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**Investigating putative depression-like states in the domestic dog: does greater
time spent displaying waking inactivity in the home kennel co-vary with negative
judgment of ambiguity?**

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23 Abstract

24

25 Exposure to chronic stressors and/or traumatic events can trigger depression-like
26 forms of waking inactivity in non-human species (mice, horses, primates) as well as
27 clinical depression in humans. This study aimed to test the hypothesis that elevated
28 levels of waking inactivity in the home environment, in tandem with exposure to chronic
29 stress and/or traumatic events, could reflect a depression-like condition in the domestic
30 dog. We tested this hypothesis in shelter dogs by investigating the association
31 between greater time spent inactive 'awake but motionless' (ABM) in the home-pen
32 and a core symptom of human clinical depression; low mood, using negative judgment
33 of ambiguity as a proxy. Subjects were 20 dogs from across three shelters (10F:10M,
34 aged 3.8 years \pm SD 2.0; 45% seized as part of legal cases, 30% found as strays, 25%
35 relinquished to the shelters). Time spent ABM was determined from 6hrs of video per
36 dog (one daily 2-hour period recorded across three consecutive days, following a day
37 and time period blocked design). To measure judgment of ambiguity, dogs were
38 trained in a location discrimination task that a bowl either contained food (positive
39 location) or was empty (negative location). Dogs were tested with one negative, one
40 positive, and one ambiguous (equidistant to the two training positions) trial. Negative
41 judgment of ambiguity manifests as longer latencies to reach the ambiguous bowl. We
42 created a positive expectancy score by adjusting the latency in the ambiguous trial to
43 the latency to approach the negative and positive locations (higher scores indicating
44 bias towards expecting more positive outcomes). Time spent ABM was compared
45 against positive expectancy scores using a multivariable GLM. Dogs were ABM for a
46 median of 2.8% of the scans (Q1: 0.75%, Q3: 4.75), with clear inter-individual variation
47 (0-20.4%). In the cognitive judgment bias, stray dogs reached the learning criterion
48 faster than those of other origins (ANOVA: $F_{19} = 4.03$, $p=0.037$; Kaplan-Meier survival
49 analyses, $\text{Chi}^2_2 = 7.88$, $p=0.019$). During the test trials, all latencies statistically differed

from each other (Negative > Ambiguous > Positive, Friedman test $\chi^2_{(2)} = 33.90$, $p < 0.001$). Cognitive expectancy scores however exhibited minimal variation and an exaggerated right-skew distribution, showing a strong bias towards expecting a positive outcome at the ambiguous location in most of the dogs, and showed no association ($p > 0.05$) with time spent ABM, therefore the hypothesis was not supported. We discuss reasons for observing such general 'optimistic' tendencies in this study, as well as further research directions.

Key words: shelter dog; affective state; depression-like state; waking inactivity; cognitive judgment bias; stray dogs

1. Introduction

Captive and domestic animals are often described as inactive, with the implicit (e.g. Broom, 1998) or explicit implication that this reduced activity is a welfare problem (e.g. McPhee and Carlstead, 2010). On the other hand, compromised inactivity, such as sleep deprivation, is also a considerable welfare problem (Ferrara and De Gennaro, 2001). Inactivity has rarely been the focus of behavioural studies. Indeed, the affective states associated with greater (or suppressed inactivity largely remain un-investigated in non-human animals (Fureix and Meagher, 2015)). In this study, we focus on a specific form of inactivity; spending greater time awake but motionless in the home environment, which, following a translational ‘from humans to animals’ rationale, has been hypothesised to reflect depression-like conditions in affected individuals (including horses, mice, dogs, non-human primates: reviewed in Fureix and Meagher, 2015).

In humans, clinical depression² is a debilitating mood disorder diagnosed by the co-occurrence of affective, cognitive, behavioural and homeostatic symptoms, of which the two core features are a low, sad mood and anhedonia (World Health Organization WHO, 1994; American Psychiatric Association APA, 2013). Chronic stress plays a major role in triggering the disease, especially in vulnerable people with predisposing genetic factors and/or experience of aversive events during their early life (APA, 2013; Hammen *et al.*, 2009; Siegrist, 2008; Capsi *et al.*, 2003). Cognitive changes are theorised to be involved in both the aetiology and maintenance of the illness. One such change is negative cognitive bias in processing information; e.g. judging ambiguity more negatively, paying more attention to, and remembering, negative information better (Beck, 1967; MacLeod and Byrne, 1996; Gotlib and Krasnoperova, 1998; Disner

² Referring here to *major depressive disorder* in the DSM-V (APA 2013) or *depressive episodes* in the ICD-10 (WHO 1994)

et al., 2011). Another cognitive feature of clinical depression is 'learned helplessness', where an individual comes to believe that desired outcomes are improbable and aversive outcomes likely, and no action on his/her part can alter this, and as such stops acting (Maier and Seligman, 1976; Abramson, Seligman and Teasdale, 1978).

Importantly, clinically depressed people often show increased inactivity in various daily activities (Baker et al., 1971; APA, 2013), which includes a reduced engagement with doing activities they once enjoyed and chores that have to be done (Knowles, 1981), as well as decreased social (APA, 2013; Baker et al., 1971; Schelde, 1998) and physical activities (Lindwall, Larsman and Hagger, 2011; Seime and Vickers, 2006). There is evidence that spending greater time awake but motionless in the home environment is associated with signs of anhedonia in horses (Fureix et al., 2015) and 'helpless' responses in laboratory mice (Fureix et al., 2016), which reinforces the symptomatic similarities between human clinical depression and greater time spent displaying waking inactivity in animals. Moreover, exposure to chronic stress and/or traumatic events appears to trigger both waking inactivity in animals, at least in some individuals (e.g. mice: Tilly, Dallaire and Mason, 2010; Fureix et al., 2016; dogs: Fox, 1968; Seligman and Altemor, 1980) and human clinical depression, particularly in vulnerable people (APA, 2013; Hammen et al., 2009; Siegrist, 2008; Capsi et al., 2003).

