latency of 30 s was allocated, and the next trial was initiated. Mean latencies followed normal distribution (Kolmogorov-Smirnov test).

Based on the study by Mendl et al. (2010), a *positive expectancy score* was calculated for each dog. That is, we adjusted each dog's mean ambivalent trial latencies ($M_{lat}A$) by taking into account its mean 'baseline' latencies to get to the positive ($M_{lat}P$) and negative ($M_{lat}N$) locations during the test phase as follows:

$$\text{positive expectancy score} = \frac{(MlatN - MlatA)}{(MlatN - MlatP)} \times 100$$

Higher scores indicate stronger positive expectancies. Positive expectancy scores followed normal distribution (Kolmogorov-Smirnov test).

Based on the results of Experiment 1, the individual placebo response could be best indicated by the relative change in the door-distress variable in Episode 1 (D/DISTR-A in the Baseline vs. Test trial). Higher relative changes are supposed to represent stronger placebo responses so the relative change of this value was calculated for each dog.

As the relationship between the placebo response values and positive expectancy scores was not linear, a logarithmic transformation was made on the placebo response values, thus the relationship could be analysed with Pearson-correlation.

3. RESULTS

3.1. Experiment 1: Conditioned placebo effect

3.1.1. Dogs' behaviour during the Conditioning trials

As the owners were present in the experimental room throughout these trials, it is not surprising that only few dogs (4 in the 'Conditioned' and 3 in the 'Control' groups) displayed any behavioural signs of distress. Dogs spent hardly any time with distress behaviours; on average 0.5% (Sedalin group) and 0.65% (Control group) of the total duration, and, this remained extremely low even after repeated trials (0.2-1% of time during the 1st, 2nd and 3rd conditioning trials in both groups). However, dogs spent much more time with passive behaviours (on average 31 and 28% in the Conditioned and the Control groups respectively) and there was no effect of repetition (treatment: χ^2= 0.2, p=0.655; repetition: χ^2=4.796, p=0.091).

3.1.2. Owners' evaluation of treatment effects

Although we did not find significant effects of Sedalin treatment on the recorded behaviour variables, the owners in the conditioned group thought more often compared to the control group that their dog received Sedalin gel in the conditioning trials (GEE analysis, group effect: χ^2=4.023, p=0.045; trial effect: χ^2=5.973, p=0.113; interaction: χ^2=2.816, p=0.421).

3.1.3. Dogs' behaviour in the Test vs. Baseline trials: the effects of conditioning

Separation episode (Episode 1)

During the separation episode dogs' passive behaviour was influenced by interaction between the trial and treatment (GEE, χ^2=6.537, p=0.011) with no significant main effects of the factors (trial: χ^2=0.356, p=0.551; treatment: χ^2=0.016, p=0.901). The change from

373 Baseline to Test trials in the *Conditioned* group was positive and significantly different from
374 the slight negative change in the *Control* group (Mann-Whitney test, $U_{(26)}$=50, p=0.027)
375 (Figure 2). Concerning passive behaviours close to the door, however, GEE analysis did not
376 show significant main effects or interaction (trial: χ^2=0.239, p=0.625; treatment: χ^2=0.017,
377 p=0.896; interaction: χ^2=1, p=0.317). The analysis of behavioural signs of distress close to the
378 door showed a significant interaction between the trial and treatment (GEE, χ^2=4.66, p=0.031)
379 with no main effects of trial (Baseline vs. Test: χ^2=0.001, p=0.985) or treatment (Sedaline vs.
380 Vitamin: χ^2=0.481, p=0.488) (Figure 3). We found significant difference between changes in
381 the *Conditioned* and the *Control* group (Mann-Whitney test, $U_{(26)}$=48, p=0.021; Figure 4).
382 Results of the separation episode are summarized in Table 2.
383
384 Episodes 2 and 3
385 There were no significant main effects or interactions for any of the behaviour
386 variables in those episodes when the owner or the experimenter was present (GEE analyses,
387 PASS-S: trial: χ^2=0.232, p=0.627; treatment: χ^2=0.052, p=0.819; interaction: χ^2=0.609,
388 p=0.435; D/PASS-S: trial: χ^2=0.061, p=0.804; treatment: χ2=0.551, p=0.458; interaction:
389 χ^2=0.055, p=0.815; D/DISTR-S: trial: χ^2=0.069, p=0.793; treatment: χ^2=2.667, p=0.102;
390 interaction: χ^2=1.736, p=0.188; PASS-O: trial: χ^2=2.291, p=0.130; treatment: χ^2=0.657,
391 p=0.418; interaction: χ^2=1.863, p=0.172; D/PASS-O: trial: χ^2=0.716, p=0.398; treatment:
392 χ^2=0.344, p=0.558; interaction: χ^2=2.270, p=0.132); D/DISTR-O: trial: χ^2=0.905 p=0.342;
393 treatment: χ^2=0.816, p=0.366; interaction: χ^2=1.249, p=0.264).
394 These results show that the two types of treatment during the conditioning phase of the
395 experiment affected dogs' later behaviour differently. After having received treatment with
396 placebo (non-sedating vitamin) before the Test trial, the behaviour of dogs in the 'dog alone'

episode depended on whether they had been treated with sedative substances during the conditioning phase.

