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6 Conditioned placebo effect in dogs decreases separation related behaviours.

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17

18 **ABSTRACT**

19

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

26 room. First, subjects did not receive any pre-treatment (Baseline trial), then they participated
27 in either of two different conditioning contexts: after having received either sedative drug
28 (Conditioned group) or non-sedating vitamin (Control group) treatment, subjects participated
29 in 3 conditioning trials on consecutive days. Finally, in the ‘Test trial’, both groups were
30 separated from their owners after receiving placebo (non-sedating vitamin). Results show
31 significant effect of the sedative drug conditioning; when comparing the change from
32 Baseline to Test trials in the *Conditioned* and the *Control* group, conditioned subjects showed
33 less active signs of distress ($U_{(26)}=48$, $p=0.021$) and more passive behaviours ($U_{(26)}=50$,
34 $p=0.027$). We also investigated the association between dogs’ susceptibility to conditioned
35 placebo effect and their expectancy bias towards positive outcomes and found a positive
36 correlation ($r_{(12)}= 0.697$ $p = 0.008$), suggesting that dogs with more positive expectations are
37 more responsive to placebo treatment. Considering previous human findings about stronger
38 responsiveness to placebo in optimistic people, our results support the validity of the
39 application of a dog model towards a better understanding of some aspects of the placebo
40 phenomena in humans.

41

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

43

44 Highlights:

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

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

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

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

49

50 **1. INTRODUCTION**

51

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

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

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

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

77 In addition to rats and other laboratory rodents that are often used to demonstrate the
78 conditioned placebo effect (see Stewart-Williams and Podd, 2004 for a review), some
79 evidence suggests a placebo-like effect in pet dogs that have undergone veterinary treatment.
80 However, it is important to note that in all placebo studies on dogs, assessment of the
81 magnitude of placebo responses has been based solely on the owners' subjective evaluation;
82 therefore, the results could be strongly influenced by the owners' expectations (Muñana et al.,
83 2010; Jaeger et al., 2005). Although the mechanism mediating the effects of placebo treatment
84 in dogs is still unclear, Cracknell and Mills (2008) investigated the role placebo treatment
85 plays in overcoming fear and anxiety. They found a significant anxiolytic effect in dogs that
86 showed excessive fear response to fireworks. This result was also based on owners' reports,
87 so further confirmation of conclusions about the role of placebo in alleviating fear or relieving
88 pain would require the collection of behavioural data through direct observations.

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

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

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

134

135 **2. MATERIALS AND METHODS**

136

137 **2.1. Experiment 1: Conditioned placebo effect**

138

139 2.1.1. Subjects

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

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

152 Thirty-one adult (> 1 year) pet dogs were included in the experiment, but 3 owners and their
153 dogs did not come back to all trials. The remaining 28 dogs (mean age \pm SD: 1.8 \pm 3.09 years,
154 15 males and 13 females from 13 different breeds and 13 mongrels) were tested and included
155 in the data analysis. Subjects were randomly assigned to either the *Conditioned* or the *Control*
156 group (N= 14-14). The two groups did not differ in their mean age ($t_{(26)}=0.905$, $p=0.374$), sex
157 ratio ($\chi^2_{(1)}=0.144$, $p=0.705$), breed distribution ($\chi^2_{(7)}=3.0$, $p=0.885$), body weight ($t_{(26)}=0.786$,
158 $p=0.439$), separation anxiety questionnaire score ($U_{(26)}=84$, $p=0.541$) and in terms of duration
159 from baseline to test trial ($t_{(26)}=1.047$, $p=0.305$), and duration from the last conditioning event
160 to test trial ($t_{(26)}=0.0$, $p>0.999$).

161

162 2.1.2. Experimental arrangement

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

167

168 2.1.3. Procedure

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

170

171 Baseline trial

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

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

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

181 Episode 1: Dog alone

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

184 Episode 2: Dog & Stranger

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

191 Episode 3: Dog & Owner

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

197

198 Conditioning trials (2-4)

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

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

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

220

221 Test trial

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

224 sprayed with water and they received one more piece of liverwurst right before the trial (Table
225 1). The procedure of this trial was identical to that described in the Baseline.
226 After the conditioning trials and test trial the owners' opinion about the type of treatment
227 (Sedalin or placebo) their dogs received was asked.