The current study aims to test the hypothesis that elevated levels of waking inactivity in the home environment, in tandem with exposure to chronic stress and/or traumatic events, could reflect a depression-like condition in the domestic dog. Pet dogs can indeed become highly inactive and unresponsive when exposed to traumatic events, such as when deprived of their owners or after the loss of a social companion (Fox, 1968). This state is commonly referred to as the dog being 'depressed' or showing 'depressive like behaviour' (e.g. Konok et al., 2015; Yeates, 2016; Gosling, Kwan and John, 2003), although this remains to be empirically confirmed. Learned helplessness, one of the cognitive features of clinical depression, has been shown in

dogs (Seligman and Altemus, 1980; Maier and Seligman, 1976), and is a phenomenon typically accompanied by an overall decrease in activity (e.g. Mineka and Hendersen, 1985). Moreover, kennelled dogs, such as those housed in research, working dog facilities or rescue shelters, can be exposed to an array of chronic stressors including minimal exercise, lack of positive social interactions, disrupted routines, high noise levels and a lack of control over their environment (Maier and Seligman, 1976; Part *et al.*, 2014; Polgár, Blackwell and Rooney, 2019; Willen, Schiml and Hennessy, 2019; Cobb *et al.*, 2019). For shelter dogs in particular, such situations could be exacerbated by the potentially traumatic abrupt loss of their previous owners, with whom they may have formed strong attachments (Fox, 1968; Willen, Schiml and Hennessy, 2019). Because the aetiology of human depression emphasises aversive life events and chronic stress as common triggers, and because of the chronic stressors and potential traumatic events dogs in rescue shelters are exposed to, we therefore propose that shelter dogs are a suitable model for testing the hypothesis that greater time spent displaying waking inactivity could reflect a depression-like condition in domestic dogs.

We evaluated this hypothesis in shelter dogs by testing for an association between greater time spent inactive 'awake but motionless' in the home-pen and a core symptom of human clinical depression; a low, sad mood (APA, 2013), using negative judgment of ambiguity as a proxy. Negative cognitive biases in processing information can be displayed by people reporting negative affect; including clinical depression; e.g. in the form of making more negative judgment about ambiguous events and stimuli (reviewed in Paul, Harding and Mendl, 2005). Similar affect-related biases in judging ambiguity have also been evidenced in non-human animals (Mendl *et al.*, 2009; Gygas, 2014; Paul, Harding and Mendl, 2005; Pryce and Seifritz, 2011; Mendl, Burman and Paul, 2010), including in dogs (Mendl *et al.*, 2010), hence the use of this cognitive measure as a proxy of affective states. We used an established paradigm commonly used to assess judgment cognitive bias in dogs (Kis *et al.*, 2015; Mendl *et al.*, 2010),

and we predicted that the dogs spending greater time inactive 'awake but motionless' in their home-pen would display the least positive judgment of ambiguity.

2. Material and methods

2.1. Ethics

The University of Bristol Animal Welfare Ethical Review Board approved the study in January 2016 (UB/15/072), and the study complied with the Animals (Scientific Procedures) Act 1986, EU directive 2010/63/EU and UK Home Office code of practice. The Head of Companion Animals Department, the Chief Veterinary Officer and the Chief Scientific Officer granted permission to approach RSPCA shelters in July 2016. Dog husbandry and care were under the management of the shelter staff.

2.2. Subjects

Subjects were 20 dogs (10F:10M) from across three shelters respectively recruited in October, November and December 2017 (**Table 1**). Inclusion criteria for selecting dogs for the study were as follows: they must not have an existing health condition (based on veterinary examination at the shelter); they were aged between 1-10 years; were not on a calorie reduced diet; and must have been in the shelter for at least 1 week (dog behaviour becomes repeatable and stable after 1 week in a shelter, Goold and Newbury, 2017). The dogs had spent on average 7.6 ± 3.9 weeks in the shelter (ranging from 1.4 to 18.1 weeks) at the time of observation. Dogs were aged $3.8 \text{ years} \pm \text{SD } 2.0$ (ranging from 1 to 7 years). Forty percent (8 dogs) of the dogs were neutered. In total, 45% (9 dogs) were seized as part of legal cases, 30% (6 dogs) were

found as strays and 25% (5 dogs) were voluntarily relinquished to the shelters. There were 12 'breeds' including crosses (**Table 1**). Due to the small numbers involved, we grouped the breeds according to American Kennel Club groups (American Kennel Club, no date) as being bred for co-operative work with humans by combining the Working / Herding / Sporting groups (n = 9 dogs) or not, by combining the Terrier / Toy / None groups (n = 11 dogs). This was done to control for differences that might be present in cognitive problem-solving abilities according to breed type, which have been evidenced previously in cognitive bias tasks (Pogány *et al.*, 2018), although we acknowledge that breed assignation may be imprecise in this context. In all shelters, the dogs were individually housed in two-compartment kennels, entirely cleaned once a day, and walked twice a day (once for 10 and once for 20 minutes) by shelter staff and/or volunteers. All dogs were fed twice a day (around 8:30 am and 4/5 pm) and provided daily with a Kong™ – a rubber toy stuffed with food (either around lunch time or around 4:30/5:00 pm). Water was provided *ad libitum*.