3.2. Experiment 2: Cognitive bias

Subjects reached the training criterion on average after 30 trials (range 12-57 trials), and P and N locations were strongly differentiated also in the test trials; dogs approached the plastic pot sooner in P than in N type test trials (paired sample t-test, $t_{(20)}=4.036$ $p<0.001$). The positive expectancy scores ranged from -12.36 to 1179.5 (mean ± SD: 124.67 ± 243.79). There was no association between the time elapsed since the conditioning of the dogs in Experiment 1 and the expectancy scores (Pearson correlation test, $r_{(20)}=0.335$ $p=0.149$). We revealed a significant positive relationship between the positive expectancy scores and placebo response values in case of the conditioned group (Pearson correlation test, $r_{(12)}= 0.697$ $p = 0.008$, Figure 6) but not in the control group ($r_{(7)}= 0.268$ $p = 0.521$).

These results indicate an association between 'cognitive bias' and 'susceptibility to placebo conditioning' measures in dogs, suggesting that dogs that have stronger positive expectancies (are more "optimistic") tend to be more responsive to the stress relieving effects of placebo treatment after conditioning with an active substance.

4. DISCUSSION

Our results provide the first behavioural evidence in dogs for the development of a conditioned placebo effect, an effect that is well-known in humans (Bendetti et al., 2003; Goebel et al., 2002) and in laboratory animals (Isaac and Isaac, 1976). In the two experimental groups (repeated treatment with sedative drug vs. non-sedating vitamin) we

observed opposite trends of changes in separation anxiety related behaviours. The effects of sedative drug conditioning manifested itself via increased passivity and decreased duration of behavioural signs of distress displayed close to the door. In contrast, dogs in the control group showed an opposite tendency in these responses. Considering that using a double dose of Acepromazine (compared to our design), Tontodonati et al. (2007) could not find any physiological or behavioural effects 16 hours after the treatment, long-term effects of acetylpromazin (Sedalin) are unlikely to explain the behaviour changes of the *Conditioned* group.

Importantly, owners were present throughout the conditioning trials in order to avoid any possibility of creating direct association between the separation from the owner and the anxiolytic effects of Sedalin. During the conditioning trials dogs had the opportunity to learn about the 'relaxed nature' of the environment but they had no opportunity to learn how to cope with separation distress under the influence of Sedalin. This procedure was designed to eliminate the possibility that dogs develop reduced behaviour signs of distress as a conditioned response. In the test trial only one aspect of the conditioning environment was changed: the presence/absence of the owner. In this new context the associative memory traces regarding the anxiolytic effects of Sedalin could have been mediated by the procedural aspects of the placebo administration and/or by the cues of the testing environment.

Our finding fits neatly into the placebo conditioning framework (McMillan 1999); therefore we assume that the repeated experience with the effects of Sedalin, as an unconditioned stimulus, could have resulted in the formation of a relaxed inner state, which was associated with some characteristic property of the pre-treatment procedure and/or with some environmental cues of the experimental set up as conditioned stimuli. As a result of this associative process, treatment procedure with the same features but without administration of Sedalin could reduce some behavioural signs of separation distress. It is worth mentioning

447 that we found no relevant differences between the *Conditioned* and *Control* groups in those
448 episodes of Test trial in which the owner or the experimenter were present (Episodes 2 and 3).
449 This suggests that the placebo effect, as a conditioned response, was specifically associated
450 with the separation from the owner, despite the fact that separation anxiety was not triggered
451 during conditioning trials where dogs were not separated.
452 These findings are in line with the notion that a wide range of placebo phenomena, even in
453 humans, is often nothing more than "contextual healing" (Miller and Kaptchuk, 2008; Di
454 Blasi and Kleijnen, 2003) because, in addition to the medicine or treatment, the situational
455 context of the healing (environmental cues and the ritual of the treatment) can also play a
456 crucial role in the process (Kaptchuk, 2002).
457 The significant conditioning effect in the Sedalin group was evident even though our
458 placebo conditioning method had some practical limitations. The liverwurst might not be an
459 ideal specific signal for the sedative drug, and the late sedative effect might also impair the
460 formation of an association. We hoped to overcome these potential problems using the water
461 spray procedure. In fact, spraying the dogs' muzzle and paws with water can be perceived as a
462 salient and unusual stimulus event that could potentially be a key component of R-S learning
463 during the conditioning phase, and thus a good mediator of the placebo effect. Using more
464 stimuli, we cannot assess to what extent the different components of the treatment triggered
465 the placebo effect, because any combination of them could be associated with the sedative
466 state. The effect of the Sedalin gel could also vary among and even within subjects.
467 Additionally, a relatively long time passed between the baseline and the test trials and there
468 were relatively few, only three, conditioning trials (we should note that the number of trials
469 affects the placebo-response in case of humans, see e.g. Colloca et al., 2010). Although
470 owners had no preliminary information about which type of treatment their dogs received, we
471 cannot exclude that they had some expectation regarding the treatment. However, since