228

229 2.1.4. Behaviour coding

230 As behaviours related to separation anxiety are typically displayed close to the
231 exit/entry door (see e.g. Prato-Previde et al. 2003, Palmer and Custance 2008, Palestini et al.
232 2005), we recorded the durations of anxiety-related behaviours while staying close (< 1 m) to
233 the doors. The two doors were not differentiated because both could be considered as a
234 potential exit by the dogs. On the other hand to examine the sedative effect of the drug the
235 time spent passively was also measured. Relative durations were recorded for both variables.
236 Definitions of the behaviour categories:

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

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

245 Door-passive: staying (standing, sitting, or lying down) in the close proximity of the door
246 (< 1 m) without physical contact with it, and/or vocalisation while alone (D/PASS-A), in the
247 presence of the stranger (D/PASS-S), or in the presence of the owner (D/PASS-O).

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

252

253 2.1.5. Data analysis

254 The relative percentage of the time spent in the above behaviours was calculated for the
255 statistical analyses. Variables did not have Gaussian distribution (Kolmogorov Smirnov test).
256 At first we analysed the data with Generalized Estimating Equation (GEE) which is an
257 extension of the GLM algorithm to accommodate the modelling of repeated measurement
258 following non-normal distribution (Hardin & Hilbe, 2003). We employed a GEE analysis to
259 examine the effect of the trial (1st, 2nd, 3rd conditioning and test trials) as within-subject factor
260 and the effect of the group (conditioned vs control) as between subject-factor on the owners'
261 opinion about the treatment. GEE analysis was also employed to examine the effect of the
262 repetition (1st, 2nd and 3rd conditioning trials) as within-subject factor and the effect of the pre-
263 treatment (administering Sedalin vs. vitamin) as between subject-factor on passive behaviour
264 of dogs during the Conditioning trials. To analyse the effect of the conditioning we used GEE
265 analysis to examine the effect of the trial (baseline vs. test) as within-subject factor and the
266 effect of the pre-treatment (administering Sedalin vs. vitamin) during the Conditioning trials
267 as between subject-factor on the dogs' behaviour. When GEE analysis revealed significant
268 trial x treatment interaction, we calculated the change in the dogs' behaviours from Baseline
269 to Test trials. We assumed that the difference in the relative durations of separation distress
270 related behaviours expressed by the Sedalin conditioned dogs would be an indicator of
271 subjects' susceptibility to the placebo effect. We subtracted the relative duration (time%) of a
272 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,

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

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

311

312 Test trials

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

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

322

323 2.2.3. Data analysis

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

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

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

336
$$\text{positive expectancy score} = \frac{(M_{latN} - M_{latA})}{(M_{latN} - M_{latP})} \times 100$$

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

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

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

346

347 **3. RESULTS**

348

349 **3.1. Experiment 1: Conditioned placebo effect**

350

351 3.1.1. Dogs' behaviour during the Conditioning trials

352 As the owners were present in the experimental room throughout these trials, it is not
353 surprising that only few dogs (4 in the 'Conditioned' and 3 in the 'Control' groups) displayed
354 any behavioural signs of distress. Dogs spent hardly any time with distress behaviours; on
355 average 0.5% (Sedalin group) and 0.65% (Control group) of the total duration, and, this
356 remained extremely low even after repeated trials (0.2-1% of time during the 1st, 2nd and 3rd
357 conditioning trials in both groups). However, dogs spent much more time with passive
358 behaviours (on average 31 and 28% in the Conditioned and the Control groups respectively)
359 and there was no effect of repetition (treatment: $\chi^2=0.2$, $p=0.655$; repetition: $\chi^2=4.796$,
360 $p=0.091$).