2.3. Home-pen activity budget

Methods related to home-pen recording activities are published in detail in Harvey *et al.* (2019). Briefly, each dog was recorded in its home-kennel using two GoPro Hero 3 cameras for one daily 2-hour period across three consecutive days, following a day (1, 2, 3) and time period (9:00-11:00; 11:30-13:30; 14:00-16:00) blocked design (totalling 6 hours of recording per dog). Behaviour was sampled from footage every 1.5-minutes *via* instantaneous sampling (Martin and Bateson, 2007), watching continuously 5 seconds either side of the scan point for the behaviours being characterised by either a lack of movement or repetition (e.g. pacing and abnormal repetitive behaviour) in order to best determine the correct action (sampling method and interval sample selection justified in Harvey *et al.*, 2019). The behaviour we

hypothesised to reflect a depression-like condition in dogs, *i.e.* being awake but motionless 'ABM', was defined as '*the dog is completely motionless (no head, body or ear movements) with eyes open apparently staring (anywhere). Dog may be lying, sitting or standing but not vocalizing. If sitting, head may be in a 'drooped' position with head lower than or level with their spine. State must last for at least 5 seconds*' (adapted from Fureix *et al.*, 2015; Fureix *et al.*, 2016, see also Harvey *et al.*, 2019). H.H. and P.J.C., two trained observers with a >95% inter-observer agreement and blind to the dogs' scores in the cognitive judgement bias at the time of the observation, scored the videos using the Behavioral Observation Research Interactive Software (Friard and Gamba, 2016).

2.4. Cognitive judgment bias test

The cognitive bias procedure was a standard spatial test based upon that detailed by Kis and colleagues (2015). Piloting was conducted with four staff owned dogs at the University of Nottingham School of Veterinary Medicine and Science. Piloting enabled refinement of the procedure, decisions regarding which food to use as a training reward, training of the experimenters and, using a stopwatch and video recordings, testing of the standard deviation in the timing of latency to approach the bowls. The final procedure contained two parts; a training phase and a testing phase. During the training phase dogs were trained to distinguish a rewarded (positive) location (bowl with food in) from an unrewarded (negative) location (bowl without food in) (**Figure 1**). Training ceased when dogs reached a criterion (detailed in 2.4.1.) that indicated they had successfully learnt to discriminate the bowl positions. The testing phase consisted of one negative trial, one positive trial and one ambiguous trial where the bowl was placed equidistant to the two previously learned positions (**Figure 1**). Prior to training, experimenters ensured that the dogs had not eaten or experienced

an event (such as a walk, volunteer kennel visit, or kennel cleaning) in the 30 minutes prior to training. Each session began with a 10-minute habituation phase, where the dog was brought into the room, let off the lead and allowed to explore the room (and meet the testers if it chose to) and was offered a bowl of water. The water was removed for training/testing and offered again at the end of the session. During this phase all dogs were offered the food to be used to bait the bowls to check that they were willing to eat it from the bowl. The dogs were also observed for behavioural indicators of stress and if trembling, avoidance or hiding was exhibited in this period (considered to indicate extreme stress, Marder *et al.*, 2013) the dog was returned to its kennel and not included in the study sample.

2.4.1. Training phase

To start the training session, the dog was held by its collar by experimenter 1 (H.H. or P.J.C.) at the predetermined starting point 3 metres from the two potential bowl locations (**Figure 1**). Experimenter 2 (C.W.) turned her back on the dog to load the bowl with a reward, or to pretend to load the bowl (dropping food into the bowl and then removing it) in the unrewarded condition – this ensured that the dog always heard the experimenter accessing the food and dropping it in the bowl, making sure the bowl also smelled of the food, and making this information of no value for predicting whether it was rewarded or not. The identity of a location as positive ('P') or negative ('N') was counterbalanced between dogs. For the first presentation only (always P), the food reward was visually shown to the dog before being placed in the bowl to motivate them to search. Bowls were presented in a pseudo-random fixed order (PPPNPPNNPNNN) repeated until criterion was reached. After baiting the bowl, experimenter 2 called the dog's name and said "Look!" until the dog looked their way, then placed the bowl on the appropriate location marker (P or N). Experimenter 2 then positioned herself facing the dog, 0.5 metres behind the bowl she had placed to ensure consistency of

presentation for all bowls and wore dark sunglasses to minimise eye contact, standing still looking straight ahead after placing the bowl. Experimenter 2 came from the same central position to place the bowl at any of the locations, as Müller *et al.* (2012) observed that the generalisation process can be biased in some way by the starting point of the experimenter. At the moment the bowl was placed, experimenter 1 released their hold of the dog's collar, and experimenter 2 started a stopwatch held at chest level, stopping it when the dog had crossed a masking tape line placed 10cm in front of the bowl locations to record the dog's latency to approach it. No communication with the dogs was allowed (no dogs required any verbal encouragement to move once being released) until the end of the trial and the dog needed to be recalled to the start position by experimenter 1. Each dog was given a maximum of 30s to visit the bowl during a trial. If they had not visited the bowl after this time, a time of 30s was recorded, and the next trial begun. If that happened five times in a row the training was stopped and restarted the next day. There were no breaks in between trials and the entire procedure was carried out on the same day where possible with the training phase limited to a 1-hour maximum. If the dog reached criterion within the hour, they were tested immediately after criterion was reached. For dogs that took longer than 1-hour to reach criterion, training was continued on the following day, and dogs were tested once criterion was reached. Criterion was indicated when the longest latency for the previous 5 positive-P trials was shorter than the latency for each of the previous 5 negative-N trials, with a difference greater than 0.4 seconds (0.4s was the standard deviation in repeat timing latencies from video footage established during piloting). Latencies were recorded live by experimenter 1 and entered into an Excel spreadsheet, which was programmed to calculate when criterion had been reached.

The reward food used for half of the dogs (50%) was Scruffy Bites™ by Misfits®, Mars Inc., as these were found to motivate the pilot dogs to continue training despite unrewarded trials, where standard balanced diet kibble did not. However, two dogs did

not eat the Scruffy Bites™ when offered in the pre-test phase, but did eat pieces of Bakers® Sizzlers® (Purina®, Nestlé Purina PetCare UK Ltd) so were trained and tested with these; three dogs needed hypoallergenic food so were trained and tested using Harringtons Hypoallergenic Grain-Free Rich in Salmon (Harringtons Pet Food) dry kibble; and five dogs in shelter 5 ate only wet food so were trained and tested with original Chappie®, (Mars Inc). To limit the calorie intake for the training sessions with differently sized dogs when using Scruffy Bites™, rewarded bowls were baited with ½ pieces of the treat for large/medium sized dogs, whilst for small/toy sized dogs they were baited with ¼ sized pieces and for extra-large breeds whole pieces were used.