owners were not present during the separation episode, this could have an indirect (if any) effect on the dogs' behaviour.

Despite the above-mentioned potential confounding factors, our results provide strong support for the existence of a conditioned placebo effect in dogs because the assessment of the behavioural change was based on behaviour observations and not on the owners' report (c.f. Munana et al., 2010; Jaeger et al., 2005; Cracknell and Mills, 2008). It is also worth mentioning that our findings concerning the conditioned placebo effect in alleviating separation anxiety have some veterinary implications and can be used to improve owners' and their dogs' daily life. Severe cases of separation anxiety often require the use of medications in addition to a behaviour modification program. Once the desired effect is achieved, the dose of the medicine may be gradually reduced and finally merely the procedure can maintain the effect. However, so far the administration method of the medicine has not been considered as important. Our results suggest that applying a specific regimen, that is, administrating the medicine always with the same environmental cues, for example with the same specific food type and with a set ritual, the real medicine can later be effectively replaced by placebo. As the anxiety relieving effect of placebo conditioning in dogs is of great applied importance, more research is needed to get a better perspective on the most efficient aspects of the treatment and the situational context that contributes to the manifestation of the placebo effect.

The results of Experiment 2 expand our knowledge on placebo conditioning in dogs and highlight the potential importance of expectancy bias on the formation of placebo responses. The finding that dogs that were more responsive to the placebo treatment tended to show stronger positive expectancy in an ambivalent situation seems to be consistent with the conclusions of human studies (Geers et al., 2005; 2007; 2010; Morton et al., 2009). Importantly however, these human studies investigated the expectancy based and not the

497 conditioned placebo effect. Although it remains unclear whether conscious learning (Stewart-
498 Williams and Podd, 2004; Kirsch, 1985) or some 'cognitively blind' physiological response
499 plays a more prominent role in the observed placebo effect, the association between dogs'
500 positive expectancy scores and the magnitude of placebo-induced responses suggests that the
501 observed placebo effect could not be entirely explained by unconscious factors.

502 In sum, the combined results of the two experiments open the door for studying the
503 mechanism of placebo responses in the dog in its own right and provide further support for
504 the validity of the application of the dog as a model species towards a better understanding of
505 some aspects of the placebo phenomena in humans.

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507

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509

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514

515 **References**

516

517 Appleby, D., Pluijmakers, J. 2004. Separation Anxiety in Dogs: The Function of Homeostasis
518 in its Development and Treatment. Veterinary Clinics of North America: Small Animal
519 Practice. 33, 321-344.

520 Bateson, M., Matheson, S.M. 2007. Performance on a categorisation task suggests that
521 removal of environmental enrichment induces “pessimism” in captive European starlings
522 (Sturnus vulgaris). Anim. Welfare 16, 33–36.
523 Bateson, M., Desire, S., Gartside, S.E., Wright, G. 2011. Agitated honeybees exhibit
524 pessimistic cognitive biases. Curr. Biol. 21, 1070–1073.
525 Benedetti, F., Colloca, L. 2004. Placebo-induced analgesia: methodology, neurobiology,
526 clinical use, and ethics. Rev. Analg. 7, 129–143.
527 Benedetti, F., Arduino, C., Amanzio, M. 1999. Somatotopic activation of opioid systems by
528 target-directed expectations of analgesia. Journal of Neuroscience. 19, 3639 –3648.
529 Benedetti, F., Pollo, A., Lopiano, L., Lanott, M., Vighetti, S. et al. 2003. Conscious
530 expectation and unconscious conditioning in analgesic, motor, and hormonal
531 placebo/nocebo responses. J. Neurosci. 23, 4315– 4323.
532 Booth, N.H., Mc Donald, L.E. 1991. Veterinary pharmacology and therapeutics. 6th ed.
533 Ames, IOWA.
534 Burman, O.H.P., Parker, R.M., Paul, E.S., Mendl, M.T. 2009. Anxiety-induced cognitive bias
535 in non-human animals. Phys. Behav. 98, 345–50.
536 Burman, O., McGowan, R., Mendl, M., Norling, Y., Paul, E. and others 2011. Using
537 judgement bias to measure positive affective state in dogs. Applied Animal Behaviour
538 Science 132, 160–168.
539 Butler, R., Sargisson, R.J., Elliffe, D. 2011. The efficacy of systematic desensitization for
540 treating the separation-related problem behaviour of domestic dogs. Appl. Anim. Behav.
541 Sci. 129, 136–145.
542 Colloca, L., Petrovic, P., Wager, T.D., Indvar, M., Benedetti, F. 2010. How the number of
543 learning trials affects placebo and nocebo responses. Pain 151, 430–439.

