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Analisi del bias di giudizio nel cane (*Canis familiaris*):
elaborazione, sviluppo e validazione di un nuovo paradigma sperimentale
per la valutazione dell'ottimismo e del pessimismo

Analysis of judgment bias in the domestic dog (*Canis familiaris*):
conceptual design, development and validation of a novel experimental paradigm
for optimism and pessimism assessment

Candidata: Dott.ssa Carlotta Burani

Relatore (Tutor): Prof.ssa Paola Valsecchi

Coordinatore del Corso di Dottorato: Prof. Michele Zoli

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Abstract

Cognitive bias theories concern the influence of emotional states on cognitive functions (such as attention, memory and judgment). In particular, judgment biases pertain to the optimistic/pessimistic interpretation of ambiguous stimuli which is typically linked to the individual's mood. Judgment bias test (JBT) represents a widespread tool to assess the optimistic/pessimistic attitude and to indirectly evaluate animals emotional state and welfare. JBT has been applied several times on dogs, mainly using a spatial test with a bowl placed in ambiguous positions located between a trained certain positive (P) location which contained a baited bowl and a trained certain negative (N) location which contained an empty bowl; the latency to approach the bowl placed in ambiguous positions is a cue of the dog expectation of a positive or a negative outcome. However, results are anything but conclusive and the link between emotions and judgment biases has not been clearly established yet in dogs.

The present PhD research programme aimed at analysing some methodological issues related to this classical protocol, and at setting up a novel procedure, in order to obtain a solid measure of optimism/pessimism, useful to investigate emotional states in dogs. The thesis consists of three studies: in the first one, results from the classical JBT protocol were thoroughly analysed following a different statistical approach than usual; the second study was carried out to test a novel JBT paradigm in dogs and the last study was conducted to validate the use of this novel protocol exploring the performance of anxious/not anxious dogs.

Study 1: the performance of 51 shelter dogs and 40 pet dogs was thoroughly analysed using the classical spatial JBT paradigm. As expected, a pattern emerged with shelter dogs behaving in a more pessimistic-like way than pet dogs. However, several methodological caveats were found. First of all, a non-negligible percentage of dogs did not pass the training phase; Second, results showed that the dogs' responses towards the trained certain locations were not consistent throughout the test, suggesting that animals may not have fully learned the association between locations and their outcomes during the training; Third, dogs did not always behave differently towards adjacent locations,

raising doubts about the animals' ability to discriminate between locations; Finally, a potential influence of the researcher's presence on dogs' performance emerged from analyses.

Study 2: the aim was to solve the methodological issues raised by the first study using a novel spatial JBT protocol. The focus was to ensure a stable discrimination between certain stimuli (P and N), i.e., two doors located at opposite sides of a test area: behind P a hallway led dogs (N=49 pets) to their owner offering a food treat; behind N, at the end of another hallway, a black cloth was blown by a fan toward dogs (the end of each hallway was not visible from the door). P and N doors were opened one at a time until dogs always entered P and always avoided N. Then 3 ambiguous doors located between P and N were opened, one at a time, to assess the dog's pessimistic/optimistic attitude (these doors led to 3 new empty hallways). A strong discrimination between P and N has been achieved: in 95% of certain trials dogs responded properly (passed P door, avoided N door). Furthermore, dogs discriminated all the 5 doors. Dogs reaction during trials were video-recorded and the analysis suggested that dogs understood the values of P and N: the more the opened door was close to N the more the dogs chose to rest at the starting position.

Study 3: it was aimed at validating this novel JBT protocol. The hypothesis tested was that anxious dogs, as anxious people, were more pessimistic than non-anxious dogs. Considering the literature regarding the anxiety trait in the domestic dog, a sample of anxious dogs (as cases) and one of non-anxious dogs (as control) were recruited using an online questionnaire (N = 487) focused on behavioural reactions potentially related to fear, anxiety and stress exhibited in dogs' everyday life. Dogs from both groups were tested with the novel JBT protocol in order to compare their performances. Contrary to expectations, dogs in a more anxious-like state (N = 19) did not show a more pessimistic attitude towards ambiguity than non-anxious dogs (N = 19). Anxiety is a multifactorial disorder and it is hard to detect, therefore the applied questionnaire might be not sensitive enough to identify the most anxious and the most non-anxious dogs; however, 7 out of 19 dogs from the anxious dogs group needed an adjustment to the JBT procedure because they were not able to stay for a few minutes without their owner, suggesting that the survey indeed found a sample of dogs who suffered of an anxiety-like state.

A further explanation of these results pertains to the potential inadequacy of the applied JBT protocol to test anxious dogs, especially regarding dogs who are in discomfort without their owner and/or in presence of stranger people: some dogs in the anxious group needed to be tested with their owner by their side, suggesting a potential influence of anxiety-like states not only on test results but also on the test performance and requiring some methodological adjustments.

Abstract – Italian version

Il fenomeno dei *bias* cognitivi riguarda l'influenza degli stati emotivi sulle funzioni cognitive. In particolare, il *bias* di giudizio è relativo all'interpretazione in senso ottimistico o pessimistico di uno stimolo ambiguo, la quale è correlata allo stato emotivo del soggetto. Lo strumento che viene comunemente utilizzato per valutare ottimismo/pessimismo e, indirettamente, lo stato emotivo, è il *judgment bias test* (JBT). Questo test è stato utilizzato in molti studi sul cane: nel protocollo classico, una ciotola viene posta in posizioni intermedie rispetto a due posizioni certe, una positiva (P) nella quale la ciotola viene sempre riempita con un boccone di cibo, una negativa (N) nella quale la ciotola è sempre vuota; la latenza a raggiungere la ciotola nelle posizioni intermedie è un indice dell'aspettativa ottimistica/pessimistica del soggetto. I risultati di questi studi non sono però del tutto coerenti tra loro e la relazione tra stato emotivo e *bias* di giudizio nel cane non è stata al momento del tutto verificata.

Lo scopo di questo progetto di ricerca è analizzare alcuni dei problemi metodologici relativi al protocollo classico e proporre una nuova procedura, in grado di fornire misure di ottimismo e pessimismo attendibili e, quindi, utili nello studio degli stati emotivi del cane.

Questa tesi si sviluppa attraverso tre differenti studi sperimentali: nel primo, i risultati di un JBT condotto con il metodo classico vengono analizzati dettagliatamente con l'aiuto di un approccio statistico nuovo rispetto a quello consueto; nel secondo studio, viene discusso l'utilizzo di una nuova procedura per lo studio del *bias* di giudizio nel cane e nell'ultimo studio, allo scopo di validare il nuovo protocollo, viene valutata la potenziale differenza nell'attitudine pessimistica e ottimistica di cani ansiosi e di cani non ansiosi.

Primo studio: lo scopo era valutare nel dettaglio il classico paradigma del JBT (N=51 cani di canile e 40 cani di proprietà). Come previsto, i cani di canile sembrano essere più pessimisti dei cani di proprietà, ma i risultati hanno evidenziato diverse problematiche metodologiche. In primo luogo, una percentuale non trascurabile di cani non è riuscita a superare il training iniziale. In secondo luogo, le risposte dei cani nei confronti delle posizioni certe non sono rimaste invariate nel corso del test, suggerendo che

l'associazione tra stimoli certi e presenza/assenza di cibo non sia stata appresa in modo solido. Inoltre, i cani non sono stati in grado di distinguere totalmente tra le posizioni adiacenti. Infine, le analisi suggeriscono che la presenza di un ricercatore nei pressi di una delle posizioni possa aver influenzato i risultati.

Secondo studio: lo studio è stato orientato a risolvere i problemi metodologici emersi e ad elaborare un nuovo protocollo sperimentale. Il focus principale è stato assicurare che i soggetti (N=49) avessero appreso in modo sicuro il valore dei due stimoli certi (P e N), cioè due porte posizionate alle due estremità dell'arena sperimentale: superata la porta P, un corridoio conduceva il cane al suo proprietario pronto ad offrirgli un boccone di cibo, superata la porta N, un analogo corridoio portava il cane a trovarsi di fronte ad un telo nero mosso da un ventilatore. Le due porte sono state aperte ripetutamente, una alla volta, fino a che i soggetti non hanno deciso di entrare solo in P. Quindi, è stata registrata la risposta all'apertura di 3 porte situate tra P ed N, collegate a 3 corridoi vuoti. I cani sono riusciti a hanno acquisito una forte capacità di discriminare tra P ed N, rispondendo nel modo corretto (entrare solo in P) nel 95% dei casi. Inoltre, sono stati in grado di discriminare completamente tra le porte adiacenti.

Terzo studio: lo scopo era validare questo nuovo protocollo per l'esecuzione del JBT nel cane. L'ipotesi da confermare era che i cani che presentano alti livelli d'ansia fossero più pessimisti dei cani non ansiosi, similmente a quanto si registra nella specie umana. È stato quindi redatto uno specifico questionario (N = 487) per selezionare cani ansiosi (come casi di studio) e cani con livelli d'ansia molto bassi (come controllo), prendendo a modello questionari presenti in letteratura.

Cani appartenenti ad entrambi i gruppi sono stati testati con il nuovo protocollo JBT per confrontare la loro attitudine nei confronti di stimoli ambigui. Diversamente dalle aspettative, i cani più ansiosi (N = 19) non hanno mostrato un maggior pessimismo rispetto ai cani non ansiosi (N = 19). L'ansia è un disturbo multifattoriale molto difficile da analizzare, di conseguenza il questionario da noi utilizzato potrebbe non essere stato adeguato ad identificare i cani più ansiosi e quelli con meno problemi d'ansia; occorre però considerare che 7 sui 19 cani testati con alti livelli d'ansia, hanno avuto bisogno

di una modifica della procedura del test perché non erano in grado di rimanere senza il loro proprietario, anche se per pochi minuti, suggerendo quindi che il questionario sia stato in realtà abbastanza sensibile da identificare i cani con maggiori problemi d'ansia.

I nostri risultati potrebbe essere dovuti al fatto che il protocollo JBT da noi utilizzato potrebbe non essere del tutto adeguato con cani ansiosi, specialmente con quei soggetti che sono molto a disagio senza il loro proprietario e/o in presenza di estranei: considerando che alcuni dei cani ansiosi hanno avuto bisogno di avere il loro proprietario al fianco per tutta la durata del test, il test stesso potrebbe aver generato in loro uno stato d'ansia e aver quindi influenzato i risultati, al posto di aver valutato uno stato d'animo ansioso generale e di lunga durata; ciò suggerisce la necessità di ulteriori modifiche al protocollo.

“Our domestic dogs are descended from wolves and jackals and though they may not have gained in cunning, and may have lost in wariness and suspicion, yet they have progressed in certain moral qualities, such as affection, trust-worthiness, temper, and probably in general intelligence.”

Charles Darwin [1]

Introduction

A dog is a man’s best friend: dogs were domesticated most probably around 35,000 years ago [2,3] and they have been selected for adaptations to human social life [4], including the ability to understand human communication and to read human emotions [5]. Dogs proved to be more able than great apes and wolves at understanding human communicative signals indicating the location of hidden food [6].

Extensive literature exists about dogs’ ability to understand human emotions [7,8]: Turcsán and colleagues demonstrated that pet dogs were able to discriminate between the happy and the neutral facial expression of their owner [9]; Merola and colleagues came to similar conclusions and they found that dogs were able to distinguish also between happy and fearful expressions of their owner [10]; dogs are also able to discriminate between happy and angry human faces [11]. Furthermore, dogs can integrate visual (human faces’ expressions) and auditory (human vocalizations’ valence) sensory emotional information and discriminate between positive and negative emotions from both humans and dogs [12]; evidences also suggest that dogs are able to empathically react to human distress and to discriminate between a person pretending to cry and a person talking or humming [13,14].

Are humans good at understanding dogs’ emotions as much as dogs are at understanding humans’ emotions? Evidences exist that humans can correctly detect some emotional expressions (linked to

happiness, sadness, anger, fear, disgust, surprise) from photographs of dog faces [15], even if this ability is related to people's experience [5] and cultural milieu [16]. Mariti and colleagues observed that more than half of dogs' owners who filled a dedicated questionnaire were able to identify the most evident behavioural indicator of stress, even if subtle behaviours (e.g., looking elsewhere, turning head, yawning and nose licking) are rarely recognised as stress signals [17]. Furthermore, dogs' owners seem to have a good comprehension of their dogs vocalizations and gross body movements, but a low appreciation of the signals that dogs send in earlier stages of emotional arousal [18]. The ability to recognize dogs' behavioural emotions, signs of stress and low welfare indicators is of primary importance: dogs share their life with humans in many contexts (pet dogs, shelter dogs, laboratory dogs, working dogs) and therefore it is human's responsibility to ensure them a good standard of living, that involves both physical health and a positive affective state [19,20]. Good welfare implies that animals experience positive emotions, such as contentment, relax and pleasure, rather than negative ones, such as fear, anxiety, pain, frustration [21].

Despite a great effort of scientific research to detect and understand animals' emotions [22] and, in particular, dogs' emotions [23], understanding the affective state of an individual that cannot verbally report how he/she is feeling is still complex.

Our knowledge of dogs' welfare is incomplete and fragmentary and most researches focused on dogs housed in laboratories and rehoming kennels, disregarding the welfare of pet dogs [20].

The development of methodologies and approaches to more reliably assess affective states, and thus welfare, in animals is therefore of great interest to researchers [21] and one of the most important question science is trying to answer on animal welfare is: how can we assess good and bad psychological welfare in animals? Animal welfare scientists use both physiological and behavioural indexes: however, the use of these kind of indicators presents challenges in measuring animal welfare, and dog welfare in particular. For example: physiological measures are usually linked to the activation of the hypothalamic pituitary adrenal (HPA) axis and of the sympathetic nervous system (heart rate, heart rate variability, adrenaline and, most commonly, cortisol), but the activation of these responses occurs with

positive and negative emotions. Moreover, considering behavioural measures, there is still uncertainty regarding behaviours that may be taken into account to reliably indicate underlying emotional state because of extreme individual variability [20,24].

Given this uncertainty, scientific research has recently shown growing interest in using cognitive approaches to infer emotional state. The focus of this PhD project is to deeply analyse a promising tool to understand animals' emotions, the judgment bias test, whose theory originates from researchers on the by-direction influences between the world of emotions and the world of cognition.

Emotion and cognition

The scientific study of emotions faces two serious problems: despite a hundred years of psychological study, we lack consensus on the definition of emotions, plus, definitions of emotions often include an implicit contrast between cognition and rationality [25].

In humans, the 'gold standard' method to analyse emotions is the linguistic self-report and research based on these reports suggest that all emotions can be defined in terms of two factors, valence and arousal, and all emotions can therefore be placed in a bi-dimensional space as shown in Fig 1.

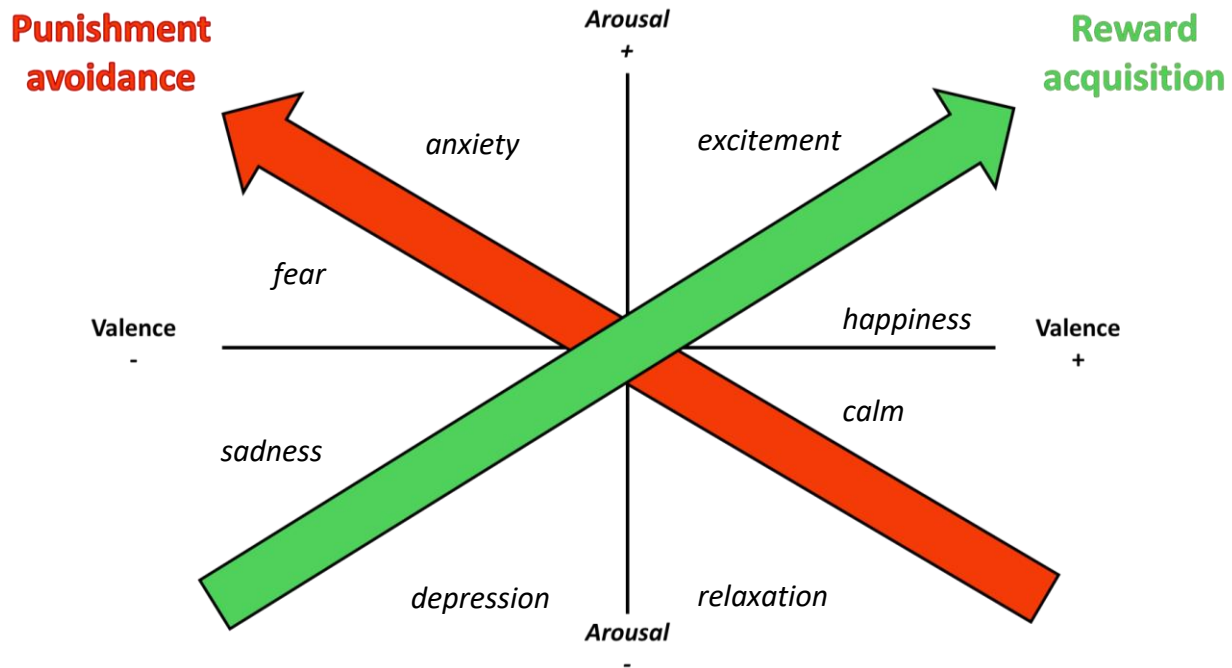


Fig 1. Adapted from [19,26,27]. Words in italics indicate possible locations of reported affective states. Positive emotions are in the right quadrants, negative ones in the left quadrants. Green and red arrows indicate the two motivational systems of reward acquisition and punishment avoidance.

It is plausible that non-human animals feel emotions that are similar to the ones reported by humans because they are linked to the motivational systems – i.e. reward acquisition and punishment avoidance – that has been selected during evolution: emotions are elicited by stimuli that are relevant for the animal survival and reproduction [28]. Furthermore, some authors hypothesized that in humans and non-human animals a bi-directional relationship exists between discrete emotions (context-dependent and short-term duration) and mood (long-term affective state): discrete emotions influence the position of the individual’s mood in the bi-dimensional space, and, vice versa, mood may influence the Position of discrete emotions.

The mood state is important in guiding animal’s decisions when selecting what stimuli are more salient, when judging uncertain stimuli and when recording the most important information from the environment. For instance, if a subject’s environment is full of threats (e.g., predators), its mood will likely be located in the upper-left quadrant (see Fig 1) as it would be advantageous to appraise

ambiguous stimuli as negative ones and thus activate the fight/flight system. Vice versa, if an animal lives in an environment with a low level of danger, the continuous activation of the alert system would be a waste of energy and opportunities. Animals' expectations (pessimistic or optimistic) are related to their environment that, in turn, influences their mood [19]. See Fig 2.

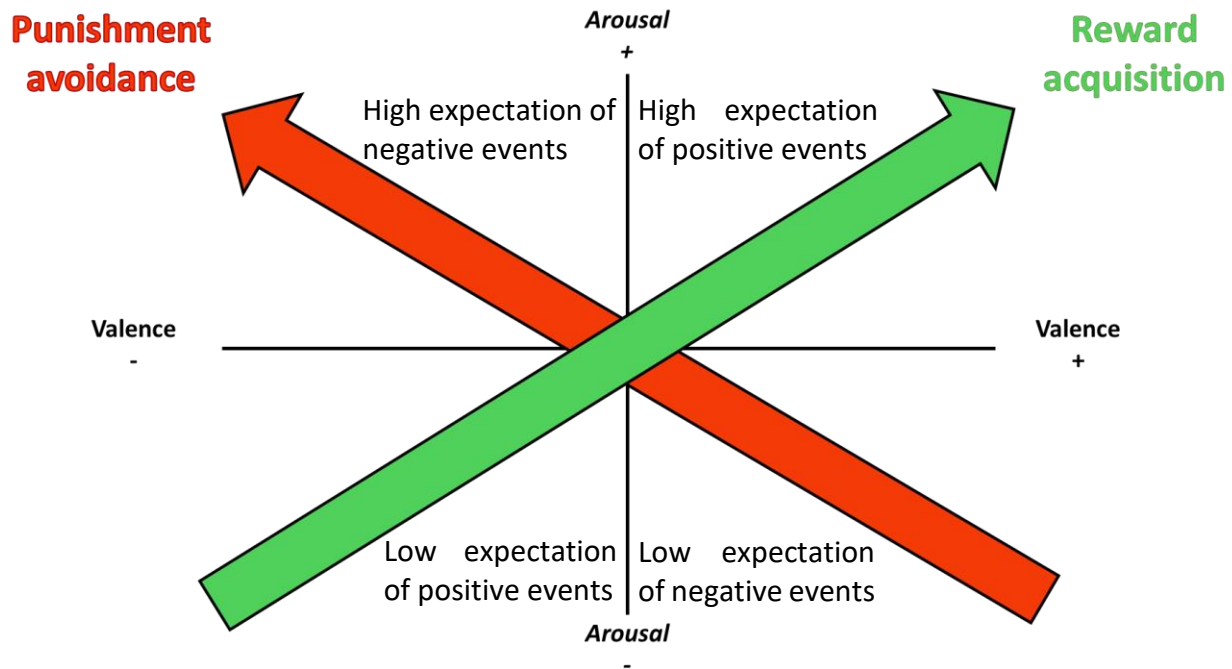


Fig 2. Animal's expectations are shown in each quadrant consistently with their affective state

Many scientific researches have demonstrated the deep relationship between emotions and cognition. An emotion requires a primary appraisal of the stimulus and therefore emotions arise from cognitive processes [29]. On the other hand, emotions give animals the ability to avoid harm/punishment and to seek valuable resources/rewards [28]; therefore, animals have to associate affective values to their environment. A significant body of work in human psychology has shown how emotions can modulate the processing of information, involving attention, memory and judgment cognitive functions. Is therefore conceivable to use objective measures of these cognitive biases as indicators of underlying, more elusive affective states that influence those biases.

These phenomena were initially studied in humans, pointing to a pre-attentive bias (selective attention towards threatening stimuli) in anxious people and a post-attentive bias (pessimistic appraisal of ambiguous stimuli) in depressed individuals [30]. Even memory is biased by emotions: moderately strong emotions improve memory performance, while extremely aroused emotions worsen it [31,32].

Judgment bias in humans

Cognitive biases can be grouped into three main categories: attention biases, memory biases and judgment biases[33].

Judgment biases pertain to the optimistic/pessimistic interpretation of ambiguous stimuli and have been traditionally studied in humans using verbal tasks (e.g., the interpretation of ambiguous semantic sentences [34] and of lexical ambiguity such as homophones), which are typically linked to the individual's mood (see for a review [35,36]). However, in recent years, non-verbal tasks have been studied in humans in an effort to allow for comparisons with other animal species. Among others, Paul and colleagues [37] applied a computer-based spatial task in which subjects were required to decide whether an ambiguous stimulus (a cross located somewhere between a positive anchor point, i.e., a smiling face, and a negative anchor point, i.e., a sad face) was nearer the positive or the negative anchor image. The authors found that subjects that presented higher "negative activation" and lower "positive activation" (i.e., a danger-oriented state and a state related to loss or absence of opportunity, respectively (measured using the PANAS scale, Positive and Negative Affect Schedule [38]), tended to interpret the cross as being nearer the negative anchor point. Anderson and colleagues [39] successfully applied a tone based task, developed in rats, to humans; participants were trained to correctly respond (left/right keypress on a serial response box) to a positive tone frequency in order to obtain rewards (money) and to a negative tone frequency in order to avoid punishment (an aversive sound clip). Subjects with higher self-reported measures of anxiety tended to respond to ambiguous tones (i.e.,

intermediate frequencies between the trained tones) in the same way they responded to the negative tone frequency [39].

Despite advancements in this field, caution is still required when using judgment bias tests (JBT) to assess the general mood of an individual. While studies using questionnaire and verbal tasks are generally suitable for this purpose, other studies, using non-verbal paradigms and measures such as response latency, may be only partially successful [40]. For example, Ilgaya and colleagues [41] did not detect a relationship between self-reported mood and judgment bias using a visual task. Similarly, Schick and associates [40] found no significant correlation between mood and interpretation bias on a tone-based challenge.

Judgment bias in animals: hoping for the best or preparing for the worse?

Harding and colleagues demonstrated for the first time the existence of cognitive biases in a non-human animal. In their test, rats were trained to press a lever in response to a tone in order to get food, and to avoid pressing the lever when hearing a different tone; after the training, half of the sample was exposed to a standard and predictable environment and the other half was housed in an unpredictable husbandry regime. When exposed to ambiguous tones (intermediate between the two tones used during training), rats housed in the unpredictable environment tended to press the lever with less frequency and a longer latency, compared to the other rats, showing therefore a lower expectation of a positive event [42]

Since that seminal study, cognitive bias paradigms borrowed from human studies have been modified and adapted several times to measure the psychological welfare of many non-human species (e.g., rats [42–47], mice [48,49], starlings [50], bees [51], sheep [52–55], macaques [56], tufted capuchin monkeys [57], pigs [58,59], horses [60], calves [61] and dogs [62–64]).

Despite this large number of studies, various questions remain to be answered about the use of JBT to evaluate the affective state of non-human animals: indeed, results are still not unequivocal and are not always in line with predictions to the point that some of those studies have led to null results or to opposite findings to those expected [41,65,66]. For example, Neville and colleagues reviewed the existing literature about the pharmacological manipulation of affect in animals and reported a high heterogeneity and a strong variability in the extent to which pharmacological manipulations of affective state alter the judgement bias [67]. Lagisz and colleagues came to similar conclusions analysing the literature about the non-pharmacological affect manipulation in animals, even though they found an overall small effect of affect manipulation on judgment bias, in agreement with predictions [68]. The experimental paradigm has been modified several times: changes regard type of stimuli (spatial, auditory, visual), trained stimuli connotation (reward versus no reward, reward versus punishment), dependent variable (lever presses, latency to reach the stimuli, go/no go responses), number of ambiguous probes, learning criterion); furthermore, statistical approaches employed to analyse the test output are not homogeneous. Therefore, the reasons underlying these contradicting results are not easy to understand and further studies are required.

Bowl half-full or half-empty? Judgment bias in dogs

JBT results on dogs are as controversial as those involving other species [24].

Mendl and colleagues [63] applied a spatial JBT on dogs for the first time and found that sheltered animals scoring higher on separation-related behaviours showed a 'pessimistic' judgment of an ambiguous cue, suggesting a negative affective state.

Titulaer and colleagues [69] employed physiological, behavioural and cognitive measures (spatial JBT) to evaluate potential differences between short-term and long-term sheltered dogs' welfare. No cognitive bias differences were found between the two samples (in addition to no differences in

physiological and behavioural measures); the authors concluded that other factors, e.g., frequency of social contacts, rather than the mere length of time spent in a shelter, can influence welfare state.

More recently, Willen and colleagues [70] found that fearful sheltered dogs showed a more pessimistic expectation towards ambiguous stimuli than non-fearful kennelled dogs and that enrichment based on positive human interaction could increase positive expectancy in fearful animals. However, they also discovered that the same enrichment had the opposite effect on JBT results in non-fearful individuals.

Despite some successful studies that found the expected relationship between emotions and judgment biases in dogs, other data did not confirm these results. Müller et al. [71] used a spatial JBT and noticed that a brief absence of the owner was not sufficient to induce a pessimistic bias in pet dogs. More recently, Walker and colleagues [72] applied a spatial JBT to sheltered dogs and noticed that separation from their kennel-mate did not induce a pessimistic mood. More broadly, with regards the relationship between not only emotions but also the welfare state and judgment bias, Owczarczak-Garstecka and colleagues [73] found that the percentage of time spent asleep during the night in sheltered dogs, was not predicted by dogs' optimistic/pessimistic bias, while Harvey and colleagues [74] found that a more pessimistic judgment bias was unrelated to the amount of time dogs were awake, but inactive, in the home environment.

Some studies even led to opposite findings to those expected: e.g., Burman and colleagues [75] found that a brief positive experience (i.e., a food-based rewarding event) induced in laboratory dogs a pessimistic bias rather than an optimistic one; the authors explained that the previous food-based event may have reduced food motivation during the JBT and/or that the interruption of the positive experience could have elicited both negative emotions and pessimistic judgment bias.

It is worth noting that other studies, whilst reporting an association between emotions and cognitive biases in dogs, presented methodological and statistical issues, such as a small sample size that makes more difficult to infer a general behavioural pattern [76,77], the employment of only a single ambiguous trial, the outcome of which could be influenced by momentary distraction [78,79] and the use of a

statistical approach that evaluates the dog's average response instead of single trial responses, thereby reducing variability in data [63,70,80,81]. Averaged measures are inevitably less accurate than raw ones and they depend on the number of repetitions: in JBTs this is usually not consistent among type of cues (more trials for each unambiguous trained cue, less trials for each ambiguous cue to minimize a potential learning effect).

These equivocal results, sometimes even opposite to predictions, the occurrence of methodological caveats in the JBT paradigm and statistical concerns in data analysis, taken together, suggest that a deeper investigation into the value of judgment bias tests as a measure of psychological welfare is necessary [24,82].

Research plan & Aims

In order to thoroughly analyse the robustness and validity of application of the judgment bias test in the canine species, a JBT, using the classical protocol, was carried out with shelter-housed dogs, a population of animals ideally suited for studies on affective state. To exclude the possibility of my results being reflective of a population bias, data from a study on family pet dogs tested with the same JBT paradigm [62] were re-analysed alongside the data collected from the sheltered dog cohort [83].

