1	Full	Title
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- 2 Does training method matter?: Evidence for the negative impact of aversive-based methods on
- 3 companion dog welfare

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- 5 Short Title
- 6 Training methods and companion dog welfare
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22 ABSTRACT

23 There is a growing number of dogs kept as companion animals, and the methods by which they 24 are trained range broadly from those using mostly positive punishment and negative 25 reinforcement (aversive-based methods) to those using primarily positive reinforcement (reward-26 based methods). Although the use of aversive-based methods has been strongly criticized for 27 negatively affecting dog welfare, these claims do not find support in solid scientific evidence. Previous research on the subject lacks companion dog-focused research, investigation of the entire 28 29 range of aversive-based techniques (beyond shock-collars), objective measures of welfare, and long-term welfare studies. The aim of the present study was to perform a comprehensive 30 evaluation of the short- and long-term effects of aversive- and reward-based training methods on 31 32 companion dog welfare. Ninety-two companion dogs were recruited from three reward-based 33 (Group Reward, n=42) and four aversive-based (Group Aversive, n=50) dog training schools. For 34 the short-term welfare assessment, dogs were video recorded for three training sessions and six 35 saliva samples were collected, three at home (baseline levels) and three after the training sessions (post-training levels). Video recordings were then used to examine the frequency of stress-related 36 37 behaviors (e.g., lip lick, yawn) and the overall behavioral state of the dog (e.g., tense, relaxed), and saliva samples were analyzed for cortisol concentration. For the long-term welfare 38 39 assessment, dogs performed a cognitive bias task. Dogs from Group Aversive displayed more stress-related behaviors, spent more time in tense and low behavioral states and more time panting 40 41 during the training sessions, showed higher elevations in cortisol levels after training and were 42 more 'pessimistic' in the cognitive bias task than dogs from Group Reward. These findings 43 indicate that the use of aversive-based methods compromises the welfare of companion dogs in 44 both the short- and the long-term.

45

46 1. Introduction

To fulfil their increasingly important role as companion animals, dogs need to be trained to behave in a manner appropriate for human households. This includes, for example, learning to eliminate outdoors or walk calmly on a lead [1,2]. The fact that dog behavior problems are the most frequently cited reason for rehoming or relinquishment of dogs to shelters and for euthanasia [2] suggests that such training is often missing or unsuccessful.

52 Dog training most often involves the use of operant conditioning principles, and dog training methods can be classified according to the principles they implement: aversive-based 53 54 methods use positive punishment and negative reinforcement and reward-based methods rely on positive reinforcement and negative punishment [3]. There is a heated debate surrounding the use 55 56 of aversive-based training methods, as studies have linked them to compromised dog welfare (e.g., [4-9]). Some aversive-based tools, such as shock collars, have indeed been legally banned 57 58 in some countries [10]. However, a recent literature review by [3] concluded that, because of 59 important limitations, existing studies on the topic do not provide adequate data for drawing firm 60 conclusions. Specifically, the authors reported that a considerable proportion of the studies relied 61 upon surveys rather than on objective measures of both training methods and welfare; that they 62 focused on sub-populations of police and laboratory dogs which only represent a small portion of 63 dogs undergoing training; and, finally, that the empirical studies have concentrated mainly on the 64 effects of shock-collar training, which is only one of several tools used in aversive-based training. In summary, limited scientific evidence exists on the effects of the entire range of dog training 65 66 techniques on companion dog welfare.

Furthermore, previous empirical studies have focused on the short-term effects of training methods on dog welfare. Behavioral and physiological indicators of welfare, such as the frequency of stress-related behaviors and the concentration of salivary cortisol, have been collected in and around the training situation (e.g., [8, 11]; see also [3]). However, the long-term welfare implications of training methods have not yet been examined. To our knowledge, only one study evaluated the long-term effects of training on welfare. Christiansen et al (2001) [12]

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found no effect of shock collar training on dog fear or anxiety; however this was based on dog owner reports of behavior and temperament tests rather than on objective and animal-based welfare indicators. Importantly, a suitable assessment of the effects of training methods on dog welfare should comprise an evaluation of both their short- and long-term effects.

77 Long-term (or chronic) stress can arise from the cumulative exposure to aversive 78 experiences [13], which may reflect the experience of dogs trained with aversive-based methods. 79 A body of research has shown that long-term stress is associated with changes in the long-term 80 affective state of animals (e.g., [14-16]). One way to assess affective states is through the cognitive bias paradigm (e.g., [16]). The cognitive bias task has been validated as an effective 81 82 tool to evaluate the affective states of non-human animals and has been extensively used with several species, including dogs [17-19]. The rationale behind the paradigm is based on theoretical 83 and empirical findings that an individual's underlying affective state biases its decision-making 84 85 and, specifically, that individuals experiencing negative emotional states make more 'pessimistic' 86 judgements about ambiguous stimuli than individuals experiencing more positive emotional states 87 [16, 18].

Therefore, the aim of the present study was to perform a comprehensive evaluation of the 88 89 short- and long-term effects of aversive- and reward-based training methods on companion dog 90 welfare. By performing an objective assessment of training methods (through the direct 91 observation of training sessions) and by using objective measures of welfare (behavioral and physiological data to assess short-term effects, and a cognitive bias task to assess long-term 92 93 effects), we addressed the question of whether aversive-based methods actually compromise the 94 well-being of companion dogs. We hypothesized that dogs trained using aversive-based methods would display higher levels of stress during training, as determined by behavioral and 95 96 physiological indicators of stress during training sessions, and more 'pessimistic' judgments of ambiguous stimuli during a cognitive bias task performed outside the training context. 97

98 Understanding the effects of training methods on companion dog welfare has important99 consequences for both dogs and humans. Both determining and applying those training methods

100	that are less	stressful for	dogs is a l	key factor to	ensure adequate	dog welfare	and to capitalize on
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the human benefits derived from interactions with dogs [20, 21].

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103 2. Materials and methods

104 2.1. Ethical Statement

All procedures were approved by ICBAS (Abel Salazar Biomedical Sciences Institute)
 ORBEA (Animal Welfare Body). All head trainers of dog training schools and owners completed
 a consent form authorizing the collection and use of the data.

108

109 2.2. Training schools

110 2.2.1. Recruitment

111 The first author (ACVC) searched on the internet for dog training schools in the 112 metropolitan area of Porto, Portugal. Based on geographical proximity and on the listed training 113 methods, ACVC posteriorly contacted eight schools through telephone. She approached the head 114 trainers of the different schools on their willingness to participate in a study that aimed to evaluate 115 dog stress and welfare in the context of training and explained the entire methodology. However, 116 it was not directly revealed that the aim of the study was to compare the effects of different 117 training methods. Seven out of the eight dog training schools agreed to participate.