544 Cracknell, N.R., Mills, D.S. 2008. A double-blind placebo-controlled study into the efficacy
545 of a homeopathic remedy for fear of firework noises in the dog (Canis familiaris). Vet. J.
546 177, 80–88.
547 Di Blasi, Z., Kleijnen, J. 2003. Context effect: Powerful Therapies or Methodological Bias?
548 Evaluation & the Health Professions. 26, 166.
549 Doyle, R.E., Vidal, S., Hinch, G.N., Fisher, A.D., Boissy, A. and others 2010. The effect of
550 repeated testing on judgement biases in sheep. Behav. Proc. 83, 349–352.
551 Geers, A.L., Helfer, S.G.,Weiland, P.E., Kosbab, K., Landry, S.J. 2005. Reconsidering the
552 role of personality in placebo effects: Dispositional optimism, situational expectations,
553 and the placebo response. J. Psychosom. Res. 58, 121-127.
554 Geers, A.L., Kosbab, K., Weiland, P.E., Helfer, S.G., Welman, J.A. 2007. Further evidence
555 for individual differences in placebo responding: An interactionist perspective. J.
556 Psychosom. Res. 62, 563-570.
557 Geers, A.L., Wellman, J.A., Fowler, S.L., Helfer, S.G., France, C.R. 2010. Dispositional
558 Optimism Predicts Placebo Analgesia. J. Pain 11, 1165-1171.
559 Goebel, M.U., Trebst, A.E., Steiner, J., Xie, Y.F., Exton, M.S. and others 2002. Behavioral
560 conditioning of immunosuppression is possible in humans. FASEB Journal. 16, 1869-
561 1873.
562 Hardin, J., Hilbe, J. 2003. Generalized Estimating Equations. London: Chapman and
563 Hall/CRC. ISBN 978-1-58488-307-4.
564 Harding, E.J., Paul, E.S., Mendl, M. 2004. Cognitive bias and affective state. Nature 427, 321.
565 Jaeger, G.T., Larsen, S., Moe, L. 2005. Stratification, blinding and placebo effect in a
566 randomized, double blind placebo-controlled clinical trial of gold bead implantation in
567 dogs with hip dysplasia. Acta Vet. Scand. 46, 57–68.

568 Kaminski, J. 2008 Dogs (Canis familiaris) are adapted to receive human communication. In:
569 Berthoz A, Christen Y (Eds.). Neurobiology of "Umwelt": how living beings perceive the
570 world. Research and perspectives in neurosciences. Springer Verlag, Berlin, pp. 103-108.
571 Kaptchuk, T.J. 2002. The Placebo Effect in Alternative Medicine: Can the Performance of a
572 Healing Ritual Have Clinical Significance? Annals of Internal Medicine. 136, 817-825.
573 Kirsch, I. 1985. Response Expectancy as a Determinant of Experience and Behavior. Am.
574 Psychol. 40, 1189-1202.
575 McMillan, F.D. 1999. The placebo effect in animals. JAVMA 215, 992-999.
576 Mendl, M., Burman, O.H.P., Parker, R.M., Paul, E.S. 2009. Cognitive bias as an indicator of
577 animal emotion and welfare: Emerging evidence and underlying mechanisms. Appl.
578 Anim. Behav. Sci. 118, 161–181.
579 Mendl, M., Brooks, J., Basse, C., Burman, O., Paul, E. and others 2010. Dogs showing
580 separation-related behaviour exhibit a " pessimistic " cognitive bias. Curr. Biol. 20, 839–
581 840.
582 Miller, F.G., Kaptchuk, T.J. 2008. The power of context: reconceptualizing the placebo effect.
583 J. Roy. Soc. Med. 101, 222–225.
584 Morton, D.L., Watson, A., El-Deredy, W., Jones, A.K.P. 2009. Reproducibility of placebo
585 analgesia: Effect of dispositional optimism. Pain 146, 194–198.
586 Müller, C.A., Riemer, S., Rosam, C.M., Schößwender, Range, F., Huber, L. 2012 Brief owner
587 absence does not induce negative judgement bias in pet dogs. Anim. Cogn. 15, 1031-
588 1035.
589 Muñana, K.R., Zhang, D., Patterson, E.E. 2010. Placebo Effect in Canine Epilepsy Trials. J.
590 Vet. Int. Med. 24, 166–170.
591 Palestrini, C., Minero, M., Cannas, S., Rossi, E., Frank, D. 2010. Video analysis of dogs with
592 separation-related behaviors. Appl. Anim. Behav. Sci. 124, 61–67.