Results showed that shelter dogs behaved in a more pessimistic-like way than pet dogs, however, this difference between the two populations was detected only analysing raw latencies to reach the locations and not the more commonly applied adjusted score (i.e., average latency values). Furthermore, several methodological caveats were found. First of all, a non-negligible percentage of dogs did not pass the training phase thus casting doubt on the appropriateness of the test. Secondly, results showed a high intra-dog variability in response to the trained locations, i.e., dogs' responses were not consistent throughout the test, suggesting that animals may not have fully learned the association between locations and their outcomes. Thirdly, dogs did not always behave differently towards adjacent locations, raising doubts about the animals' ability to discriminate between locations.

Finally, a potential influence of the researcher's presence on dogs' performance emerged from analyses.

Considering all these issues, I attempted to improve the JBT classical protocol, adapting a JBT protocol originally developed for sheep [53,54]: two corridors, P (leading to the owner offering a food treat) and N (leading to a black cloth blowing), located at two opposite sides of a test area, represented the certain stimuli; the ambiguous stimuli are represented by 3 (empty) corridors located between P and N [84].

My aims were to obtain more reliable measures and my hypotheses were that this novel protocol, increasing the difference between the payoff of the two trained stimuli (P and N), might ease the learning process and improve the discrimination among stimuli: this has been confirmed by results on 50 pet dogs. Furthermore, I set a stronger learning criterion compared to the classical protocol and I reduced the number of trials and their length, to prevent dogs' tiredness and distraction and to raise the consistency in responses.

This protocol turned out to be reliable and capable to provide stable and consistent measures; therefore, I focused the last experimental procedure in evaluating the reliability of my protocol results as optimism/pessimism indexes: I compared performances of anxious dogs and of non-anxious dogs in a judgment bias test (JBT) [85]. Anxiety is a reaction related to the anticipation of future danger and does not necessarily have a specific object of threat [86], it is elicited in potentially (from the individual point of view) dangerous situations. Anxiety disorders represent the main cause of many canine behavioural problems [86–89] and considering that anxiety can impair welfare, from a psychological and physical (anxiety could influence dogs' lifespan [90]) point of view, efforts should be made to better understand canine anxiety and to decrease its prevalence in dog population [91].

Considering that cognitive biases are linked to long-term mood and emotional state and not to brief and discrete emotions [19], I aimed at recruiting a sample of dogs characterised by anxiety trait (i.e. the individual tendency to experience anxiety across time and context [92]) and not by state anxiety

(anxiety experienced at one particular time and context [93]); therefore, I built an online questionnaire focused on behavioural reactions potentially related to fear, anxiety and stress in dogs everyday life.

After recruiting dogs that likely, on the basis of questionnaire results, were experiencing an emotional state characterised by anxiety (as cases) and dogs in an alleged non-anxious state (as control), I presented them with the novel spatial JBT protocol.

Eysenck and colleagues demonstrated that clinically anxious humans interpret ambiguous sentences in a more pessimistic sense than either recovered clinically anxious subjects and normal control subjects [94]. Therefore, my expectations were that dogs in a more anxious-like state would show a more pessimistic attitude towards ambiguity than non-anxious dogs, as found in humans [95–97] (in particular regarding attention bias [30,98]) and non-humans animals [99].

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Study 1

Using judgment bias test in pet and shelter dogs (*Canis familiaris*): methodological and statistical caveats

Carlotta Burani^{1, 2*}, Shanis Barnard^{3, #a}, Deborah Wells^{3*}, Annalisa Pelosi¹, Paola Valsecchi²

¹ Dipartimento di Medicina e Chirurgia, Università degli Studi di Parma, Parma, Italia

² Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università degli Studi di Parma, Parma, Italia

³ Animal Behaviour Centre, School of Psychology, Queen's University Belfast, Belfast, UK

^{#a} Department of Comparative Pathobiology, College of Veterinary Medicine, Purdue University, West Lafayette, Indiana, USA

*Corresponding authors

E-mail: carlotta.burani@studenti.unipr.it (CB); D.Wells@qub.ac.uk (DW)

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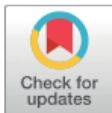
RESEARCH ARTICLE

Using judgment bias test in pet and shelter dogs (*Canis familiaris*): Methodological and statistical caveatsCarlotta Burani^{1,2*}, Shanis Barnard^{3a}, Deborah Wells^{3*}, Annalisa Pelosi¹, Paola Valsecchi²

1 Dipartimento di Medicina e Chirurgia, Università degli Studi di Parma, Parma, Italia, **2** Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università degli Studi di Parma, Parma, Italia, **3** Animal Behaviour Centre, School of Psychology, Queen's University Belfast, Belfast, United Kingdom

^a Current address: Department of Comparative Pathobiology, College of Veterinary Medicine, Purdue University, West Lafayette, Indiana, United States of America

* carlotta.burani@studenti.unipr.it (CB); D.Wells@qub.ac.uk (DW)



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Abstract

It is now widely agreed that a positive affective state is a crucial component of animal well-being. The judgment bias test represents a widespread tool used to assess animals' optimistic/pessimistic attitude and to evaluate their emotional state and welfare. Judgment bias tests have been used several times with dogs (*Canis familiaris*), in most cases using a spatial test with a bowl placed in ambiguous positions located between a relatively positive trained location (P) which contains a baited bowl and a relatively negative trained location (N) which contains an empty bowl. The latency to approach the bowl in the ambiguous locations is an indicator of the dog's expectation of a positive/negative outcome. However, results from such tests are often inconclusive. For the present study, the judgment bias test performance of 51 shelter dogs and 40 pet dogs was thoroughly analysed. A pattern emerged with shelter dogs behaving in a more pessimistic-like way than pet dogs. However, this difference between the two populations was detected only when analysing the raw latencies to reach the locations and not the more commonly applied adjusted score (i.e. average latency values). Furthermore, several methodological caveats were found. First of all, a non-negligible percentage of dogs did not pass the training phase, possibly due to the experimental paradigm not being fully suited for this species. Second, results showed a high intra-dog variability in response to the trained locations, i.e. the dogs' responses were not consistent throughout the test, suggesting that animals may not have fully learned the association between locations and their outcomes. Third, dogs did not always behave differently towards adjacent locations, raising doubts about the animals' ability to discriminate between locations. Finally, a potential influence of the researcher's presence on dogs' performance emerged from analyses. The implications of these findings and potential solutions are discussed.

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Introduction

It is now widely agreed that a positive affective state (comprising both the longer term background mood state and shorter-term discrete emotions [1]), and not only physical health, is a crucial component of animal well-being [1]. Animal welfare science and biomedical research have attempted to establish scientific and reliable measures of affective states in non-human animals, with the cognitive bias approach being one of the most commonly applied tests across species (for reviews see [2–5]; for critical analyses see [6,7]; for a meta-analysis on how pharmacological manipulations influence judgment bias see [8]). Cognitive bias theories concern the influence of emotional states on cognitive functions (such as attention, memory and judgment) [3,7]. These phenomena were initially studied in humans, pointing to a pre-attentive bias (selective attention towards threatening stimuli) in anxious people and a post-attentive bias (pessimistic appraisal of ambiguous stimuli) in depressed individuals [9].

Cognitive biases can be grouped into three main categories: attention biases, memory biases and judgment biases[2]. Judgment biases pertain to the optimistic/pessimistic interpretation of ambiguous stimuli and have been traditionally studied in humans using verbal tasks (e.g., the interpretation of ambiguous semantic sentences [10] and of lexical ambiguity such as homophones), which are typically linked to the individual's mood (see for a review [11,12]). However, in recent years, non-verbal tasks have been studied in humans in an effort to allow for comparisons with other animal species. Among others, Paul and colleagues [13] applied a computer-based spatial task in which subjects were required to decide whether an ambiguous stimulus (a cross located somewhere between a positive anchor point, i.e., 😊, and a negative anchor point, i.e., 😞) was nearer the positive or the negative anchor image. The authors found that subjects that presented higher “negative activation” and lower “positive activation” (i.e., a danger-oriented state and a state related to loss or absence of opportunity, respectively (measured using the PANAS scale, Positive and Negative Affect Schedule [14]), tended to interpret the cross as being nearer the negative anchor point. Anderson and colleagues [15] successfully applied a tone based task, developed in rats, to humans; participants were trained to correctly respond

(left/right keypress on a serial response box) to a positive tone frequency in order to obtain rewards (money) and to a negative tone frequency in order to avoid punishment (an aversive sound clip). Subjects with higher self-reported measures of anxiety tended to respond to ambiguous tones (i.e., intermediate frequencies between the trained tones) in the same way they responded to the negative tone frequency [15].

Despite advancements in this field, caution is still required when using judgment bias tests (JBT) to assess the general mood of an individual. While studies using questionnaire and verbal tasks are generally suitable for this purpose, other studies, using non-verbal paradigms and measures such as response latency, may be only partially successful [16]. For example, Ilgaya and colleagues [17] did not detect a relationship between self-reported mood and judgment bias using a visual task. Similarly, Schick and associates [16] found no significant correlation between mood and interpretation bias on a tone-based challenge.

Cognitive bias paradigms borrowed from human studies have been modified and adapted several times to measure the psychological welfare of many non-human species (e.g. rats [18], starlings [19], bees [20], sheep [21], macaques [22], pigs [23], horses [24], calves [25]), including the domestic dog, *Canis familiaris* [26–28]. Despite the large number of studies that have used JBT as the golden standard to evaluate the affective state of non-human animals, various questions remain to be answered; results are still not unequivocal and are not always in line with predictions to the point that some of those studies have led to null results or to opposite findings to those expected [7,17,29].

JBT results on dogs are as controversial as those involving other species. Some studies have found the expected relationship between emotions and judgment biases. For instance, Mendl and colleagues [27] applied a spatial JBT on dogs for the first time and found that sheltered animals scoring higher on separation-related behaviours showed a ‘pessimistic’ judgment of an ambiguous cue, suggesting a negative affective state. Titulaer and colleagues [30] employed physiological, behavioural and cognitive measures (spatial JBT) to evaluate potential differences between short-term and long-term sheltered dogs’ welfare. No cognitive bias differences were found between the two samples (in addition to no

differences in physiological and behavioural measures); the authors concluded that other factors, e.g., frequency of social contacts, rather than the mere length of time spent in a shelter, can influence welfare state. More recently, Willen and colleagues [31] found that fearful sheltered dogs showed a more pessimistic expectation towards ambiguous stimuli than non-fearful kennelled dogs and that enrichment based on positive human interaction could increase positive expectancy in fearful animals. However, they also discovered that the same enrichment had the opposite effect on JBT results in non-fearful individuals.

Studies on dogs have not always reported the expected association between emotions and cognition. For example, Burman and colleagues [32], using a visual JBT, found that a brief positive experience (i.e., a food-based rewarding event) induced an unexpected pessimistic bias rather than an optimistic one; the authors explained that the previous food-based event may have reduced food motivation during the JBT and/or that the interruption of the positive experience could have elicited both negative emotions and pessimistic judgment bias. Müller et al. [33] used a spatial JBT and noticed that a brief absence of the owner was not sufficient to induce a pessimistic bias in pet dogs. More recently, Walker and colleagues [34] applied a spatial JBT to sheltered dogs and noticed that separation from their kennel-mate did not induce a pessimistic mood. More broadly, with regards the relationship between welfare state and judgment bias in sheltered dogs, Owczarczak-Garstecka and colleagues [35] found that the percentage of time spent asleep during the night was not predicted by dogs' optimistic/pessimistic bias, while Harvey and colleagues [36] found that a more pessimistic judgment bias was unrelated to the amount of time dogs were awake, but inactive, in the home environment.

Some studies in this area, whilst reporting a significant association between emotions and cognitive biases in dogs, have reported methodological and statistical issues, including a small sample size that makes it hard to infer a general behavioural pattern [37,38], the employment of a single ambiguous trial, the outcome of which could be influenced by momentary distraction [39,40] and the use of a statistical approach that evaluates the average dog's response instead of single trial responses, thereby reducing variability in the data [27,31,41]. Averaged measures are inevitably less accurate since, during

the test, trials are repeated for each dog and for each type of cue and the number of repetitions is not consistent among cues (more trials for each trained cue, less trials for each ambiguous cue to minimize a potential learning effect).

Taken together, these equivocal results, sometimes even opposite to predictions, the occurrence of methodological caveats in the JBT paradigm and statistical concerns in data analysis suggest that a deeper investigation into the value of judgment bias tests as a measure of psychological welfare is necessary [42]. Given that the experimental paradigm has been modified several times (e.g., type of stimuli (spatial, auditory, visual), trained stimuli connotation (reward versus no reward, reward versus punishment), dependent variable (lever presses, latency to reach the stimuli, go/no go responses), number of ambiguous probes, learning criterion) and that statistical approaches employed to analyse the test output are not homogeneous, the reasons underlying these contradicting results are not easy to understand and further studies are required (for a review see [6,7]).

Research on dogs is important as it allows us to investigate the basic emotional systems shared by social mammals [2] and offers the opportunity to compare different populations (i.e., pets, shelter, stray, laboratory, working dogs) that are often involved in welfare studies [43–49]. For the present research, a JBT with spatial stimuli was carried out with shelter-housed dogs, a population of animals ideally suited for studies on affective state. Entering a shelter is a stressful event for dogs [45], and the kennel environment can potentially induce negative emotions in more vulnerable individuals (due to stressors including noise, social deprivation, confinement). To exclude the possibility of our results being reflective of a population bias, data from a study on family pet dogs tested with the same JBT paradigm [26] were re-analysed alongside the data collected from the sheltered dog cohort.

The aim of this research was to evaluate the robustness and validity of the spatial JBT in dogs. To achieve this aim, the following points were thoroughly investigated:

- analysis of latencies to reach the five locations to ensure that dogs behaved differently towards them;

- analysis of the variability in latencies to reach the ambiguous locations to detect a potential learning effect over the trials;
- evaluation of dogs' responses towards trained locations to check that subjects had correctly learned the association between the location and its outcomes.

Furthermore, a comparison between the two populations (sheltered vs. pet dogs) was made using both raw latencies to reach the bowl and an adjusted score of optimism/pessimism calculated using average latencies.

Materials and methods

Subjects

Shelter dogs: Fifty-one healthy sheltered dogs were selected for the study following two criteria: being in the shelter for at least 6 days and being aged between 1-11 years old. Thirty-five of these dogs passed the training phase (see later) and thus performed the JBT. These dogs varied in breed and were aged between 1-10 years old (average age: $4.19 \pm \text{sd } 3.01$ years). Twelve of the subjects were female (75% spayed) and 23 were male (39% neutered). Their average time spent in the shelter was $143 \pm \text{sd } 301.38$ days (min 6 days, max 1460 days).

Twenty-four dogs were recruited from the charity kennel "Benvardin Animal Rescue Kennels" (BARK), in County Antrim, Northern Ireland (UK) and twenty-seven dogs enrolled were from the Italian Shelter "La cuccia e il nido" ruled in Calvatone (CR) by ANPANA (Associazione Nazionale Protezione Animali Natura Ambiente). In both shelters, dogs were either single or pair-housed. At BARK, dogs spent most of their day in outside enclosures (approximately 4x5 metres) and rested in smaller indoor pens (approximately 2x2 metres) during the night. In ANPANA shelter, dogs stayed in an indoor pen

(approximately 3x2 metres) during the day and night and were released for exercise once or twice a day into outside enclosures (approximately 5x5 metres).

Pet dogs: Forty dogs were recruited among the students and staff of the School of Psychology, Queen's University Belfast, and by word of mouth. Thirty-one of these dogs succeeded in the training phase (see later) and were therefore tested with ambiguous trials. These dogs, of various breeds, were between 1-10 years of age (average age: $4.55 \pm \text{s.d } 2.57$ years). Eighteen were females (78% spayed) and 13 were males (92% neutered). Owners provided consent for their pet to take part in the study and all of the animals were healthy. For a detailed report of dogs' characteristics see Tables A and B in Supporting information, S1 Appendix.

Judgment bias test

To avoid distractions, the JBT was carried out in a barren indoor area (approximately 6x6 m) within the two shelter facilities, and, for the pet dogs, in a testing room (5x5 m) at the Animal Behaviour Centre, Queen's University Belfast. Data for the pet dogs were collected for another study [26] and raw data were re-analysed for this study.

The JBT protocol was the same as that adopted by others with dogs [27,28]. In brief, it consisted of a spatial task with two trained cues (P-positive and N- Negative) and 3 ambiguous cues (NP-Near Positive, M-Middle, NN-Near Negative). Latency to reach the cue was evaluated: short latency indicates a potential anticipation of a positive outcome (food), i.e. an 'optimistic' judgment, whereas longer latency is reflective of a potential 'pessimistic' judgment. The test consisted of 2 phases: training and testing.

1) Training

Each dog was trained to discriminate between a positive location (P) in which a bowl baited with a piece of palatable food was placed on the ground, and a negative location (N) in which the bowl was always

empty (see Fig 1 for locations of the bowl). The food used was a piece of sausage; to adapt the food intake to the dog's size, we baited the bowl in positive trials with 1/2 slice of sausage for small/toy sized dogs, one slice of sausage (approximately weight = 3 gr) for medium sized dogs and two slices of sausage for large sized dogs.

The dog was presented with only one bowl per trial, placed in either the P or N position following a pseudo-random order, with no more than two trials of the same type performed consecutively. The location of the positive cue was balanced for the sample (i.e., for 50% of the dogs the positive cue was on the left-hand side of the room and for the remaining dogs was on the right-hand side). A researcher placed the baited/unbaited bowl on the ground in the P or N position while each dog was led on a leash to the starting position by an assistant (Fig 1).

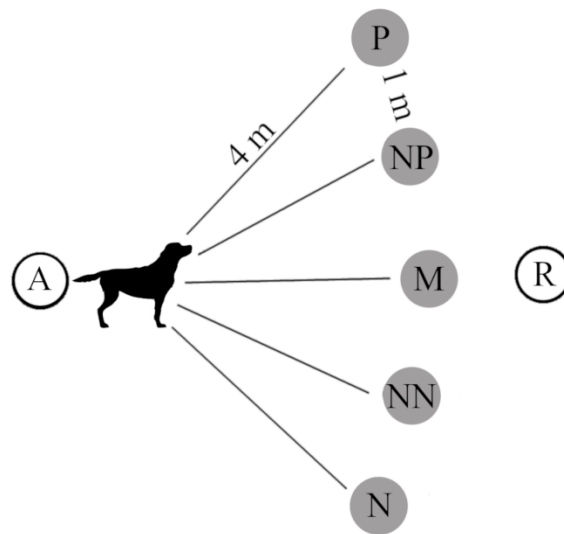


Fig 1. Experimental set-up. A (assistant), R (the researcher baiting/not baiting the bowl). Five bowl positions: P (Positive), NP (Near Positive), M (Middle), NN (Near Negative), N (Negative). Dog's silhouette created using resources from Freepik.com

The researcher pretended to bait the bowl to be placed in N, thereby reproducing the same movements and sound generated when the bowl was baited and then placed in P. The dog was allowed to watch the bowl baiting and positioning during the first 4 trials only (2P and 2N trials) to increase its motivation

to check the bowl content. In all the other trials, the dog was led by the assistant behind a barrier during the bowl baiting in order to ensure that it could not observe what the researcher was doing; when the bowl was positioned, the researcher stood a couple of steps behind the M position, looking straight ahead and avoiding eye contact with the dog. The assistant then led the leashed dog to the starting position and released it after it was ensured that the animal had seen the bowl. Latency to reach the bowl (i.e., length of time from the point of release to the moment the dog's nose was within 10 centimetres of the bowl) was measured using a stopwatch. The maximum latency to reach the bowl for each trial was set at 30 seconds; when this time elapsed, a latency of 30 seconds was recorded. The assistant then leashed the dog and led it to the starting position for the next trial.

Dogs had a minimum of 15 and a maximum of 40 trials to reach the learning criterion. This was set on the basis of other studies [26,27,32], so that for the preceding 3 positive trials and the preceding 3 negative trials, the longest latency to reach P was at least 0.5 seconds shorter than any of the latencies to reach N; this criterion was evaluated with each additional trial of training (rolling criteria). For each dog, the number of training trials required to reach the learning criterion was recorded.

2) Testing

The testing phase took place immediately after the dog reached the learning criterion. Dogs were presented with an unbaited bowl placed in one of three ambiguous locations located between P and N, spaced equally along an arc, 4 metres from the starting position (see Fig 1). All probe locations were presented three times, separated by 4 standard training trials (i.e., P, N), following the order: M NP NN - NP NN M - NN M NP (each probe location was presented first, second or third in each block of three test trials). Overall, the testing phase included 41 consecutive trials.

At the end of the testing phase, an empty bowl was placed in the P position to make sure that the dogs were relying on spatial cues and not on the treat's odour (42nd trial, "false positive" bowl).

Overall, the judgment bias test (training + testing) lasted nearly one hour per dog.

Statistical analyses

All analyses were performed using the software R, version 3.3.1. and packages DAAG (version 1.22), nlme (version 3.1-128), multcomp (version 1.4-6), MuMIn (version 1.40.4).

Analysis of the training phase

The effects of canine age (years), sex (male, female), reproductive status (neutered, intact) and position of positive bowl (left/right) on the number of training trials required to achieve the learning criterion was investigated using Spearman's correlation tests for continuous variables and Mann-Whitney U tests or permutation tests for factor variables (a Levene test was used to check for homogeneity of variances). Data were analysed separately for the two populations of dogs (sheltered, pets). Furthermore, for the kennelled dogs only, the effect of length of stay (days) in the shelter and shelter location (Italy, N. Ireland) was explored using the same statistical analyses.

In addition, a Mann-Whitney U test was carried out to compare the number of training trials required to achieve the learning criterion between the sheltered and pet dogs.

Analysis of the testing phase

Effect of the treat's smell. In order to ensure that dogs' decision-making processes relied on bowl position and not on the odour of the treats, a Wilcoxon test for paired samples was employed to compare, for each dog, the median latency to reach the baited bowls placed in P during the test phase and the latency to reach the empty bowl placed in P on the last trial ("false positive" trial). This analysis was carried out separately for sheltered and pet dogs.

Influence of dogs' characteristics on latencies to reach the bowl. As recommended by Gygas [42], a Linear Mixed-Effect model was applied (separately for sheltered and pet dogs), with untransformed latencies for each single trial (including the positive and negative cues used as anchor to estimate the slope across the different ambiguous cues) set as the dependent variable; a maximum-likelihood estimation was employed to account for designed imbalance in the data. This statistical approach has been criticized by Bateson and colleagues [50] who claimed that judgment bias regards only ambiguous stimuli and not the trained ones. Moreover, these authors considered ambiguous trials to be different from P/N ones because they are fewer (9 ambiguous trials vs. 32 P/N trials) and never reinforced. They therefore suggest not to pooling data from ambiguous and unambiguous trials and, rather, using data from the trained stimuli as a covariate in the analysis to account for differences in running speed between subjects. However, as pointed out by Gygas [42], the latencies to reach the trained locations are needed as anchors (dogs' responses to trained locations are expected to be stable) to estimate a slope across the different ambiguous locations. Furthermore, proper random effects and a maximum-likelihood estimation can handle a designed imbalance in the data (i.e., different number of ambiguous and unambiguous trials) [51]. Using the dog's identity as a random effect factor also takes into account the potential difference in running speed between subjects; in addition, using the average latency to reach the trained location as a covariate reduces the variability in this variable that was notable as regards to N location (see Results).

The Bayesian Information Criterion (BIC) was used to select the best combination of random effects. As expected, both in shelter and in pet dogs, the best models entailed that intercepts vary incorporating all the necessary hierarchical levels in the random effect (i.e., bowl location nested in dog identity). Model selection using BIC also showed that the model better fit the data if the bowl position variable was coded as a factor and not as a continuous variable; this could be ascribed to a deviation from the expected graded output (i.e., gradually higher latencies if the bowl was moved towards the negative

position, see results for further explanations). Therefore, bowl position variable was coded as a factor in the analyses.

A backwards approach was applied to test the fixed effects of canine age, sex, reproductive status on the subjects' latencies and, for the kennelled dogs only, the effects of length of stay in the shelter and shelter location (Italy, N. Ireland). These fixed effects were tested in interaction with bowl position and the interaction was removed from the model if not significant.

Since shelter location had no effect on latencies to reach the locations, the two kennelled populations were pooled for further analyses.

Analysis of latencies to reach the five locations. To assess whether the dogs behaved differently towards the bowl placed in the five positions, a Linear Mixed-Effects model ("lme" function) was created with bowl positions nested in dogs' identity as a random effect, latency to reach the bowl as a dependent variable and bowl position as a fixed effect. To compare the behavioural responses of the two population, the dogs' population (shelter/pet dogs) was included in the model in interaction with the bowl location variable.

Post-hoc tests with Bonferroni correction were used for pairwise comparisons between latencies to reach adjacent locations (P-NP, NP-M, M-NN, NN-N) and the two trained locations (P and N) separately for each population of animals. Furthermore, post-hoc tests with Bonferroni correction were used for pairwise comparison of latencies to reach the same location between the two populations.

Variability in latencies to reach the ambiguous locations: looking for a learning effect. The analysis of latencies to reach the five locations (see results) showed that the variability in latencies was very high, suggesting a need for further analysis. With regard to ambiguous locations, variability in latencies could point to a learning effect, i.e., dogs after the first or the second trial may have learnt that the bowl

placed in the ambiguous locations was empty; in that case, only the first trial would be a real ambiguous one and therefore only the first trial would be a reliable measure of optimism/pessimism. In order to explore for a potential learning effect, a Linear Mixed-Effects model was built for each ambiguous location (NP, M and NN), with dogs' identity as a random effect, latency to reach the bowl as the dependent variable and the trial number (3 trials for each location, factor variable) in interaction with the dog's population (shelter vs. pet dogs) as fixed effects; the interaction was removed from the model where not significant.

Variability in latency to reach the trained locations: intra and inter-dog components. Consistency in dogs' responses (estimated using the intra-dog variability in responses) was evaluated to check that subjects had correctly learned the association during the training. Based on the assumption that dogs should be confident in their responses towards trained locations whose outcomes they had previously learned, intra-dog variance in latencies to reach trained locations would be expected to be smaller than inter-dog variance. To evaluate the variability in dogs' responses, two Linear Mixed-Effects models (one for P and one for N location) were built, with dogs' identity as a random effect and latency to reach the bowl as the dependent variable. These models were used to split the total variance in the inter-dog (estimated variances between the random-effects terms in the linear Mixed-Effects model) and intra-dog (within-group error variance) components. Inter and intra-dog variances were compared for each trained location, separately for each population of animals.

Dogs' responses to the trained locations. To determine how the subjects responded towards the trained stimuli (P and N), we evaluated the number of "go trials" and "no go trials". A "go trial" is one in which the dog reaches the bowl within 30 s., a "no go trial" is a trial in which the dog does not reach the bowl within 30 s.

We calculated the percentage of “go trials” on the total number of negative/positive trials (number of N “go trials”/total number of N trials and number of P “go trials”/total number of P trials). A Pearson's Chi-squared test with Yates' continuity correction was also applied to evaluate if there was an association between the occurrences of “go/no go trials” and the dogs’ population (shelter vs. pet dogs), for both the P and N location. Standardised residuals and Cramér’s V effect size were evaluated.

Optimism/pessimism adjusted scores. Finally, to compare our results with previous studies, for each dog and each type of ambiguous location (NP, M and NN) an “adjusted score” of optimism/pessimism was calculated. We took into account each subject’s ‘baseline’ latencies to get to the trained stimuli (P and N), according to the following formula [27,28]:

$$\text{adjusted score} = \frac{\text{mean latency to probe location} - \text{mean latency to Positive location}}{\text{mean latency to Negative location} - \text{mean latency to Positive location}} \times 100$$

An adjusted score near 0 means that the subject reacted to the probe location in a similar way to how it reacted to the positive location, i.e., it considered the ambiguous cue as potentially rewarding (optimistic bias); an adjusted score near 100 means that the subject reacted to the probe location in a similar way to how it reacted to the negative location, perceiving the ambiguous cue as potentially unrewarding (pessimistic bias).

We compared the optimism/pessimism adjusted score between sheltered and pet dogs using a Linear Mixed-Effects model with the dogs’ identity as a random effect, the adjusted score as the dependent variable and the bowl location in interaction with the dogs population variable as fixed effects; the interaction was removed from the model if not significant.

Ethics statements

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed were approved by the School of Psychology Research Ethics Committee, Queen’s University Belfast (Ethical approval reference number No 90-2015-16). Special permission to use shelter dogs in such behavioural studies is not required in Italy (Decreto legislativo 4 marzo 2014, n. 26, Art. 2).

Results

Analysis of the training phase

None of the tested variables were significantly related to the minimum number of training trials required to reach the learning criterion, neither in sheltered, nor in pet dogs (see Table 1 for detailed results).

Table 1. Influence of dogs’ characteristics on the minimum number of training trials required to reach the learning criterion

Tested variable	Population	Test statistic	P-value
Age	Sheltered dogs	Spearman’s correlation rho = -0.21	p = 0.23
	Pet dogs	Spearman’s correlation rho = -0.27	p = 0.15

Sex	Sheltered dogs	Permutation test with 50000 simulations	p = 0.17
	Pet dogs	Mann-Whitney U test W = 77	p = 0.11
Reproductive status	Sheltered dogs	Mann-Whitney U test W = 157	p = 0.91
	Pet dogs	Mann-Whitney U test W = 93.5	p = 0.13
Positive bowl location	Sheltered dogs	Mann-Whitney U test W = 174	p = 0.50
	Pet dogs	Mann-Whitney U test W = 128.5	p = 0.75
Length of stay in the shelter (days)	Sheltered dogs	Spearman's correlation rho = -0.21	p = 0.23
Shelter location (Italy vs. N. Ireland)	Sheltered dogs	Mann-Whitney U test W = 147	p = 0.86

The minimum number of training trials required to reach the learning criterion did not differ significantly between sheltered and pet dogs (mean \pm sd 20.14 \pm 6.33 for sheltered dogs, 23.97 \pm 8.87 for pet dogs, Mann-Whitney U test $W = 402.5$: $p = 0.07$).