The training schools had different class structures and training sites; however, the types
of behaviors trained was fairly standard across training schools. These characteristics are
described in detail in Appendix S1.

121

122 2.2.2. Classification of training methods

123 After securing seven participating schools, we performed an objective assessment of the 124 training methods used by each school. We videotaped four training sessions at each training 125 school using a video camera on a tripod. Afterwards, we analyzed the videos for the frequency of 126 aversive-based operant conditioning procedures utilized, namely positive punishment and 127 negative reinforcement (see Appendix S1 for the specific definitions). The analysis was performed by AVCV using The Observer XT software, version 10.1 (Noldus Information 128 129 Technology Inc, Wageningen, The Netherlands). The schools were classified as aversive-based 130 if they used any positive punishment and/or negative reinforcement training techniques, and as 131 reward-based if they did not use any of these techniques. Schools A, C, D and F were classified 132 as aversive-based, and Schools B, E and G were classified as reward-based (see Appendix S1).

133

134 2.3. Subjects

The head trainer of each training school was asked to indicate at least fourteen dogs fitting our inclusion criteria, and we then approached the owners to ask if they were willing to participate. The information about the study given to the owners was the same that was given to the head trainers of the schools. The inclusion criteria for the dogs were: 1) to have attended the training school for less than two months, in order to mitigate habituation to training methods, and 2) to be free of behavioral problems (e.g., aggression, fearfulness and separation anxiety, as determined by the owner and ACVC), in order to prevent any confounding stress.

142 Over the course of the study, which was conducted between October 2016 and March 143 2019, the owners of 122 companion dogs agreed to participate. However, 30 dog owners dropped 144 out of the training schools before any meaningful data could be collected. Specifically, these 145 subjects dropped out before meeting our requirement that at least two training sessions were video 146 recorded and that the owner completed a written questionnaire. Hence, our final sample comprised 147 92 subjects, 50 recruited from aversive-based schools, hereafter 'Group Aversive' (Schools A, D 148 and F: 14 dogs, School C: 8 dogs), and 42 from reward-based schools, hereafter 'Group Reward' 149 (School B and G: 15 dogs, School E: 12 dogs).

150	As for subjects' demographics, the average age was $11.9 (\pm 9.3)$ months, 54% were male
151	and 35% were neutered/spayed. Thirty-four percent were mixed-breed dogs and the remaining
152	66% belonged to a FCI-recognized breed group: 18% belonged to Group 1: Sheepdogs and
153	Cattledogs (except Swiss Cattledogs), 13% to Group 2: Pinscher and Schnauzer - Molossoid and
154	Swiss Mountain and Cattledogs, 5% to Group 3: Terriers, 4% to Group 6: Scent hounds and
155	related breeds, 2% to Group 7: Pointing dogs, 20% to Group 8: Retrievers - Flushing Dogs -
156	Water Dogs, and 3% to Group 9: Companion and Toy Dogs.
157	
158	2.4. Data collection
159	The study had two phases. The goal of Phase 1 was to evaluate the welfare of dogs within
160	the training context, and the goal of Phase 2 was to evaluate the welfare of these same dogs outside
161	the training context. These aimed to represent measures of the short- and long-term impact of
162	training methods on the welfare of dogs.
163	
164	2.4.1. Phase 1 – Evaluating welfare within the training context
165	In order to evaluate behavioral indicators of welfare during training, each dog was
166	videotaped for the first 15 minutes of three training sessions using a video camera on a tripod (one
167	Sony Handycam HDR-CX405 and two Sony Handycam DCR-HC23). Five experimenters were
168	responsible for data collection (ACVC, Danielle Fuchs - DF, Stefania Pastur - SP, and two
169	undergraduate students, Margarida Lencastre and Flávia Canastra). The cameras were positioned
170	to get an optimal view of the specific participant without interfering with training. The day and
171	time of the training sessions were determined by the training schools and by the participants'
172	availability.

To obtain physiological data on stress during training, six saliva samples were collected per dog to allow assay of salivary cortisol [8, 22]. Three samples were collected 20 min after each training session (PT – post-training samples) and three were collected at home on days when no 176 training took place, approximately at the same time as PT samples (BL - baseline samples). 177 Owners were asked not to give their dog water in the 20 minutes preceding each sample collection, 178 nor a full meal in the hour preceding each sample collection, respectively. The timing for sample 179 collections, as well as other recommendations regarding saliva collection for cortisol analysis, 180 were drawn from previous relevant research on dogs' cortisol responses to potentially stressful 181 stimuli [8, 22-24]. ACVC collected the first sample of every subject (PT1) while simultaneously 182 demonstrating proper sample collection to the owners. The following samples were always 183 collected by the owners. A synthetic swab (Salivette®) was rubbed in the dogs' mouth for about 184 2 minutes to collect saliva. For samples collected at the training schools (PT), the swab was placed 185 back into the provided plastic tube and immediately stored on ice. It was then transferred to a -186 20°C freezer as soon as possible. For samples collected at home (BL), owners were instructed to place the swab back into the plastic tube and immediately store it in their home freezer. Owners 187 188 were provided with ice-cube plastic makers to transport the BL samples to the training school 189 during the next scheduled training session without them unfreezing, and they were stored at -20°C 190 as soon as possible. Owners were also provided with detailed written instructions for saliva 191 collection and ACVC's cell phone number in case any owners had questions related to sample 192 collection. For standardization purposes, we ensured that Phase 1 did not last more than three 193 months for each dog.

194

195 2.4.2. Phase 2 - Evaluating welfare outside the training context

After finishing data collection for Phase 1, dogs moved to Phase 2, which consisted of a spatial cognitive bias task. For standardization purposes, we ensured that 1) dogs had attended the training school for at least one month prior to moving to Phase 2 and that 2) the cognitive bias task was conducted within one month of completing Phase 1. Due to limited owner availability, 13 subjects either dropped out of the study or did not meet the criteria for Phase 2, resulting in 79 (44 from Group Aversive and 35 from Group Reward) of the original 92 dogs participating in bioRxiv preprint doi: https://doi.org/10.1101/823427. this version posted October 29, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY 4.0 International license.

202 Phase 2. The tests were scheduled according to owners' availability, both on weekdays and203 Saturdays.

The test was conducted in an indoor room (7.7 x 3 meters) within a research building at the Abel Salazar Biomedical Sciences Institute (ICBAS), University of Porto in Portugal. All dogs were unfamiliar with the room prior to testing. Two experimenters conducted the test while the dog's owner(s) sat in a chair in a corner area of the room (see Figure 1). Dog owners were asked not to look into the dog's eyes or to speak to the dog during the test, unless the experimenters instructed otherwise. The entire test took place over one meeting for each dog. The room was cleaned with water and liquid detergent at the end of each test.