593 Palestrini, C., Prato Previde, E., Spiezio, C., Verga, M. 2005. Heart rate and behavioural
594 responses of dogs in the Ainsworth's Strange Situation: A pilot study. Appl. Anim.
595 Behav. Sci. 94, 75–88.
596 Palmer, R., Custance, D. 2008. A counterbalanced version of Ainsworth's Strange Situation
597 Procedure reveals secure-base effects in dog–human relationships. Appl. Anim. Behav.
598 Sci. 109, 306–319.
599 Prato-Previde, E., Custance, D.M., Spiezio, C., Sabatini, F. 2003. Is the dog–human
600 relationship an attachment bond? An observational study using Ainsworth's Strange
601 Situation. Behaviour 140, 225–254.
602 Price, D.D., Finniss, D.G., Benedetti, F. 2008. A comprehensive review of the placebo effect:
603 Recent advances and current thought. Ann. Rev. Psychol. 59, 565–90.
604 Petrovic, P., Kalso, E., Petersson, K.M., Ingvar, M. 2002. Placebo and opioid analgesia—
605 imaging a shared neuronal network. Science 295, 1737–1740.
606 Stewart-Williams, S., Podd, J. 2004. The placebo effect: dissolving the expectancy versus
607 conditioning debate. Psychol. Bull. 130, 324-340.
608 Tontodonati, M., Fasdelli, N., Moscardo, E., Giarola, A., Dorigatti, R. 2007. A canine model
609 used to simultaneously assess potential neurobehavioural and cardiovascular effects of
610 candidate drugs. J. Pharm. Tox. 56, 265–275.
611 Topál, J., Gácsi, M. 2012. Lessons we should learn from our unique relationship with dogs: an
612 ethological approach. In: Birke L, Hockenhull J (Eds.). Crossing boundaries. Brill
613 Academic Press Leiden the Netherland pp. 163-187.
614 Topál, J., Miklósi, Á., Csányi, V., Dóka, A. 1998. Attachment Behavior in Dogs (Canis
615 familiaris): A New Application of Ainsworth's (1969) Strange Situation Test. J. Comp.
616 Psychol. 112, 219-229.

617 Voudouris, N.J., Peck, C.L., Coleman, G. 1990. The role of conditioning and verbal
618 expectancy in the placebo response. Pain 43, 121–128.
619 Wager, T.D., Rittling, J.K., Smith, E.E., Sokolit, A., Casey, K.L. and others 2004. Placebo-
620 induced changes in fMRI in the anticipation and experience of pain. Science 303, 1162–
621 1167.
622 Wright, J.C., Nesselrote, M.S. 1987 Classification of Behavior Problems in Dogs:
623 Distributions of Age, Breed, Sex and Reproductive Status. Applied Animal Behaviour
624 Science 19, 169-178.
625

626 Table 1

	Baseline trial	Conditioning (trials 2-4)	Test trial
Conditioned group (N=14)	*Separation* No pre-treatment	*No separation* Sedative pre-treatment (Sedalin) Water spray	*Separation* Non-sedating pre-treatment (vitamin) Water spray
Control group (N=14)		*No separation* Non-sedating pre-treatment (vitamin) Water spray	

627

628 **Table 1. Experimental design of Experiment 1.**

629 All types of pre-treatments contained the additional water spraying and a piece of liverwurst
630 right before the trials.

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640 Table 2

	GEE analysis (based on raw data)			Mann-Whiney test (change from baseline to test trial)
	trial (baseline vs. test)	treatment group (conditioned vs. control)	trial x treatment interaction	conditioned vs. control group
D/distr-A	χ^2=0.001, p=0.985	χ^2=0.481, p=0.488	χ^2=4.66, p=0.031	$U_{(26)}$=48, p=0.021
PASS-A	χ^2=0.356, p=0.551	χ^2=0.016, p=0.901	χ^2=6.537, p=0.011	$U_{(26)}$=50, p=0.027
D/PASS-A	χ^2=0.239, p=0.625	χ^2=0.017, p=0.896	χ^2=1, p=0.317	$U_{(26)}$=79, p=0.401

641

642 Table 2. Summary of the statistical analyses (separation episode, Experiment 1).

643

Figure captions

Figure 1. Schematic layout of the experimental arrangement in Experiment 1.