361

362 3.1.2. Owners' evaluation of treatment effects

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

367

368 3.1.3. Dogs' behaviour in the Test vs. Baseline trials: the effects of conditioning

369 Separation episode (Episode 1)

370 During the separation episode dogs' passive behaviour was influenced by interaction
371 between the trial and treatment (GEE, $\chi^2=6.537$, $p=0.011$) with no significant main effects of
372 the factors (trial: $\chi^2=0.356$, $p=0.551$; treatment: $\chi^2=0.016$, $p=0.901$). 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: $\chi^2=0.239$, $p=0.625$; treatment: $\chi^2=0.017$,
377 $p=0.896$; interaction: $\chi^2=1$, $p=0.317$). The analysis of behavioural signs of distress close to the
378 door showed a significant interaction between the trial and treatment (GEE, $\chi^2=4.66$, $p=0.031$)
379 with no main effects of trial (Baseline vs. Test: $\chi^2=0.001$, $p=0.985$) or treatment (Sedaline vs.
380 Vitamin: $\chi^2=0.481$, $p=0.488$) (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: $\chi^2=0.232$, $p=0.627$; treatment: $\chi^2=0.052$, $p=0.819$; interaction: $\chi^2=0.609$,
388 $p=0.435$; D/PASS-S: trial: $\chi^2=0.061$, $p=0.804$; treatment: $\chi^2=0.551$, $p=0.458$; interaction:
389 $\chi^2=0.055$, $p=0.815$; D/DISTR-S: trial: $\chi^2=0.069$, $p=0.793$; treatment: $\chi^2=2.667$, $p=0.102$;
390 interaction: $\chi^2=1.736$, $p=0.188$; PASS-O: trial: $\chi^2=2.291$, $p=0.130$; treatment: $\chi^2=0.657$,
391 $p=0.418$; interaction: $\chi^2=1.863$, $p=0.172$; D/PASS-O: trial: $\chi^2=0.716$, $p=0.398$; treatment:
392 $\chi^2=0.344$, $p=0.558$; interaction: $\chi^2=2.270$, $p=0.132$); D/DISTR-O: trial: $\chi^2=0.905$ $p=0.342$;
393 treatment: $\chi^2=0.816$, $p=0.366$; interaction: $\chi^2=1.249$, $p=0.264$).

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'

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

399

400 **3.2. Experiment 2: Cognitive bias**

401

402 Subjects reached the training criterion on average after 30 trials (range 12-57 trials), and P
403 and N locations were strongly differentiated also in the test trials; dogs approached the plastic
404 pot sooner in P than in N type test trials (paired sample t-test, $t_{(20)}=4.036$ $p<0.001$). The
405 positive expectancy scores ranged from -12.36 to 1179.5 (mean \pm SD: 124.67 ± 243.79).
406 There was no association between the time elapsed since the conditioning of the dogs in
407 Experiment 1 and the expectancy scores (Pearson correlation test, $r_{(20)}=0.335$ $p=0.149$). We
408 revealed a significant positive relationship between the positive expectancy scores and
409 placebo response values in case of the conditioned group (Pearson correlation test, $r_{(12)}= 0.697$
410 $p = 0.008$, Figure 6) but not in the control group ($r_{(7)}= 0.268$ $p = 0.521$).

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

415

416 **4. DISCUSSION**

417

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

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

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

440 Our finding fits neatly into the placebo conditioning framework (McMillan 1999); therefore
441 we assume that the repeated experience with the effects of Sedalin, as an unconditioned
442 stimulus, could have resulted in the formation of a relaxed inner state, which was associated
443 with some characteristic property of the pre-treatment procedure and/or with some
444 environmental cues of the experimental set up as conditioned stimuli. As a result of this
445 associative process, treatment procedure with the same features but without administration of
446 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

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

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

491 The results of Experiment 2 expand our knowledge on placebo conditioning in dogs
492 and highlight the potential importance of expectancy bias on the formation of placebo
493 responses. The finding that dogs that were more responsive to the placebo treatment tended to
494 show stronger positive expectancy in an ambivalent situation seems to be consistent with the
495 conclusions of human studies (Geers et al., 2005; 2007; 2010; Morton et al., 2009).
496 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.