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Figure 1. Schematic representation of the cognitive bias task.

213

214 2.4.2.1. Familiarization period

Prior to the start of the cognitive bias task, the dogs were given the opportunity to familiarize with the test room and the researchers. This consisted of a 10-min period during which the dog was allowed to freely explore the room and engage with the researchers and the owner(s).

218

219 2.4.2.2. Training phase

The methodology of Phase 2 was based on [19]. During the training phase, dogs were trained to discriminate between a 'positive' (P) location of a food bowl, which always contained a food reward, and a 'negative' (N) location, which never contained a food reward. At the start of each trial, the dog was held by the 'handler' (played by SP, DF, Margarida Lencastre, Flávia Canastra or Joana Guilherme-Fernandes) behind a barrier (2 x 2 m, see Figure 1), while the 'timer' (ACVC) baited (or did not bait, depending on the type of trial) the bowl with a piece of sausage (approximately 1.25 g for smaller dogs and 2.5 g for larger dogs). To ensure that the dog, the owner and the 'handler' were blind to whether or not the bowl contained food during each trial, the bowl was baited out of their sight, on the opposite side of the barrier. Additionally, the food reward was rubbed onto the food bowl before every trial to prevent the influence of olfactory cues. The height of the food bowl was such that visual cues to the presence or absence of food could not be judged by the dog at the start position.

232 After baiting (or not baiting) the bowl, the 'timer' placed it at one of the two training 233 locations. The 'timer' then determined the start of the trial, by verbally signaling to the 'handler', 234 upon which the 'handler' led the dog to the start position and released him. The 'handler' always led the dog to the start position on her left side. Because we found that dogs had some difficulty 235 236 noticing the bowl at the end of the room during pilot tests, the 'handler' walked towards the bowl 237 and pointed it out to the dog in the first four trials. For the remaining trials, the 'handler' simply walked the dog to the start position and released him. After the dog reached the food bowl and 238 239 (when applicable) ate the reward, the 'handler' collected him and led him behind the barrier to 240 start the next trial. The latency to reach the bowl, defined as the time elapsed between release at 241 the start position and the dog putting his head in line with the edge of the bowl, was recorded for 242 each trial by the 'timer' using a stopwatch.

The position of the 'positive' and 'negative' locations was counterbalanced across subjects and training schools, such that for half of the dogs from each training school, the 'positive' location was on the right hand side as they faced the test area, and for the other half it was on the left. Initially, each dog received two consecutive 'positive' trials (bowl placed in the 'positive' location) followed by two 'negative' trials (bowl placed in the 'negative' location). Subsequently, 'positive' and 'negative' trials were presented in a pseudorandom order, with no more than two trials of the same type being presented consecutively.

All dogs received a minimum of 15 training trials to learn the discrimination between bowl locations. Dogs were considered to have learnt an association between bowl location and food (the learning criterion) when, after a minimum of 15 trials, the longest latency to reach the 'positive' location was shorter than any of the latencies to reach the 'negative' location for the preceding three 'positive' trials and the preceding three 'negative' trials. Each trial lasted a maximum of 20 seconds. If the dog did not reach the bowl by that time, the trial automatically ended and a latency of 20 seconds was recorded.

All but two dogs were able to complete the training phase. For the two dogs that failed to complete training, one did not show any interest in the food reward and the other was foodmotivated but could not focus on the task. These two dogs belonged to Group Aversive. Therefore, the total number of subjects completing Phase 2 in Group Aversive was 42.

261

262 2.4.2.3. Test phase

263 Testing began once the learning criterion was achieved. Test trials were identical to 264 training trials except that the bowl (empty) was placed at one of three ambiguous locations equally spaced along an arc 4 m from the dog's start position, between the 'positive' and 'negative' 265 266 locations. The three test locations were: 'near-positive' (NP: one third of the way along the arc 267 from the 'positive' location), 'middle' (M: half way along the arc), 'near-negative' (NN: one third of the way along the arc from the 'negative' location). Three test trials were presented at each test 268 269 location (nine test trials in total) in the following order for all dogs: 270 M,NP,NN,NP,NN,M,NN,M,NP (each location was presented first, second or third in each block 271 of three test trials). Each test trial was separated from the next one by two training trials identical to those conducted in the training phase (one 'positive' and one 'negative' trials presented in a 272 273 random order), in order to maintain the associations between the 'positive' and 'negative' 274 locations and the presence or absence of food, respectively. Thus, the test phase included a further 275 sixteen training trials interspersed in blocks of two between the nine test trials.

To end the test phase, a final trial was conducted by placing an empty bowl in the 'positive' location to determine whether dogs ran to the empty bowl as quickly as they did to the baited bowl. This was meant to establish that the dogs were not relying on olfactory or visual cues

11

during the test. During the entire test, each trial was kept as similar as possible in terms ofpreparation time and activity, and dogs were handled in the same way throughout the test.

Due to circumstances beyond our control, namely people speaking loudly and other dogs barking in the building during some of the tests, some subjects were clearly distracted and disengaged from the task during some trials. Whenever this happened, no latency was recorded for that trial. The experimenters waited for the dog to resettle and moved to the following trial.

285

286 2.5. Questionnaire

All owners were asked to complete a brief written questionnaire regarding dog demographics and background, and owner demographics and experience with dogs and dog training. The questionnaire was based on [9].

290

291 2.6. Data analysis

292 2.6.1. Phase 1 – Evaluating welfare within the training context

293 2.6.1.1. Behavior coding

We developed two ethograms based on previous literature to record the frequency of different stress-related behaviors and the time spent in different behavioral states and panting during the training sessions [8, 9, 25]. The behaviors and their definitions are described in Appendix S2.

Behavior coding was conducted by three observers (ACVC, DF and SP). DF and SP were blind to how the training schools had been classified according to training methods. Each video was coded twice, once with the ethogram for stress-related behaviors, using a continuous sampling technique (by ACVC and DF, see Appendix S2), and a second time with the ethogram for overall behavioral state and panting, by scan-sampling at 1 minute intervals (by ACVC and SP, see Appendix S2). The Observer XT software, version 10.1 (Noldus Information Technology Inc, Wageningen, The Netherlands) was used to code for stress-related behaviors and Windows
Movie Player and Microsoft Excel to code for overall behavioral state and panting.