A chair and some toys were present in the experimental room. Door 1 was used by the stranger to enter; Door 2 was used by the owner to enter. The areas near the door are indicated with broken lines. SP & OP were places marked with adhesive tape on the floor where the stranger (SP) and the owner (OP) stood (see Procedure).

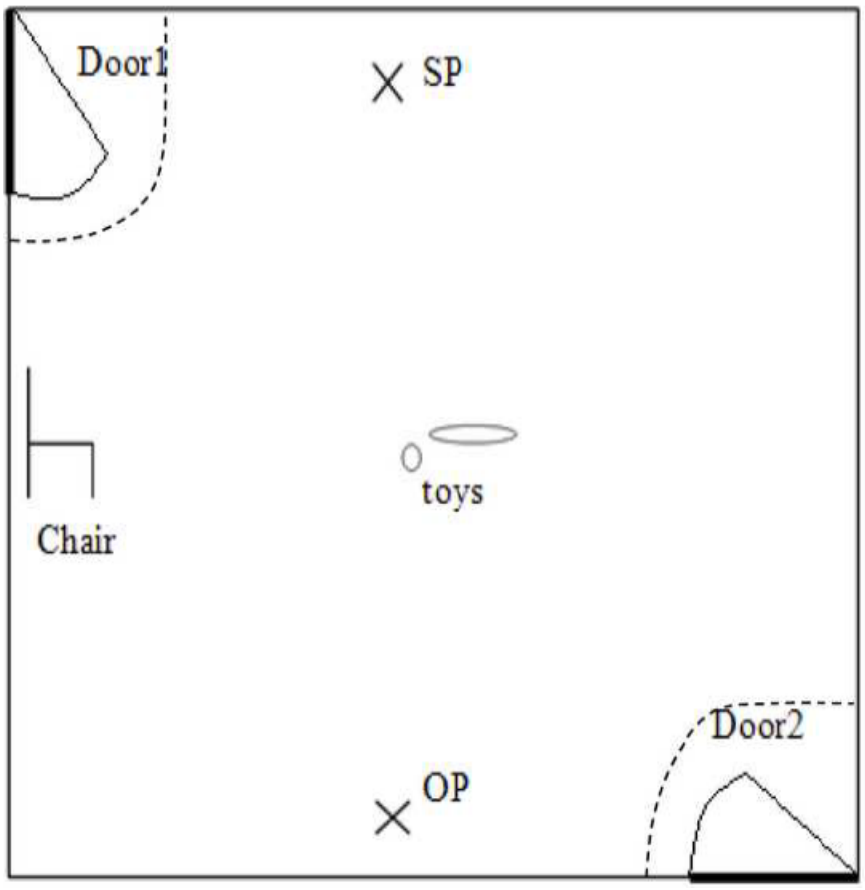

Figure 2. Relative duration of passive behaviours in Episode 1

Dogs in the two groups showed different changes in passive behaviour after the conditioning. * indicates significant ($p < 0.05$) trial (Baseline vs. Test) x treatment (administering Sedalin vs. vitamin) interaction.