Analysis of the testing phase

Effect of the treat's smell

Wilcoxon tests for paired samples showed no statistically significant difference between median latencies to reach the baited bowls located in P and the latency to reach the empty bowl located in P in the last trial, either in sheltered dogs (z-value = 0.42, $p = 0.39$) or pets (z-value = 0.46, $p = 0.20$), confirming that the dogs' decision-making processes relied on bowl location and not on the treat's smell.

Influence of dogs' characteristics on latencies to reach the bowl

Linear Mixed-Effects models did not reveal any significant effect of age, sex, reproductive status, length of stay (days) in the shelter or shelter location on latencies to reach the various bowl locations (see Table 2 for detailed statistical results).

Table 2. Analysis of the influence of dogs' characteristics on latencies to reach the bowl

Dogs' characteristic	Population	F-value	df	P-value
Age	Sheltered dogs	0.5533	29	$p = 0.46$
	Pet dogs	0.35980	27	$p = 0.55$

Sex	Sheltered dogs	3.0975	29	p = 0.09
	Pet dogs	0.01773	27	p = 0.90
Reproductive status	Sheltered dogs	0.2656	29	p = 0.61
	Pet dogs	0.00157	27	p = 0.97
Bowl location * Age	Sheltered dogs	1.6450	116	p = 0.17
	Pet dogs	0.15006	108	p = 0.96
Bowl location * Sex	Sheltered dogs	0.8575	116	p = 0.49
	Pet dogs	0.55721	108	p = 0.69
Bowl location * Reproductive status	Sheltered dogs	0.1479	116	p = 0.96
	Pet dogs	0.36174	108	p = 0.84
Length of stay (days)	Sheltered dogs	0.0576	29	p = 0.81
Shelter location (Italy vs. N. Ireland)	Sheltered dogs	3.5648	29	p = 0.07
Bowl location * Length of stay (days)	Sheltered dogs	0.3587	116	p = 0.84
Bowl location * Shelter location	Sheltered dogs	0.4476	116	p = 0.77

* indicates the interaction between variables.

Analysis of latencies to reach the five locations

There was a significant interaction between bowl location and dogs' population (shelter/pet dogs) on the animals' latencies to reach the bowl (F-value = 6.7681, df = 256, p-value <.0001). Post-hoc contrasts analysis revealed that sheltered dogs behaved differently towards adjacent bowl positions, except for bowls located in P and NP (see Table 3). In contrast, pet dogs did not behave differently towards adjacent bowl positions, except for bowls located in the NN and N locations (see Table 3). Both sheltered and pet dogs successfully distinguished between bowls located at P and N (see Table 3).

Table 3. Pairwise comparisons between latencies to reach trained locations and adjacent ones

Pairwise comparison	Population	Estimate	P-value
P – N	Sheltered dogs	-18.80	p <0.0001 ***
	Pet dogs	-13.65	p <0.0001 ***
P – NP	Sheltered dogs	0.43	p = 1.00
	Pet dogs	-0.13	p = 1.00
NP – M	Sheltered dogs	-4.37	p = 0.007 **
	Pet dogs	-2.41	p = 1.00
M – NN	Sheltered dogs	-5.47	p = 0.0002 ***
	Pet dogs	-2.86	p = 0.47

NN – N	Sheltered dogs	-14.42	p <0.0001 ***
	Pet dogs	-8.25	p <0.0001 ***

Simultaneous Tests for General Linear Hypotheses, Multiple Comparisons of Means. P = Positive location, NP = Near Positive location, M = Middle location, NN = Near Negative location, N = Negative location. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05, adjusted p values reported, Bonferroni method

Post-hoc contrasts analysis revealed that latencies to reach P, NP and M locations were not significantly different between sheltered and pet dogs, but a significant difference was found in the animals' latencies to reach the NN and N locations (latencies were higher in shelter dogs, see Fig 2 for a graphic depiction and Table 4 for detailed statistical results).

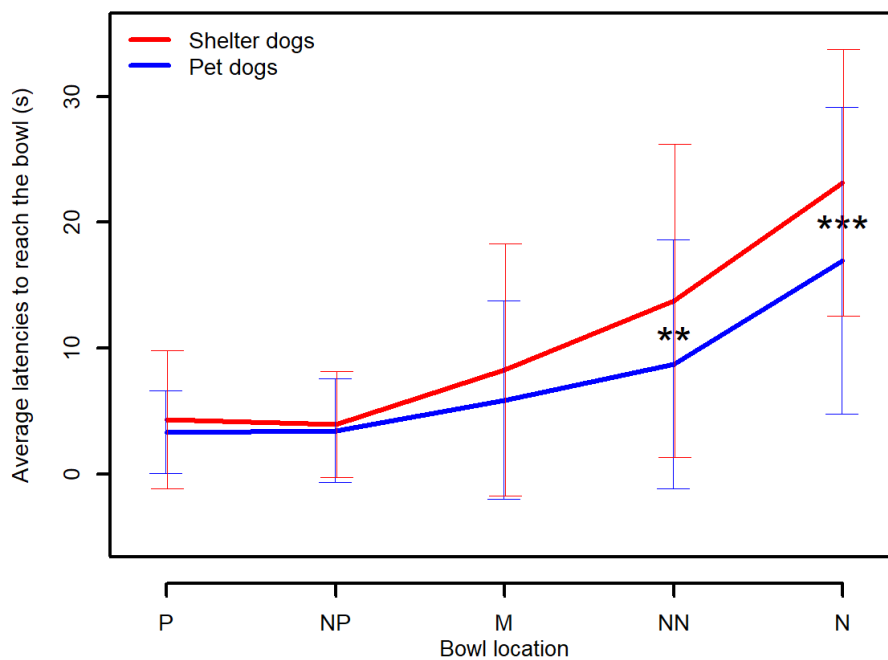


Fig 2. Average latencies to reach the five locations ± standard deviations, for sheltered and pet dogs. Positive (P), Near Positive (NP), Middle (M), Near Negative (NN) and Negative (N) location. * shows a significant difference (significance codes: 0 '***' 0.001 '**' 0.01 '*')

Table 4. Pairwise comparisons between pet and sheltered dogs' latencies to reach the five locations

Location	Estimate	P-value
P	-1.0198	p = 1.00
NP	-0.4603	p = 1.00
M	-2.4237	p = 1.00
NN	-5.0333	p = 0.008 **
N	-6.1674	p <0.0001 ***

Simultaneous Tests for General Linear Hypotheses, Multiple Comparisons of Means. P = Positive location, NP = Near Positive location, M = Middle location, NN = Near Negative location, N = Negative location. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05, adjusted p values reported, Bonferroni method

Despite many studies on JBT using mean values to graphically show an increasing general trend in latencies from P to N location [18,27,30,32,34], we used median values as these are more reliable and robust measures than means in cases of non normally distributed variables and extreme values (such as a 30 s latency recorded for a No-go response whereas the majority of trials presented a quick Go response). The general trend in median latencies to reach the five locations is graphically shown in Fig 3. It is worth noticing that the median latency to reach M, expected to be the most ambiguous location, is extremely short (3,48 s for shelter dogs, 2,96 s for pet dogs), almost equivalent to the median latency to reach P (2,84 s for shelter dogs, 2,66 s for pet dogs), suggesting a potential optimistic bias (see Discussion for an extensive explanation). Furthermore, Fig 3 shows that the variability in latencies is very high, suggesting the need for more in-depth analysis (see later).

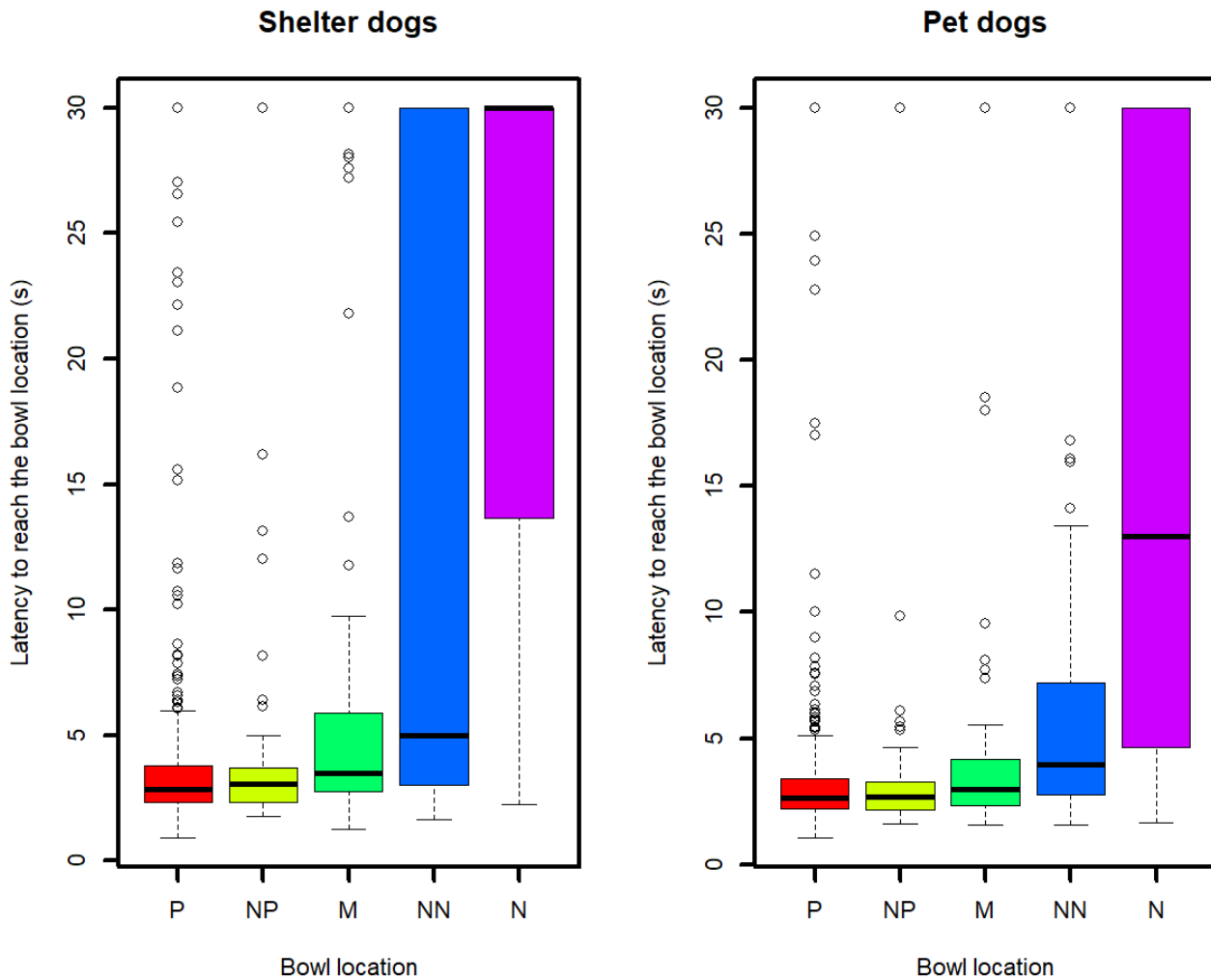


Fig 3. Boxplots of latencies to reach the five locations. Positive (P), Near Positive (NP), Middle (M), Near Negative (NN) and Negative (N) location. The graph shows medians (bar within the box), upper and lower quartiles (borders of box), lowest and highest cases within 1.5 times the IQR (bottom and top whiskers) and outliers (circles).

Variability in latencies to reach the ambiguous locations: looking for a learning effect

Trial number was not found to have a significant effect on dogs' latencies to reach the bowl placed at the NP location. However, trial number had a significant effect on both the sheltered and pet dogs'

latencies to reach the bowl positioned at M (df = 130, F-value = 5.17740, p-value = 0.007). Post-hoc contrasts analysis revealed that latencies to reach M were significantly higher in trial 2 than in trial 1 (estimate = -4.109848, df = 130, p-value = 0.003); latencies did not differ significantly, however, between trial 2 and trial 3 (see Fig 4).

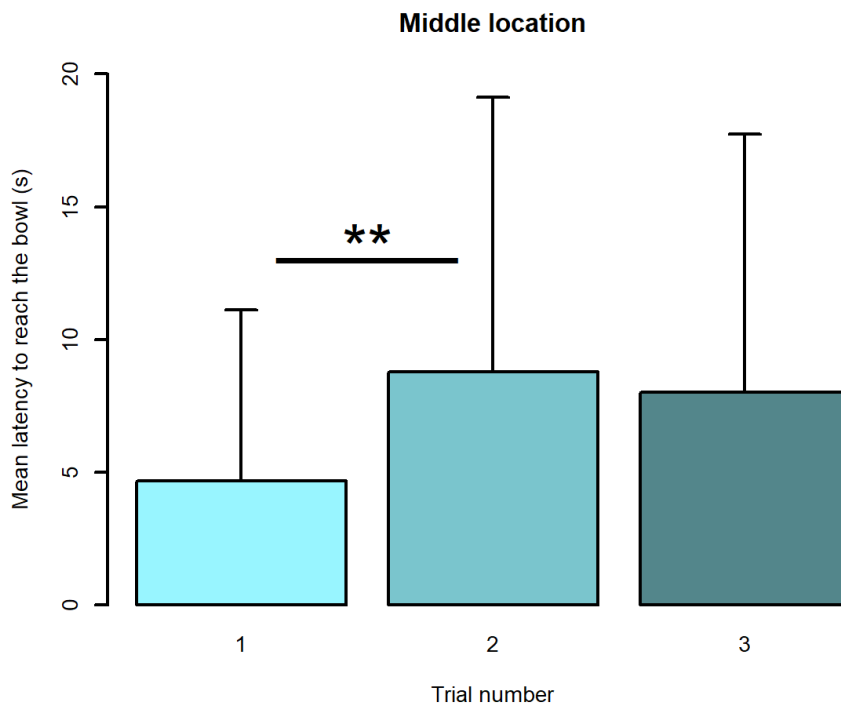


Fig 4. Barplot of latencies to reach the M location during the three trials. Means \pm sd are shown. ** shows a significant difference, $p < 0.01$

Dog population was found to have a significant effect on animals' latencies to reach the bowl positioned at NN, with higher latencies in sheltered dogs than in pets (estimate = 5.033266, df = 64, p-value = 0.01), according to the previous analysis (see analysis of latencies to reach the five locations). Latencies to reach the bowl in this position were also significantly related to trial number (df = 130, F-value = 3.68575, p-value = 0.03). Post-hoc contrasts analysis revealed that latencies to reach NN were not statistically different between trial 1 and trial 2, but were significantly higher in trial 3 than in trial 2

(estimate = 3.942879, df = 130, p-value 0.03, see Fig 5). The interaction trial * dog's population was not significant.

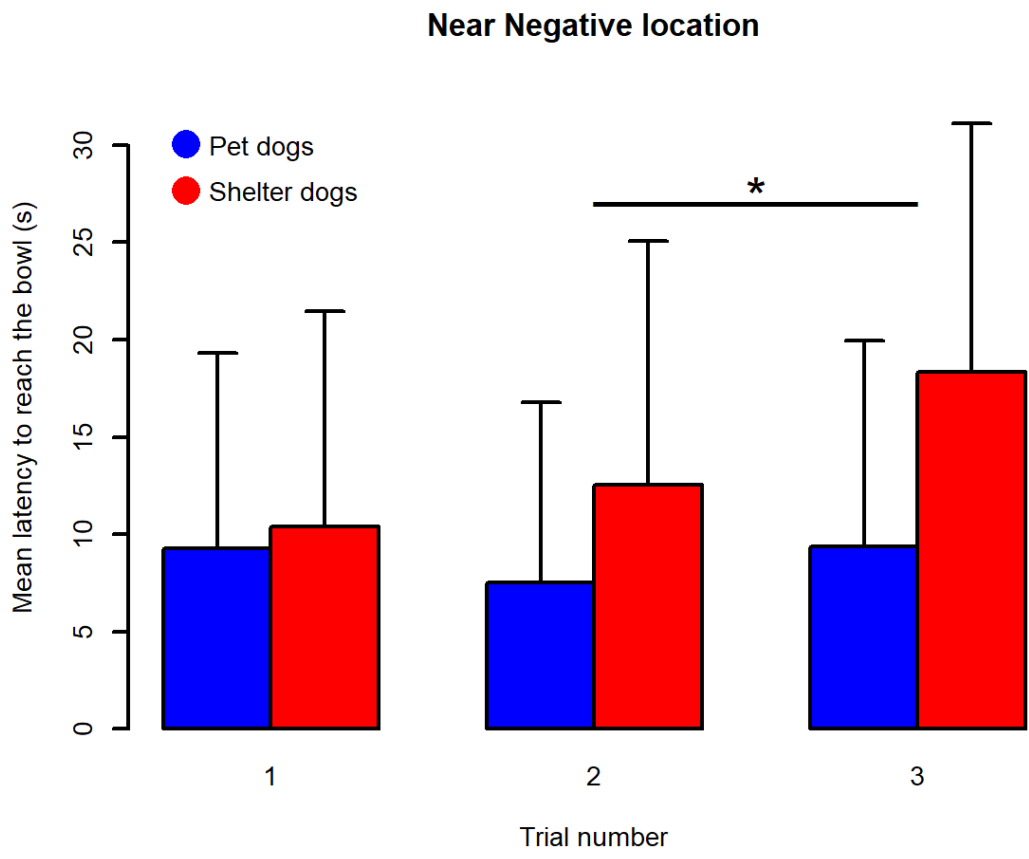


Fig 5. Barplot of latencies to reach the NN location during the three trials. Means ± sd are shown. * shows a significant difference, $p < 0.05$

Variability in latency to reach the trained locations: intra and inter-dog components

We compared intra-dog and inter-dog variability in latencies to reach the bowl placed in the trained locations. Contrary to expectations, intra-dog variability was significantly higher than inter-dog

variability, both for P location (in shelter dogs p-value < 0.0001, in pet dogs p-value < 0.0001) and N location (in sheltered dogs p-value < 0.0001, in pet dogs p-value < 0.001). Results are shown in Fig 6.

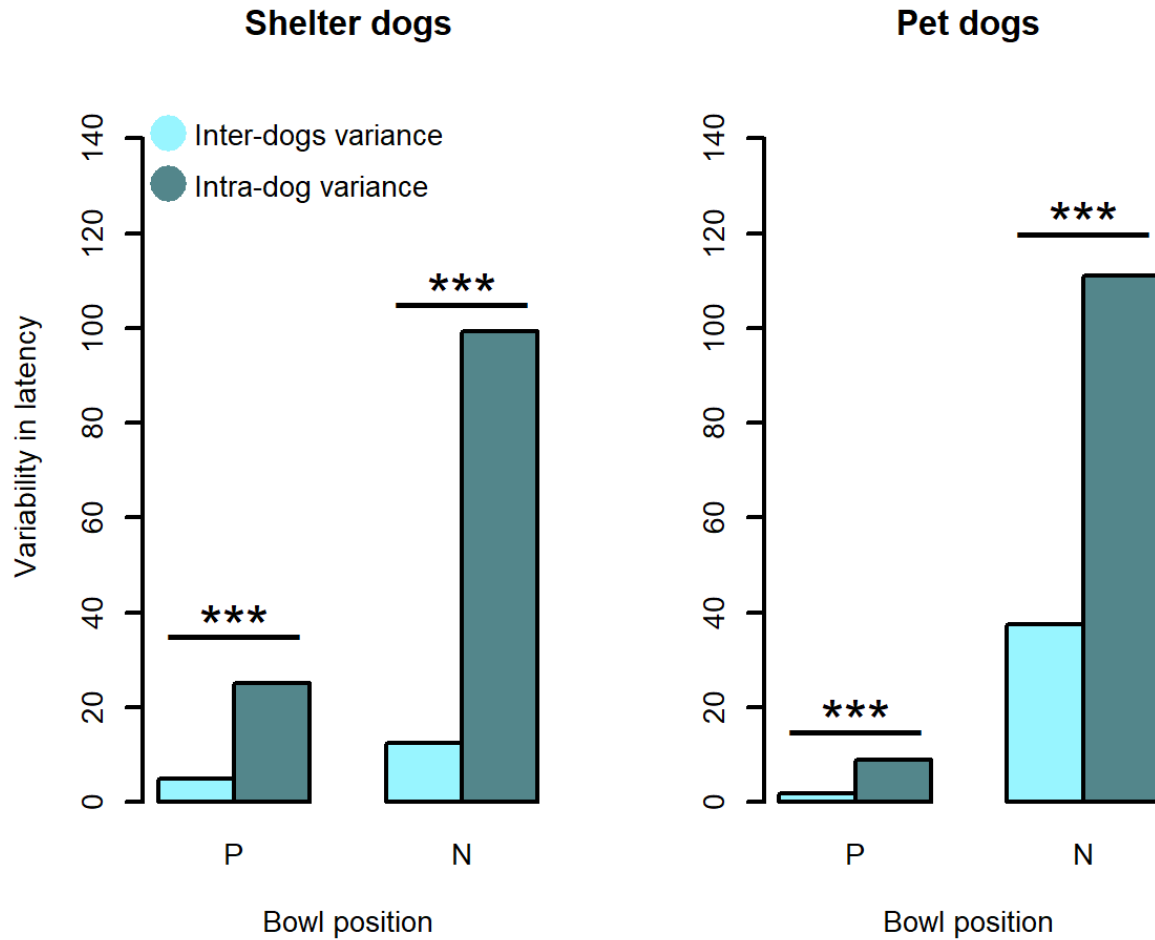


Fig 6. Inter and intra-dog variability in latency to reach the trained locations. Inter-dog (lighter bars) and intra-dog (darker bars) variance of latencies to reach the trained locations: Positive (P) and Negative (N). *** means p-value < 0.001

Dogs' responses to the trained locations

During the testing phase, the percentage of 'go trials' for the P location was 96,79% for sheltered dogs and 99.19% for pet dogs (average latencies in 'go trials'± sd were 3.50 ± 2.93 s and 3.12 ± 2.21 s for

shelter and pet dogs respectively). The percentage of ‘go trials’ for the bowl positioned at N was 33.57% for sheltered dogs and 56.05% for pet dogs (average latencies in ‘go trials’ \pm sd were 9.60 ± 7.54 s and 6.78 ± 5.30 s for sheltered and pet dogs respectively).

Pearson's Chi-squared test with Yates' continuity correction revealed a significant association between occurrences of “go/no go trials” and the population of animals (sheltered dogs vs. pet dogs), for both P and N locations: (**P location:** X-squared = 6.3418, df = 1, p-value = 0.01; **N location:** X-squared = 52.99, df = 1, p-value < 0.0001). Standardised residuals showed that pet dogs exhibited more “go” responses than sheltered dogs, for both trained locations. However, Cramér’s V effect size coefficients revealed that, although the result was statistically significant for both P and N location, the association between the dogs’ behaviour (“go/no go” trials occurrences) and the population was weak regarding P location (Cramér’s V effect size = 0.07) and that the association was instead moderate regarding N location (Cramér’s V effect size = 0.22). Results are shown in Fig 7.

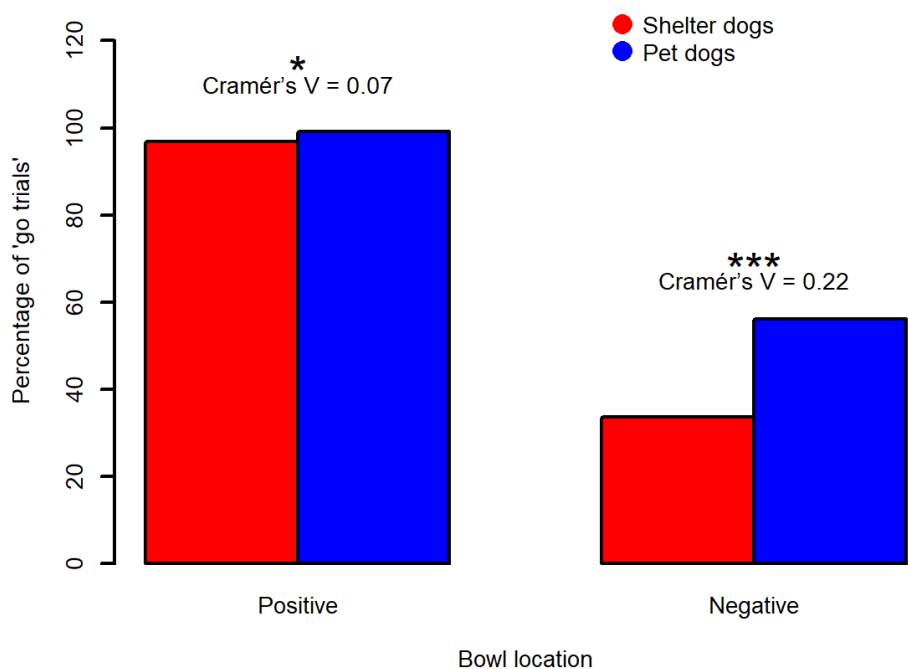


Fig 7. Percentage of “go trials” for the trained locations. A “go trial” is a trial in which the tested dog reached the bowl in less than 30 s. The percentage of “go trials” was calculated on the total number of trials of each type. Data are shown in percentage terms to take into account that the sheltered dog sample (N=35) was larger than the pet dog sample (N=31) and therefore the total number of trials was higher in the sheltered than in the pet dog sample. Significance codes: 0 ‘***’ 0.001, 0.01 ‘*’ 0.05.

Optimism/pessimism adjusted scores

The adjusted scores' frequency distributions are graphically illustrated in Fig 8.

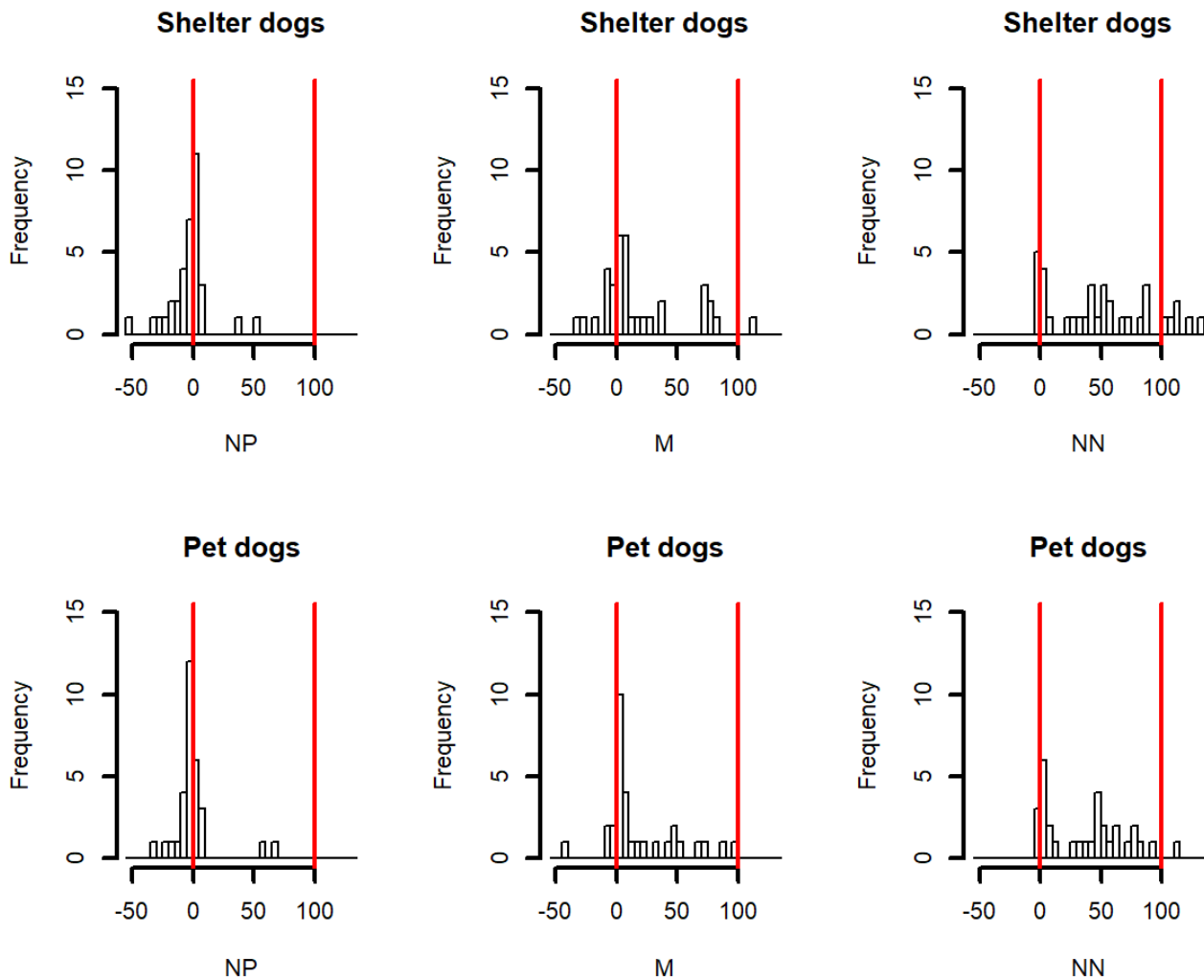


Fig 8. Optimism/pessimism adjusted score. Adjusted score's frequency distribution for the three ambiguous locations: Near Positive (NP), Middle (M) and Near Negative (NN). Red vertical lines help finding 0 and 100 values.