306 Before coding independently, observers DF and SP were trained to become familiar with 307 the ethograms, and inter-observer reliability was assessed. Inter-observer reliability was tested by 308 having DF (for the ethogram for stress-related behaviors) and SP (for the ethogram for overall 309 behavioral state and panting) code sets of four videos in parallel with ACVC. Cohen's Kappa 310 coefficient was calculated using The Observer XT. Where there was poor agreement (r<0.80), 311 observers received further training. Values of r>0.80 were assumed to indicate strong agreement 312 (see, for example, [8]), and once this level was attained the observers began coding videos 313 independently. ACVC coded 77% of the videos with the ethogram for stress-related behaviors 314 and 65% with the ethogram for overall behavioral state and panting. For each ethogram, the videos 315 were distributed randomly between observers, with the exception that each observer coded a 316 similar number of videos from Group Aversive and Group Reward.

317

318 2.6.1.2. Cortisol analysis

319 Two dogs (one from School B and one from School E, both from Group Reward) did not cooperate with the saliva collection procedure and, as such, no saliva samples were extracted 320 321 from them. For the remaining 90 dogs, only 23 dog owners (11 from Group Reward and 12 from Group Aversive) were able to appropriately collect six saliva samples. The samples from these 322 323 subjects were selected for analysis. An additional 40 dog owners (15 from Group Reward and 25 324 from Group Aversive) were able to properly collect at least four saliva samples. From these 40 325 dogs, eight were randomly selected to have their samples analyzed (four from Group Reward and 326 four from Group Aversive). In the end, 16 dogs from Group Aversive and 15 dogs from Group 327 Reward had their samples selected for analysis (Schools A, C, D, E and F: n=4; School B: n=5; 328 School G: n=6). These samples were sent to the Faculty of Sport Sciences and Physical Education 329 of the University of Coimbra, Coimbra, Portugal, where they were assayed for cortisol 330 concentration using standard ELISA kits (Salimetrics®).

331

332 2.6.2. Phase 2 – Evaluating welfare outside the training context

For each dog, we calculated the average latency to reach the food bowl during each of the three types of test trials (NP, M, NN) as well as the average latency to reach the 'positive' and 'negative' training locations during the test phase. Afterwards, to control for individual variation in running speeds, we adjusted each dog's test latencies by taking into account their mean latencies to get to the 'positive' and 'negative' training locations during the test phase as follows [19]:

339

340 $adjusted \ latency = \frac{mean \ latency \ to \ test \ location - mean \ latency \ to \ positive \ location}{mean \ latency \ to \ negative \ location - mean \ latency \ to \ positive \ location} * 100$

341

This adjusted score expresses all test latencies as a percentage of the difference between each dog's mean latencies to the 'positive' and 'negative' locations [19].

344 Seventy-three dogs completed the cognitive bias task. From these 73 subjects, 14 345 disengaged from the task for some trials due to noise outside the test room. Thirteen disengaged 346 for one test trial (Group Reward: one dog at location M, three dogs at location NP, and one dog at location NN; Group Aversive: five dogs at location NP, and three dogs at location NN), and 347 348 one (Group Reward) for three test trials (one at each test location). For these dogs, the average 349 latencies to the test locations were calculated from the remaining test trials. Of the remaining four 350 dogs, one (from School G) completed the first seven test trials (at locations 351 M,NP,NN,NP,NN,M,NN), two (one from School A and one from School E) completed the first 352 five test trials (at locations M,NP,NN,NP,NN), and one (from School G) completed the first three 353 test trials (at locations M,NP,NN); then they stopped cooperating with the task. Their average 354 latencies to the test locations were calculated from these trials.

355

356 2.7. Statistical analyses

All statistics were conducted using the software SPSS[®] Statistics 25.0. All data were analyzed using the Shapiro-Wilk test to check for normality. Except for the number of trials required to reach the learning criterion in the cognitive bias task, the data were not normally distributed; hence, except for the former variable, non-parametric tests were used for analysis. Namely:

- Chi-square tests were used to compare the two groups (Reward and Aversive) for dog
 demographics and background, and owner demographics and experience with dogs
 and dog training (variables collected with the questionnaire, see Appendix S3), in
 order to investigate whether the two groups differed in these factors.
- Kruskal-Wallis and Mann-Whitney tests were used to verify if the dog-related
 variables age, FCI breed group, and age of separation from the mother (which differed
 between Group Reward and Group Aversive, see Appendix S3) affected the
 dependent variables measured.
- Friedman's ANOVA and Wilcoxon signed-rank tests were used to analyze the
 frequency of stress-related behaviors, the percentage of scans in the different
 behavioral states and the percentage of scans panting across the three training
 sessions, in order to examine whether there was an effect of sampling period. Where
 no effect of training session was found, the data for each dog were averaged across
 the three sessions.
- Mann-Whitney tests were used to compare the frequency of stress-related behaviors,
 the percentage of scans in the different behavioral states, the percentage of scans
 panting, the difference in cortisol concentrations and the adjusted latencies for the
 cognitive bias task between Group Reward and Group Aversive, in order to
 investigate whether the groups differed in these different indicators of welfare.
- A Wilcoxon signed-rank test was used to compare the post-training with the baseline
 levels of cortisol within both Group Reward and Group Aversive, with the aim of

examining whether the concentration of salivary cortisol changed as a result oftraining for each group.

- A Mann-Whitney test was used to compare the number of training classes attended
 by the dogs before moving to Phase 2, to verify if these did not differ between groups.
 A t-test for independent samples was used to compare the number of trials needed to
 reach the learning criterion in the cognitive bias task between Group Reward and
 Group Aversive, in order to explore whether the groups differed in learning speed.
- A Wilcoxon signed-rank test was used to compare, in the cognitive bias task, the
 latency to reach the P location during test trials and during the final trial, when the
 bowl contained no food, to verify whether dogs were relying on olfactory or visual
 cues to discriminate between bowl locations.
- Spearman correlation coefficients were used to examine the correlation between the number of aversive stimuli used in the different aversive-based schools and the frequency of stress-related behaviors, the percentage of scans in the different behavioral states, the percentage of scans panting, the difference in cortisol concentrations, and the adjusted latencies in the cognitive bias task; in order to investigate whether there was a correlation between the frequency of aversive stimuli used in training and the different indicators of welfare.

401

With the exception of the analyses conducted to test for the effect of the sampling period on behavioral data and the analysis performed to verify if dogs were relying on olfactory or visual in the cognitive bias task, which were within-subjects, all remaining analyses were betweensubjects. Two-tailed tests were used for all but correlations analyses, for which one-tailed tests were conducted. The level of significance was set at $\alpha = 0.05$.

407

408 3. Results

409 3.1. Questionnaire

410 3.1.1 Dog demographics and background

Concerning dog demographics, the two groups did not differ in sex and neuter status ratios, but they differed with regards to age $[X^2(3)=10.361, p=0.016]$ and FCI breed group $[X^2(7)=19.586, p=0.007]$. As for dog background, the groups differed only in the age of separation from the mother $[X^2(7)=19.041, p=0.003, see Appendix S3 for full results]$. However, statistical analysis performed for each training method group revealed that age, FCI breed group and age of separation from the mother did not affect any of the dependent variables measured in the present study (Appendix S3).