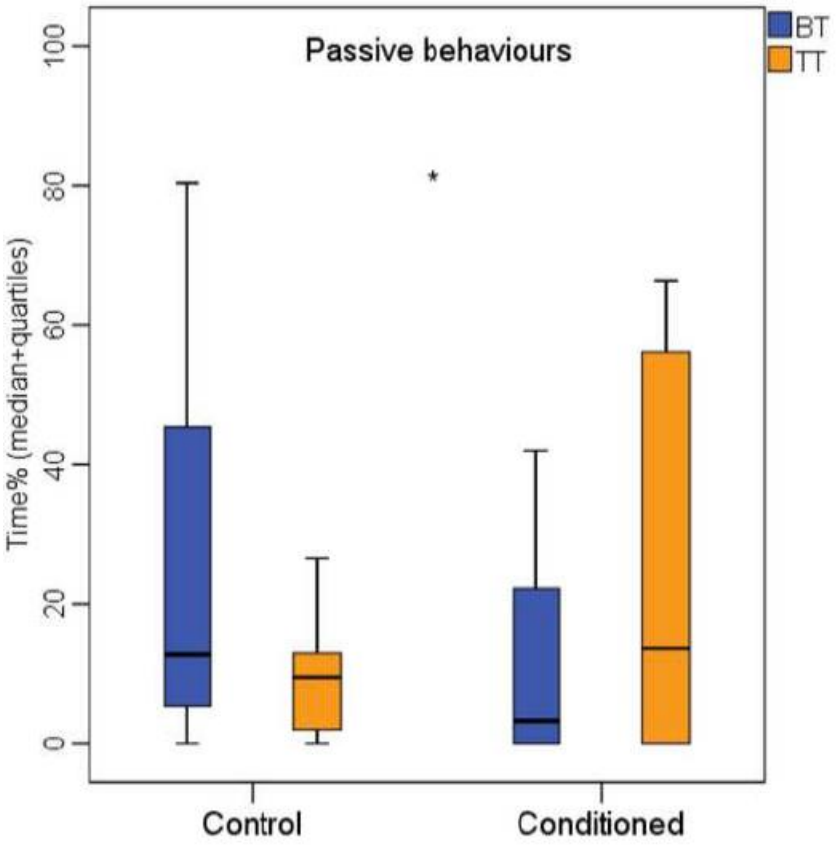

Figure 3. Relative duration of distress close to the door in Episode 1

Dogs in the two groups showed different changes in distress signs close to the door after the conditioning. * indicates significant ($p < 0.05$) trial (Baseline vs. Test) x treatment (administering Sedalin vs. vitamin) interaction.

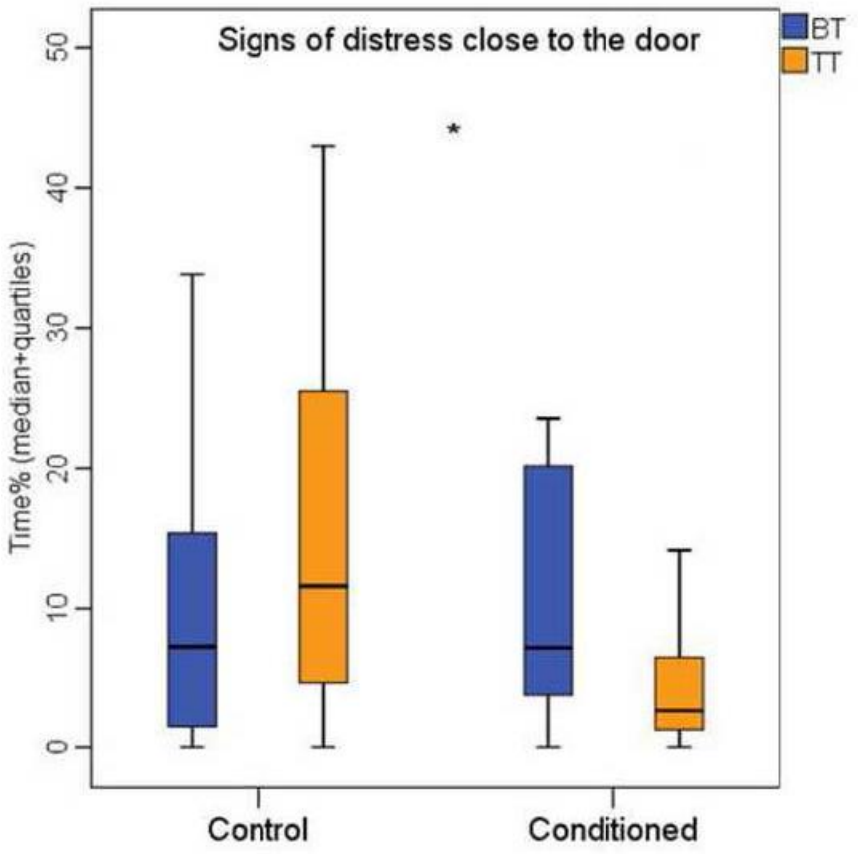

Figure 4. Difference values of sign of distress close to the door

Dogs in the Conditioned group have higher difference scores (compared to the Control group), which represent higher placebo response (higher change in distress). * indicates significant ($p < 0.05$) between group difference (median+quartiles+outlier data).