506

507

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509

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514

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625

626 Table 1

	Baseline trial	Conditioning (trials 2-4)	Test trial
Conditioned group (N=14)	<i>Separation</i>	<i>No separation</i>	<i>Separation</i> Non-sedating pre- treatment (vitamin) Water spray
Control group (N=14)		No pre-treatment <i>No separation</i> Non-sedating pre- treatment (vitamin) Water spray	

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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	$\chi^2=0.001$, p=0.985	$\chi^2=0.481$, p=0.488	$\chi^2=4.66$, p=0.031	$U_{(26)}=48$, p=0.021
PASS-A	$\chi^2=0.356$, p=0.551	$\chi^2=0.016$, p=0.901	$\chi^2=6.537$, p=0.011	$U_{(26)}=50$, p=0.027
D/PASS- A	$\chi^2=0.239$, p=0.625	$\chi^2=0.017$, p=0.896	$\chi^2=1$, p=0.317	$U_{(26)}=79$, p=0.401

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

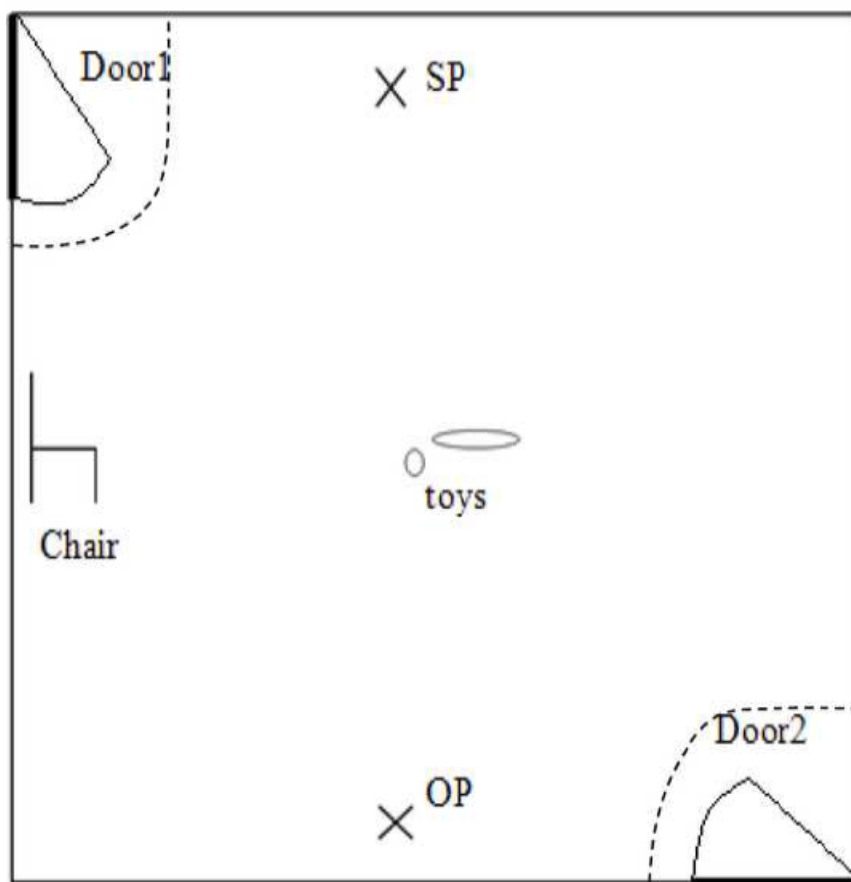
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644 **Figure captions**