418

419 3.1.2. Owner demographics, experience with dogs and dog training

Regarding owner demographics, the two groups did not differ in owner age, family household size and whether they had children, but they differed in owner sex $[X^2(1)=8.360,$ p=0.006]. Regarding owner experience with dogs and dog training, the groups did not differ in whether owners had attended training classes with a previous dog, but they differed in whether owners had had other dog(s) in the past $[X^2(1)=4.658, p=0.037]$ and in the information they relied on for choosing the dog training school $[X^2(4)=11.656, p=0.016, see Appendix S3 for full results].$

426

427 3.2. Phase 1 – Evaluating welfare within the training context

428 3.2.1. Behavioral data

429 3.2.1.1. Stress-related behaviors

For each dog, we first summed all the occurrences of each stress-related behavior as defined in the ethogram for stress-related behaviors. A comparison between the two groups revealed that dogs from Group Aversive displayed significantly more stress-related behaviors

433	than dogs from Group Reward [Group Aversive: M(±SEM)=57.06±5.98 vs Group Reward:
434	M(±SEM)=10.56±1.04; U=1974.50, p<0.001; M – Mean, SEM – Standard Error of the Mean].
435	When analyzing each of the stress-related behaviors separately, we found that dogs from
436	Group Aversive showed a significantly higher frequency of body turn (U=1833.50, p<0.001),
437	move away (U=1308.50, p=0.042), crouch (U=1630.50, p<0.001), salivating (U=1211.50,
438	p=0.019), yawn (U=1555.50, p<0.001) and lip lick (U=2004, p<0.001) than dogs from Group
439	Reward. Additionally, there was a tendency for dogs from Group Aversive to exhibit more lying
440	on side/back (U=1134, p=0.062), yelp (U=1174, p=0.072) and paw-lift (U=1258.50, p=0.094)
441	behaviors. Finally, there were no differences regarding the average frequency of whine
442	(U=1141.50, p=0.353), body shake (U=1194.50, p=0.247), and scratch (U=972, p=0.482)
443	behaviors. Fear-related elimination was never displayed during this study (Figure 2).

444

Figure 2. Number of occurrences of each stress-related behavior averaged across the three training sessions for Group Reward (filled bars) and Group Aversive (empty bars). Vertical bars show the SEM. * stands for statistically significant differences at α =0.05.

448

Because we observed a difference in the frequency of aversive stimuli used during training among the different aversive-based schools (see Appendix S3), we further analyzed the relationship between the number of stress-related behaviors displayed by the dogs and the number of aversive stimuli used in the different schools. We found a strong positive correlation between the number of aversive stimuli used and the total number of stress-related behaviors shown by the animals (Spearman correlation coefficient, r_s=0.833, p<0.001).

455 Moreover, when examining the correlation between the frequency of each individual 456 stress-related behavior and the number of aversive stimuli used by each training school, we found 457 positive correlations for body turn ($r_s=0.653$, p<0.001), move away ($r_s=0.200$, p=0.028), crouch 458 ($r_s=0.567$, p<0.001), lying on side/back ($r_s=0.273$, p=0.004), salivating ($r_s=0.334$, p=0.001), yawn

	459	$(r_s=0.533, p<0.6)$	001), paw lift	$(r_s=0.224, p$	=0.016) and lip	$p \text{ lick } (r_s = 0.850, 1)$	p<0.001) behaviors. I	Foi
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460 the remaining behaviors there were no significant correlations (yelp: $r_s=0.107$, p=0.155; whine:

461 $r_s=0.111$, p=0.147; body shake: $r_s=0.127$, p=0.114; scratch: $r_s=-0.151$, p=0.075).

462

463 3.2.1.2. Overall behavioral state

464 For each training session of each dog, we calculated the percentage of scans spent in each overall behavioral state (Tense, Low, Relaxed, Excited). A Friedman's ANOVA conducted on 465 466 the percentage of scans spent in each behavioral state across the three training sessions revealed that there was a significant effect of session in the percentage of scans in the Excited state 467 468 $[\chi^2(2)=12.105, p=0.002]$, but not in the remaining states [Tense: $\chi^2(2)=3.645, p=0.162$; Low: $\chi^2(2)=1.077$, p=0.584; Relaxed: $\chi^2(2)=4.244$, p=0.120]. Specifically, the percentage of scans in 469 470 the Excited state decreased across training sessions - Session 1: M(±SEM)=50.21±3.51, Session 2: M(±SEM)=42.94±3.59, Session 3: M(±SEM)=41.13±3.55. When analyzing the two 471 472 training groups separately, we found that this pattern was statistically significant for Group 473 Aversive $[\chi^2(2)=9.490, p=0.009]$ but not for Group Reward $[\chi^2(2)=3.674, p=0.159]$.

474 When comparing between training groups, dogs from Group Aversive showed a 475 significantly higher percentage of scans in Tense state than dogs from Group Reward for all three 476 training sessions [Session 1: U=1769, p<0.001; Session 2: U=1820.50, p<0.001; Session 3: 477 U=1592, p<0.001] and a significantly higher percentage of scans in Low states for Session 1 and Session 2 [Session 1: U=1239, p=0.002; Session 2: U=1176.50, p=0.011; Session 3: U=925, 478 479 p=0.211]. Additionally, dogs from Group Aversive showed a significantly lower percentage of 480 scans in Excited [Session 1: U=296.500, p<0.001; Session 2: U=287.500, p<0.001; Session 3: U=157, p<0.001] and Relaxed [Session 1: U=628, p<0.001; Session 2: U=793, p=0.042; Session 481 3: U=525, p=0.001] states for all three training sessions than dogs from Group Reward (see 482 483 Figure 3).

484

485 Figure 3. Average percentage of scans in the different behavioral states in training sessions 1 (S1),

486 2 (S2) and 3 (S3) for Group Reward (left) and Group Aversive (right).

487

488 When averaging the results of all three training sessions, dogs from Group Aversive 489 showed a significantly higher percentage of scans in Tense [Group Aversive: 490 M(±SEM)=40.50±3.07 vs Group Reward: M(±SEM)=4.18±0.74; U=2010.50, p<0.001] and Low 491 [Group Aversive: M(±SEM)=2.18±0.63 vs Group Reward: M(±SEM)=0.16±0.12; U=1138, 492 p<0.001] states and a lower percentage of scans in Relaxed [Group Aversive: M(±SEM)=10.03±1.95 vs Group Reward: M(±SEM)=16.24±2.06; U=652, p=0.002] and Excited 493 494 [Group Aversive: M(±SEM)=24.92±3.27 vs Group Reward: M(±SEM)=68.43±2.81; U=170, 495 p<0.001] states than dogs from Group Reward.