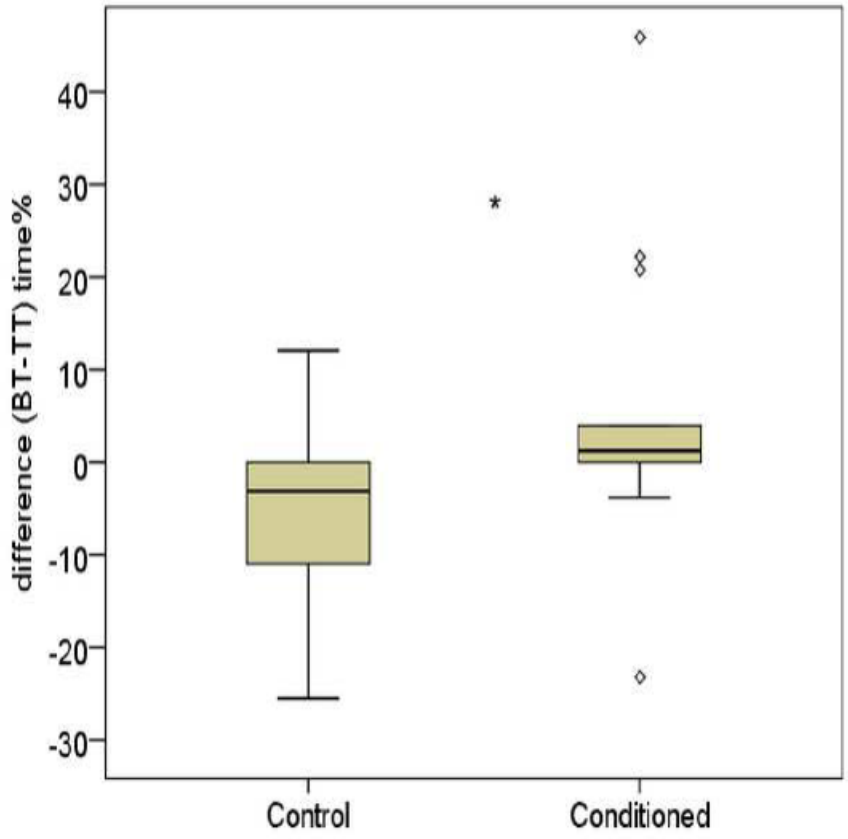

Figure 5. Experiment 2: Arrangement of the cognitive bias test.

The experimenter standing behind the dog and the owner baited (or did not bait, depending on trial type) a plastic pot with a piece of sausage. Then she placed the food bowl at one of three pre-determined locations (negative, ambivalent, positive), then she went behind the owner, and the dog was released to approach the bowl.

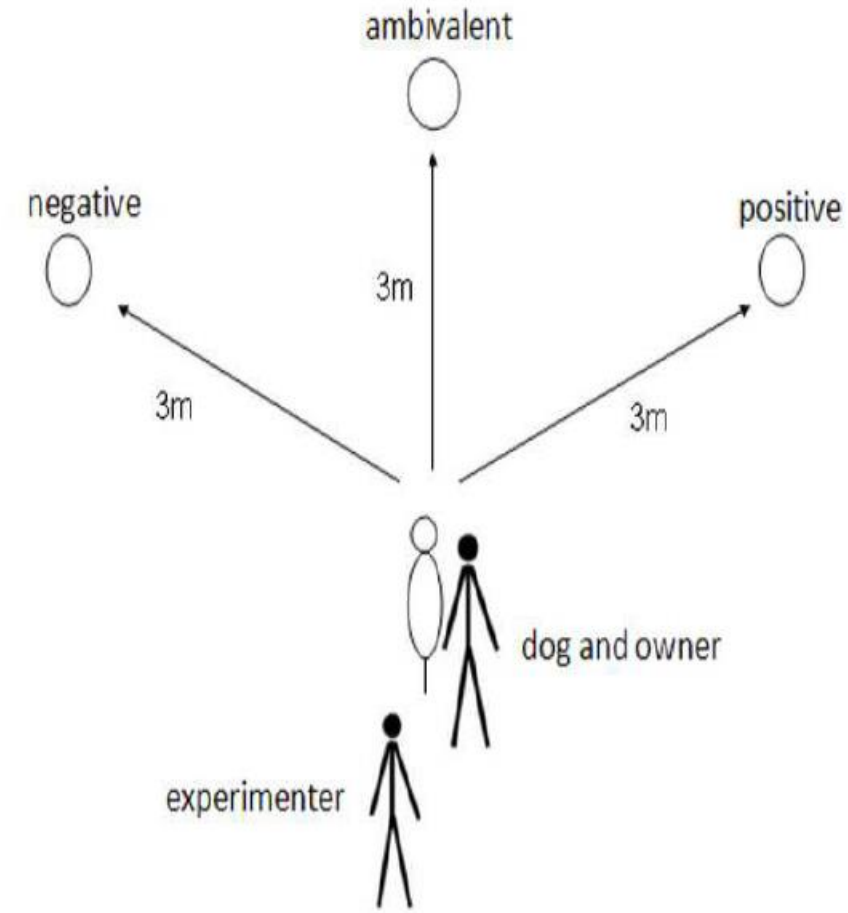

Figure 6. Relationship between the individual placebo response and positive expectancy

There is a logarithmic relationship between the positive expectancy scores and the placebo response values (r=0.697 p=0.008) in the conditioned group.