Overall, as expected, dogs appeared to have a more optimistic attitude (lower adjusted scores) the closer to the positive position the bowl was located. The Linear Mixed-Effects model showed that the bowl location had a significant influence on the optimism/pessimism scores ($df = 130$, $F = 37.11543$, P -value $< .0001$). Post-hoc contrasts analysis revealed that NP adjusted scores were significantly lower than M adjusted scores (estimate = -20.438 , Adjusted P -value, Bonferroni method = 0.0002) and that M adjusted scores were significantly lower than NN adjusted scores (estimate = -25.140 , Adjusted P -value, Bonferroni method < 0.0001). No significant differences in the adjusted scores were found between sheltered and pet dogs.

It is worth noting that some adjusted scores values were lower than 0, i.e., the mean latency to reach the ambiguous location was lower than the mean latency to reach the trained positive location and that some adjusted scores values were higher than 100, i.e., the mean latency to reach the ambiguous location was higher than the mean latency to reach the trained negative location.

Discussion

The judgment bias test (JBT) represents a promising tool to assess the optimistic/pessimistic attitude of animals, a cognitive measure linked to emotional state and welfare. However, studies on dogs have not clearly established the link between emotions and judgment biases, with some studies yielding contradicting results [30,32–34]. The reasons underlying these controversial results are not easy to determine given that the experimental paradigm and the statistical analysis of the data have been modified several times (see for a review [6,7]).

The aim of the present paper was to evaluate the original paradigm of the judgment bias test on dogs [27] using an extensive statistical investigation (e.g., analysis of the training phase, responses to the

trained locations during the testing phase, behaviour towards adjacent locations, variability in latencies to reach the bowl). To our knowledge, these issues have never been considered altogether. To make sure that our results were not biased by testing sheltered dogs (a population of animals under stress), all the analyses were mirrored on a population of pet dogs previously tested with an identical JBT paradigm by the same research group [26]. Where appropriate, a comparison between these two populations was also investigated.

Given that our dog populations were heterogeneous, we firstly verified the absence of potential influences of dogs' characteristics (i.e., age, sex, reproductive status and, for sheltered dogs only, shelter location and length of stay in the shelter) on learning (i.e., minimum number of training trials required to reach the learning criterion) and on latencies to reach the bowls during the testing phase. No significant differences related to these variables emerged and learning was not influenced by the location of the reinforced bowl (left/right). Furthermore, results suggested that sheltered and pet dogs did not differ in learning skills in JBT (no differences in minimum number of training trials).

In line with previous studies [26–28,30], results confirmed that dogs' decision-making processes during the testing phase relied on the bowl position and not on the smell of treats.

The first critical point, detected through a proper model for repeated measures, regarded the dogs' behaviour towards the bowl placed in adjacent locations. Latencies to reach the P and NP locations did not differ significantly in either population of animals (which is consistent with previous studies on dogs, [27,30,35]). Contrary to what one might have expected, latencies to reach the NP-M and M-NN locations also did not differ significantly, at least in the pet dog sample. This may reflect an optimistic judgment bias in pet dogs, but it also suggests that these dogs may have struggled to discriminate between adjacent locations and that some adjustments to the experimental design are required to facilitate discrimination (perhaps by increasing the distance between the locations). Some studies, according to dimensional models of affect, propose that the JBT is a useful tool to discriminate not only emotions with different valences, but also emotions with the same valence, but with different levels of arousal (see for a review [3]). For example, depression is characterized by a lower expectation of a

positive event, potentially resulting in pessimistic responses to ambiguous stimuli that are similar to the trained positive cue [18,52]; anxiety is instead characterized by a higher expectation of a negative event, resulting in reduced detection-latencies for cues appearing near the trained negative cue [53,54]. To obtain this level of detail in the evaluation of emotions experienced by the individual, it is fundamental that dogs are able to discriminate properly among all the cues used during the test.

A second critical point regarded the median latency to reach the M location, expected to be the most ambiguous one. As can be seen in Fig 3, this latency was extremely short in both populations. Although this could be ascribed to a strong positive judgment bias, it must be considered that dogs could have reached this location so quickly because they were interested in the researcher who stood behind the bowl placed in M, rather than in the bowl itself. Previous studies highlighted this methodological caveat and provided evidence that the presence of a researcher can influence the dogs' responses in the JBT. For instance, in Muller and colleagues' study [33] the researcher did not stand behind M (the paradigm was slightly different from ours because dogs were able to see the researcher placing the bowl on the ground); these authors reported that the direction from which the researcher came when placing the bowl in M influenced the animals' latencies (i.e., the latencies to reach M were shorter if the researcher approached the M position from the side of the positive location compared to the latencies measured when the researcher approached it from the side of the negative location). More recently, Kis and colleagues [39] compared dogs' responses in spatial JBT when the researcher who placed the bowl on the ground was standing behind the M location (i.e., visible to the animal) and when the researcher was out of sight (i.e., invisible to the animal): they found that dogs reached the bowl faster if the researcher were visible. The strong bond between dogs and humans means that the presence of a person might have a confounding influence on test outcomes, especially towards dogs who experience infrequent human contact, and that they might get considerably aroused by human interaction [6]. Sheltered dogs often suffer from poor welfare due to social isolation [55–57], which could increase their motivation towards human social contacts.

Another element that was thoroughly analysed was the variability in dogs' latencies to reach the bowl. Specifically, we explored for evidence of a learning effect between trials on the ambiguous locations (i.e., dogs after the first or the second trial may have learnt that the bowl placed in the ambiguous locations was empty). Analyses revealed no significant difference between trials in the animals' latencies to reach the NP location, higher latencies to reach M in the second trial than in the first one and higher latencies to reach NN in the third trial compared to the second one. If a learning effect existed, then one would expect a similar pattern of response to all three ambiguous locations; the results from this study therefore suggest that there was not a proper learning effect between trials. Furthermore, it appears unlikely that dogs had learnt the association between the location of the bowl and its outcome in only one or two trials, given that the training phase required an average of 20 and 24 trials for sheltered and pet dogs respectively to learn this association. The differences in latencies between trials 1-2 (for the M location) and 2-3 (for the NN location) suggested instead a potential different optimistic/pessimistic attitude; for example more optimistic subjects may be inclined to keep reaching the bowl quickly, even after they did not find food in the first or second trial. On the basis of this result, we think that it is important to evaluate dogs' latencies to approach each ambiguous location for all trials, rather than focusing solely on the first trial.

Regarding the trained locations, the Linear Mixed-Effect model confirmed an expected result: both sheltered and pet dogs behaved differently between P and N locations, supporting the hypothesis that dogs perceive these cues in a different light. However, it is worth noting that the variability in latencies was very high (see Fig 3); surprisingly, for both sheltered and pet dogs, the intra-dog variance (within-group error variance) was higher than inter-dog variance (between-group error variance) for both trained locations (see Fig 6). Whilst the inter-individual variability in latencies could be ascribed to differences in dogs' personality, motivation or physical characteristics (such as the running speed), a high intra-individual variability entails inconsistency in behavioural responses by the same dog to the same stimulus. It is also worth taking into account that the nature of our data (censored at 30 seconds) may have caused a large intra-individual variance in latencies if only a few responses were different from the others (i.e., few 'no go' responses on P location or few 'go' responses on N location). A sporadic

“no go” response towards P could be due to distraction, confusion or lack of interest [58]; a sporadic “go” response towards N could be due to the low risk involved in actually finding something ‘unpleasant’. However, intra-individual variability in latencies to reach N was extremely high. It should be pointed out that the JBT applied in the current study lasted nearly one hour per dog and this relatively long length of time (42 trials overall only in the testing phase) may have contributed to the observed inconsistency in behavioural responses, with some dogs maybe becoming tired or losing interest in the task.

Learning entails relatively permanent changes in behaviour and stable responses to the stimulus [59,60]. In JBT, the consistency in latencies to reach the trained stimuli assures us that the dogs have indeed learned the association between the trained locations/outcomes and have reached a stable level of discrimination between P and N [61]. Our results suggest that, even if dogs behaved differently towards the two trained locations (mixed-effect models, see results), they might not have completely learned the association between the bowl location and the presence/absence of food, in particular with regards to the N location. By comparing the behaviour of the two populations during the testing phase, it emerged that pet dogs reached the bowl in N on more occasions and faster than sheltered dogs (see Figs 2 and 7); it might be hypothesized that in pet dogs, the association between the absence of food and the N location was weaker than in sheltered dogs. Rather than being a trained location, N could therefore have represented an ambiguous cue and pet dogs might be more optimistic subjects, taking a chance on the bowl placed in N (they also seemed more optimistic than sheltered dogs towards the NN location, see Fig 2).

The high intra-dog variability in latencies to reach N might also be due to the two stimuli (P and N) having a very different payoff. The baited bowl in P was a strong positive reinforcement, whereas the empty bowl in N could be considered to have held a mild negative salience. There was a low cost involved in a 4-metre walk to check the contents of the bowl placed in N and, in addition, it could be hypothesized that dogs had a strong prior conditioning that caused them to expect food in a bowl placed on the ground. As stated by Mendl and colleagues [3], when the “negative” outcome is mild

(e.g., absence of an attractive stimulus as opposed to the presence of an aversive stimulus), subjects might take a chance on every location. As a matter of fact, dogs in the current study tended to reach the bowl even when in N (the percentage of ‘go trials’ was 34% for shelter dogs, 56% for pet dogs), and in these “go trials” dogs reached the bowl quickly (latencies were on average 9.6 and 6.78 s, for shelter and pet dogs respectively), although not as quickly as in P trials. This suggests that dogs behaved differently towards the two trained locations, probably due to a gradual extinction of the “go” response from the P to the N location.

We suggest that the difference between the payoff of the two stimuli was insufficient, therefore learning the reinforced/unreinforced nature of the trained stimuli could be difficult [62]. Given this methodological caveat, the assessment of pessimistic/optimistic attitude in JBT becomes arduous, since responses to ambiguous and trained cues are compared in order to detect a pessimistic/optimistic expectation of the negative/positive outcome previously experienced and learned. Therefore, in future studies it is important to make sure that subjects’ responses to trained stimuli are stable and well defined. To make this learning experience more reliable, one option may be to provide a negative cue that is more aversive or, perhaps, provide longer training sessions with a stronger learning criterion; a difference of 0.5 seconds between the slowest approach to P and the fastest approach to N in the last six training trials may be too slight. The learning criterion could entail a higher and consistent difference between the slowest approach to P and the fastest approach to N, for more than 6 training trials. However, this way the duration of the training phase would inevitably be extended, with the disadvantage of lengthening the duration of the test even more.

Another concern that can be raised when analysing data from the training phase, is whether this paradigm is truly suitable for dogs. During training, 69% of sheltered dogs and 78% of pet dogs fulfilled the learning criterion and were able to move to the testing phase. Müller and colleagues [33], using the same paradigm, reported a similar percentage (75%) of dogs who achieved criterion. Even if these figures still represent the majority of dogs, an inclusion bias could be hypothesised; that is, dogs who struggle to reach the criterion and are therefore excluded from the test, may be the ones experiencing

a negative, or vice versa, a positive mood. As an example, Willen and colleagues [31] found that fearful dogs needed more training trials than non-fearful ones to reach a similar learning criterion. We suggest that the paradigm may not be suited for dogs who, for whatever reason, struggle to learn the association between locations and outcomes. A stronger difference between the payoff of trained stimuli could shorten and simplify the learning process and strengthen the association between trained locations and their outcomes.

Despite the highlighted methodological caveats, sheltered dogs appeared to be more pessimistic than pet dogs when comparing the raw latency to reach the five locations using a proper mixed-effects model (see Fig 2). In order to compare our results with previous studies, an “adjusted score” of optimism/pessimism was calculated in line with Mendl and colleagues [27]. Overall, as expected, dogs appeared to have a more optimistic attitude (lower adjusted scores) the closer to the positive position the bowl was located. However, some patterns of response deserved a critical analysis. First of all, this approach implies that latencies are averaged across several trials of the same type and therefore this approach reduces variability in the data (which, as we discussed above, should not be ignored) and does not take into account the dependencies in the repeated measures. Secondly, some scores were lower than 0, indicating that dogs reached the ambiguous locations faster than the positive one, whereas some scores were higher than 100, indicating that dogs reached the ambiguous locations slower than the negative one. In addition, considering the high intra-individual variability in latencies to reach P and N (used as anchor in the formula to calculate adjusted scores), we recommend that the interpretation of these adjusted scores as measures of optimism/pessimism should be cautious. Furthermore, statistical analysis applied on the pessimism/optimism adjusted score did not detect a more pessimistic attitude in shelter dogs towards the NN location compared to pet dogs. Therefore, the statistical approach that analyses raw latencies to reach the bowl in each trial seems to be preferable.

Conclusions

The aim of this study was to consider a spatial JBT widely applied on dogs (e.g. [30,32–34]) to investigate methodological and statistical criticalities highlighted by previous reviews [7,42,58] and to propose possible solutions and improvements. Our analyses revealed that there are some caveats in the judgment bias tests methodology. Firstly, dogs who passed the training did not behave differently towards bowls placed in adjacent locations; secondly, a great inconsistency in response to the trained stimuli was found, perhaps because dogs did not fully learn the association between the bowl location and the presence/absence of food. Finally, dogs' responses may have been influenced by the presence of the researcher behind the M position.

These outcomes point to a necessity of methodological adjustments to improve the experimental set-up: a bigger difference between payoff of the trained locations could help subjects to learn the association between the bowl location and its outcome, may allow more individuals to pass the training and thus take part in the test [7].

All of this is essential to properly detect optimistic and pessimistic responses to ambiguous cues and therefore to infer the emotional state of subjects. Despite the highlighted methodological caveats, by analysing the dogs' behaviour using raw latencies and a proper mixed-effects model, sheltered dogs appeared to be more pessimistic than pet dogs. However, the significant difference between the two populations, even towards the N location, raises some doubts about the reliability of this JBT paradigm. In this paper, we suggest some improvements that could be implemented in an effort to establish a valid cognitive bias tool for dogs. Such an approach could be particularly valuable for sheltered animals, allowing individuals at risk of reduced emotional welfare to be identified and targeted for interventions aimed at improving quality of life.

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Supporting information

S1 Appendix

Table A. Characteristics of shelter dogs tested with judgment bias test

Name	Breed	Age (years)	Sex	Reproductive status	Shelter	Permanence (days)
Abbey	Mixed breed	4	F	Neutered	BARK	30
Athos	German Shepherd	10	M	Entire	ANPANA	180
Blackjack	Mixed breed	2	M	Neutered	BARK	7
Bobby	Lhasa Apso	2	M	Neutered	BARK	16
Chinotto	Mixed breed	1	M	Entire	ANPANA	10
Dodger	Mixed breed	1,5	M	Neutered	BARK	21
Enjoy	Mixed breed	2	M	Entire	ANPANA	23
Eric	Mixed breed	9	M	Entire	ANPANA	180
Fudge	Lhasa Apso	8	M	Entire	BARK	29
Honeycomb	Mixed breed	2	F	Neutered	BARK	7
Jack	Mixed breed	3	M	Entire	ANPANA	38
Lea	Mixed breed	2	F	Entire	ANPANA	55
Lucky_shelter	Mixed breed	10	F	Neutered	BARK	227
Lyca	Mixed breed	1	F	Entire	ANPANA	60
Max_shelter	Mixed breed	1	M	Entire	ANPANA	60
Miriam	Mixed breed	4	F	Entire	ANPANA	365
Monty	Border Collie	6	M	Neutered	BARK	14
Pepe	Mixed breed	2	M	Neutered	ANPANA	150
Pepper	Mixed breed	1	F	Neutered	BARK	17
Pippin	Mixed breed	5	F	Neutered	BARK	10
Reilly	English Springer Spaniel	1	M	Neutered	BARK	12
Ringo	Mixed breed	2	M	Entire	ANPANA	150

Rocco	Mixed breed	5	M	Entire	ANPANA	1095
Rocky	Mixed breed	10	M	Entire	ANPANA	1460
Roxy	Lhasa Apso	8	F	Neutered	BARK	28
Rufus	Griffon Bruxelloise	1	M	Neutered	BARK	25
Rum	Mixed breed	3	M	Neutered	BARK	15
Ryder	German Shepherd	9	M	Entire	ANPANA	60
Scatoletta	Mixed breed	6	M	Entire	ANPANA	116
Shame	Mixed breed	3	M	Entire	BARK	7
Socks	Mixed breed	3	M	Entire	ANPANA	90
Soda	English Springer Spaniel	4	F	Neutered	BARK	44
Sooty	Jack Russell Terrier	4	F	Neutered	BARK	26
Trixie	Labrador Retriever	8	F	Neutered	BARK	14
Tyson	Mixed breed	3	M	Neutered	ANPANA	365

Table B. Characteristics of pet dogs tested with judgment bias test

Name	Breed	Age (years)	Sex	Reproductive Status
BebeP	Mixed Breed	1,5	F	Entire
Buddy	Mixed Breed	8,0	M	Neutered
Ellie	Mixed Breed	4,5	F	Neutered
Finn	Mixed Breed	5,0	M	Neutered
Finn_R	Schnauzer	8,5	M	Neutered
Fionn	Mixed Breed	5,0	M	Neutered
Gina	Mixed Breed	4,5	F	Neutered
Loki	Mixed Breed	2,0	M	Neutered
Lola	Mixed Breed	5,0	F	Neutered
LouieP	Miniature Schnauzer	3,0	M	Neutered
Lucky	Jack Russell Terrier	3,0	F	Neutered

Luna	Mixed Breed	10,0	F	Neutered
Maisie	Dachshund	6,0	F	Neutered
Max	Mixed Breed	7,0	M	Neutered
Max_C	Labrador Retriever	2,5	M	Entire
Mia	Mixed Breed	6,5	F	Neutered
Penny	Labrador Retriever	3,0	F	Neutered
Poppy	Staffordshire Bull Terrier	3,5	F	Neutered
PoppyP	Mixed Breed	1,0	F	Entire
Riggins	Mixed Breed	2,5	M	Neutered
Romeo	Mixed Breed	4,0	M	Neutered
Ruby	Labrador Retriever	6,0	F	Neutered
Ruby_L	Labrador Retriever	7,0	F	Entire
Ruby_V	Hungarian Short-haired Pointer (Vizla)	6,0	F	Entire
Rupert	Mixed Breed	1,0	M	Neutered
Sophie	Dachshund	9,0	F	Neutered
Suzie	Mixed Breed	1,0	F	Neutered
Tara	Irish Red Setter	2,0	F	Neutered
TobyP	Yorkshire Terrier	8,0	M	Neutered
Wizard	Mixed Breed	3,0	M	Neutered
Zara	Mixed Breed	2,0	F	Neutered

Study 2

A novel spatial judgment bias test provides promising results in dogs (*Canis familiaris*)

Carlotta Burani^{1, 2*}, Annalisa Pelosi¹, Paola Valsecchi²

¹ Dipartimento di Medicina e Chirurgia, Università degli Studi di Parma, Parma, Italia

² Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università degli Studi di Parma, Parma, Italia

*Corresponding author

E-mail: carlotta.burani@studenti.unipr.it

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Abstract

Scientific and reliable measures of affective states in non-human animals are essential to evaluate the psychological well-being, with the judgment bias approach representing one of the most commonly applied; it allows to evaluate animals' optimistic/pessimistic attitude and to infer their emotional and welfare state accordingly.

Judgment bias tests have been used several times with dogs (*Canis familiaris*), in most cases using a spatial test with a bowl placed in ambiguous positions (i.e., located between two certain positions). However, results from such tests are often inconclusive and highlight methodological and statistical criticalities: dogs struggle to learn the association between the certain positions and their outcomes, they hardly discriminate between adjacent locations and they may have been influenced by the presence of researchers during the test.

In this study we propose a novel spatial go/no-go paradigm suitable for dogs, aimed at easing the learning process and to achieve more reliable measures: the difference between payoff of the trained locations is increased, the number of trials and the length of them are reduced and the potential influence of researcher towards one location is removed.

Introduction

Scientific and reliable measures of affective states in non-human animals (hereafter animals) are essential to evaluate the psychological well-being, which, along with the physical health, represents a fundamental component of good welfare [1,2]. The assessment of animal affective state is still challenging, with the cognitive bias approach being one of the most commonly applied across species (for reviews see [3–6]; for critical analyses see [7,8]). This approach is based on a phenomenon initially studied in humans [9]: emotional states could bias cognitive functions [4,8]. Thus, it should be possible to infer an individual's emotional state from the evaluation of cognitive biases.

Cognitive biases can be grouped into three main categories: attention, memory and judgment biases [3]. The latter pertain to the optimistic/pessimistic interpretation of ambiguous stimuli and has been traditionally studied in humans using verbal tasks (e.g., the interpretation of ambiguous semantic sentences [10] and of lexical ambiguity such as homophones, see for a review [11,12]). In recent years, non-verbal tasks have been studied in humans in an effort to develop tools suitable for assessing affective states both in humans and in animals and for making comparisons among species [13]. Among others, Paul and colleagues [14] developed a non-verbal spatial task: subjects were required to decide whether a stimulus (a cross located in random positions) was nearer a positive (i.e. a smiling face) or a negative anchor image (i.e. a sad face). If the cross were located in ambiguous positions between the two anchor points, subjects with lower “positive activation” (state related to loss or absence of opportunity) and higher “negative activation” (a danger-oriented state, see the PANAS scale, Positive and Negative Affect Schedule [15]), tended to interpret the cross as being nearer the negative anchor point, suggesting a pessimistic attitude.

Despite advancements in this field, caution is still required when using non-verbal judgment bias tests (JBTs) to assess the general mood in humans. While verbal tasks are generally suitable for this purpose, non-verbal tasks were only partially successful, for example Ilgaya and colleagues [16] employed a non-

verbal JBT and they did not detect a relationship between results and the mood reported by participants. Similarly, Schick and associates [17] found no significant correlation between mood and interpretation bias measured using a non-verbal, tone-based JBT.

Nevertheless, non-verbal paradigms are the only available tool to assess judgment biases in non-human animals, therefore various protocols have been developed and adapted for several animal species (e.g. rats [18–22], starlings [23], bees [24], sheep [25–27], macaques [28], pigs [29], horses [30], calves [31]), including the domestic dog, *Canis familiaris* [32–36]. Despite of the large number of studies that have used JBTs to evaluate the welfare and the affective state in animals, doubts remain about its reliability: results are not always in line with predictions, with some studies even leading to null results or to opposite findings to those expected [8,16,37]. For example, Neville and colleagues reviewed the existing literature about the pharmacological manipulation of affect in animals and reported a high heterogeneity and a strong variability in the extent to which pharmacological manipulations of affective state alter the judgement bias [38]. Lagisz and colleagues came to similar conclusions analysing the literature about the non-pharmacological affect manipulation in animals, even though they found an overall small effect of affect manipulation on judgment bias, in agreement with predictions [6].

Judgment bias studies with dogs offer the opportunity to compare different populations (e.g., pets, shelter, stray, laboratory, working dogs) that are often involved in welfare studies [39–45]; however, JBTs results are as controversial as those from other species. Some studies detected the expected relationship between emotions and judgment biases. For instance, Mendl and colleagues [33] applied a spatial JBT on dogs for the first time and found that sheltered animals scoring higher on separation-related behaviours showed a ‘pessimistic’ judgment of an ambiguous cue, suggesting a negative affective state. More recently, Willen and colleagues [46] found that fearful sheltered dogs showed a more pessimistic expectation towards ambiguous stimuli than non-fearful ones and that enrichment based on positive human interaction could increase positive expectancy in fearful animals. However, they also discovered that the same enrichment had the opposite effect on JBT results in non-fearful individuals, raising doubts about the enrichment’s influence on judgment bias and mood.

Ambiguous or null results are not an exception in JBTs studies with dogs: e.g., Titulaer and colleagues [47] did not find differences in the optimistic/pessimistic attitude between short-term and long-term sheltered dogs (expected to be in a more negative affective state), however, also physiological and behavioural measures were not different between the two samples. Müller *et al.* [48] noticed that the brief absence of the owner did not induce a pessimistic bias in pet dogs. More recently, Walker and colleagues [49] reported that the separation from the kennel-mate did not induce a pessimistic mood in shelter dogs. Regarding the relationship between welfare state and judgment bias in sheltered dogs, Owczarczak-Garstecka and colleagues [50] found that dogs' optimistic/pessimistic bias was not related to the percentage of time spent asleep during the night (a potential welfare index), while Harvey and colleagues [51] found that a more pessimistic judgment bias was unrelated to the amount of time dogs were awake, but inactive, in the home environment (a potential index of a depression-like state).

Some studies even led to opposite findings to those expected: e.g., Burman and colleagues [52] found that a brief positive experience (i.e., a food-based rewarding event) induced in laboratory dogs a pessimistic bias rather than an optimistic one.

It is worth noting that other studies, whilst reporting an association between emotions and cognitive biases in dogs, presented methodological and statistical issues, such as a small sample size that makes more difficult to infer a general behavioural pattern [53,54], the employment of only a single ambiguous trial, the outcome of which could be influenced by momentary distraction [35,55] and the use of a statistical approach that evaluates the dog's average response instead of single trial responses, thereby reducing variability in data [33,46,56]. Averaged measures are inevitably less accurate than raw ones and they depend on the number of repetitions: in JBTs this is usually not consistent among type of cues (more trials for each unambiguous trained cue, less trials for each ambiguous cue to minimize a potential learning effect).

In a recent study [57], we deeply analysed the robustness and validity of the original spatial JBT protocol usually used with dogs [32–34]. In brief, it consisted of a training phase aimed at training dogs to discriminate between a positive location (P) in which a baited bowl was placed, and a negative location

(N) in which an empty bowl was placed; this phase was followed by a testing phase, aimed at measuring the optimistic/pessimistic attitude: the latency to reach a bowl placed in 3 novel locations (NP-Near Positive, M-Middle, NN-Near Negative) interspersed between P and N, was an index of dog's expectations. In line with predictions, our results showed that sheltered dogs appeared to be more pessimistic than pet dogs: entering a shelter is a stressful event [41] and the kennel environment can potentially induce negative emotions (due to stressors including noise, social deprivation, confinement).

However, our study highlighted some methodological caveats that raised doubts about the experimental protocol. Firstly, not all dogs reached the learning criterion and were able to move on to the testing phase (78% of pet dogs succeeded [57], percentage which is in line with other studies [48]), suggesting the possibility that an inclusion bias existed: e.g., dogs who struggle to reach the criterion and are excluded from the test, may be the ones experiencing a negative or a positive mood. Moreover the fact that several dogs failed to meet the learning criterion suggests that the paradigm may not be fully suited for this species; potentially, a stronger difference between the payoff of trained stimuli could simplify and shorten the learning process [58].

In addition to this, the analysis of the testing phase also suggests that the difference between the payoff of the two stimuli may not be sufficient: learning entails relatively permanent changes in behaviour and stable responses to the learned stimulus [59,60], but the intra-individual variability in latencies to reach N was extremely high. This inconsistency in responses to the N stimulus, suggesting an unstable learning, might be due to the different payoff of the two trained stimuli (P and N): the baited bowl in P was a strong positive reward, whereas the empty bowl in N could be considered to have a mild negative salience. When the negative outcome is mild, subjects might take a chance on every location and respond positively even to negative cues, because the cost of doing so is negligible [4,6]. In addition, it could be hypothesised that dogs were accustomed to find food in a bowl placed on the ground by their owner; this strong prior conditioning could have influenced dogs' expectations, leaving open the possibility of the bowl being baited in the next trial.

In JBTs, responses to ambiguous cues are compared to responses to trained cues, in order to understand if the former ones indicate the expectation of the negative or of the positive outcome previously experienced and learned; however, when responses to trained cues are inconsistent, the assessment of pessimistic/optimistic attitude becomes arduous.

A further critical point was that dogs' responses did not entirely differ between adjacent locations (pairwise comparisons, i.e., P/NP, NP/M, M/NN and NN/N), suggesting that dogs may have struggled to discriminate between them. Discrimination between all the stimuli is very relevant because JBTs have the potential benefit of discriminating not only emotions with different valences, but also emotions with the same valence and different levels of arousal (see for a review [4]). According to the dimensional core affect model [61], depression is characterized by a lower expectation of a positive event (reward) [62], potentially resulting in pessimistic responses to the near positive (NP) location [18,63]; anxiety is instead characterized by a higher expectation of a negative event (punisher) [62], resulting in reduced detection-latencies for cues appearing near the trained negative cue (NN location) [64,65]. To obtain this level of detail in the evaluation of emotions using JBTs, it is fundamental that dogs can discriminate properly among all the stimuli.

Finally, we detected a potentially influence of the presence of a researcher, as highlighted also by others [36,48,55]: the median latency to reach the M location was extremely short, potentially because dogs were interested in the researcher who stood behind the bowl placed in M, rather than in the bowl itself.

Taken together, equivocal results of judgment bias studies in dogs and the occurrence of methodological and statistical caveats, suggest a reconsideration of the protocol.

In the current study we adapted a JBT protocol originally developed for sheep [26,27] and we applied it to 50 pet dogs. Our aims were to obtain more reliable measures and to ease the learning process : two corridors, P (leading to the owner offering a food treat) and N (leading to a black cloth blowing), located at two opposite sides of a test area, represented the certain stimuli; the ambiguous stimuli are represented by 3 (empty) corridors located between P and N.

Our hypotheses are that this novel protocol could raise the difference between the payoff of the two trained stimuli (P and N) and ease the learning process and the discrimination among stimuli: in this case, we expect that a great proportion of dogs will be able to discriminate among P and N and to pass the training phase. Furthermore, in this novel protocol the researcher is positioned near each tested location, in order to remove her potential influence on dogs' responses to the M location; we hypothesize that the latency to reach this ambiguous stimulus would not be as short as in the classical protocol, leading to a graded output (i.e., gradually higher latencies to enter corridors, from P to N corridor).