645

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

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



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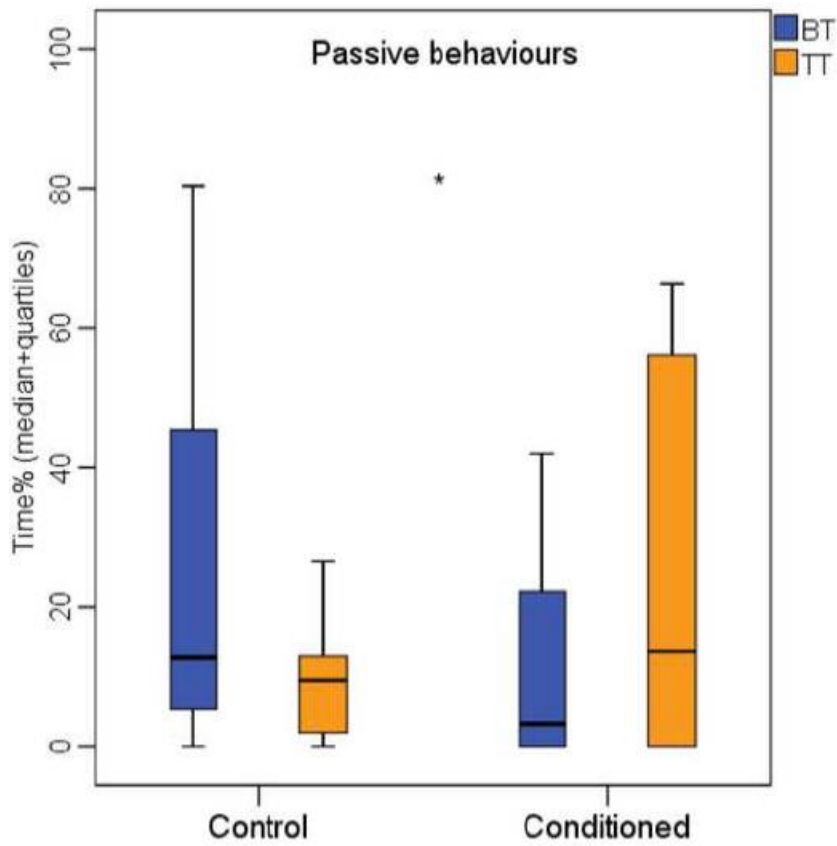
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658 **Figure 2. Relative duration of passive behaviours in Episode 1**

659 Dogs in the two groups showed different changes in passive behaviour after the conditioning.

660 * indicates significant ($p < 0.05$) trial (Baseline vs. Test) x treatment (administering Sedalin

661 vs. vitamin) interaction.



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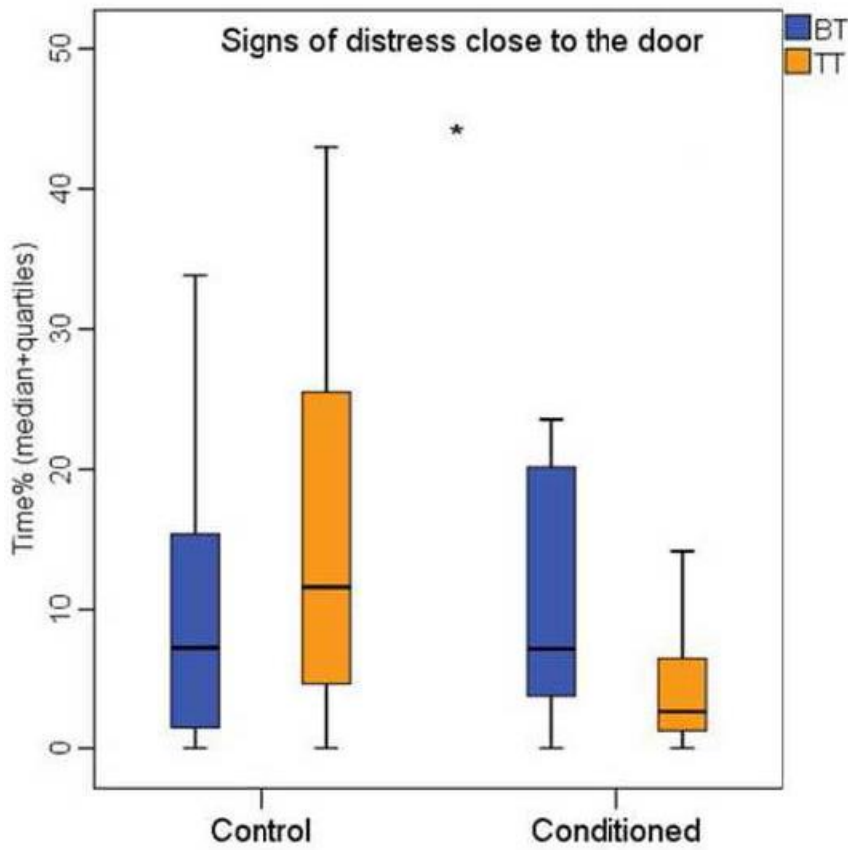
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672 **Figure 3. Relative duration of distress close to the door in Episode 1**