2.4.2. Test phase

Once criterion was reached, a camera (GoPro HERO 3, White edition) pointed towards the bowls (side on) was turned on and the dogs were presented with three trials ending with presentation of the ambiguous bowl, in the order N-P-A. The ambiguous bowl was not baited with a food reward. All conditions of the protocol remained the same as in the training phase. Latencies were timed live and were additionally recorded by C.W. from video footage in triplicate (from the moment the bowl touched the floor to the dog crossing the tape marker) and the mean latency was utilised for data analysis.

2.5. Statistical analyses

Statistical analysis was conducted using SPSS v. 22 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were calculated to summarise the behavioural variables observed during the home-pen and cognitive judgment bias test. Time spent ABM and model residuals were not normally distributed (and two dogs were considered extreme

on a stem-and-leaf plot), so ABM was transformed into a logarithmic scale (after adding 1 to remove zeros). The logarithmically transformed variable is indicated with 'lgABM'. Bivariate analysis of variance (ANOVA) were used to investigate potential associations between time taken to reach the learning criterion in the cognitive judgment bias task (normally distributed dependent variable) and each independent variable: sex, neuter status, shelter, origin, and American Kennel Club working/herding/sporting breed (yes/no), whilst linear regression models (LM) were used to compare against age and the length of time each dog had been in the shelter (weeks).

To check that the dogs latency to approach the bowl differed according to the bowl type in the test phase, a Friedman test was used (for data that violates the assumptions of normality of residuals and sphericity for a within-subjects repeated measures ANOVA) followed by Wilcoxon signed rank post-hoc tests with Bonferroni correction (Siegel and Castellan, 1988). In order to investigate the relationship between the time dogs spent ABM and their judgment of ambiguity, we calculated for each dog its Positive Expectancy Score (PES) according to Kis *et al.* (2015) as follows:

PES = 100 – Cognitive Bias Score, where

$$\text{Cognitive Bias Score} = \frac{(\text{latency to reach ambiguous location} - \text{latency to reach positive location})}{(\text{latency to reach negative location} - \text{latency to reach positive location})} * 100$$

Higher PES indicate a bias towards expecting more positive outcomes; where the latency to reach the ambiguous location falls in-between the positive and negative the PES value will range from 0-100, and where the latency to reach the ambiguous location is faster than the positive the PES value will be above 100. One dog (ID 76 in **Table 1**) had to be excluded from calculating the PES because it ran faster to the negative location than it did the ambiguous, giving it a PES score of -505, which

violates the assumptions of the score. For the remaining 19 dogs, the PES scores were not normally distributed and PESs could not be logarithmically transformed, so it was recoded into a binary variable around the median to group dogs into low ($n = 10$) and high ($n = 9$) PES groups. Bivariate comparisons between PES group and (lg)ABM, sex, age, neuter status, breed type, length of time each dog has been in the shelter for, the dog's origin, the dog's weight and the shelter using Mann-Whitney U tests for continuous variables, Fisher's exact tests for binary variables and Chi-square tests for categorical variables. All variables were then tested in multivariable logistic regression models against PES group, using both forwards and backwards selection methods in case any variables interacted with each other to stratify the data. Significance was set at $p < 0.05$.

3. Results

3.1. Awake but Motionless (ABM) data

Being awake but motionless was displayed for a median time of 2.8% of the scans (1st quartile: 0.75%, third quartile: 4.75%), with clear variation between individual dogs (from 0 to 20.4% of scans).

3.2 Number of trials required to reach learning criterion in the cognitive judgment bias assay (training phase)

The mean number of training trials taken to reach learning criterion in the cognitive judgment bias was 43 (SD \pm 18, median 43.5), with considerable individual variation (from 12 to 83 trials). There was a significant difference in time taken to reach learning criterion according to the origin of the dogs (ANOVA: $F = 4.03$, $p = 0.037$, $df = 19$). This finding was confirmed with a Kaplan-Meier survival analysis, which showed that stray dogs reached criterion significantly faster than those that were relinquished or seized

(**Figure 2**, $\text{Chi}^2 = 7.88$, $p = 0.019$, $df = 2$). Dogs in the working/herding/sporting group appeared to reach criterion quicker than the others, however this difference did not reach statistical significance (ANOVA: $F = 1.97$, $p = 0.177$, $df = 19$), and no other variable predicted the number of trials taken to reach learning criterion (LM: age, $t = 0.766$, $p = 0.456$; the length of time each dog had been in the shelter, $t = -0.32$, $p = 0.756$; ANOVA: sex, $F = 0.20$, $p = 0.660$; neuter status, $F = 2.24$, $p = 0.142$ and shelter, $F = 1.36$, $p = 0.283$).

3.3 Latency to reach the test bowls and PES scores in the cognitive judgment bias assay (testing phase)

The median and interquartile range for the raw latencies to reach the positive, ambiguous and negative locations during the cognitive judgment bias test trials are shown in **Figure 3** (see also **Table 2**). There was a statistically significant difference in the dogs' latencies to reach the different types of bowl during the test phase (Friedman Chi-square₍₂₎ = 33.90, $p = <0.001$). Post-hoc analysis with Wilcoxon signed rank tests resulted in significant differences between latency for all three bowl types after Bonferroni correction resulting in a significance threshold of $p < 0.017$. Latencies for the negative-N bowl were significantly longer than both the positive-P ($Z = 3.92$, $p = <0.001$) and the ambiguous-A latencies ($Z = 3.58$, $p = <0.001$), whilst the ambiguous-A latencies were significantly longer than the positive-P latencies ($Z = 3.22$, $p = 0.001$).