Finally, we hypothesize that reducing the number of trials and their length, the dogs' tiredness and distraction could be prevented or reduced, raising the consistency in responses and allowing a stable learning of the trained stimuli; for the same reason, we set a stronger learning criterion compared to the classical protocol.

The main aim of our study was to elaborate a trustworthy protocol capable to provide stable and consistent measures; in future studies the influence of emotions on these measures and their reliability as optimism/pessimism indexes might be evaluated, comparing dogs living in conditions able to generate different emotional states (e.g., enriched versus barren environment).

Materials and methods

Subjects

Fifty dogs were recruited by an announcement posted on the Facebook page of our research group and by word of mouth. One dog appeared extremely stressed during the procedure, probably due to the temporary separation from its owner, and it did not succeed in the training phase (see later); therefore,

the tested sample was of 49 dogs (various breeds; mean age \pm sd : 5.9 \pm 3.6 years; 30 females and 19 males; see Table A in Supplementary Materials for details). All the dogs have been living with their owner at least for 1 year at the time of testing. Training level was recorded as following: level 0 for dogs who have never experienced any type of training, level 1 for dogs who attended some basic training lessons and level 2 for dogs who experienced at least one type of dog sport/problem-solving/nose-work activity in addition to basic training lessons. All the owners consented to take part in the study and stated that their dogs were healthy and well socialized with unfamiliar people.

Judgment bias test (JBT)

The judgment bias test (JBT) was carried out in a barren outdoor area (approximately 10x5 metres) fenced with wire mesh and covered with a shade cloth to ensure dog safety and to avoid potential distractions. The JBT protocol was adapted from Verbeek and colleagues [26,27]: the apparatus consisted of five adjacent corridors (named P, NP, M, NN, N) made of 2-m-high plastic walls; each corridor was closed at the end with a wire mesh and a shade cloth and it was closed at the beginning with a wooden door locked with a metal hook latch. The testing arena and the experimental set-up are shown in Fig 1.

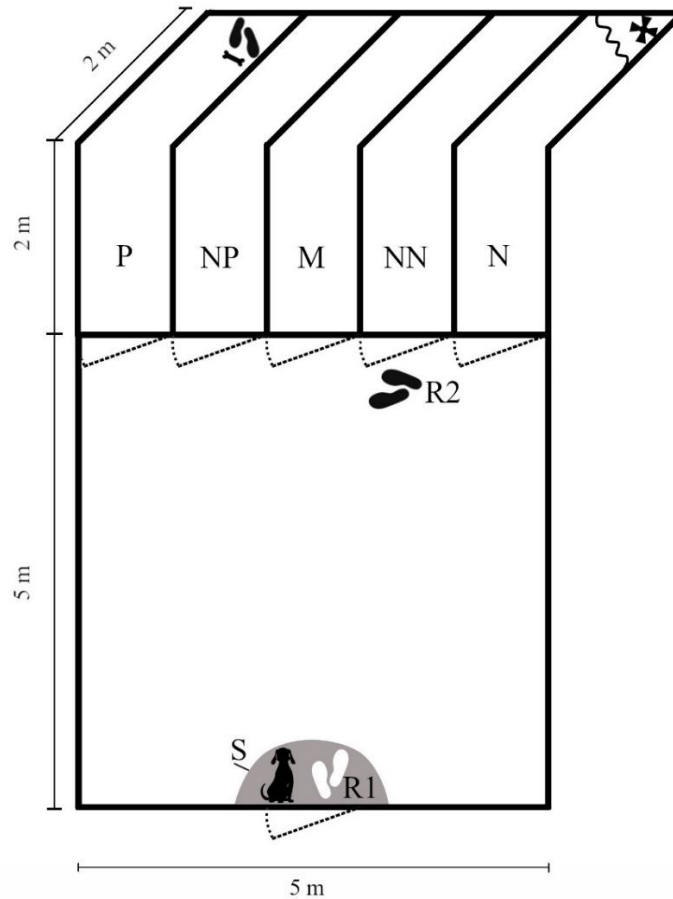


Fig 1. Experimental set-up. R1 (researcher #1) led the dog at S (starting position, grey area) at the beginning of each trial; R2 (researcher #2) opened the doors of the five corridors: P (Positive), NP (Near Positive), M(Middle), NN (Near Negative), N (Negative). This image was designed using Freepik.com resources. NP, M and NN corridors were empty, whereas at the end of the P corridor there was the dog's owner, instructed to greet its dog and to give it a piece of chicken sausage, at the end of the N corridor there was a black cloth blown by the fan behind it.

Each corridor was 4-m-long: starting from the door, the first half of the corridor (2-m-long) was straight, whereas the second half (2-m-long) was curved by a 45° angle to the right. From the starting position (S in the Fig 1), which is 5 m from the M door, it was impossible to see the end of the corridors without entering them.

The protocol consisted of 3 phases: familiarization, training and testing.

1) Familiarization

The dog and the owner were welcomed by two researchers who explained the whole procedure to the owner and asked him/her to sign an agreement to participate in the study and to let us register their personal data and contact details (name, dog's data, email address and phone number); after that, the dog was taken, using a leash, by the researcher #1 (R1) for a 5-minute walk in a fenced grassy area, along with the owner, to get the dog accustomed to be led by R1. The dog was then led to the testing arena and R1 offered it three pieces of chicken sausage to check that the dog liked it.

To familiarize the dog with the apparatus, it was led by R1, using the leash, at the starting position and its owner was asked to stand by it; researcher #2 (R2) called the dog by name to get its attention, opened the first door on the left corner of the arena and the dog was then allowed to explore the newly opened corridor, along with R1 and the owner. If the dog hesitated to enter the corridor, the owner was asked to encourage it. When the dog had explored the corridor, it was led back to the starting position by R1, together with the owner, while R2 closed the door. This procedure was repeated for each corridor. The dog was then led by R1 out of the testing arena, R2 gave to the owner some slices of chicken sausage in a hermetically closed container and accompanied the owner to a chair located at the end of the P corridor; then R2 placed a fan at the end of the N corridor and a black cloth right in front of the fan, very close to it and finally R2 closed all the doors. The location of the positive corridor (P) was balanced in the sample (i.e., for 50% of the dogs the positive corridor was on the left-hand side and for the remaining dogs was on the right-hand side of the arena). R2 called back R1 who came with the dog and held it in the starting position.

2) Training

Each dog was trained to discriminate between a positive stimulus (P corridor) and a negative stimulus (N corridor). In case the dog entered the P corridor, it found its owner who was instructed to greet the dog using a joyful and sweet tone, to cuddle it and to give it a piece of chicken sausage. To adapt the

food intake to the dog's size, we instructed the owner to give half slice of sausage to small/toy sized dogs, one slice of sausage (approximately weight = 3 gr) to medium sized dogs and two slices of sausage to large sized dogs. In case the dog entered the N corridor, R1 activated the fan using a remote control, so that the dog faced the black cloth suddenly blown by the fan behind it. This way, the noise of the fan was not audible, the cloth was not waving and was not visible from the starting position when the door was opened, but only when the dog had already entered the N corridor.

During the training, the dog was presented with only one accessible corridor per trial (either P or N), following a pseudo-random order, making each corridor accessible no more than twice consecutively. The applied procedure was the following: R1 held the dog using the leash in the starting position, R2 called the dog by name and opened the P or N door while R1 released the dog who was free to explore the testing arena for 15 seconds. R2 stood next to the open door and R1 stood in the starting position looking straight ahead, both ignoring the dog; R2 used a stopwatch to measure the latency to enter the corridor, i.e., the time elapsed from the moment of release to the moment in which the dog passed the door line with its all four paws. If the dog did not enter the corridor within 15 s, the door was closed and a latency of 15 seconds was registered. A trial ended when the dog passed the door line or when 15 seconds elapsed; at this point R1 gently called the dog by name, leashed it, led it to the starting position for the next trial and R2 closed the door. Given that a previous study [48] on dogs reported that the direction from which the researcher came influenced latencies (i.e. latencies to reach an ambiguous probe were shorter if the researcher approached it from the side of the positive location compared to the latencies measured when the researcher approached it from the side of the negative location), at the beginning of each new trial R2 stood for 2 seconds ahead of the M door (see Fig 1) facing the dog.

Dogs had to enter each trained corridor (P and N) at least once and they had a minimum of 10 and a maximum of 35 trials to reach the learning criterion. The learning criterion is reached when for 6 consecutive trials dogs entered the P corridor and did not enter the N corridor. This criterion was evaluated with each additional trial of training (rolling criteria). Once the learning criterion was reached,

we could suppose that the testing arena became a conditioned environment for the tested dog, with a positive stimulus (P corridor) and a negative one (N corridor) at the two ends of the line of doors.

The number of training trials required to reach the learning criterion was recorded for each dog.

3) Testing

The testing phase took place immediately after the dog reached the learning criterion. The procedure was the same of the training phase, except for the fact that dogs were presented also with the three ambiguous corridors, located between P and N, i.e., NP (Near Positive corridor), M (Middle corridor) and NN (Near Negative corridor), (see Fig 1). These corridors were empty and each of them was presented three times, always separated by one P and one N trials in order to confirm the previous conditioning (see the scoring sheet for the trial sequence – Table B in Supplementary Materials). In order to counteract any effect of previous trials on ambiguous trials, dogs had equal presentation of positive and negative trained stimuli before the ambiguous ones as done by Murphy and colleagues with pigs [66].

Overall, the testing phase included 25 consecutive trials. At the end of the testing phase, the dog was led by R1 out of the testing arena, the owner was accompanied by R2 at the end of the N corridor, R2 closed all the doors and call back R1 who came with the dog and held it in the starting position. Then, using the same procedure described above, R2 called the dog by name and opened the door of the P corridor. This last trial, named “false positive”, was run to make sure that dogs were relying on spatial cues and not on the treat’s and/or the owner’s odour.

Latency to cross the door was measured by R2 using a stopwatch. Latency was evaluated as a cue of optimistic/pessimistic attitude: a short latency indicates a potential anticipation of a positive outcome (owner and food), i.e., an ‘optimistic’ judgment, whereas a longer latency is reflective of a potential ‘pessimistic’ judgment.

Overall, the judgment bias test (familiarization + training + testing) lasted nearly 40 minutes per dog.

Behavioural analyses

In order to thoroughly investigate the dog's reactions towards the different types of trials (P, NP, M, NN and N trial), the test was video recorded with a camera positioned on a tripod. Videos were analysed using a specific ethogram and scoring was carried out using the Software Solomon Coder beta (17.03.22 version, Copyright © 2006-2017 by András Péter). The detailed ethogram is reported in Table 1. The test was videorecorded for a subsample of 32 dogs.

Table 1. Ethogram

Durations	
Checking P door:	The dog walks towards the P door and enters the related corridor (even without passing the door line with its four paws) if the door is open or tries to open it if it is closed or the dog walks towards the P door, stops close to it and observes/smells it
Checking NP door:	The dog walks towards the NP door and enters the related corridor (even without passing the door line with its four paws) if the door is open or tries to open it if it is closed or the dog walks towards the NP door, stops close to it and observes/smells it
Checking M door:	The dog walks towards the M door and enters the related corridor (even without passing the door line with its four paws) if the door is open or tries to open it if it is closed or the dog walks towards the M door, stops close to it and observes/smells it

Checking NN door:	The dog walks towards the NN door and enters the related corridor (even without passing the door line with its four paws) if the door is open or tries to open it if it is closed or the dog walks towards the NN door, stops close to it and observes/smells it
Checking N door:	The dog walks towards the N door and enters the related corridor (even without passing the door line with its four paws) if the door is open or tries to open it if it is closed or the dog walks towards the N door, stops close to it and observes/smells it
Resting close to P door	The dog is sitting or lying down close to the P door
Resting close to NP door	The dog is sitting or lying down close to the NP door
Resting close to M door	The dog is sitting or lying down close to the M door
Resting close to NN door	The dog is sitting or lying down close to the NN door
Resting close to N door	The dog is sitting or lying down close to the N door
Resting close to the starting position	The dog is sitting or lying down close to the starting position
Resting elsewhere	The dog is sitting or lying down elsewhere (far from any of the doors or from the starting position)
Gazing R2	The dog is gazing at R2 from a stationary position
Gazing R1	The dog is gazing at R1 from a stationary position
Other	The dog is displaying any other behaviour or it is out of sight

Displacement behaviour	The dog is eating the grass or digging. These are the only replacement behaviours observed during the experiment. Self-directed behaviours are included in stress related behaviours (see below) and their frequencies were recorded.
Frequencies (instant events)	
Gazing R2	The dog is gazing at R2 from a stationary position
Gazing R1	The dog is gazing at R1 from a stationary position
Vocalization	The dog is barking or whining
Stress-related behaviour	The dog is yawning, scratching, shaking, startles or it is keeping a paw lifted from the ground. We decided to not consider panting, drinking and lips/nose licking in this category because temperatures were high and dogs drank frequently
False start	The dog walks towards the open door but it stops before entering the related corridor

Inter-observer agreement was assessed by means of independent parallel coding of a random sample of 11 dogs (34% of the sample). The agreement between two observers was assessed by Spearman correlations and it was significant for all the variables (p values ranged from 0.04 to values < 0.001, Rho values ranged from 0.61 to 1; see Table C in Supplementary Materials for detailed results). Inter-observer agreement was evaluated on raw durations measured in seconds and, for instant events, on the total number of occurrences.

Durations

We calculated for every dog the percentage of time spent exhibiting different behaviours during each of the five types of trial, e.g., the percentage of time exhibiting “checking P door” during P trials by dog#1 = (seconds spent exhibiting “checking P door” during P trials by dog#1/total length of P trials for dog#1)*100.

Frequencies

We calculated for every dog the rate of occurrences of different behaviours during each of the five types of trial, e.g., the rate of occurrences of “stress-related behaviours” during P trials for dog#1 = occurrences of “stress-related behaviours” during P trials for dog#1/total length of P trials for dog#1.

Statistical analyses

All analyses were performed using the software R, version 3.3.1., with packages nlme (version 3.1-128) for multilevel analysis, multcomp (version 1.4-6) for multiple comparisons in linear mixed effects models, moments (version 0.14) to calculate skewness of distributions and MuMIn (version 1.40.4) for model selection.

Analysis of the training phase

The relationship between canine age (years) and the number of training trials required to achieve the learning criterion was investigated using a Spearman’s correlation test; effects of dogs’ sex (male/female), training level (0/1/2) and of the position of the positive corridor (left/right) on the number of training trials were investigated using Mann-Whitney U tests and a Kruskal-Wallis rank sum test.

Analysis of the testing phase

Effect of the treat/owner’s smell. To ensure that dogs’ decision-making processes relied on corridor position and not on the odour of the treats/owner, a Wilcoxon test for paired samples was employed

to compare, for each dog, the mean latency to reach the P corridor during the testing phase and the latency to reach the same corridor during the last trial (“false positive” trial), when the owner and treats were in the N corridor.

Influence of dogs’ characteristics on latencies to enter the corridor. As recommended by Gyax [67] a Linear Mixed-Effect model was applied, setting as dependent variable the untransformed latencies to enter the corridor in each single trial, including also latencies recorded in P and N trials, used as anchors to estimate the slope across the different ambiguous cues (dogs’ responses to trained corridors P and N are expected to be stable); a maximum-likelihood estimation was employed to account for designed imbalance in the data (3 trials for NP,M and NN corridor vs. 8 trials for P and N corridor)[68].

A backwards approach was applied to test the fixed effects of canine age, sex and training level on the latencies to enter the corridors. These fixed effects were tested in interaction with corridor position variable and the interaction was removed from the model if not significant.

Analysis of latencies to enter the five corridors. To assess whether the dogs behaved differently towards the five corridors, a Linear Mixed-Effects model was created with corridor position variable nested in dogs’ identity as a random effect, latency to enter the corridor as a dependent variable and corridor position as a fixed effect. The corridor position variable was coded as a factor in this analysis.

Dogs’ responses to the trained corridors (P and N). The percentage of correct responses (enter the P corridor in P trials, not enter the N corridor in N trials) during the testing phase was evaluated to check that the learned association between the stimulus and its outcome was maintained during the testing phase: a stable association is fundamental to obtain reliable measures of optimism and pessimism.

Furthermore, to determine how the subjects responded towards the trained stimuli (P and N corridor), we evaluated the number of “go trials” and “no go trials” towards these stimuli. A “go trial” is a trial in which the dog enters the corridor within 15 s, a “no go trial” is a trial in which the dog does not enter the corridor within 15 s. We calculated the percentage of “go trials” on the total number of P/N trials (number of P “go trials”/total number of P trials, number of N “go trials”/total number of N trials).

Dogs' responses to the ambiguous corridors (NP, M, NN): looking for a learning effect. To determine how the subjects responded towards the ambiguous stimuli, for each type of corridor, we calculated the percentage of “go trials” on the total number of trials, as done for the P and the N corridor.

Furthermore, latencies to enter the ambiguous corridors showed a high variability (see results); this could suggest a great amount of uncertainty in dogs' responses towards these stimuli, but it could also point to a learning effect, i.e., dogs after the first or the second trial may have learnt that the ambiguous corridor was empty. In that case, only the first trial would be a real ambiguous one and therefore only the first trial would be a reliable measure of optimism/pessimism. In order to explore for a potential learning effect, a Linear Mixed-Effects model was built for each ambiguous corridor (NP, M and NN), with dogs' identity as a random effect, latency to enter the corridor as the dependent variable and the trial number (1,2 or 3 for each corridor, factor variable) as fixed effect.

The number of training trials required to achieve the learning criterion, during the training phase, was included in the analysis as fixed effect to take into account the dogs' learning skills: if there is a learning effect over the 3 ambiguous trials, this has to be more noticeable in dogs who have learnt faster the positive/negative value of the P/N corridor during the training. This variable was included in the model in interaction with the trial number variable (1,2 or 3); the interaction was removed from the model where not significant.

Dogs' responses to the ambiguous corridors: optimism/pessimism index? Even if averaged measures are inevitably less accurate than raw ones, to compare our results with previous studies and to evaluate the potential usefulness of our protocol in measuring optimism and pessimism, we calculated an “adjusted score” of optimism/pessimism, according to the following formula, originally developed by Mendl and colleagues [33]:

$$\text{adjusted score} = \frac{\text{mean latency to probe location} - \text{mean latency to Positive location}}{\text{mean latency to Negative location} - \text{mean latency to Positive location}} \times 100$$

The adjusted score was calculated for each dog and each type of ambiguous location (NP,M and NN). An adjusted score near 0 means that the subject reacted to the probe location in a similar way to how it reacted to the positive location (i.e., it considered the ambiguous cue as potentially rewarding - optimistic bias); vice versa an adjusted score near 100 indicates a pessimistic bias.

We compared the adjusted score among the three ambiguous corridors using a Linear Mixed-Effects model with the corridor location as fixed effect and the dogs' identity as a random effect.

Ethics statements

The ethical committee for animal testing of the University of Parma declared that a special permission to use pet dogs in this behavioural study is not required in Italy (Decreto legislativo 4 marzo 2014, n. 26, Art. 2). Furthermore, the committee reported that this study accomplishes ethical requirements in compliance with the law about the use of animals in clinical and zootechnical studies (PROT.N. 11/CE/2019).

Results

Analysis of the training phase

The minimum number of training trials required to reach the learning criterion was on average 18.29 trials \pm sd 7.21 trials. None of the tested variables was significantly related to the minimum number of training trials required to reach the learning criterion (see Table 2 for detailed results, males, mean \pm sd : 19.68 \pm 8.16, females: 17.4 \pm 6.54 trials; P at left side: 16.62 \pm 8.17, P at right side: 20.17 \pm 5.54

trials; training level “0”: 19.25 ± 9.43, training level “1”: 18.22 ± 7.65, training level “2”: 18.19 ± 6.88 trials).

Table 2. Influence of dogs’ characteristics on the minimum number of training trials required to reach the learning criterion

Tested variable	Test statistic	P-value
Age	Spearman’s correlation rho = 0.02	p = 0.89
Sex	Mann-Whitney U test W = 243	p = 0.39
Positive door location	Mann-Whitney U test W = 201	p = 0.05
Training level	Kruskal-Wallis rank sum test $\chi^2_{(2)} = 0.06$	p = 0.97

Analysis of the testing phase

Effect of the treat/owner’s smell

Wilcoxon test for paired samples showed that the latency to enter the P corridor in the last “false positive” trial (mean ± sd 2.22 ± 1.33 s), when the dog’s owner was in the N corridor, was statistically significantly shorter than the mean latency (mean ± sd 2.55 ± 1.19 s) to enter it during the test (z-value

= 0.5, $p < 0.001$, the result is graphically shown in Fig 2): this confirms that the dogs' decision-making processes relied on the door location and not on the treat/owner's smell.

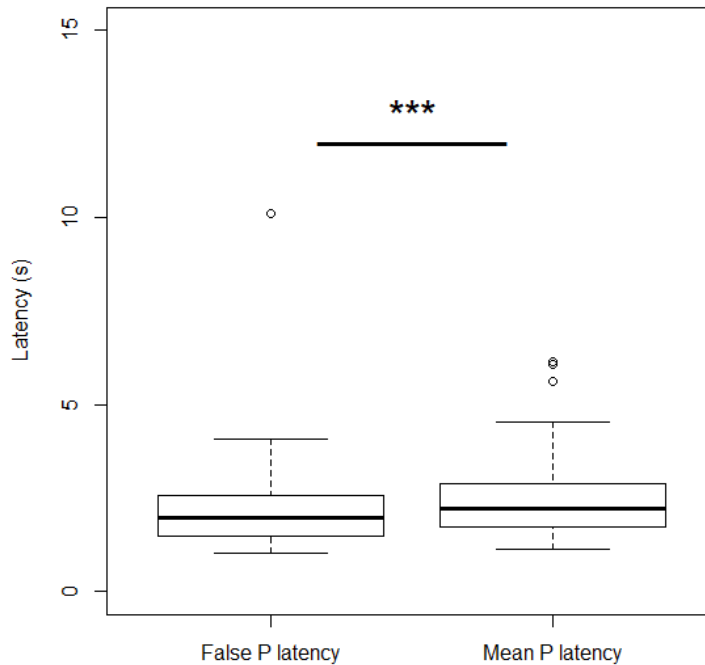


Fig 2. Boxplots of latencies to enter the P corridor in the last “false positive” trial and of the mean latency to enter it during the test. This graph shows medians (bar within the box), upper and lower quartiles (borders of box), lowest and highest cases within 1.5 times the IQR (bottom and top whiskers) and outliers (circles). *** shows a significant difference, $p < 0.0001$

Influence of dogs' characteristics on latencies to enter the corridor

The Bayesian Information Criterion (BIC) was used to select the best combination of random effects: as expected, the best model entailed that intercept vary incorporating all the necessary hierarchical levels (i.e., corridor location nested in dog identity). Using the dog's identity as a random effect factor takes into account the potential differences in running speed between subjects. Model selection using BIC also showed that the model better fit the data if the corridor position variable was coded as a continuous variable and not as a factor, in agreement with the expected graded output (i.e., gradually higher latencies to enter the corridor if it was close to the N corridor). However, the corridor position

variable was coded as a factor in the following analyses in order to detect potential different influences of dogs' characteristics on latencies to enter different corridors.

Linear Mixed-Effects models did not reveal any significant effect of the fixed effects of sex and training level on latencies to enter the various corridors (see Table 3 for detailed statistical results; males, mean \pm sd : 8.5 ± 6.22 , females: 8.85 ± 6.02 s; training level "0": 9.78 ± 5.7 , training level "1": 8.82 ± 6.09 , training level "2": 8.48 ± 6.16 s). The age variable has a mild influence ($p = 0.02$, estimate = 0.14 s) on latencies, regardless of the corridor location; even if it is realistic that older dogs were slower than younger ones, the comparison between a model with the age as fixed effect (model1, M1) and a model without this variable (model2, M2) revealed that the effect of the age variable is probably negligible (BIC M1=6320.433, BIC M2=6318.664) and that therefore a simpler model (M2) is preferable. Furthermore, the dog's speed to enter corridors is probably influenced by many individual factors, including, among others, joint mobility and dog's size; the mixed-effect model takes already into account personal differences among dogs by setting the dog's identity as a random effect.

Table 3. Analysis of the influence of dogs' characteristics on latencies to enter the corridor

Fixed effects	F-value	numDF	denDF	P-value
Corridor location	403.15	4	176	$P < 0.0001$
Age (years)	5.96	1	44	$p = 0.02$
Sex	0.30	1	44	$p = 0.59$
Training Level	1.16	2	44	$p = 0.32$
Corridor location x Age	1.94	4	176	$p = 0.11$
Corridor location x Sex	0.62	4	176	$p = 0.65$

Corridor location x Training level	1.38	8	176	p = 0.21
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“x” indicates the interaction between variables.

Analysis of latencies to enter the five corridors

There is a significant effect of the location variable on latencies to enter the corridor (F-value_(4, 192) = 349.69, p-value < 0.0001). Post-hoc pairwise comparisons revealed that dogs behaved differently towards P and N corridors and towards adjacent ones (see Table 4), with latency significantly increasing from P to N corridor (mean latency ± sd P: 2.55 ± 1.82 s, NP: 5.26 ± 4.63 s, M: 8.99 ± 5.65 s, NN: 12.77 ± 4.28 s, N: 14.54 ± 1.92 s).

Table 4. Pairwise comparisons between latencies to enter trained corridors and adjacent ones

Pairwise comparison	Estimate	P-value
P – N	-11.99	p <0.0001
P – NP	-2.69	p <0.0001
NP – M	-3.75	p <0.0001
M – NN	-3.78	p <0.0001
NN – N	-1.77	p <0.0001

Simultaneous Tests for General Linear Hypotheses, Multiple Comparisons of Means. P = Positive corridor, NP = Near Positive corridor, M = Middle corridor, NN = Near Negative corridor, N = Negative corridor. Adjusted p values reported, Bonferroni method

Despite many studies on JBT using mean values to graphically show an increasing trend in latencies from P to N [18,33,47,49,52], we reported here (Fig 3) median values as these are more reliable and

robust measures in case of non-normally distributed variables and extreme values (such as, e.g., if a 15 s latency was recorded in one trial whereas the majority of trials presented a quick go response). To better highlight that responses towards ambiguous corridors presented a high variability than responses to the trained corridors, we report variances of latencies to enter each door: 3.32 (P), 21.39 (NP), 31.91 (M), 18.31 (NN), 3.68 (N).

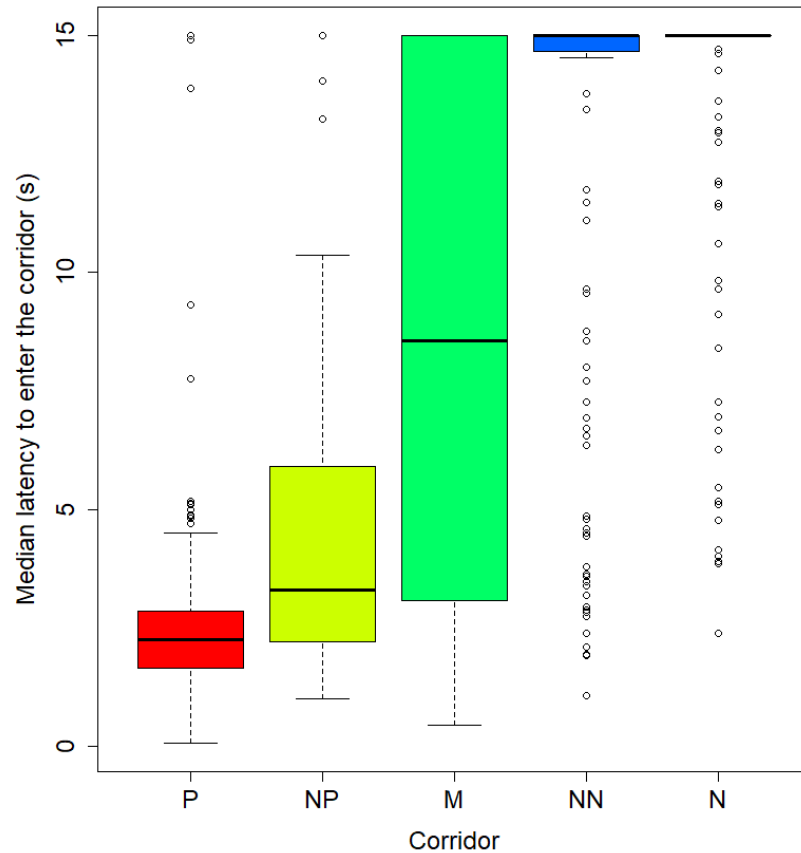


Fig 3. Boxplots of latencies to enter the five corridors. This graph shows medians (bar within the box), upper and lower quartiles (borders of box), lowest and highest cases within 1.5 times the IQR (bottom and top whiskers) and outliers (circles). Positive (P), Near Positive (NP), Middle (M), Near Negative (NN) and Negative (N) corridor.

Dogs' responses to the trained corridors (P and N)

Overall, 95.66% of responses to the trained corridors was correct (enter the P corridor in P trials, not enter the N corridor in N trials) during the testing phase. In particular, almost all of the responses to P trials were correct (98.98%) and only 3 dogs out of 49 made the wrong choice (not enter P) at least in one trial. The percentage of correct responses to N trials was slightly lower (92.35%) and 20 dogs out of 49 entered the N corridor at least one time. Regarding specifically the 'go trials' events, during P "go trials" the average latency \pm sd was 2.42 ± 1.32 s; during N "go trials" trials the average latency \pm sd was 8.98 ± 3.87 s.