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



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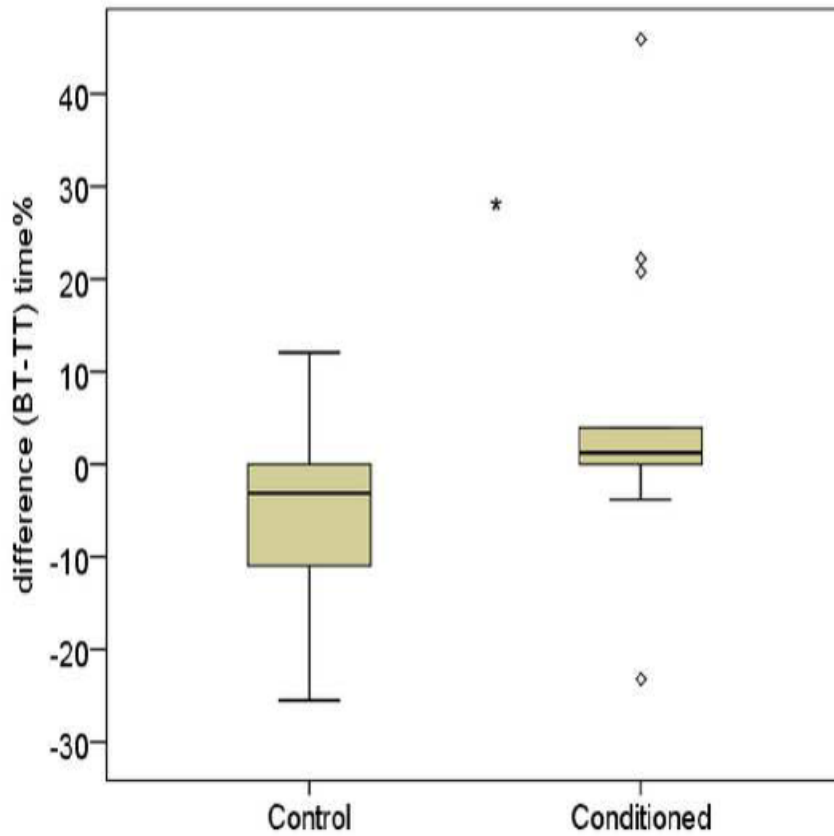
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686 **Figure 4. Difference values of sign of distress close to the door**