Although the ambiguous-A latencies were significantly longer than the positive-P latencies, as shown in **Figure 3** there was very little variation in the ambiguous-A latencies in general. As such, the PES scores calculated from these raw latencies exhibited minimal variation and an exaggerated right-skew distribution (**Figure 4**): 50% of the data fell between 81.8 and 99.6 and the median score was 96.7, showing a strong bias towards expecting positive outcome at the ambiguous location in most of the dogs.

3.4 Comparisons between PES scores and time spent ABM

Bivariate comparisons revealed no associations between PES group and (lg)ABM (Mann-Whitney U: $Z = 0.00$, $p = 1.000$), sex (Fisher's exact test $p = 0.586$), age (Mann-Whitney U: $Z = -0.22$, $p = 0.830$), neuter status (Fisher's exact test $p = 0.255$), breed type (Fisher's exact test $p = 0.414$), the length of time each dog had been in the shelter (Mann-Whitney U: $Z = -0.29$, $p = 0.775$), the dog's origin (Chi-square = 2.82, $p = 0.244$), weight (Mann-Whitney U: $Z = -0.08$, $p = 0.935$) or the shelter they were tested at (Chi-square = 1.59, $p = 0.451$). All variables were tested together in multivariable logistic regression models against PES group, using both forwards and backwards stepwise selection methods in case of stratifying effects of interacting variables. However, no model could be found where any variable was significantly ($p < 0.05$) associated with PES group.

3.5 Extreme scoring dogs

There was only one dog (ID 86 in Table 1) whose PES score could be considered to reflect a negative judgement bias, with a PES of 25. However, nothing of note stood out when their data was examined; they were in the shelter for only four weeks, were seized as part of a legal case along with four other dogs from the same property that were also in the study, but did not show different than average behaviour (i.e. their scores were not outside of the standard percentile range) for any behaviour measurements including ABM.

Dogs 80 and 69 in Table 1 had the highest ABM scores, spending 20.4% and 13.0% of scans ABM, respectively. These were considered extreme values on a stem-and-leaf plot, with the rest of the dogs spending between 0% and 7.4% of their time ABM. Both dogs had high PES scores; 81.8 for dog 80 and 99.9 for dog 69. Dog 80, who spent the most time ABM, was a relinquished dog that had been in the shelter for

6 weeks. This dog also spent the most time whining (17.9% of scans compared to an interquartile range of 0 to 3.08%) out of all dogs and took the second longest number of trials to reach training criterion for the judgement bias test (75 trials, interquartile range 31 to 57), but did not stand out as different for any other behavioural variable. Dog 6 was a stray who had been at the shelter for 2.1 weeks and their behaviour did not stand out as distinctly different from the rest of the studied dogs for any other measured variable.

4. Discussion

This study aimed to test the hypothesis that greater time spent displaying waking inactivity in the home environment could reflect a depression-like condition in kennelled domestic dogs. We tested this hypothesis in shelter dogs by investigating the association between greater time spent inactive 'awake but motionless' in the home-pen (ABM) and negative judgment of ambiguity (a proxy for low mood, e.g. Mendl et al., 2009; Gygas, 2014; Paul, Harding and Mendl, 2005; Pryce and Seifritz, 2011). Results show that the dogs displayed ABM, with clear variation between individual dogs, although this variation was not predicted by the dog's sex, neuter status, age, breed type, weight, each dog's origin, the shelter they were tested at, or the length of time each dog had been in the shelter for. The dogs successfully learnt to discriminate between the positive and negative locations in the judgment cognitive bias task, with stray dogs reaching learning criterion significantly faster than dogs that were relinquished by their owners or seized as part of welfare cases. Stray dogs have been shown to differ cognitively from pet dogs in ways that characterise reduced ability to follow human gestural cues (Udell, Dorey and Wynne, 2008; Udell, Dorey and Wynne, 2010), so the fact that strays learnt the discrimination task faster than pet dogs in this non-social learning paradigm may indicate that stray dogs have a higher

propensity for independent learning, which likely favours survival under feral life conditions. Contrary to our prediction however, greater time spent ABM did not predict lower positive expectancy scores in the cognitive judgment bias test. In fact, most dogs scored very highly on their positive expectancy scores, resulting in very little variation between subjects. The results therefore do not support the study hypothesis. That the amount of time spent ABM overall appears relatively short (median 2.8%, third quartile 4.75%) seems unlikely to explain this lack of support. Indeed, this time varies between individuals, with some dogs spending considerably longer in this state. Moreover, in both horses and mice; in which greater time spent displaying putatively similar forms of waking inactivity have been shown to predict depression-like symptoms (Fureix *et al.* 2015, 2016); the average times spent displaying waking inactivity were not higher than 4% of the scans. On the other hand, the unexpected lack of variation between individual dog's responses in the cognitive judgment bias test might invalidate the performance in the test as a comparison measure. We thus discuss below tentative reasons for observing such a general 'optimistic' tendency in our study, as well as further research directions.

As discussed in Burman (2014), and confirmed by an updated literature screening (**Table 2**), very similar methodologies have been adopted in studies measuring dogs' judgment cognitive biases. In fact, most of these studies (including the current one) adapted the spatial go / no go version of the paradigm originally developed in rats (Burman *et al.*, 2008a), and first adjusted to dogs by Mendl *et al.* (2010). The values we observed, from the number of trials required to reach the learning criterion to the dogs' test latencies, fall within the range of reported values from similar studies (**Table 2**) [with the exception of Kis *et al.*, 2015 that reports shorter latencies overall]. The general 'optimistic' tendency observed in our study is therefore unlikely to be explained by dogs failing to discriminate locations overall.