Finally, when examining the correlation between the percentage of scans in each behavioral state and the number of aversive stimuli used in training, we found positive correlations for Tense ($r_s=0.881$, p<0.001) and Low ($r_s=0.472$, p<0.001) states, and negative correlations for Relaxed ($r_s=-0.472$, p<0.001) and Excited ($r_s=-0.821$, p<0.001) states.

500

501 3.2.1.3. Panting

502 For each training session of each dog, we also calculated the percentage of scans spent 503 panting. A significant effect of training session was found for Group Reward [$\gamma^2(2)=7.043$, 504 p=0.030], but not for Group Aversive [$\chi^2(2)$ =0.294, p=0.863]. However, there was no systematic 505 increase or decrease in panting across sessions for Group Reward; instead, panting increased from 506 Session 1 to Session 2 [Session 1: $M(\pm SEM) = 13.93 \pm 2.91$ vs Session 2: $M(\pm SEM) = 20.66 \pm 3.99$; 507 T=234, p=0.054] and then decreased slightly, although not significantly, from Session 2 to 508 Session 3 [Session 2: M(±SEM)= 20.66±3.99 vs Session 3: M(±SEM)=16.17±3.22; T=116.50, 509 p=0.213]. When comparing between training groups, dogs from Group Aversive panted more

510	than dogs from Group Reward during all training sessions (Session 1: U=1516.500, p<0.001;
511	Session 2: U=1359, p=0.008; Session 3: U=1223.500, p=0.001, see Figure 4).
512	
513	Figure 4. Average percentage of scans spent panting in training sessions 1 (S1), 2 (S2) and 3 (S3)
514	for Group Reward (left) and Group Aversive (right).

515

When averaging the results of the three training sessions, we also found that dogs from
Group Aversive panted more overall than dogs from Group Reward [Group Aversive:
M(±SEM)=37.71±3.56 vs Group Reward: M(±SEM)=16.99±2.71; U=1540, p<0.001].

519 Finally, the percentage of scans spent panting was positively correlated with the number 520 of aversive stimuli used in training ($r_s=0.407$, p<0.001).

521

522 3.2.2. Physiological data

523 In order to investigate potential changes in salivary cortisol concentration as a result of 524 training methods, we averaged the baseline sample values (BL) and the post-training sample 525 values (PT). Afterwards, we computed the difference between the average post-training 526 concentration and the average baseline concentration. The average results for each training 527 method are depicted in Figure 5. There was a significant difference between groups, with dogs 528 from Group Aversive showing an average increase of 0.10 µg/dL in salivary cortisol 529 concentration after training and dogs from Group Reward showing, on average, no changes in 530 cortisol concentration (U=196, p=0.002).

531

Figure 5. Average difference in cortisol concentration (PT: post-training average concentration,
BL: baseline average concentration) for Group Aversive and Group Reward. Vertical bars show
the SEM.

535

536	Additional analysis within each group revealed that the increase in cortisol concentration
537	for Group Aversive was statistically significant [Baseline: M(±SEM)=0.14±0.01 vs Post-training:
538	M(±SEM)=0.24±0.03 μ /dL; T=134, p=0.001], whereas no significant differences existed for
539	Group Reward [Baseline: M(±SEM)=0.13±0.02 vs Post-training: M(±SEM)=0.13±0.02 µ/dL;
540	T=51, p=0.609]. Moreover, whereas there were no differences between groups regarding baseline
541	levels (U=151.50, p=0.216), the two groups differed significantly for post-training levels
542	(U=192.50, p=0.003).
543	The average difference in cortisol concentrations also showed a positive correlation with
544	the number of aversive stimuli used in training ($r_s=0.512$, p<0.002).
545	
546	3.3. Phase 2 – Evaluating welfare outside the training context
547	Before performing the cognitive bias task, dogs from Group Reward had attended, on
548	average, 6.07 training classes (SEM=0.36) and dogs from Group Aversive had attended, on
549	average, 6.66 (SEM=0.39), with no statistically significant differences observed between groups
550	(U=1232.50, p=0.147).
551	
552	3.3.1. Training phase
553	On average, dogs took 27.14 (SEM=0.85) trials to reach the learning criterion. Dogs from
554	the Group Reward took significantly fewer trials to reach the learning criterion than dogs from
555	Group Aversive [Group Reward: M(±SEM)=24.80±1.26 vs Group Aversive:
556	M(±SEM)=29.10±1.08, t=-2.612, p=0.011].

558 3.3.2. Test phase

Figure 6 shows the average adjusted latencies for the two training stimuli (P, N) and the three test stimuli (NP, M, NN) for Group Reward and Group Aversive. As noted in the figure, whereas there were no significant differences between groups for either the NP (U=755.50, p=0.834) or the NN (U=874.50, p=0.154) stimuli, there was a statistically significant difference for the M stimulus, with dogs from Group Aversive taking longer to approach this bowl location than dogs from Group Reward (U=987, p=0.01).

565

Figure 6. Average adjusted latency to reach the food bowl as a function of location: P - 'positive', NP - 'near positive', M - 'middle', NN - 'near negative', N - 'negative', for Group Reward (black circles) and Group Aversive group (white circles). Vertical bars show the SEM. * stands for statistically significant differences at α =0.017.

570

Additionally, we found a positive correlation between the number of aversive stimuli used in training and the adjusted latency for the M stimulus ($r_s=0.258$, p=0.012). No correlations were found between the number of aversive stimuli used in training and the adjusted latencies for the NP ($r_s=-0.013$, p=0.454) and the NN ($r_s=0.076$, p=0.256) stimuli.

Lastly, an analysis comparing the average latency to reach the P location during test trials and the latency to reach this same location during the final trial (when the bowl contained no food) revealed no significant differences (T=1295.50, p=0.328), confirming that the dogs were not relying on olfactory or visual cues to discriminate between bowl locations.

579

580 4. Discussion

This was the first empirical study to systematically investigate the short- and long-term effects of aversive- and reward-based training methods on the welfare of companion dogs. We objectively classified training methods, extended the study of aversive-based methods to other techniques and tools besides shock collars, and used objective and validated measures for the assessment of both the short- (behavioral and physiological stress responses during training) and long-term welfare (cognitive bias task outside the training context) of companion dogs. Overall, our results showed that dogs trained with aversive-based methods displayed poorer indicators of both short- and long-term welfare as compared to those trained using reward-based methods.