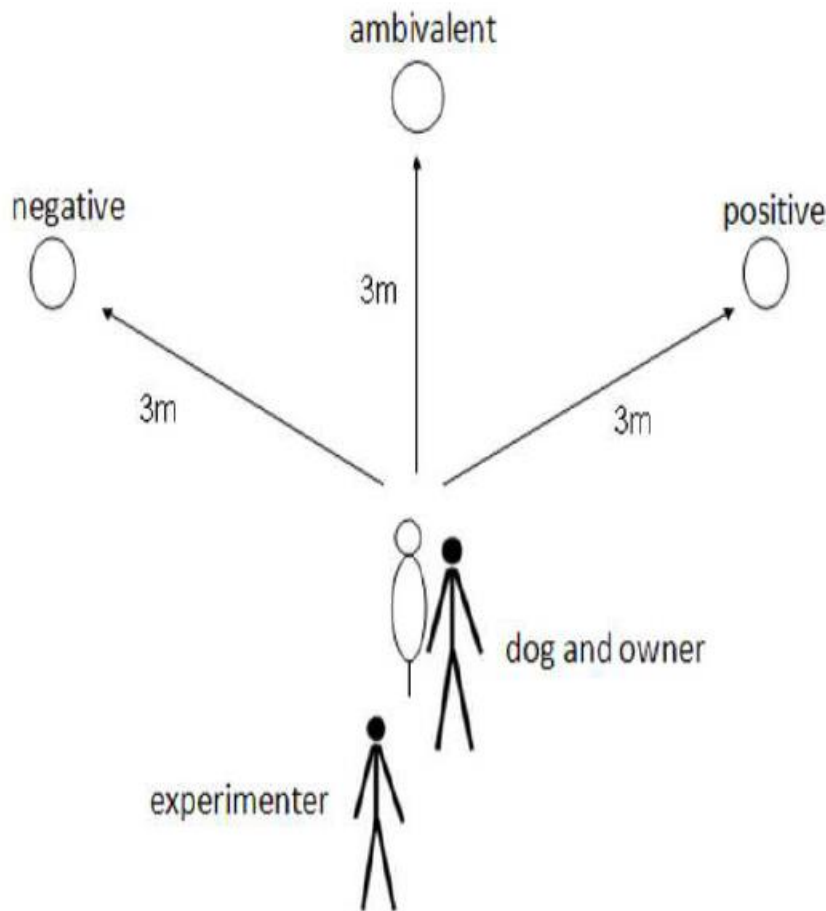
687 Dogs in the Conditioned group have higher difference scores (compared to the Control
688 group), which represent higher placebo response (higher change in distress). * indicates
689 significant ($p < 0.05$) between group difference (median+quartiles+outlier data).



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

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



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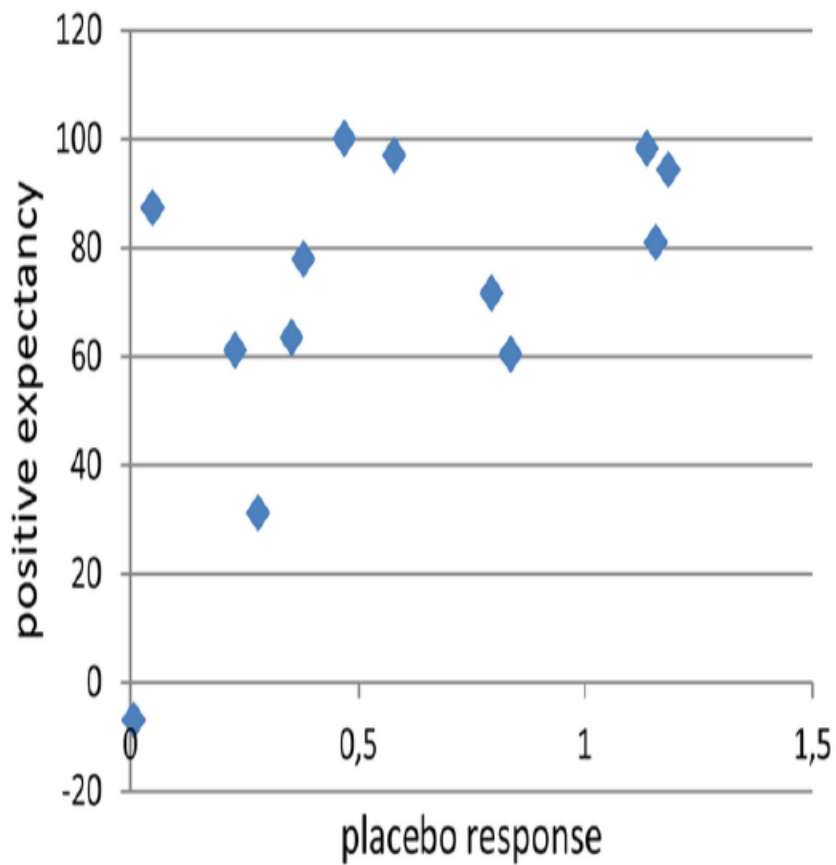
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711 **Figure 6. Relationship between the individual placebo response and positive expectancy**

712 There is a logarithmic relationship between the positive expectancy scores and the placebo

713 response values ($r=0.697$ $p=0.008$) in the conditioned group.



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