Dogs' responses to the ambiguous corridors (NP, M, NN): looking for a learning effect

Overall, the percentages of 'go trials' in NP, M and NN trials were 85,62%, 59,18% and 25,85%, respectively. Average latencies in these 'go trials' \pm sd were 3.62 ± 2.5 s, 4.84 ± 3.40 s and 6.37 ± 3.94 s, respectively. As regards to the presence of a learning effect over the 3 trials, the number of training trials required to reach the criterion during the training has not a significant effect on latencies to enter the NP, M or NN corridor. However, the "trial number" variable (1,2 or 3) had a significant effect on latencies to enter the NP ($F\text{-value}_{(2, 95)} = 15.61$, $p\text{-value} < 0.0001$) and the M corridor ($F\text{-value}_{(2, 96)} = 35.47$, $p\text{-value} < 0.0001$). Post-hoc pairwise comparisons revealed that latencies to enter NP corridor were significantly higher in trial 3 than in trial 2 (means difference = 3.17, $t\text{-value}_{(95)} = 4.12$, $p\text{-value} < 0.0001$, see Fig 4a), and that latencies to enter M corridor were significantly shorter in trial 1 than in trial 2 (means difference = - 6.12, $t\text{-value}_{(96)} = -7.49$, $p\text{-value} < 0.0001$, see Fig 4b).

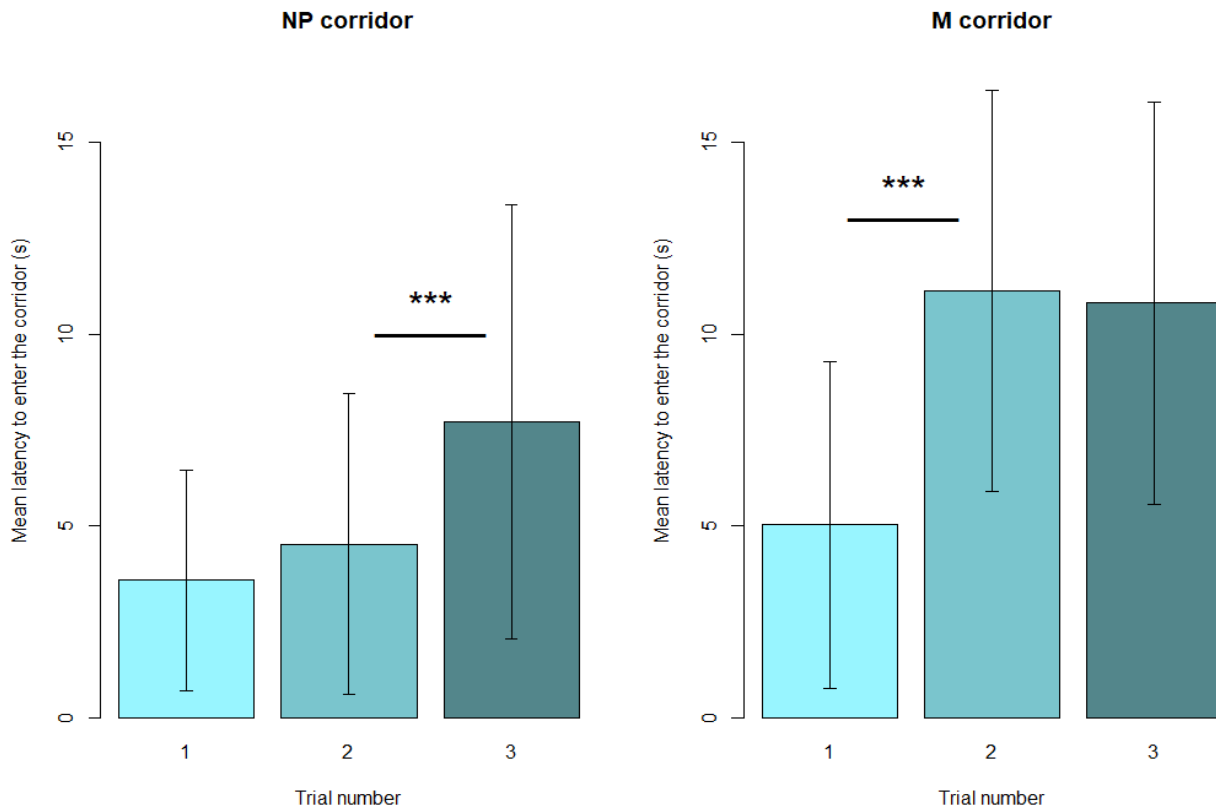


Fig 4a. Barplot of latencies to enter the NP corridor during the three trials. Means \pm sd are shown. *** shows a significant difference, $p < 0.0001$ **Fig 4b. Barplot of latencies to enter the M corridor during the three trials.** Means \pm sd are shown. *** shows a significant difference, $p < 0.0001$

The percentage of dogs who entered the corridors during each of the three trials was consistent with these results: regarding NP trials, 96% of dogs entered it in the first and in the second trial, 63% of dogs entered it in the third trial; regarding M trials, 92% of dogs entered it in the first trial, 41% and 45% of dogs entered it in the second and in the third trial. Only 31%, 24% and 22% of dogs entered the NN corridor in the first, second and third trial, respectively.

Dogs' responses to the ambiguous corridors: optimism/pessimism index?

The adjusted scores' frequency distributions are graphically illustrated in Fig 5.

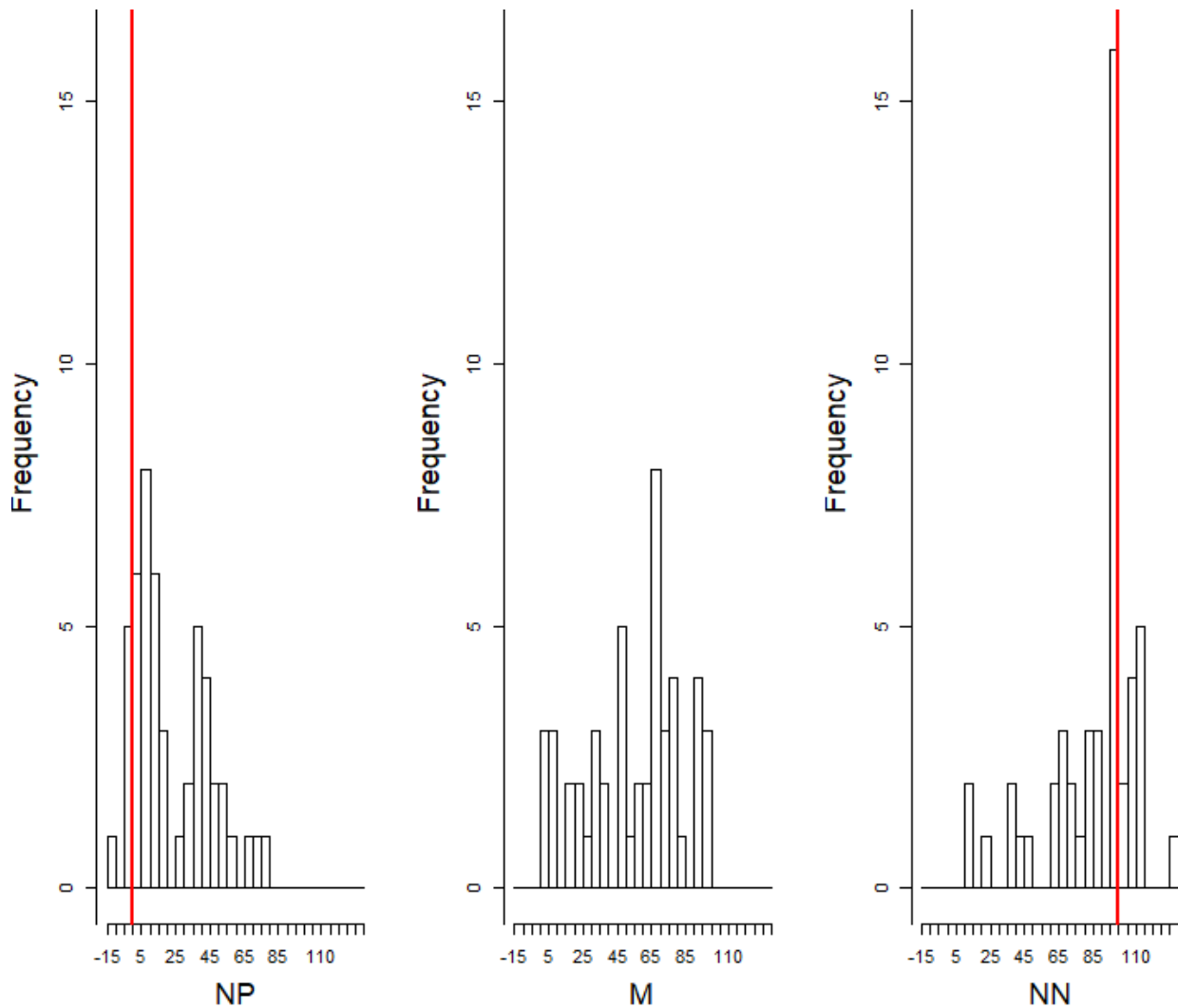


Fig 5. Optimism/pessimism adjusted score. Adjusted score's frequency distribution for the three ambiguous corridors: Near Positive (NP), Middle (M) and Near Negative (NN). Red vertical lines help finding 0 and 100 values. Mean values \pm sd and skewness of distribution are: 22.46 ± 22.71 , for NP with skewness = 0.6; 54.01 ± 28.98 for M with skewness = -0.32; 85.75 ± 27.51 for NN corridor with skewness = -1.21.

As expected, dogs had a lower adjusted score (index of a more optimistic attitude) the closer to P the ambiguous corridor was located (Linear Mixed-Effects model, $F_{(2, 96)} = 119.73$, P-value < 0.0001). Post-hoc contrasts analysis revealed that NP adjusted scores were significantly lower than M ones (means

difference = -31.55, $t\text{-value}_{(96)} = -7.71$, Adjusted P-value, Bonferroni method < 0.0001) and that M adjusted scores were significantly lower than NN ones (means difference = -31.74, $t\text{-value}_{(96)} = -7.76$, Adjusted P-value, Bonferroni method < 0.0001).

Behavioural analyses

Durations

The following behaviours were never showed and therefore they were not considered in the analysis: “Resting close to NP door”, “Resting close to NN door”, “Resting close to N door”. Average percentages of time spent exhibiting “Gazing” behaviours were very low, likely dogs gave researchers only short glances. Therefore, only the rate of occurrences was evaluated. Only behaviours exhibited on average for more than 5% of time, in at least one type of trial, are discussed below.

During P trials, the focus of dogs was on the open P door: they spent on average 96,5% of time investigating it or entering the related corridor. It is worth noting that the interest in P door (i.e., “checking door” behaviour) is maintained also during the other type of trials, when the P door was closed; this did not occur for the other doors. Detailed average percentages of time are shown in Table 5.

The interest in the open door decreased from P to N trials (Linear Mixed-Effects model with dogs’ identity as random effect, $F\text{-value}_{(4, 124)} = 124.84$, $p\text{-value} < 0.0001$, see Table D in Supplementary Materials for detailed results), whereas average percentages of time spent exhibiting “other behaviours” (Linear Mixed-Effects model with dogs’ identity as random effect, $F\text{-value}_{(4, 124)} = 44.03$, $p\text{-value} < 0.0001$, see Table E in Supplementary Materials for detailed results) and “resting close to the starting position” (Linear Mixed-Effects model with dogs’ identity as random effect, $F\text{-value}_{(4, 124)} = 10.77$, $p\text{-value} < 0.0001$, see Table F in Supplementary Materials for detailed results) increased from P to N trials.

Table 5. Average percentages of time spent exhibiting each behaviour, during each type of trial.

Behaviour	P trials	NP trials	M trials	NN trials	N trials
Checking P door:	96.50%	3.53%	3.41%	3.46%	3.19%
Checking NP door:	0%	75.46%	0.92%	0.25%	0.17%
Checking M door:	0%	0%	37.38%	0.14%	0.21%
Checking NN door:	0%	0%	0%	16.90%	0.14%
Checking N door:	0%	0%	0.19%	0.08%	4.10%
Resting close to the starting position	0.45%	1.90%	16.89%	21.41%	22.83%
Other	2.74%	17.06%	38.46%	53.68%	64.17%
Resting elsewhere	0%	0.60%	0.64%	0.29%	1.93%
Displacement behaviour	0.24%	0.90%	0.38%	1.49%	1.19%

Only behaviours exhibited on average for more than 1% of time, in at least one type of trial, are reported. Grey cells point out that the “checking door” behaviour is related to an open door. Displacement behaviours were exhibited only by 2 dogs.

Frequencies (rate of occurrences)

Vocalizations were mostly exhibited by only one dog who displayed 177 vocalizations out of 186. For this reason, we decided to not consider vocalizations in our analysis. Detailed average rate of occurrences (events/min) are shown in Table 6.

Table 6. Average rate of occurrences (events/min) for each behaviour, during each type of trial.

Behaviour	P trials	NP trials	M trials	NN trials	N trials
Gazing R2	0.03	0.57	0.63	0.68	0.27
Gazing R1	0.04	0.13	0.56	0.60	0.95
Stress-related behaviour	0	0.09	0.36	0.08	0.44
False start	0.04	0.78	1.21	1.05	0.50

Rate of occurrences of “gazing R2” was very low and did not differ among trials (Linear Mixed-Effects model with dogs’ identity as random effect, $F\text{-value}_{(4, 124)} = 2.01$, $p\text{-value} = 0.1$).

Rate of occurrences of “gazing R #1” differed among trials (Linear Mixed-Effects model with dogs’ identity as random effect, $F\text{-value}_{(4, 124)} = 5.91$, $p\text{-value} < 0.0001$): contrast analysis revealed that dogs glanced R1 more during N trials than during P (z value = 4.26, std. error = 0.21, $p\text{-value} = 0.0002$ with Bonferroni correction, estimate = 0.92) and NP trials (z value = 3.85, std. error = 0.21, $p\text{-value} = 0.001$ with Bonferroni correction, estimate = 0.83).

Rate of occurrences of “stress-related behaviour” differed among trials (Linear Mixed-Effects model with dogs’ identity as random effect, $F\text{-value}_{(4, 124)} = 3.18$, $p\text{-value} = 0.02$): contrast analysis revealed that dogs exhibited more stress-related behaviours during N trials than during P trials (z value = -2.92, std. error = 0.15, $p\text{-value} = 0.01$ with Bonferroni correction, estimate = -0.44).

Rate of occurrences of “False start” differed among trials (Linear Mixed-Effects model with dogs’ identity as random effect, $F\text{-value}_{(4, 124)} = 6.58$, $p\text{-value} < 0.0001$): contrast analysis revealed that dogs exhibited more false starts during ambiguous trials (NP, M and NN) than during P trials (z values range

from 4.64 to 2.95, std. error = 0.25, p-values range from 0.03 to <0.0001 with Bonferroni correction, estimates ranges from 1.17 to 0.74).

Discussion

In our previous paper we identified in the classical judgment bias protocol several methodological and statistical issue [57]; thus, in the present paper, we propose a novel protocol of the judgment bias test (JBT), suitable for dogs, aimed at obtaining measures of optimistic/pessimistic attitude more reliable than those previously published (see [47–49,52] for some contradicting results of JBT in dogs). The apparatus consisted of five adjacent corridors (named P, NP, M, NN, N) closed with a wooden door. At the end of the positive corridor (P) dogs found their owner, while at the end of the negative corridor (N, located at the opposite side of the arena) dogs found a black cloth slowly blowing. After the training to discriminate between P and N corridor, ambiguous stimuli (NP, M and NN corridors, empty and located between P and N) are presented, along with the previously learned ones (25 consecutive trials): the latency to enter each corridor and dogs' behaviours during the test were analysed.

Present data will be compared to the ones previously published by our research group [57], therefore in following statements we are referring to this study when mentioning our previous paper.

All dogs, except one, succeeded in the training phase (98% present data *versus* 78% previous data); furthermore, dogs needed on average less trials ($18.29 \pm \text{sd } 7.21$ present data *versus* $23.97 \pm \text{sd } 8.87$ previous data) to reach a learning criterion that was even more severe than the one used in the classical protocol: this is an indicator that the novel protocol improved the dogs' performance.

Results of statistical analysis show that neither dogs' characteristics (i.e., age, sex and training level) nor the position of the positive corridor (left/right) influenced the number of training trials required to reach the learning criterion or the latencies to enter the corridors during the testing phase.

Furthermore, dogs were not influenced by the treat/owner's smell, as the latency to enter the P corridor in the last "false positive" trial, was significantly shorter than the mean latency to enter the same corridor when the dog's owner was in it. The short break in the testing necessary to allow the owner to move to the N corridor, could have raised the dog's motivation to reach its owner.

In line with our expectations, since the difference between the payoff of the two trained stimuli (P and N) was raised in the new protocol, the dogs' learning of the outcomes of the P and the N corridors appears more stable and solid: statistical analyses demonstrated that dogs behaved differently between P and N corridor and that the variability of latencies to enter these corridors was extremely lower than the variability to enter ambiguous locations. Even if the variability in latencies could be ascribed to differences in dogs' personality, motivation or physical characteristics, a stable learning entails a relatively consistent behaviour towards the learned stimuli [59,60]; *vice versa* NP, M and NN corridors have an ambiguous connotation and therefore we found a higher variability in responses, as expected. According to this ambiguous connotation, dogs seemed to be more hesitant towards these corridors: they exhibited more false starts during ambiguous trials (NP, M and NN) than during P trials.

A low variability in latencies to reach the trained stimuli suggests that dogs have indeed learned the association between entering the trained corridors and the relative outcome [69]. In our previous experiment, the variability to reach the N trained location was, instead, extremely high, suggesting that dogs might not have completely learned the association. The shorter duration of our novel protocol (26 trials in the testing phase *versus* 42 trials, maximum length of 15 s for each trial *versus* 30 s) may have contributed to solve this learning issue, facilitating dogs' concentration and preventing them to be tired or bored. As discussed above, the learning process could have also been improved by an increased difference between the payoff of the two trained stimuli: the owner and the treats have a strong positive value, whereas the black fluttering cloth represented a potential negative stimulus.

Behavioural analyses confirmed these results: when the P door was open, dogs spent almost all the trial time investigating/entering the P corridor; furthermore, the interest in the P door is maintained even during the other trials, when the P door was closed, suggesting that the P corridor was perceived as a very pleasant location: dogs' motivation towards this corridor was so high that subjects entered it in 98.98% of P trials and only 3 dogs out of 49 did not enter the P corridor (in at least one trial).

During the testing phase dogs entered the N corridor only in 7.65% of trials (*versus* 56% for pet dogs in our previous experiment using the classical protocol). Twenty dogs out of 49 took a chance to enter this corridor at least once and during these "go trials" the average latency to enter the corridor was high (8.98 s on average \pm 3.87 sd), suggesting that the negative stimulus was not extremely aversive, but also that the dogs' motivation towards the N corridor was most likely low (long latency). This is in line with behavioural analyses: dogs exhibited more stress-related behaviours during N trials than during P trials, suggesting that they perceived the N corridor as an unpleasant stimulus, though frequencies of stress-related behaviours were not worrying (0.44 events/min). Overall, the interest in the opened door decreased from P to N trials, along with an increase of time spent exhibiting other behaviours and resting close to the starting position, suggesting that dogs' behaviours were consistent with their decisions: dogs were deeply interested in the P corridor but their motivation to enter corridors decreased from P to N, resulting in other behaviours as exploring the environment or remaining in the starting position and glancing the researcher at their side (dogs glanced R1 with a higher frequency during N trials than during P and NP trials).

As expected, dogs behaved differently also towards each pair of adjacent corridors and they entered the corridors with significantly increasing latency from P to N. On the contrary, in our previous study, using the classical JBT protocol, latencies to reach adjacent locations did not completely differ, suggesting that dogs struggled to discriminate between them. In particular, latencies did not differ between P-NP (in agreement with other studies [33,47,50]), NP-M and M-NN locations. As discussed in the introduction, a full discrimination between all the stimuli is important in JBTs because it allows a more accurate evaluation of emotions (see for a review [3]) and makes it possible to discriminate

emotions with the same valence and different levels of arousal, such as anxiety (higher expectation of a negative event - reduced detection-latencies for cues near the trained negative one, i.e., NN location [62,64,65]) and depression (lower expectation of a positive event - pessimistic responses to the NP location [18,62,63]). To obtain this level of detail in the evaluation of emotions using JBTs, it is fundamental that dogs can discriminate properly among all the stimuli.

Regarding in detail responses to ambiguous stimuli, percentage of “go trials” decreased from NP to NN, with increasing average latencies to enter these corridors in the “go trials”, suggesting that dogs’ motivation to enter them decreased from NP to NN, together with an increase in dogs’ hesitation: given that usually subjects are faster to respond to unambiguous stimuli than to ambiguous ones [13,14], a longer time of decision could be an indicator of uncertainty [13,14]. However, the frequency of false starts, another indicator of hesitation, did not increase from NP to NN, suggesting that the increase in latency was ascribable to a decrease in motivation, rather than to an increase in the perceived ambiguity.

The number of ambiguous trials was small to avoid a learning effect [14], i.e. dogs might learn that ambiguous corridors were empty; however, the variability in latencies to enter the ambiguous corridors was remarkable (see Fig 3) and statistical analyses reveal that latencies to enter the NP corridor were significantly shorter in trial 2 than in trial 3 and that latencies to enter M corridor were significantly shorter in trial 1 than in trial 2. The percentage of dogs who entered the corridors during each of the 3 trials was consistent with these results: almost all dogs entered the NP corridor in the first and in the second trial, whereas only 63% of them entered it in the third trial; almost all dogs entered the M corridor in the first trial, whereas less than half of them entered it in the second and in the third trial. However, the number of training trials required to reach the criterion during the training (an index of dogs’ learning skills) has not a significant effect on latencies to enter the ambiguous corridors during the 3 trials. If there were a learning effect over the trials, this should have been more pronounced in dogs who had better learning skills. Furthermore, it is reasonable that a learning effect would be similarly expressed in all three ambiguous stimuli and not only towards NP and M corridors. Finally, it

appears unlikely that dogs had learnt that ambiguous corridors were empty in only one or two trials, given that it took them on average 18 trials to learn the association between the trained stimuli and their related reward/punishment. In conclusion, a learning effect over ambiguous trials did likely not exist and differences in responses between trials 2-3 (for the NP corridor) and 1-2 (for the M corridor) could be ascribed to differences in the optimistic/pessimistic attitude: e.g., more optimistic dogs may be the ones who keep entering the NP corridor in the third trial and the ones who keep entering the M corridor in the second trial. It is therefore recommended to run more than one single trial for each ambiguous corridor and to take into account the number of trial (first, second or third) in the statistical analysis.

Overall, this new spatial JBT protocol solves the methodological issues previously identified in the classical protocol and it fits for the purpose of obtaining stable and consistent measures. To compare our results with previous studies we calculated an “adjusted” score of optimism/pessimism; it shows that responses to the same ambiguous stimulus (NP, M or NN corridor) differ among dogs, suggesting that the present JBT is enough sensitive to detect differences in the optimistic/pessimistic attitude in a dog population. Furthermore, as expected, dogs appeared more optimistic (significantly lower adjusted score) toward the NP corridor than toward the M one and toward the M corridor than toward the NN one. However, it is worth noticing that some scores were lower than 0, i.e., the mean latency to enter the ambiguous corridor was lower than the mean latency to enter P, whereas some scores values were higher than 100, i.e., the mean latency to enter the ambiguous corridor was higher than the mean latency to enter N. Furthermore, the statistical approach behind these adjusted scores implies that latencies are averaged across trials, and it reduces variability in the data; therefore, we recommend caution in the interpretation of these scores as indexes of optimism/pessimism and we opt in favour of analyses of raw latencies using a mixed-effect model. In future studies the robustness and reliability as optimism/pessimism indexes of these measures might be evaluated, e.g., by comparing dogs living in conditions that potentially influence their emotional state with positive and negative valence.

Conclusions

In the present paper, we proposed a novel protocol of the judgment bias test (JBT) in order to obtain reliable and consistent measures of optimism/pessimism and to solve methodological and statistical issue observed in the classical protocol [57]. The difference between payoff of the trained locations was increased leading to a stable learning of the association between these locations and their outcomes; the number of trials and the length of them were reduced and the potential influence of researcher was removed, thus obtaining stable measures of optimism/pessimism that are consistent with expectations and that suggest a promising effectiveness of this protocol to assess judgment biases in dogs.

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Supplementary Materials

Table A. Characteristics of dogs tested with the judgment bias test

Name	Breed	Age (years)	Sex	Reproductive status	Training level
Altea	Labrador Retriever	12,25	F	Neutered	2
Argo	Mixed breed	9,48	M	Neutered	2
Argo_2	Australian Shepherd	2,35	M	Entire	2
Ariel	Border Collie	7,35	F	Neutered	1
Bea	Jack Russell Terrier	1,24	F	Entire	1
Birra	Mixed breed	2,55	F	Neutered	2
Charlie	Mixed breed	5,65	M	Neutered	2
Chubby	Mixed breed	4,84	M	Entire	1
Ciro	Labrador Retriever	2,73	M	Neutered	2
Ciuchino	Mixed breed	2,48	M	Neutered	2
Cometa	Lagotto Romagnolo	2,58	F	Neutered	1
Diana	Mixed breed	11,15	F	Neutered	2
Eolo	Maltese	3,10	M	Neutered	1
Fetta	Australian Shepherd	2,68	F	Neutered	2
Indi	Border Collie	7,10	F	Neutered	2
Iris	Labrador Retriever	8,12	F	Neutered	2
Iris_2	Bernese Mountain Dog	1,24	F	Entire	2
Isotta_2	Mixed breed	11,72	F	Neutered	2
Jack	West Highland White Terrier	11,26	M	Neutered	1
Kubi	Border Collie	4,83	M	Entire	2
Lily	Beagle	4,64	F	Neutered	2
Lissi	Mixed breed	10,31	F	Neutered	0
Lola	German Shepherd	3,46	F	Neutered	2

Luna	Golden Retriever	3,35	F	Neutered	1
Marta	Mixed breed	7,41	F	Neutered	2
Matilde	Hungarian Hound	11,13	F	Neutered	1
Maya	Golden Retriever	3,17	F	Entire	2
Miranda	Mixed breed	15,53	F	Neutered	1
Myrtille	Cavalier King Charles Spaniel	3,26	F	Neutered	1
Oliver	Chihuahua	4,07	M	Neutered	1
Oliver_2	Mixed breed	6,40	M	Entire	2
Oscar	Mixed breed	2,10	M	Neutered	0
Otto	Jack Russell Terrier	8,54	M	Entire	1
Pallina	Mixed breed	11,45	F	Neutered	0
Pimpa	Mixed breed	10,73	F	Neutered	0
Pioggia	German Wire-haired Pointing Dog	3,36	F	Neutered	1
Rashka	Australian Shepherd	4,74	F	Neutered	2
Rufus	Mixed breed	1,18	M	Entire	2
Saki	Mixed breed	3,52	M	Neutered	1
Scilla	Dachshund	9,82	F	Neutered	1
Shira	Australian Shepherd	2,59	F	Entire	2
Spindle	Border Collie	6,80	M	Entire	2
Thelonius	Mixed breed	9,82	M	Neutered	1
Tilde	Mixed breed	2,22	F	Entire	2
Toby	Miniature Pinscher	3,59	M	Entire	1
Tommaso	Pomeranian	5,87	M	Entire	2
Viki	Mixed breed	5,51	F	Neutered	1
Wendy	Golden Retriever	2,80	F	Neutered	2
Zelda	Border Collie	7,72	F	Entire	2

Table B. Scoring sheet and trial sequence for the judgment bias test

Training trials		
Trial	Cue	Seconds
1	P	
2	P	
3	N	
4	N	
5	P	
6	N	
7	P	
8	P	
9	N	
10	P	
11	N	
12	N	
13	P	
14	N	
15	P	
16	P	
17	N	
18	N	
19	P	
20	N	
21	P	
22	N	
23	N	
24	P	
25	P	
26	N	
27	P	
28	P	
29	N	
30	P	
31	N	
32	N	
33	P	
34	N	
35	P	

Test trials		
Trial	Cue	Seconds
1	M	
2	P	
3	N	
4	NP	
5	P	
6	N	
7	NN	
8	P	
9	N	
10	NP	
11	P	
12	N	
13	NN	
14	P	
15	N	
16	M	
17	P	
18	N	
19	NN	
20	P	
21	N	
22	M	
23	P	
24	N	
25	NP	

26	False Positive	
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During the training phase, the dog was presented with only one accessible corridor per trial (either P or N), following a pseudo-random order, making each corridor accessible no more than twice consecutively. Latency to enter the corridor, i.e., the time elapsed from the moment of release to the moment in which the dog passed the door line with its all four paws, was recorded. If the dog did not enter the corridor within 15 s, the door was closed and a latency of 15 seconds was registered. Dogs had a minimum of 10 and a maximum of 35 trials to reach the learning criterion.

The testing phase took place immediately after the dog reached the learning criterion. The procedure was the same of the training phase, except for the fact that dogs were presented also with the three ambiguous corridors NP (Near Positive corridor), M (Middle corridor) and NN (Near Negative corridor); each of them was presented three times, following the order: M NP NN - NP NN M - NN M NP (each corridor was presented first, second or third in each block of three ambiguous trials). The ambiguous corridors trials were separated by one P and one N trial in order to confirm the previous conditioning.