A plausible explanation for the general 'optimistic' tendency we observed here might instead come from the limited stimulation and social contact with humans received by shelter dogs (Burman *et al.*, 2011; Part *et al.*, 2014; Willen, Schiml and Hennessy, 2019; Müller *et al.*, 2012). Research has shown that in some cases shelter dogs can be so impoverished of stimulation from human contact that they can form attachment bonds with unfamiliar humans in a matter of minutes (Gacsi *et al.*, 2001) and even when they appear stressed by the presence of an unfamiliar human, they choose to remain in close proximity to them, where pet dogs do not (Barrera *et al.*, 2010). It has also been shown that interaction with humans can have long lasting positive effects on the behaviour and physiology of shelter dogs (Bergamasco *et al.*, 2010; Willen, Schiml and Hennessy, 2019). Being handled by the experimenters throughout the training period, receiving not only food treats and cognitive stimulation from training but also time out of their home-pen and interaction with people, might thus have provided an important source of positive excitement, potentially elevating the dogs' affective state and overshadowing a more negative 'baseline mood' that may have been present in the comparatively more barren home kennel (Burman *et al.*, 2011; Burman, 2014; Willen, Schiml and Hennessy, 2019). Interestingly, the vast majority of the dogs tested here scored in a similar range to pet dogs that had been artificially dosed with oxytocin, a hormone inducing positive expectation biases in dogs (Kis *et al.*, 2015). Elevated oxytocin levels might thus be a physiological mechanism at stake in the general 'optimistic' tendency we observed here, although this suggestion remains to be tested.

Such a high sensitivity to human contact and access to stimulation in shelter dogs (perhaps elevating their mood, perhaps *via* increased oxytocin levels) is however unlikely to be the sole explanation for the general 'optimistic' tendency we observed here. Indeed, several independent studies have been conducted using an analogous paradigm in comparable shelter dog populations, in which the authors did observe

inter-individual variation in the dogs' responses, irrespective of whether these differences supported the studies hypothesis or not (Willen, Schiml and Hennessy, 2019; Owczarczak-Garstecka and Burman, 2016; Titulaer *et al.*, 2013; Mendl *et al.*, 2010). We therefore discuss in the next paragraph some methodological aspects that differ between the task we applied here and the ones reported in the literature that could also partially explain our results, *i.e.* the number of probe test trials and of ambiguous location tested, as well as individual tailoring in the reward food used.

Following Kis *et al.* (2015), we conducted one ambiguous probe test trial (*versus* 3 or more) in the middle position only (*versus* also assessing 'near positive' and 'near negative' locations). We chose to do so to prevent the dogs from learning that the probe stimulus was unrewarded, or conversely that, in the absence of punishment, there was no real cost in approaching ambiguous locations (see *e.g.* Mendl *et al.*, 2009 and Burman, 2014 for further discussion of these limitations). Practically speaking, it also allowed us to keep the test as short as possible due to time-related constraints (*e.g.* availability of the room at the shelter). However, conducting a single probe test trial at the middle location only comes with its own limitations. One may for instance wonder if the dogs' reaction to the probe test trial did reflect a generalisation effect (probe being perceived as closed enough to the positive location to approach it fast), or an excitement / curiosity response to a novel location; a potential confound that cannot be investigated with only one trial. Moreover, using only the intermediate position did not allow testing for finer predictions, such as depressed individuals being specifically less optimistic (*i.e.* effect specifically observed on the Near Positive location) while anxious individuals appeared to be more pessimistic (*i.e.* stronger effect observed at the Near Negative location) (Salmeto *et al.*, 2011; although supports for this differential prediction are mixed: Müller *et al.*, 2012; Burman, 2014).

Lastly, we tailored the reward used in the test for 50% of the dogs due to health-related constraints or to ensure the dogs were motivated to perform in the training. To

our best knowledge, no investigation has been performed so far to test whether training animals with their *preferred* reward in a judgment cognitive bias paradigm can induce more 'optimistic' response to ambiguity than when being tested with a 'given' reward (*i.e.* rewarding but not necessarily preferred). Using preferred vs less preferred rewards nonetheless appears to influence the animal's performance in cognitive tasks, *e.g.* piglets demonstrate greater level of impulse control in a delay-of-gratification paradigm to gain their highly preferred reward (Zebunke *et al.*, 2018), and monkeys (*Macaca fuscata*) appear more motivated to perform in a working memory task to gain their preferred reward, while displaying neurobiological signs (increased in dopamine release in the prefrontal cortex) suggesting that performing the task for the less preferred reward could be 'mildly stressful' (Kodama *et al.*, 2014). Although *preference* for used rewards was not empirically tested in our study, tailoring the reward might have strongly boosted our subjects' motivation for acquiring the treat (*e.g.* Riemer *et al.*, 2018; see also Bentosela *et al.*, 2009), which may partly explain the general 'optimistic' tendency observed here, since greater motivation to gain a food reward has been associated with trait 'optimism' in rats (Rygula *et al.*, 2015).

Going back to the study predictions, our results do not support the hypothesis that greater time spent displaying waking inactivity in the home environment reflects a depression-like condition in kennelled dogs, although we believe that, in the unexpected absence of variation between individual dog's responses in the cognitive judgment bias measure, methodological refinements and complementary investigations are required before it is possible to safely reject this hypothesis. Such refinements would target the above-discussed aspects; *e.g.* conducting a judgment bias assay testing for more probe tests locations and with several trials per location while implementing *e.g.* variable reinforcement during training, and investigate whether using preferred rewards might induce greater 'optimism' than non-preferred rewards to take an informed decision on whether tailoring reward used would confound

the results. Other methodological refinement would include assessing different types of cognitive measurements as proxies for low mood, which might also be easier to perform within the dog's home kennel, in an automated way to reduce the impact of interacting with humans on the dogs' affective state at the time of the test. This could for example be done using an incentive contrast paradigm, since sensitivity to reward loss appears greater in clinically depressed people and in rats exposed to environmental enrichment removal (see e.g. Burman *et al.*, 2008b), and the incentive contrast effect has been reported in domestic dogs (Bentosela *et al.*, 2009, although see Riemer *et al.*, 2016 for mixed support of these results). Enhanced sensitivity to negative feedback has also been shown in people with clinical depression and in rats displaying 'pessimistic' trait (e.g. Rygula *et al.*, 2016). Probabilistic reversal-learning tasks allow for measuring propensity of the subject to shift behavioural response following (and hence sensitivity to) negative and positive feedback, and applying such a task to dogs could provide a complementary cognitive measurement as a proxy for low mood.