589 Short-term welfare was assessed during training sessions. Here, dogs from Group 590 Aversive spent more time in Tense and Low behavioral states as well as more time panting than 591 dogs from Group Reward. Tense and low body postures reflect states of stress and fear in dogs 592 (e.g., [26]), and panting has also been associated with acute stress in dogs (e.g., [8, 27]). Dogs 593 from Group Aversive also displayed more stress-related behaviors than dogs from Group Reward. 594 More specifically, these dogs exhibited more lip licks, yawns, body turns, moves away, crouches 595 and instances of salivating. There was also a tendency for dogs from Group Aversive to engage 596 in more yelping, paw-lifting and lying on side/back. In previous studies, high levels of lip licking 597 and yawning behaviors have been consistently associated with acute stress in dogs (e.g., [9, 23]). 598 Importantly, lip licking has been associated with stressful social situations [23]. This most likely 599 explains the large magnitude of this behavior observed in Group Aversive, as aversive-based 600 training methods comprise social and physical confrontation with the dog. Paw lifting, salivating 601 and yelping have previously been interpreted as responses to pain and stress (e.g., [23, 25, 28]). 602 The display of avoidance behaviors such as body turn, move away, crouch and lying on side/back, 603 specifically in response to training techniques, highlights the aversive nature of the training 604 sessions at the aversive-based schools. Notably, lying on side/back was only displayed in 605 aversive-based schools (and mostly in School A, the school employing the highest frequency of 606 aversive stimuli). Finally, no differences were found between groups for body shake, scratch and 607 whine. Previous studies on dog training methods have also failed to identify significant 608 differences regarding these behaviors [8, 9], suggesting that these behaviors may not be reliable 609 indicators of stress, at least in the context of training. In support of this view, whining has also 610 been associated with social solicitation, attention seeking and food begging behavior in dogs [29,

611 30]. It is possible that body shaking and scratching may also be associated with excitement and 612 arousal rather than 'negative' stress. Hence, the present study shows a strong association between 613 the use of aversive-based training methods and an increased frequency of stress-related behaviors 614 during companion dog training. These results strengthen and extend the findings of previous 615 studies on companion dogs, which suggested a positive correlation between the use of both shock 616 collars [8] and other negative reinforcement techniques [9], and stress behaviors in the context of 617 dog training.

618 With regards to physiological measures of stress, whereas Group Reward showed no 619 differences in the concentration of salivary cortisol between baseline and post-training samples, 620 dogs from Group Aversive exhibited a statistically significant increase in the concentration of 621 salivary cortisol in post-training samples. Previous studies investigating cortisol levels in dogs in 622 relation to training have yielded contradictory results. Schalke et al (2007) [31] found significant 623 differences in the cortisol levels of three groups of laboratory dogs trained using shock collars 624 with different degrees of shock predictability (the lower the predictability, the higher the cortisol 625 levels). However, studies comparing aversive- and reward-based training methods have found 626 either no significant differences or the opposite pattern. Namely, [8] and [32] found no elevation 627 in cortisol levels after the use of shock collars and lemon-spray bark collars when compared to control groups, and [33] found that a negative punishment training method (a quitting signal) 628 629 resulted in higher levels of cortisol than the use of a pinch collar (aversive-based technique). 630 Hence, the present study is the first to report a significant increase in cortisol levels in dogs trained 631 with aversive-based methods as compared to dogs trained with reward-based methods.

Meanwhile, the increase in cortisol levels observed in the present study (M=0.10 μ g/dL) was smaller than that reported in other studies that found significant increases after dogs were exposed to aversive stimuli (0.20-0.30 μ g/dL in [31] and 0.30-0.40 μ g/dL in [23]). One possible explanation for this difference in magnitude may be related to the nature of the stimuli used in the different studies. Whereas the reported elevations in cortisol in [31] and [23] appeared after the presentation of non-social stimuli (shocks in [31], and shocks, sound blasts and a falling bag in [23]), the stimuli used during training in the present study where mainly of a social nature (i.e.:
leash jerks, physical manipulation or yelling at the dog). Stimuli administered in a social context
may be more predictable or better anticipated and, therefore, generate less acute stress responses
[23]. In support of this view, [23] did not find elevations in cortisol after the presentation of social
stimuli (physical restraint and opening an umbrella).

643 When considering long-term welfare, the present results were also in accordance with our 644 hypothesis. Dogs from Group Aversive displayed a more 'pessimistic' judgment of the 'middle' 645 ambiguous test location in the cognitive bias task, revealing less positive underlying affective 646 states (and hence poorer welfare) than the dogs from Group Reward. The only other study that, 647 to our knowledge, addressed the long-term welfare effects of training methods in dogs was 648 performed by Christiansen et al (2001). In this study, hunting dogs were trained not to attack 649 sheep using shock collars. No general effect of the use of shock collars on dog fear and anxiety 650 was found one year after training took place. However, unlike the test used by [12], which was a 651 modified version of a temperament test used by the Norwegian Kennel Club, the cognitive bias 652 approach used in the current study is a widely established and well-validated method for 653 evaluating animal welfare (e.g., [17, 18]). Hence, to our knowledge, this is the first study to 654 reliably assess and report the effects of aversive- and reward-based training methods in the long-655 term affective states of dogs.

656 Additionally, dogs from Group Reward learned the cognitive bias task faster than dogs 657 from Group Aversive. Similar findings were observed previously by [34], who found a positive 658 correlation between the reported use of reward-based training methods and a dog's ability to learn 659 a novel task (touching a spoon with the nose). In another study, [35] found that dogs with high-660 level training experience were more successful at opening a box to obtain food than dogs that 661 have received either none or only basic training. Although the authors reported that all subjects' 662 training included positive reinforcement methods, they did not specify whether positive 663 punishment and/or negative reinforcement were used in combination. Altogether, previous 664 research suggests that training using positive reinforcement may improve the learning ability of 665 dogs. However, in all previous studies cited above, animals were required to perform a given 666 behavior in order to obtain a positive reinforcer. It is unclear whether the same effect would stand 667 if the dogs had to learn a task whose goal was, for example, to perform a behavior to escape from 668 an unpleasant situation. It may be the case that dogs trained with positive reinforcement develop 669 a specific 'learning set' [36] for tasks involving positive reinforcement, but that dogs trained with 670 aversive-based methods perform better in tasks involving some sort of aversive stimuli. Further 671 research is needed to clarify the relationship between training methods and learning ability in 672 dogs.