Table C. Inter-observer agreement

Behavioural variable	p-value	Rho
Checking P door	P = 0.005	0.77
Checking NP door	P < 0.001	0.94
Checking M door	P < 0.001	0.89
Checking NN door	P < 0.001	0.95
Checking N door	P < 0.001	0.92
Resting elsewhere	P = 0.005	0.77
Resting close to P door	P < 0.001	1
Resting close to the starting position	P < 0.001	0.96
Gazing R#2 (Duration)	P = 0.04	0.61

Gazing R#1 (Duration)	P < 0.001	0.99
Other	P < 0.001	0.95
Displacement behaviour	P < 0.001	1
Stress-related behaviour (frequency)	P < 0.001	1
False start (frequency)	P = 0.003	0.81

Inter-observer agreement assessed by means of independent parallel coding of a random sample of 11 dogs out of 32 (34% of the sample). The agreement between two observers was assessed by Spearman correlations and it was significant for all the variables. Inter-observer agreement was evaluated on raw durations measured in seconds and, for instant events, on the total number of occurrences. Results are not reported for the remaining variables (Resting close to NP, M, NN, N door and Vocalization) because these behaviours were never recorded by neither of the two observers.

Table D. Interest in the open door – pairwise comparisons

Comparison	Estimate	Std. Error	z value	p-value
P-N	92.40	4.86	19.00	< 0.0001
P-NP	21.04	4.86	4.33	< 0.0001
NP-M	38.08	4.86	7.83	< 0.0001
M-NN	20.48	4.86	4.21	0.0001
NN-N	12.81	4.86	2.63	0.04

P = Positive location, NP = Near Positive location, M = Middle location, NN = Near Negative location, N = Negative location. Linear Mixed-Effects model with the percentage of time spent exhibiting “checking the open door” (i.e., door P during P trials, door NP during NP trials, etc.) as a dependent variable, location as fixed effect, dogs’ identity as a random effect. Post-hoc tests with Bonferroni correction were used for pairwise comparisons.

Table E. Other behaviours – pairwise comparisons

Comparison	Estimate	Std. Error	z value	p-value
P-N	-61.43	5.32	-11.54	< 0.0001
P-NP	-14.32	5.32	-2.69	0.04
NP-M	-21.39	5.32	-4.02	0.0003
M-NN	-15.22	5.32	-2.86	0.02
NN-N	-10.49	5.32	-1.97	0.24

Linear Mixed-Effects model with the percentage of time spent exhibiting “other behaviours” as a dependent variable, location as fixed effect, dogs’ identity as a random effect. Post-hoc tests with Bonferroni correction were used for pairwise comparisons.

Table F. Resting close to the starting position – pairwise comparisons

Comparison	Estimate	Std. Error	z value	p-value
P-N	-22.38	4.56	-4.91	< 0.0001
P-NP	-1.45	4.56	-0.32	1
NP-M	-14.99	4.56	-3.29	0.005
M-NN	-4.53	4.56	-0.99	1
NN-N	-1.42	4.56	-0.31	1

Linear Mixed-Effects model with the percentage of time spent exhibiting “resting close to the starting position” as a dependent variable, location as fixed effect, dogs’ identity as a random effect. Post-hoc tests with Bonferroni correction were used for pairwise comparisons.

Study 3

Should I stay or should I go? Influence of anxiety on judgment bias test performance in dogs

Carlotta Burani^{1, 2*}, Paola Valsecchi²

¹ Dipartimento di Medicina e Chirurgia, Università degli Studi di Parma, Parma, Italia

² Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università degli Studi di Parma, Parma, Italia

*Corresponding author

E-mail: carlotta.burani@studenti.unipr.it

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Abstract

Anxiety is a fundamental emotion that prepare the individual to detect and to deal with danger and it has been naturally selected to maximize survival and reproduction. However, anxiety may turn into a pathological trait when dysregulated and generalized and it can jeopardize canine welfare.

In the present paper, performances of anxious dogs and of non-anxious dogs in a judgment bias test (JBT) are compared. The judgment bias approach represents one of the most commonly applied methodology to evaluate animals' optimistic/pessimistic attitude and to infer their emotional and welfare state accordingly.

A sample of anxious (as cases) and non-anxious dogs (as control) were recruited using an online questionnaire focused on behavioural reactions potentially related to fear, anxiety and stress exhibited in dogs' everyday life. Contrary to expectations dogs in a more anxious-like state (N = 19) did not show a more pessimistic attitude towards ambiguity than non-anxious dogs (N = 19). Anxiety is a multifactorial disorder and it is hard to detect, therefore the applied questionnaire might be not enough sensitive to identify the most anxious and the most non-anxious dogs; however, 7 out of 19 dogs from the anxious dogs group needed an adjustment in the JBT procedure because they were not able to remain without their owner for few minutes, suggesting that the survey indeed found a sample of dogs who suffer of an anxiety-like state.

A further explanation of these results pertains to the potential inadequacy of the applied JBT protocol to test anxious dogs, especially regarding dogs who are in discomfort without their owner and/or in presence of stranger people: some dogs in the anxious group needed to be tested with their owner by their side, suggesting a potential influence of anxiety-like states not only on test results but also on the test performing and recommending some methodological adjustments.

Introduction

Anxiety is a reaction related to the anticipation of future danger and does not necessarily have a specific object of threat[1]; as in humans, anxiety in dogs is accompanied by somatic symptoms of tension, including physiological (e.g., increased respiratory and heart rates, vasomotor changes, trembling or paralysis, increased salivation, gastrointestinal disturbances) [2] and behavioural signs (e.g., immobility, pacing, circling, restlessness or changes in appetite) [3] related to the activation of the hypothalamic-pituitary-adrenal axis (HPA).

Anxiety is among the most fundamental emotions required to survive in potentially dangerous situations and its function is to prepare the individual to detect and to deal with threats; from an evolutionary point of view, anxiety had been naturally selected to maximize survival and reproduction. However, this fundamental emotion, may turn into a pathological trait when prolonged, dysregulated and generalized. Indeed, anxiety disorders are among the most common mental illnesses in humans [4] and they also represent the main cause of many canine behavioural problems [1,5,6]. Anxiety-proneness and anxious symptoms are distributed along a continuum, and the boundary between the adaptive function and the pathology is not simple to detect [4,7]. However, considering that anxiety can impair welfare, efforts should be made to better understand canine anxiety and to decrease its prevalence in dog population [8].

The aim of the present paper is to analyse and to compare performances of anxious dogs and of non-anxious dogs in a judgment bias test (JBT). The JBT is one of the most applied tool to measure affective states in non-human animals (for reviews see [9–12]; for critical analyses see [13,14], for application in the canine species see [15–21]) and it relies on the by-directional influence between emotions and cognitive functions [10,14]. The JBT measures the animal's attitude towards uncertainty (optimism/pessimism) and consequently infer data about the animal's affective state: our expectations are that dogs in a more anxious-like state would show a more pessimistic attitude towards ambiguity than non-anxious dogs, as found in humans [22,23] (in particular regarding attention bias [24,25]) and non-humans animals [26].

Considering that cognitive biases are linked to long-term mood and emotional state and not to brief and discrete emotions [27], we aimed at recruiting a sample of dogs characterised by anxiety trait (i.e. the individual tendency to experience anxiety across time and context [28]) and not by state anxiety (anxiety experienced at one particular time and context [29]); therefore, we built an online questionnaire focused on behavioural reactions potentially related to fear, anxiety and stress in dogs everyday life. Even if surveys results, compared to behavioural test, could be more influenced by owners' subjectivity and by their ability to recognise dogs' behaviours, questionnaires offer the possibility to collect data from a larger population and could provide information about dogs' attitude in many everyday life conditions [30,31].

After recruiting dogs experiencing an emotional state characterised by anxiety (as cases) and dogs in an alleged non-anxious state (as control), we presented them with a novel spatial JBT protocol that we previously developed and applied on 50 dogs without behavioural problems [32]: the hypothesis is that more anxious dogs, identified through the survey, would show a more pessimistic attitude towards ambiguous stimuli compared to non-anxious dogs.

Materials and methods

Questionnaire

To recruit a sample of anxious dogs and a sample of non-anxious ones, we realized an online questionnaire, based on previously validated ones ([7,8,30,33]), aimed at detecting and analyse behavioural reactions potentially related to fear, anxiety and stress, to understand in which situations these reactions were elicited, their frequency and the time for recovery from discomfort. There were 70 questions in the survey and it required an average of 20 minutes to be completed (see Appendix 1 in Supplementary Materials for the survey text).

The first section of the survey aimed at collecting information on dog's characteristics (such as age, sex, breed, health issues) and about daily routine and life conditions (i.e., exercise, training, environment).

The following sections were focused on dog's behaviour in various situations related to most frequent forms of canine anxiety: meeting an unfamiliar person, meeting an unfamiliar dog, being in a new environment/situation, hear a loud noise and being at home alone [7,8,30]. Finally, some questions were aimed at detecting stereotypic behaviour and reactions potentially related to stress and anxiety when dogs were presented with heights and particular surfaces (such as a metal grid or a shiny floor).

To reduce subjectivity as much as possible, we asked the owners to report if he/she had ever observed one or more behaviours from a fixed list of behaviours potentially related to anxiety and we avoided asking whether the owner thought that the dog was scared or in discomfort in a certain situation (as previously done in other studies [5,30]). Furthermore we asked about frequency of these behaviours and about time of recovery (as previously done by Salonen and colleagues [8]).

The survey was publicized by a post on our research group's Facebook page and by word of mouth, with the collaboration of dog trainers and veterinarians.

Were considered only data resulting from dogs older than 1 year and who had been leaving with their owner for at least 6 months.

The final sample was made of 487 dogs from various breeds (281 dogs were pure breed and 206 dogs were mixed breed), their mean age \pm sd was 6.0 ± 3.3 years, 269 dogs were females and 218 were males. Training level was recorded as following: level 0 for dogs who had never experienced any type of training (N = 103), level 1 for dogs who attended some basic training lessons and/or practiced with dog puzzle toys (N = 57) and level 2 for dogs who experienced at least one type of dog sport/nose-work activity in addition to basic training lessons and/or puzzle toys (N = 327). All the owners gave their consent to participate in the study.

Dogs were classified as Anxious (A group) and Non-Anxious (NA group) on the basis of survey results (see statistical analyses section for details), with the aim to test animals from both groups in the Judgment Bias Test (JBT). Dogs who suffered of severe health issues that could influence JBT results or that were treated with medications capable of influencing their behaviour were not considered.

Dogs were selected for the judgment bias test on the basis of their questionnaire data and of the owners' willingness to take part in the study at our laboratory; furthermore, to maximize the procedure's safety, owners were contacted by phone to make sure that dogs were not extremely aggressive towards unfamiliar people.

Subjects

Overall, 42 dogs were recruited for the JBT. Two dogs (1 from A Group, 1 from NA Group) lost interest in the test after a few trials and therefore they failed to pass the training phase. Two dogs from the A group were too scared of researchers to be tested.

Hence, the tested sample was of 38 dogs of various breeds and mixed breed: 19 were assigned to the Anxious Group (mean age \pm sd 5.89 ± 3.21 years, 8 females and 11 males, 5 dogs with "0" level training, 6 with "1" level training, 8 with "2" level training) and 19 were assigned to the Non-Anxious Group (mean age \pm sd 5.72 ± 3.31 years, 10 females and 9 males, 3 dogs with "0" level training, 5 with "1" level training, 11 with "2" level training), see Table B in Supplementary Materials for other details.

Judgment bias test (JBT)

The judgment bias test (JBT) was carried out in a grassy outdoor area (approximately 10x5 metres) fenced with wire mesh and covered with a shade cloth to ensure dog safety and to avoid potential distractions. The JBT protocol was adapted from Verbeek and colleagues [34,35] and it was the same we previously applied in a research on pet dogs without behavioural problems (for a more detailed description see [32]): the apparatus consisted of five adjacent corridors (named P, NP, M, NN, N), each of them was closed at the end and it was accessible at the beginning through a wooden door (see Fig

1). Each corridor was 4-m-long and it was designed so that from the starting position, which is 5 m from the M door, it was impossible to see the end of the corridor without entering it. The testing arena and the experimental set-up are shown in Fig 1.

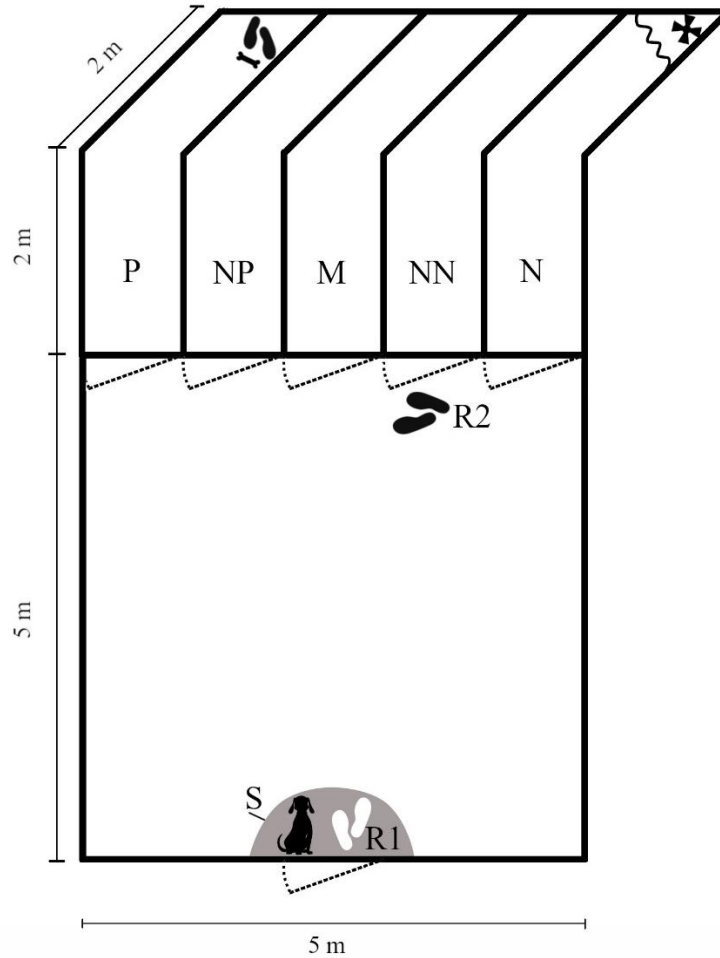


Fig 1. Experimental set-up. R1 (researcher #1) led the dog at S (starting position, grey area) at the beginning of each trial; R2 (researcher #2) opened the doors of the five corridors: P (Positive), NP (Near Positive), M(Middle), NN (Near Negative), N (Negative). NP, M and NN corridors were empty, whereas at the end of the P corridor there was the dog’s owner, instructed to greet its dog and to give it a piece of chicken sausage; at the end of the N corridor there was a black cloth blown by the fan behind it. This image was designed using Freepik.com resources.

The protocol consisted of 3 phases: familiarization, training and testing.

1) Familiarization

The dog and the owner were welcomed by two researchers who explained the whole procedure to the owner and asked him/her to sign an agreement to participate in the study; after that, the dog was taken, using a leash, by researcher #1 (R1) for a 5-minute walk in a fenced grassy area, along with the owner, to get the dog accustomed to be led by R1. The dog was then led to the testing arena and R1 offered it three pieces of chicken sausage to check that the dog liked it.

To familiarize the dog with the apparatus and with the procedure, it was led by R1, using the leash, at the starting position, then researcher #2 (R2) called the dog by name to get its attention, opened the first door on the left corner of the arena and the dog was then allowed to explore the newly opened corridor, along with R1 and the owner. If the dog hesitated to enter the corridor, the owner was asked to encourage it. When the dog had explored the corridor, it was led back to the starting position by R1, while R2 closed the door. This procedure was repeated for each corridor. The dog was then led by R1 out of the testing arena, R2 gave to the owner some slices of chicken sausage in a hermetically closed container and accompanied him/her to a chair located at the end of the P corridor; then R2 placed a fan at the end of the N corridor and a black cloth right in front of the fan, very close to it and, finally, R2 closed all the doors. The location of the positive corridor (P) was balanced in the sample (i.e., for 11 dogs from the Anxious Group and for 10 dogs from the Non-Anxious group the positive corridor was on the left-hand side, for the remaining dogs it was on the right-hand side of the arena). R2 called back R1 who came with the dog and held it in the starting position.

2) Training

Each dog was trained to discriminate between a positive stimulus and a negative stimulus: the P corridor, where the owner was waiting, ready to cuddle the dog and to feed it with chicken sausage, and the N corridor, where the black cloth was suddenly blown by the fan behind it (R1 activated the fan using remote control only when the dog entered the N corridor).

The procedure was as follows: R1 held the dog using the leash in the starting position, R2 called the dog by name and opened the P or N door, following a pseudo-random order (each corridor was accessible no more than twice consecutively), while R1 released the dog who was free to explore the testing arena for 15 seconds. R2 stood next to the open door and R1 stood in the starting position, both ignoring the dog; R2 used a stopwatch to measure the latency to enter the corridor, i.e., the time elapsed from the moment of release to the moment in which the dog passed the door line with all its four paws. If the dog did not enter the corridor within 15 s, the door was closed by R2 and a latency of 15 seconds was registered. At the end of each trial R1 gently led the dog to the starting position for the next trial and R2 closed the door.

Dogs had to enter each trained corridor (P and N) at least once and they had a minimum of 10 and a maximum of 35 trials to reach the learning criterion, i.e., for 6 consecutive trials dogs entered the P corridor and did not enter the N corridor. The number of training trials required to reach this criterion was recorded for each dog.

Seven dogs (all of them from the Anxious group) appeared very stressed at the beginning of the training phase, probably due to the temporary separation from their owners, and therefore a change to the standard procedure was necessary: luckily these dogs came with two owners each, one of them was positioned as usual at the end of the P corridor, the other one stood at the starting position, so that the dog was never alone with researchers. This adjustment was considered in the statistical analysis to control for potential influences on JBT results.

3) Testing

The procedure was the same of the training phase, except for the fact that dogs were presented also with the three ambiguous corridors, located between P and N, i.e., NP (Near Positive corridor), M (Middle corridor) and NN (Near Negative corridor, see Fig 1). These corridors were empty and each of them was presented three times, always separated by one P and one N trial in order to confirm the previous conditioning.

Overall, the testing phase included 25 consecutive trials. At the end of the testing phase, a “false positive” trial was executed, to make sure that dogs were relying on spatial cues and not on the treats’ and/or the owner’s odour: a P trial was run as usual, but the owner was hidden at the end of the N corridor.

Latency to cross the door was measured by R2 using a stopwatch. Latency was evaluated as a cue of optimistic/pessimistic attitude: a short latency indicates a potential anticipation of a positive outcome (owner and food), i.e., an ‘optimistic’ judgment, whereas a long latency indicates a potential ‘pessimistic’ judgment.

Overall, the judgment bias test (familiarization + training + testing) lasted nearly 40 minutes per dog.

Statistical analyses

All analyses were performed using the software R, version 3.6.2 with packages nlme (version 3.1-142) for multilevel analysis, multcomp (version 3.6.3) for multiple comparisons in linear mixed effects models, moments (version 0.14) to calculate skewness of distributions and MuMIn (version 1.43.17) for model selection.

Questionnaire analysis

Owners’ answers were converted in numerical values (see Table A in Supplementary Materials, for the conversion table); these values were used to calculate dogs’ scores in five categories:

- **Unfamiliar people:** this value resulted from the sum of scores of items 20 (frequency of barking/extreme excitement when someone rang the doorbell), 23 and 24 (frequency of stress-related behaviours when meeting an unfamiliar person and time for recovery). This value ranges from 0 to 12: dogs with a value above 6 were classified as Anxious dogs in this category, whereas dogs with a value below 6 were classified as Non-Anxious.

- **Unfamiliar dogs:** this value resulted from the sum of scores of items 27 and 28 (frequency of stress-related behaviours when meeting an unfamiliar dog and time for recovery). It ranges from 0 to 8: dogs with a value above 4 were classified as Anxious dogs in this category, whereas dogs with a value below 4 were classified as Non-Anxious.

- **New environments/situations:** this value resulted from the sum of scores of items 31 and 32 (frequency of stress-related behaviours when in a new environment/situation and time for recovery). It ranges from 0 to 8: dogs with a value above 4 were classified as Anxious dogs in this category, whereas dogs with a value below 4 were classified as Non-Anxious.

- **Sound sensibility:** this value resulted from the average value of scores related to subcategories of reaction to storms (items 38 and 39 - frequency of stress-related behaviours during a storm and time for recovery), fireworks (items 43 and 44 - frequency of stress-related behaviours during fireworks and time for recovery), shots (items 48 and 48 - frequency of stress-related behaviours when shots are audible and time for recovery) and other noises (items 52 and 53 - frequency of stress-related behaviours when the dog hears other noises, such as the vacuum cleaner, sirens, alarms, and time for recovery). This value ranges from 0 to 11: dogs with a value above 5 were classified as Anxious dogs in this category, whereas dogs with a value below 6 were classified as Non-Anxious.

- **Separation anxiety:** this value was the one of item 64 (frequency of stress-related behaviours when at home alone). It ranges from 0 to 4: dogs with a value above 2 were classified as Anxious dogs in this category, whereas dogs with a value below 2 were classified as Non-Anxious.

Regarding the “separation anxiety” category, an adjustment was done to increase data reliability: if the owner answered that the dog did not show any behaviour potentially related to stress when at home alone, but he/she could not demonstrate it for sure (i.e., the owner did not declare to control the dog with a remote camera), the score of that category was transformed in a missing value. A research by Van Rooy and colleagues [36] demonstrated that there is a positive correlation between the percentage of time spent in anxiety-related behaviours (assessed via footage) and the owner-reported separation-related behaviours that are more evident (destruction, whining, howling) but subtle anxiety-related behaviours are less likely to be noticed.

The aim of questionnaire analyses was to find the most anxious individuals as cases and those with no marked anxious reactions as controls. Therefore, dogs who resulted to be Anxious in at least 3 categories out of 5 were classified as Anxious dogs (A group) and the ones who resulted to be Non-Anxious in at least 3 categories out of 5 were classified as Non-Anxious dogs (NA group).

Questions related to dogs' reactions towards surfaces and heights (items 55, 56, 57, 58, 59) were not considered because many owners did not answer them (404 missing values overall); questions related to stereotypic behaviours (items 65, 66, 67, 68) were not considered because they were observed only sporadically by owners (mean value = 0.3, 0.2, 0.2 and 0.9 for chasing-tail behaviour, invisible-things-catching, pacing and self-mutilation, all scores ranging from 0 to 4).

JBT: Analysis of the training phase

The effects of canine characteristics and of test procedure on the number of training trials required to achieve the learning criterion were investigated using Spearman's correlation test (age – years), Mann-Whitney U tests (sex - male/female; group - A/NA; position of the positive corridor - left/right; procedure - standard/modified with one owner at the starting position) and of the) and Kruskal-Wallis test (training level - 0/1/2).

JBT: Analysis of the testing phase

Effect of the treat/owner's smell. To ensure that dogs' decision-making processes relied on the corridor position and not on the odour of treats/owner, a Wilcoxon test for paired samples was employed to compare, for each dog, the mean latency to reach the P corridor during the testing phase and the latency to reach the same corridor during the last trial ("false positive" trial), when the owner and treats were moved in the N corridor.

Influence of dogs' characteristics and test procedure on latencies to enter the corridor. As recommended by Gyax [37] and as done in our previous researches [32,38], a Linear Mixed-Effect model was applied, setting as dependent variable the untransformed latencies to enter the corridor in each single trial, including also latencies recorded in P and N trials, used as anchors to estimate the slope across the different ambiguous cues (dogs' responses to trained corridors P and N are expected to be stable); a maximum-likelihood estimation was employed to account for designed imbalance in the data (only 3 trials for NP, M and NN corridor vs. 8 trials for P and N corridor) [39].

A backwards approach was applied to test the fixed effects of canine age, sex, training level, group (A/NA) and test procedure (standard/modified) on latencies to enter the corridors. These fixed effects were tested in interaction with the corridor's position variable and the interaction was removed from the model if not significant.

"Go/No-Go" responses. To better analyse the decision-process towards the different corridors, we evaluated the number of "go trials" and "no go trials" towards these stimuli. A "go trial" is a trial in

which the dog enters the corridor within 15 s, a “no-go trial” is a trial in which the dog does not enter the corridor within 15 s. Considering that each dog was tested only 3 times with each ambiguous corridor and 8 times with each trained one, we calculated the percentage of “go trials” on the total number of trials of each type (i.e., number of P “go trials”/total number of P trials). To check for a group effect, a Chi-squared test (or a Fisher’s exact test in case expected frequencies were not more than 5 in at least 80% of the cells [40]) was run for each location on the contingency tables of “go/no-go” trials between Anxious and Non-Anxious dogs. The same analysis was run between dogs tested with the standard/modified procedures.

Optimism/pessimism adjusted score. Even if averaged measures are inevitably less accurate than raw ones, to compare our results with previous studies and to evaluate the potential usefulness of our protocol in measuring optimism and pessimism, we calculated an “adjusted score” of optimism/pessimism, according to the following formula, originally developed by Mendl and colleagues [15]:

$$\text{adjusted score} = \frac{\text{mean latency to probe location} - \text{mean latency to Positive location}}{\text{mean latency to Negative location} - \text{mean latency to Positive location}} \times 100$$

The adjusted scores were calculated for each dog and each type of ambiguous location (NP,M and NN). An adjusted score near 0 means that the subject reacted to the ambiguous corridor in a similar way to how it reacted to the positive one (i.e., it considered the ambiguous cue as potentially rewarding - optimistic bias); vice versa an adjusted score near 100 indicates a pessimistic bias.

To compare the adjusted scores between Anxious and Non-Anxious dogs and between dogs tested with the standard/modified procedure, we run a Linear Mixed-Effects model: the dogs’ identity was set as a random effect, the adjusted scores as dependent variable and group and test procedure, in interaction with the corridor locations, as fixed effects.

Ethics statements

The ethical committee for animal testing of the University of Parma declared that a special permission to use pet dogs in this behavioural study is not required in Italy (Decreto legislativo 4 marzo 2014, n. 26, Art. 2). Furthermore, the committee reported that this study accomplishes ethical requirements in compliance with the law about the use of animals in clinical and zootechnical studies (PROT.N. 11/CE/2019).

Results

Questionnaire analysis

Following data refers to the entirety of results of the survey (N = 487).

Overall, 52 dogs (10.68% of our sample) were classified as Anxious dogs (A group), whereas 222 dogs (45.59% of our sample) were classified as Non-Anxious dogs (NA group).

The mean values for each category \pm sd were: 4.86 ± 2.69 for “unfamiliar people” category, 3.51 ± 1.91 “unfamiliar dogs”, 2.20 ± 2.22 “new environments/situations”, 2.62 ± 2.59 “sound sensibility”, 1.41 ± 1.41 “separation anxiety”.

Percentages of dogs classified as anxious and non-anxious in each category are reported in Table 1.

Table 1. Percentages of dogs classified as anxious and non-anxious in each category

Category	Anxious dogs	Non-anxious dogs
Unfamiliar people	31.21%	55.44%
Unfamiliar dogs	31.62%	40.86%
New environments/situations	16.84%	60.37%
Sound sensibility	20.33%	82.75%
Separation anxiety	14.99%	31.01%

Percentages of dogs classified as Anxious in the categories “new environments/situations”, “sound sensibility” and “separation anxiety” were in line with previous researches [3,7,41–43].

JBT: Analysis of the training phase

The minimum number of training trials required to reach the learning criterion was on average $15.7 \pm$ sd 5.6 trials and none of the tested variables was significantly related to it (see Table 2 for statistical values; males, mean \pm sd : 15 ± 6.17 , females: 16.39 ± 5.04 trials; training level “0”: 16.75 ± 6.11 , training level “1”: 15.09 ± 6.59 , training level “2”: 15.53 ± 5.08 trials; Group A: 15.89 ± 5.58 , Group NA: 15.42 ± 5.83 trials; Procedure standard: 16.16 ± 5.99 , Procedure modified: 13.43 ± 3.05 ; P at left side: 16.62 ± 8.17 , P at right side: 20.17 ± 5.54 trials).

Table 2. Influence of dogs' characteristics and test procedure on the minimum number of training trials required to reach the learning criterion

Tested variable	Test statistic	P-value
Age	Spearman's correlation rho = - 0.05	p = 0.75
Sex	Mann-Whitney U test W = 222	p = 0.22
Training level	Kruskal-Wallis rank sum test $X^2_{(2)} = 0.59$	p = 0.74
Group	Mann-Whitney U test W = 197.5	p = 0.63
Procedure	Mann-Whitney U test W = 86	p = 0.40
Positive door location	Mann-Whitney U test W = 148.5	p = 0.38

JBT: Analysis of the testing phase

Effect of the treat/owner's smell

Wilcoxon tests for paired samples showed no statistically significant difference between the mean latency to enter the P corridor during the test (mean \pm sd 2.45 \pm 1.43 s) and the latency to enter it in the last "false positive" trial (mean \pm sd 2.36 \pm 2.11 s), confirming that the dogs' decision-making process relied on the door location and not on the treat/owner's smell (z-value = 0.46, p = 0.22), in line with our previous research [32].