Should such future studies be performed with refined methodologies demonstrate that greater time spent awake but motionless predicts cognitive proxies for low mood (e.g. negative judgment of ambiguity, greater sensitivity to loss and to negative feedback), this would *support* the hypothesis that affected individuals could be in a depression-like state, but would not be sufficient to *fully demonstrate* it. Further investigations targeting several forms of validation are required as discussed in detail in Harvey *et al.* (2019), Fureix *et al.* (2015) and Ferdowsian *et al.* (2011), that include assessing the co-variation of ABM with other symptoms of depression, as well as its similarities with clinical depression in terms of risk and curative factors.

Concerning covariation of ABM with other depression-like symptoms, recent research tested the hypothesis that greater time spent ABM in shelter dogs would, if representing a depression-like state, predict a sign of anhedonia, using reduced

interest for, and consumption of, palatable 'treat' foods as a proxy for anhedonia (Harvey *et al.*, 2019). The hypothesis that ABM reflects a depression-like syndrome was not supported either, although as discussed in Harvey *et al.* (2019) methodological refinements and complementary investigations are also required before it is possible to safely reject this hypothesis. It is after all plausible that in the random samples of shelter dogs from both studies, no dogs (or too few dogs) were in a depression-like state for us to detect such an association statistically.

Risk factors for greater time spent displaying ABM also require further investigation. As per the study from Harvey *et al.* (2019) conducted on a bigger sample size, none of the following factors; the dog's age, breed type, weight, sex, neuter status, the shelter they were tested at, nor time spent in the shelter, were associated with time spent showing ABM behaviour. In the Harvey *et al.* (2019) study, only one measure from the anhedonia test (reflecting greater interest) was associated with more time spent ABM; but only when time spent in the shelter was included as a co-variate (very tentatively suggesting that greater time ABM could be associated with another negative aversive state: boredom, as discussed in the 2019 study). The length of time each dog has been in the shelter for did not however predict the time spent ABM directly in either study; neither was it associated with the cognitive bias results. Further research is therefore warranted on the role of length of time in the shelter as an influencer of affective state, as well as on other possible predictors of ABM that have not been investigated yet. These would include for instance assessing personality aspects related to abilities to cope with challenges, as well as previous experience of living with other dogs, which could add isolation from conspecifics to the stressors for the current dogs all housed singly.

Finally, complementary investigations focusing on refining definitions or measurement of inactive behaviours relevant to hypotheses under test; such as using continuous recording of the behaviour (allowing for the measurement of precise

595 durations and number of bouts) and refining postural aspects of its definition; would
596 help in addressing cross-study and cross-species comparisons.

598 5. Conclusions

600 This study aimed to test the hypothesis that greater time spent displaying waking
601 inactivity in the home environment could reflect a depression-like condition in domestic
602 dogs. We tested this hypothesis in shelter dogs by investigating the association
603 between greater time spent inactive 'awake but motionless' in the home-pen and
604 negative judgment of ambiguity (a proxy for low mood, a core symptom of human
605 clinical depression). Results incidentally demonstrated an effect of the dog's origin on
606 learning abilities, with stray dogs reaching learning criterion significantly faster than
607 dogs that were relinquished by their owners or seized as part of welfare cases. Results
608 however do not support the study hypothesis, as positive expectancy scores from the
609 judgement bias test showed no association with time spent ABM. Positive expectancy
610 scores nevertheless exhibited minimal variation and an exaggerated right-skew
611 distribution, showing a strong bias towards expecting positive outcome at the
612 ambiguous location in most of the dogs, and such an unexpected lack of variation
613 between individual dog's responses might invalidate the performance in this test as a
614 comparison measure. Methodological refinements and complementary investigations
615 are thus required before it is possible to safely reject the study hypothesis. Further
616 research directions include refining the current cognitive assay and measurement of
617 the inactive behaviour; assessing different types of cognitive measurements as proxies
618 for low mood; assessing the co-variation of ABM with other symptoms of depression,
619 and its similarities with clinical depression in terms of risk and curative factors; and
620 investigating other potential affective states associated with ABM (e.g. boredom).

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Table legends

Table 1: Individual characteristics of the 20 dogs included in this study. **MD** = Missing data. Breed classifications for analyses are based on American Kennel Club groups and further grouped into being a breed bred for co-operative work with humans (working/herding/sporting '**WHS**') or not (**Others**). Shelters are numbered after Harvey

et al. (2019). Breed information was obtained from pedigrees (when available) or visual inspection (which for the latter might involve some overestimation of Staffordshire Bull Terrier crosses, see e.g. Gunter, Barber and Wynne, 2018).

Table 2: Number of trials required to reach learning criterion (training) and raw latencies to reach positive, ambiguous and negative locations (testing) observed in the current study and those reported from publications similarly assessing judgment cognitive bias in dogs. Both the number of trials and raw latencies observed in the current study fall into the range of reported values from the literature, with the exception of Kis *et al.* (2015) which reports shorter latencies overall. All figures and tables cited in this table refer to figures and tables numbers used within the cited publication. \approx : number visually approximated from figures provided in publications.