673 Notably, we found that the frequency of aversive stimuli used in training (which differed 674 among the aversive-based schools) was correlated with all the welfare indicators measured in the 675 present study. Specifically, we found that the higher the frequency of aversive stimuli used in 676 training, the greater the impact on the short- and on the long-term welfare of dogs. This result is in line with the findings of a previous survey study by [7], whose results showed that a higher 677 678 frequency of punishment was correlated with higher anxiety and fear scores. However, even if 679 only comparing the schools with the lowest frequency of aversive stimuli used during training 680 (C and F) to Group Reward, differences in welfare were found (Appendix S4). Dogs trained at 681 schools C and F showed more stress-related behaviors, spent more time in Tense and Low 682 behavioral states and more time panting during training than dogs trained at reward-based schools, 683 and they also took more trials to learn the cognitive bias task. However, the difference in cortisol 684 concentration between post-training and baseline samples was only marginally significant when 685 dogs from schools C and F were compared with dogs trained with reward-based methods, and no 686 differences were found in the latencies to reach the ambiguous stimuli in the cognitive bias task. 687 This seems to suggest that, although dogs trained in 'least aversive' schools show poorer indicators of welfare than dogs trained in reward-based schools, these are not as poor as those of 688 689 dogs trained in 'most aversive' schools. Notably, schools C and F also used positive reinforcement 690 frequently during their training sessions alongside aversive-based methods, which was rarely the 691 case in schools A and D. Nonetheless, caution is required when interpreting these results due to the reduced sample size for analysis of only two training schools. Future studies should further 692

693 compare the effects on dog welfare of reward-based training and what can be called 'balanced' 694 training, or the use of reward-based methods alongside aversive-based methods. Despite the fact 695 that the results of the present study show that reward-based methods are better for dog welfare, 696 the efficacy of training should also be examined. Presently, there is a lack of scientific evidence 697 regarding the efficacy of different training methods (see also [3]), and thus further research in the 698 topic is required. If reward-based methods are, as the current results show, better for dog welfare 699 than aversive-based methods, and also prove to be more effective or equally effective to 700 aversive-based methods, there is no doubt that owners and dog professionals should use 701 reward-based training practices. If, on the other hand, aversive-based methods prove to be more 702 effective, we would advise using aversive stimuli as infrequently as possible during training, and 703 use them in combination with reward-based techniques, due to the implications for dog welfare.

704 Ultimately, some limitations of the present study must be considered. Firstly, because this 705 was an empirical rather than an experimental study, we cannot infer a true causal relationship 706 between training methods and dog welfare. To do so would require a randomized control trial. 707 Because we did not randomly allocate dogs to the two treatments (training methods), we cannot 708 discard the possibility that dogs enrolling training at aversive-based schools had higher stress 709 levels a priori, or that there are other significant differences between dog-owner pairs that lead 710 some owners to choose an aversive-based school and others to choose a reward-based school. The 711 fact that the baseline levels of cortisol did not differ between groups weakens the former possibility. Regarding the latter, however, we did indeed find differences between groups in 712 713 owner sex, previous experience with dogs and in the information they relied on for choosing the 714 dog training school. However, conducting an experimental study would raise ethical concerns 715 (but see [8]), as previous studies have already suggested an association between the use of 716 aversive-based methods and indicators of stress in dogs (see [3] for a review), as well as with the 717 quality of dog-owner attachment [37]. Secondly, we need to consider the possibility for a 718 volunteer bias and hence any generalization of the present results must take this in account.

719

720 5. Conclusions

721 Our results show that companion dogs trained using aversive-based methods experienced 722 poorer welfare as compared to companion dogs trained using reward-based methods, at both the 723 short- and the long-term level. Specifically, dogs attending schools using aversive-based methods 724 displayed more stress-related behaviors and body postures during training, higher elevations in 725 cortisol levels after training, and were more 'pessimistic' in a cognitive bias task than dogs 726 attending schools using reward-based methods. Moreover, we found that the higher the frequency 727 of aversive stimuli used in training, the greater the impact on the short- and the long-term welfare 728 of dogs. To our knowledge, this is the first comprehensive and systematic study to evaluate and report the effects of dog training methods on companion dog welfare. Critically, our study points 729 730 to the fact that the welfare of companion dogs trained with aversive-based methods appears to be 731 at risk.

732

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843 Supporting information captions

844

Appendix S1. Information on training schools and training methods.

Table S1a. Characterization of the training schools by types of behaviors trained, training sites

- and class structure.
- Table S1b. Definition of the aversive-based operant conditioning procedures used to classify the
- dog training schools as aversive-based or reward-based. The schools were classified as aversive-
- 850 based if they used some sort of positive punishment and/or negative reinforcement and as reward-
- based if they did not use any of these techniques.

852	Table S1c. Frequency (mean \pm standard deviation) of positive punishment and negative
853	reinforcement used during the four training sessions videotaped at the dog training schools.
854	Schools A, C, D and F were classified as aversive-based and Schools B, E and G were classified
855	as reward-based.

856

- 857 Appendix S2. Ethograms used for the analysis of the video recordings of dog training sessions.
- 858 Table S2a. Ethogram for stress-related behaviors.
- Table S2b. Ethogram for overall behavioral state and panting.
- 860

Appendix S3. Questionnaire data and relationship with the dependent variables measured in thepresent study.

Table S3a. Variables obtained from the questionnaire (dog demographics and background, and owner demographics and experience with dogs and dog training). Chi-square tests were used to compare the two groups (Reward and Aversive).

866 Table S3b. Statistical results for the effects of the dog-related variables that differed between the 867 Reward and the Aversive groups (age, FCI breed group and age of separation from the mother) 868 on the different dependent variables measured in the current study. The variable age comprised 869 five categories (< 6 months; 6 -11 months; 1-3 years; 4-7 years; >7 years), the FCI breed group 870 variable comprised eleven (Mixed breed; Sheepdogs and Cattledogs, except Swiss Cattledogs; 871 Pinscher and Schnauzer - Molossoid and Swiss Mountain and Cattledogs; Terriers; Daschunds; 872 Spitz and primitive types; Scent hounds and related breeds; Pointing dogs; Retrievers, Flushing 873 Dogs and Water Dogs; Companion and Toy Dogs; Sight Hounds), and the variable age of 874 separation from the mother comprised nine [less than 1 month; 1 - 1.5 months (inclusive); 1.5 - 1.5875 2 months (inclusive); 2 - 2.5 months (inclusive); 2.5 - 3 months (inclusive); 3 - 4 months 876 (inclusive); 4 - 5 months (inclusive); more than 5 months, don't know]. Kruskal-Wallis and 877 Mann-Whitney tests were used to compare more or less than two categories, respectively.

878	Significant differences were found for the frequency of paw-lift and percentage of scans in Tense
879	state in Group Aversive and for the percentage of scans panting in Group Reward; however, post-
880	hoc pairwise comparisons with adjusted Bonferroni's correction revealed no differences between
881	the different categories.
882	
883	Appendix S4. Statistical results for the comparison of the different welfare indicators between
884	Group Reward and the two schools from Group Aversive that used the lowest frequency of
885	aversive stimuli during training (C and F).
886	

Appendix S5. Raw data underlying all the analyzes performed in the current research paper.









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Difference in cortisol concentraion (PT-BL)