Figure legends

Figure 1. Schematic representation of the cognitive judgment bias test set-up. The two training locations are shown as circles with a solid line, and the ambiguous test location as a circle with a dotted line. The dog's location at the start of the training is shown as a patterned circle. The side of the positive (rewarded) and negative (unrewarded) locations was counterbalanced across dogs.

Figure 2. Survival plot showing the distribution of time taken to reach training criterion between the three types of dogs based upon whether they were relinquished to the shelter ($n = 5$), seized as part of legal cases ($n = 9$) or found as strays ($n = 6$). The dotted line to the left of the plot shows that all the stray dogs reached learning criterion faster than the relinquished or seized case dogs.

Figure 3. Boxplot showing the distribution (median and interquartile ranges) of raw latencies during the test trials when the bowl was located at the positive (rewarded), negative (unrewarded) and ambiguous (equidistant to the two training positions) locations. $N = 20$ dogs. All latencies were statistically different from each other (Friedman test $\text{Chi-square}_{(2)} = 33.90$, $p < 0.001$).

Figure 4. Histogram of Positive Expectancy Scores (PES) calculated from the raw latencies during the test trials. $N = 19$ dogs (dog ID 76 was excluded as it ran faster to the negative location than it did the ambiguous, see methods for details). The PES scores exhibited minimal variation and an exaggerated right-skew distribution, showing a strong bias towards expecting positive outcome at the ambiguous location in most of the dogs.

687 **Table 1**

688

ID	Shelter	Sex	Neutered status	Age (years)	Breed (<i>Classification according to American Kennel Club groups</i>)	Weight (Kg)	Origin
64	5	Male	Neutered	2	German shepherd dog x Akita (<i>WHS</i>)	35.4	Stray
65	5	Male	Neutered	5	Mixed (<i>Other</i>)	16.85	Relinquished
66	5	Male	Neutered	7	Staffordshire bull terrier (<i>Other</i>)	17.45	Stray
67	5	Female	Neutered	2	Mixed (<i>Other</i>)	26.85	Relinquished
68	5	Male	Neutered	2	Akita (<i>WHS</i>)	27.3	Stray
69	5	Female	Intact	4	Akita (<i>WHS</i>)	33.85	Stray
70	5	Female	Neutered	4	Border Collie (<i>WHS</i>)	15.5	Stray
73	5	Male	Intact	6	German shepherd dog (<i>WHS</i>)	29.65	Stray
74	6	Female	Intact	2	Yorkshire Terrier (<i>Other</i>)	4.9	Case
75	6	Female	Intact	7	Yorkshire Terrier (<i>Other</i>)	5.1	Case
76	6	Female	Intact	1	Pug x Bichon frise (<i>Other</i>)	2.1	Case
77	6	Female	Intact	4	Chihuahua (<i>Other</i>)	2.4	Case
79	6	Female	Neutered	6	Staffordshire bull terrier (<i>Other</i>)	13.7	Relinquished
80	6	Female	Intact	4	Staffordshire bull terrier (<i>Other</i>)	21.6	Relinquished
81	6	Male	Intact	5	Labrador (<i>WHS</i>)	37.6	Case
82	6	Female	Intact	3	Shih Tzu cross (<i>Other</i>)	5.6	Case
83	7	Male	Intact	MD	Newfoundland (<i>WHS</i>)	44.55	Case
84	7	Male	Neutered	2	Staffordshire bull terrier (<i>Other</i>)	16.75	Relinquished
86	7	Male	Intact	MD	Newfoundland (<i>WHS</i>)	47.3	Case
87	7	Male	Intact	MD	Newfoundland (<i>WHS</i>)	45	Case

689

690 **Table 2.**

Publication	Number of trials to reach learning criterion	Raw latency to reach <u>positive</u> location during the test trials (s)	Raw latency to reach <u>middle</u> location during test trials (s)	Raw latency to reach <u>negative</u> location during test trials (s)
Current study	12-83 ($\bar{X} = 43 \pm 18$)	2 \pm SD 0.6	2.5 \pm SD 1.3	14.5 \pm 12.4
Mendl <i>et al.</i> , 2010	21-61 ($\bar{X} = 29.42 \pm 9$)	\approx 5 [Fig. 1B]	\approx 10 [Fig. 1B]	\approx 23 [Fig. 1B]
Müller <i>et al.</i> , 2012	30-90 ($\bar{X} = 42$)	Not found in publication	\approx 58 [Fig.1]	Not found in publication
Titulaer <i>et al.</i> , 2013	NF	$\bar{X} =$ 3.75 \pm 1.97	$\bar{X} =$ 7.65 \pm 6.34	$\bar{X} =$ 20.34 \pm 4.36
Kis <i>et al.</i> , 2015 [from control group in the communicative context, i.e. the closest group to the tested dogs in current study]	12-36 ($\bar{X} = 23 \pm$ SD 6)	$\bar{X} =$ 1.83 \pm SD 0.49 (1.0 – 2.8)	$\bar{X} =$ 2.26 \pm SD 0.57 (1.4-3.4)	$\bar{X} =$ 2.68 \pm SD 0.88 (1.6-4.4)
Owczarczak-Garstecka & Burman, 2016	16-51 ($\bar{X} = 24.12 \pm 8.95$) [Table S2]	$\bar{X} =$ 2.64 \pm SE 0.64 [Table 6]	$\bar{X} =$ 10.98 \pm SE 2.66 [Table 6]	$\bar{X} =$ 23.94 \pm SE 5.81 [Table 6]
Wells <i>et al.</i> , 2017	$\bar{X} = 24.10$ (95% CI 21.23-27.43)	\approx 3 [Fig. 2, Table 1]	\approx 6 [Fig. 2, Table 1]	\approx 18 [Fig. 2, Table 1]
Willen <i>et al.</i> , 2019	30-50	\approx 5	\approx 13	\approx 27

[Fig. 2]

[Fig. 3]

[Fig. 3]

[Fig. 3]

Accepted Manuscript

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