Influence of dogs' characteristics and test procedure on latencies to enter the corridor

The Bayesian Information Criterion (BIC) was used to select the best combination of random effects: as expected, the best model entailed that intercept vary incorporating all the necessary hierarchical levels in the random effect (i.e., corridor location nested in dog identity). Using the dog's identity as a random effect factor takes into account the potential differences in running speed between subjects. Model selection using BIC also showed that the model better fit the data if the corridor position variable was coded as a continuous variable and not as a factor, in agreement with the expected graded output (i.e., gradually higher latencies to enter the corridor if it was close to the N corridor) and with our previous research [32]. However, the corridor position variable was coded as a factor in the following analyses in order to detect potential different influences of dogs' characteristics on latencies to enter different corridors.

Linear Mixed-Effects models did not reveal any significant effect of age, sex, group and test procedure on latencies to enter the various corridors (see Table 3 for detailed statistical results; males, mean \pm sd : 8.36 ± 6.18 , females: 8.66 ± 6.24 s; Anxious, mean \pm sd : 8.15 ± 6.21 , Non-Anxious: 8.84 ± 6.2 s; standard procedure, mean \pm sd : 8.52 ± 6.2 , modified procedure: 8.41 ± 6.25 s). The training level (training level "0" mean \pm s.d.: 8.69 ± 6.11 , training level "1": 8.83 ± 6.27 , training level "2": 8.23 ± 6.21 s), in interaction with the corridor position, had an influence on latencies ($p = 0.006$); however, the comparison between a model with training level in interaction with corridor location as fixed effects (model1, M1) and a model with only the corridor location as fixed effect (model2, M2) revealed that the effect of the training level is probably negligible (BIC M1= 5089.58, BIC M2= 5038.40) and that therefore a simpler model (M2) is preferable.

The fixed effect of corridor location is strongly significant ($F\text{-value}_{(4, 124)} = 352.12$, p value < 0.0001 ; mean \pm sd = 2.45 ± 2.10 s – P corridor; 4.77 ± 4.66 s – NP corridor; 9 ± 5.77 s – M corridor; 12.66 ± 4.38 s – NN corridor; 14.2 ± 2.74 s – N corridor).

Table 3. Analysis of the influence of dogs' characteristics and test procedure on latencies to enter the corridor

Fixed effects	F-value	numDF	denDF	P-value
Corridor location	352.12	4	124	$P < 0.0001$
Age (years)	1.05	1	31	$p = 0.31$
Sex	0.36	1	31	$p = 0.55$
Training Level	1.03	2	31	$p = 0.37$

Group	2.44	1	31	p = 0.13
Test procedure	0.33	1	31	p = 0.57
Corridor location x Age	1.46	4	124	p = 0.22
Corridor location x Sex	0.92	4	124	p = 0.46
Corridor location x Training level	2.83	8	124	p = 0.006
Corridor location x Group	1.40	4	124	p = 0.24
Corridor location x Test procedure	1.42	4	124	p = 0.23

“x” indicates the interaction between variables.

Post-hoc pairwise comparisons revealed that dogs behaved differently towards P and N corridors and towards adjacent ones (see Table 4), with latency significantly increasing from P to N corridor, in line with our previous research [32].

Table 4. Pairwise comparisons between latencies to enter trained corridors and adjacent ones

Pairwise comparison	Estimate	P-value
P – N	-11.75	p <0.0001
P – NP	-2.31	p <0.0001
NP – M	-4.23	p <0.0001
M – NN	-3.65	p <0.0001
NN – N	-1.54	p = 0.005

Simultaneous Tests for General Linear Hypotheses, Multiple Comparisons of Means. P = Positive corridor, NP = Near Positive corridor, M = Middle corridor, NN = Near Negative corridor, N = Negative corridor. Adjusted p values reported, Bonferroni method.

“Go/No-Go” responses

Almost all (99 %) of responses during P trials were correct, i.e., these responses were “go trials”, whereas the percentage of correct responses, i.e., “no-go trials”, during N trials was slightly lower (90.46%), in line with our previous research [32].

The number of “go trials” during N trials was different between Anxious and Non-Anxious dogs: the former entered this corridor more frequently than expected ($X^2_{(1)} = 12.35$, p-value = 0.0004, effect size V di Cramer = 0.20), see Fig 2.

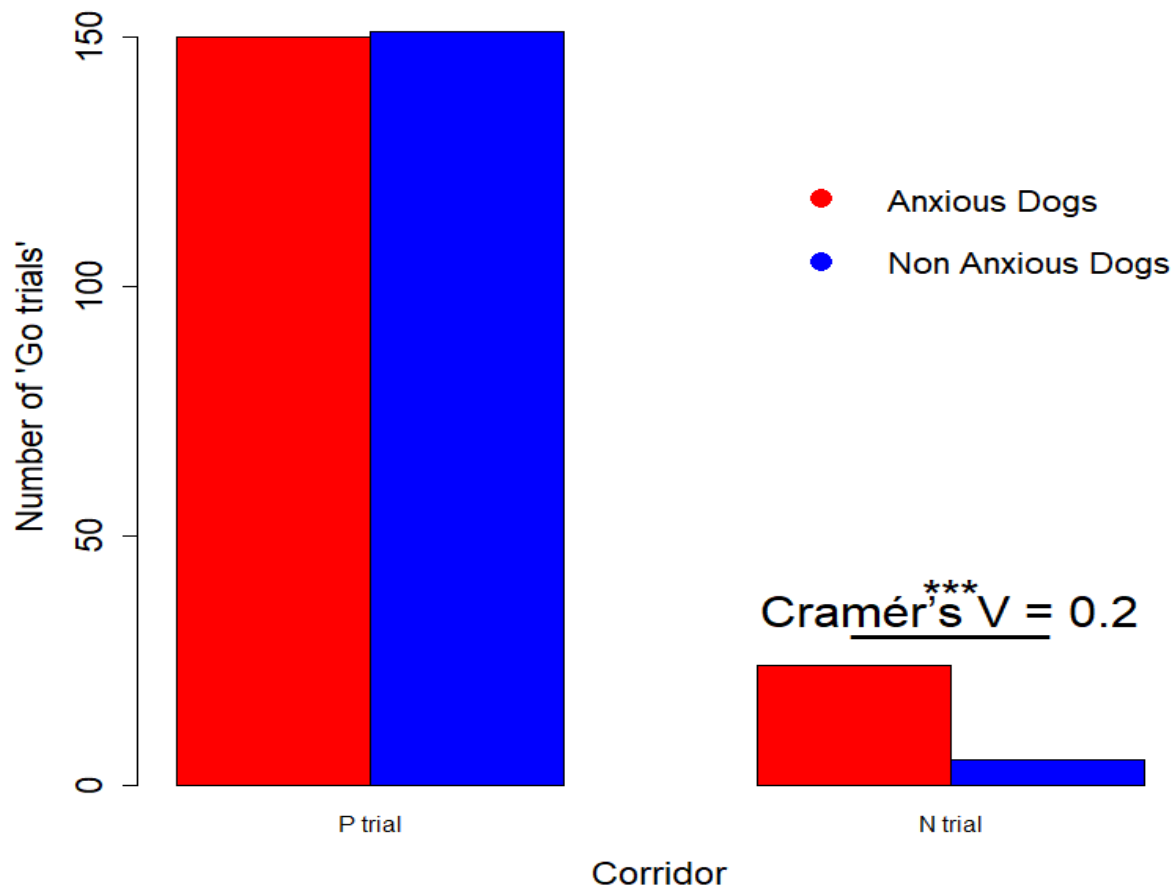


Fig 2. Number of “go trials” for the trained locations (P and N). A “go trial” is a trial in which the dog enters the corridor in less than 15 s. “***” indicates a p value < 0.001

The percentages of ‘go trials’ in NP, M and NN trials were 85.96%, 55.26% and 24.56%, respectively, in line with our previous research [32]. The number of “go trials” was not different between Anxious and Non-Anxious dogs during ambiguous trials ($X^2_{(1)} = 0.07$, p-value = 0.79 for NP trials; $X^2_{(1)} = 0$, p-value = 1 for M trials; $X^2_{(1)} = 2.32$, p-value = 0.13 for NN trails) and during P trials (Fisher’s exact test).

Similarly, the number of “go trials” was not different between dogs tested with the standard/modified procedure, nor during training trials (P - Fisher’s exact test, p-value = 0.09; N - $\chi^2_{(1)} = 2.53$, p-value = 0.11), nor during ambiguous trials (NP - Fisher’s exact test, p-value = 0.49; M - $\chi^2_{(1)} = 2.43e-30$, p-value = 1; NN - $\chi^2_{(1)} = 6.96e-31$, p-value = 1).

Optimism/pessimism adjusted score

The adjusted scores' frequency distributions are graphically illustrated in Fig 3.

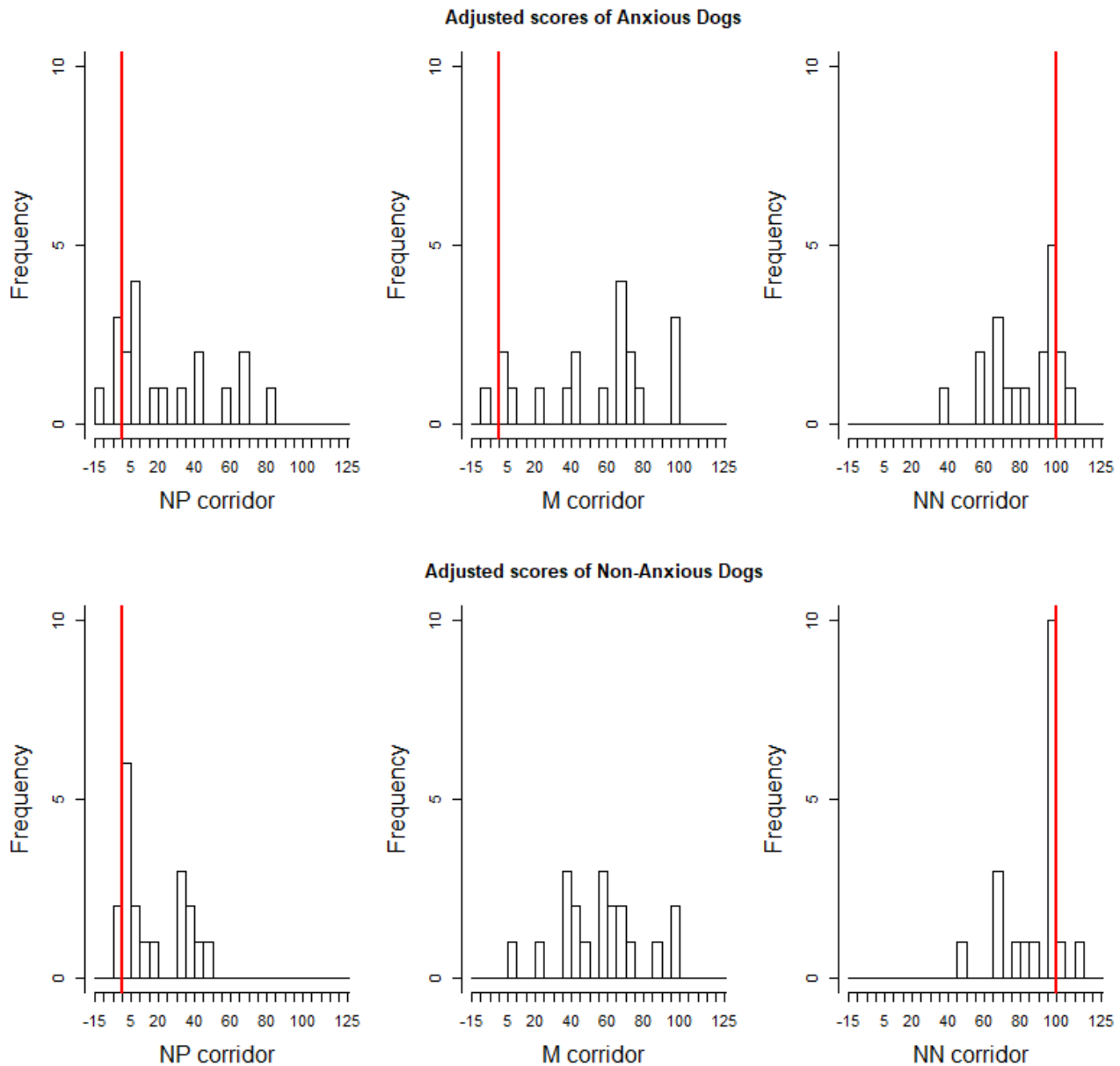


Fig 3. Optimism/pessimism adjusted scores. Adjusted score's frequency distribution for the three ambiguous corridors: Near Positive (NP), Middle (M) and Near Negative (NN), between the two groups (Anxious and Non-Anxious dogs). Caution is required when interpreting these adjusted scores, that are calculated using average values, because this approach reduces the variability in the data and led to unexpected results: some scores were lower than 0, whereas some scores were higher than 100, suggesting that the mean latencies to enter ambiguous

corridors were lower than the mean latency to enter P or higher than the mean latency to enter N, respectively (red vertical lines help finding 0 and 100 points).

Mean values \pm sd and skewness of distribution are: A group: 23.20 ± 28.15 , for NP with skewness = 0.7; 52.43 ± 33.92 for M with skewness = -0.3; 83.84 ± 19.86 for NN corridor with skewness = -0.64 — AN group: 16.94 ± 17.32 , for NP with skewness = 0.54; 56.34 ± 24.24 for M with skewness = 0.12; 89.91 ± 16.72 for NN corridor with skewness = -1.12.

As expected, dogs had a lower adjusted score (index of a more optimistic attitude) the closer to P the ambiguous corridor was located (Linear Mixed-Effects model, the corridor location has a significant influence on adjusted scores, $F_{(2, 74)} = 92.75$, P-value < 0.0001). Post-hoc pairwise comparisons revealed that NP adjusted scores were significantly lower than M ones (means difference = -34.31, $t\text{-value}_{(74)} = -6.99$, Adjusted P-value, Bonferroni method, $p < 0.0001$) and that NN adjusted scores were significantly higher than M ones (means difference = 32.50, $t\text{-value}_{(74)} = 6.62$, Adjusted P-value, Bonferroni method, $p < 0.0001$), in line with our previous research [32].

No significant differences were found between the adjusted scores of Anxious and Non-Anxious dogs or between dogs who were tested with standard/modified procedures.

Discussion

The aim of the present research was to compare the Judgment Bias Test (JBT) performance of dogs in a negative affective state (i.e., dogs that show in everyday life behaviours suggesting an anxiety-like state) with the performance of dogs in a positive emotional state: the hypothesis was that the former would show a more pessimistic bias than the latter.

To analyse the dogs' judgment bias, we used a novel JBT protocol we previously developed and applied on dogs without behavioural problems ([32], in following statements we are referring to this study when mentioning our previous data): this protocol enhanced robustness and reliability of results and solved several methodological issues we detected in the classic protocol ([38], see also [44–47] for some contradicting results of JBT in dogs). The experimental set-up consisted of five adjacent corridors (named P, NP, M, NN, N) closed with a wooden door. At the end of the positive corridor (P) dogs found their owner, while at the end of the negative corridor (N, located at the opposite side of the arena) dogs found a black cloth slowly blowing. After a training to discriminate between P and N corridor, ambiguous stimuli (NP, M and NN corridors, empty and located between P and N) are presented: the latency to enter each corridor was analysed as index of optimism/pessimism attitude.

We verified that dogs were not influenced by the treat/owner's smell, and that dogs have perfectly learned the association between the trained stimuli (P and N corridors) and their outcomes: during the testing phase dogs behaved differently towards the two corridors and their behaviour is consistent among trials; almost all of responses during P trials were "go trials", suggesting that the P corridor was perceived as a very pleasant location and that dogs were very motivated to enter it, whereas dogs entered the N corridor only in 9.54% of N trials, suggesting that subjects have indeed learned what there was at the end of this corridor and that they were not interested in entering it. These results confirm that dogs learned the association between the trained corridors and their outcomes, which is a necessary requirement in JBT because responses to ambiguous cues are compared to responses to the trained ones in order to assess the pessimistic/optimistic attitude.

To recruit a sample of anxious dogs (cases) and a sample of non-anxious ones (control) we realized an online questionnaire, based on previously validated ones ([7,8,30,33]). In the survey, pet owners were asked to report if they had ever observed, in different situations, their dog showing one or more behaviours from a fixed list of behaviours potentially related to anxiety, to tell the frequency of these behaviours and the time of recovery. As extensively reported in the results section, in our sample (N = 487) the majority of behaviours potentially related to anxiety was elicited by unfamiliar people and unfamiliar dogs.

Seven dogs from the Anxious group tested with JBT (N = 19) were extremely stressed between the end of the familiarization phase and the beginning of the training phase, when the dog was separated from the owner, taken out from the testing arena for few minutes and then led back to the it, where the owner was not in sight, hidden at the end of P corridor; a change to the standard procedure solved this issue: luckily these dogs came with two owners each, one of them stood at the starting position during all the test. This adjustment allowed to test these dogs and it is a potential index of the effectiveness of our survey in detecting the most anxious dogs, or at least we ones who struggle the most to be with stranger people, without their owner and/or in new environments.

This procedural adjustment was considered in statistical analyses and it did not influence neither the learning process (minimum number of training trials required), nor latencies to enter the corridors. Indeed, none of the test variables (age, gender, training level, group, standard/modified procedure), except the corridor location, had a remarkable effect on latencies to enter corridors. Dogs behaved differently towards adjacent corridors, with latency significantly increasing from P to N corridor, in line with our previous outcomes: this suggests that all dogs were able to fully discriminate among the five corridors; this level of discrimination is especially desirable in JBTs that can potentially discriminate not only positive and negative emotional states, but also emotions characterised by the same valence and different levels of arousal (see for a review [10]). Example given, according to the dimensional core affect model [48], a low expectation of a reward characterises depression [49] and therefore this state might result in pessimistic responses towards the NP location [50,51]; instead, anxiety is characterized by a high expectation of a punishment [49], and it might result in pessimistic responses towards the NN location (e.g. reduced detection-latencies for cues appearing near the trained negative cue [52,53]).

Therefore, a high level of discrimination among ambiguous corridors allows a more accurate evaluation of emotions; thus, in the present study the expectations were that Anxious dogs might behave towards the NN corridor in a more pessimistic way compared to Non-Anxious dogs. On the contrary, statistical analyses showed that this is not the case and that even dogs tested with the modified procedure (potentially the most anxious ones) did not behave differently towards the NN corridors compared to dogs tested with the standard procedure.

Similarly, Anxious and Non-Anxious dogs' adjusted scores of optimism/pessimism were not different; likewise, these adjusted scores were not different between dogs tested with the standard or the modified procedure. However, the general pattern is in line with expectations: the closer to P the ambiguous corridor was, the lower were the adjusted scores (index of a potential optimistic bias), i.e., dogs appeared more optimistic towards stimuli closer to P. Analyses done using linear-mixed effect model confirmed that dogs' motivation to enter ambiguous corridors decreased from NP to NN with increasing latencies to enter the corridor the closer it was to N; even the percentage of "go trials" decreased from NP to NN, suggesting that subjects might have a more pessimistic expectation towards stimuli closer to N.

In line with results obtained using linear mixed-effect models on raw latencies and using adjusted scores, we did not detect a difference between Anxious and Non-Anxious dogs in the number of "go trials" during ambiguous trials. Overall and contrary to expectations, from this pilot study we can't infer that anxious dogs have a more pessimistic attitude than non-anxious ones: this result could be ascribed to the inadequacy of our protocol to detect dogs' cognitive biases, even if, as expected, latencies to enter the corridors show a gradual increase the closer to N were the corridors and dogs' responses demonstrate a full learning of the association between the trained stimuli and their outcomes and an exact discrimination between all pairs of adjacent corridors. More generally, despite of the large number of studies that have used JBTs to evaluate the affective state in animals, results are not always in line with predictions, with some studies even leading to null results or to opposite findings to those expected (in non-human animals [12,14,54–56] and in particular in dogs [44–47,57,58]), raising some doubts about the real meaning of JBT results.

A further explanation of our results could be related to issues in detecting anxious dogs: anxiety is a multifactorial disorder, it is among the most difficult behavioural problems to diagnose [1], clinical monitoring of anxiety tend to depend on not validated methods and there is no validated general scale to assess the severity of anxiety [59].

Our questionnaire might be not enough sensitive to identify the most anxious and the most non-anxious dogs; 7 out of 19 dogs from the A group needed an adjustment in the test procedure because they were not able to remain without their owner for few minutes, suggesting that our survey indeed found a sample of dogs who suffer of an anxiety-like state. However, questionnaire results could be biased by owners' biases, emotions, expectations and misinterpretations and therefore they could provide less reliable data than behavioural test [30].

Among others, Tiira and Lohi [30] developed a questionnaire aimed at studying canine anxiety and compared the results with behavioural test outcomes: even if they found a good correlation between questionnaire results about fear towards humans/situations and related behavioural test (reaction to a stranger approaching and novel object test), they did not find any relationship between survey outcomes and a novel-space-exploration test, a behavioural test similar to the open field test. The latter is a test routinely used in rodents personality studies and it consists of subjecting an animal to an unknown environment from which escape is prevented: a long latency to enter the central part of the arena, a long time spent near the walls, a great number of fecal boli and of risk assessment postures are indexes of anxiety [60,61]. This test has been applied also on dogs: Araujo and colleagues found that hearing a thunderstorm during the open field test increased laboratory Beagle dogs' inactivity (i.e. freezing behaviour) and time spent near the exit door (suggesting attempts to escape) [62]. Gruen and colleagues found similar results in Labrador retrievers selected for training as improvised explosive device detection dogs [63] and Wormald and colleagues discovered that Greyhound dogs from the Melbourne University Canine Blood Bank treated with diazepam increased locomotion and mean distance travelled during the open field test [29]. However, it must be considered that all these studies were carried out with laboratory dogs that may lack to exposure to natural sounds (such as a thunderstorm) and that live in conditions different from pet dogs [64]. Furthermore, even if the open field test represents a promising tool in research on canine anxiety, it is worth discussing that anxiety in this test is triggered by social isolation (from social group) and agoraphobia (the arena is very large compared to rodents' cages or natural environment, such as small tunnels) and therefore that this test might be suitable only for gregarious species who show fear of open spaces, and not for a neophilic predator, such as the domestic dog [60].

An interesting and unexpected result of the present study is that the number of "go trials" during N trials was different between Anxious and Non-Anxious dogs: the former entered this corridor more than expected (see Fig 2). A possible explanation for this result could be that the performance in discrimination of A dogs was worse than the one of NA dogs. In humans, it has been demonstrated that anxiety disorders are associated with altered cognitive performances, such as difficulties with concentrating and impairments in working memory functioning [65–67] and in spatial navigation [68,69]. Evidences exist also in non-human animals: mice characterised by nonadaptive anxiety-related behaviour showed a higher level of short-term memory errors and less cognitive flexibility compared to control [70], dogs affected with noise reactivity had a lower performance on a puzzle box test than non-affected dogs [71], and dogs with a diagnosis of anxiety-related problems took longer to solve the initial solvable trial of the classic unsolvable task paradigm and showed more stress-related behaviours compared to dogs without behavioural problems [72]. However, A and NA dogs did not differ in learning abilities (minimum number of training trials required) nor in their latencies to enter the corridors. Furthermore, the number of "go trials" during N trials was not different between dogs tested with the standard and with the modified procedure: thus, the potentially most anxious dogs, who needed at least one owner always by their side during the test, did not enter the N corridors more times than the other subjects.

An alternative explanation of this result could be that dogs in A group took more chances to look for their owner even in the N corridor, even more considering that the cost of an approximately 5-metre walk is probably low. When A dogs were tested with the modified procedure, they did not feel the urgency to reach the owner who was out of sight due to the presence of the other owner. This evidence raises doubts about the adequacy of this JBT protocol to test anxious dogs, especially regarding dogs who are in discomfort without their owner and/or in presence of stranger people: the JBT purpose is to assess the optimistic/pessimistic attitude and the long-term emotional state associated to this attitude, and not to elicit discrete emotions, such as stress or anxiety state, that could bias test results itself. Given that 7 out of 19 dogs in A group needed to be tested with their owner by their side, whereas none of the 19 dogs in the NA group needed this adjustment, a consideration must be suggested on a potential influence of anxiety-like states on the test performing and not only on test results. A detailed analysis of dogs' behaviour during the test (as done in our previous research) could help to find out if the testing protocol generated stress and anxiety in dogs: the expectation is that stress-related behaviours would be more frequent in anxious dogs than in non-anxious ones and that no difference would be found between dogs tested with the modified/standard procedure.

Some methodological adjustments that could be proposed to make more sensitive dogs feel comfortable consist in applying the modified procedure to all subjects (which would require all dogs to come accompanied by two owners) or in always assigning the starting position to the dog's owner, replacing the positive reinforcement at the end of the P corridor with only a food reward (in which case only food motivated dogs could be tested).

Conclusions

The aim of this study was to better understand the attitude of anxious dogs towards uncertainty, with the hypothesis that these dogs would have a more pessimistic attitude than non-anxious ones. Contrary to expectations, even if judgment bias test (JBT) results were robust and consistent, anxious dogs' performance in JBT did not differ from the performance of non-anxious dogs. Considering that some dogs in the anxious group needed an adjustment in the JBT procedure because they were not able to remain without their owner for few minutes, suggesting a potential influence of anxiety-like states on the test performing, some improvements in the protocol are suggested to establish a valid tool to analyse dogs' long-term emotional state and pessimistic/optimistic attitude, even with more sensitive and fragile subjects.

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Supplementary Materials

Appendix 1. Questionnaire – English version

1. I have read the privacy notice

Having seen

2. CONSENT TO THE PROCESSING OF PERSONAL INFORMATION REGULATION (EU)2016/679 AND
CONSENT TO PARTICIPATE IN THE RESEARCH

I agree

1. GENERAL INFORMATION

3. Name and surname of the dog's owner:

4. Phone number:

5. Email address:

6. Dog's name:

7. Dog's breed:

8. Date of birth of the dog:

9. Sex of the dog:

Male

Female

10. Has the dog been neutered?

Yes

No

11. How long has the dog lived with you?

less than 6 months

6 months - 1 year

1 year or more

12. Where does your dog live?

Always at home

Always outside

A little at home, a little outside

Other:

13. How long does your dog exercise (games, walks, sports and training activities) on a typical day?

- 3 hours or more
- 2-3 hours
- 1-2 hours
- 30 minutes- 1 hour
- less than 30 minutes

14. Do you practice some activities with your dog (even at home)? What? (Multiple options can be selected)

- Obedience, Alabama
- Agility
- IGP
- Search and rescue
- Hunting training
- Sheepdog
- Mobility
- Dog dance
- Basic education and/or tricks
- Nosework
- Dog puzzles
- Other:

15. If you have selected at least one option from the previous ones, how often do you carry out these activities with your dog?

- Once a year
- 1-2 times a month
- 1-2 times a week
- 2-4 times a week
- Almost every day
- Several times a day

16. Are you willing to come to the University with your dog to perform a behavioral test (lasting about 1 hour)?

- Yes
- No

17. Have you already participated with your dog in any of our studies?

- Yes
- No

18. Does your dog have any health problems? If so, which ones?

19. Is your dog taking medication? If so, which and why?

2. MEETING WITH UNKNOWN PEOPLE

20. Is your dog barking excessively or is it extremely excited when someone rings the doorbell or knocks on the door?

- Always or almost every time
- Often
- Sometimes
- Seldom
- I do not know (e.g., if the dog lives in the garden and never hears the bell)

21. Does your dog ever meet unknown people? *

- Yes
- No

If you replied "NO" go to the next section

22. Have you ever observed one or more of these behaviors when your dog meets an unknown person, regardless of this person's behavior? (You can select multiple options.) If your dog has never behaved in this way, move on to the next section

- It walks away
- It barks (without moving towards the person)
- It growls (without moving towards the person)
- It Barks and/or growls and moves towards the person
- Low tail/between paws
- It stays close to the owner
- It avoids eye contact with the unknown person (e.g., sniffing on the ground)
- Piloerection on its back, neck or tail

23. How often does the dog show these behaviors when meeting an unknown person?

- Always or almost every time
- Often
- Sometimes
- Seldom

24. How long is the dog aroused/worried after the stranger has left? The dog returns to normality:

- Straight after
- In a few minutes
- In 15 minutes - 1 hour

In 1-5 hours

3. MEETING WITH UNKNOWN DOGS

25. Does your dog ever meet unknown dogs? *

Yes

No

If you replied "NO" go to the next section

26. Have you ever observed one or more of these behaviors when your dog meets an unknown dog, regardless of the behavior of this dog? (You can select multiple options.) If your dog has never behaved in this way, move on to the next section

It walks away

It barks (without moving towards the dog)

It growls (without moving towards the dog)

It Barks and/or growls and moves towards the dog

Low tail/between legs

It stays close to the owner

It avoids eye contact with the unknown dog (e.g., sniffing on the ground)

Piloerection on its back, neck or tail

It is usually friendly, but it responds aggressively if another dog shows aggressive behavior

It is usually friendly, but sometimes a fight begins with the other dog

27. How often does the dog show these behaviors when meeting an unknown dog?

Always or almost every time

Often

Sometimes

Seldom

28. How long is the dog aroused/worried after the unknown dog has gone away? The dog returns to normality:

Straight after

In a few minutes

In 15 minutes - 1 hour

In 1-5 hours

4. NEW SITUATIONS AND ENVIRONMENTS

29. Does your dog ever experience new situations or new environments? *

- Yes
- No

If you replied "NO" go to the next section

30. Have you ever observed one or more of these behaviors when your dog is in a new situation or environment? Do not consider your dog's reaction when you go to the veterinarian. (You can select multiple options.) If your dog has never behaved in this way, move on to the next section

- It tries to escape from the new environment
- It barks
- It has its tail low or between its paws
- It pants
- it trembles
- If some food is offered to it, it does not accept
- It startles if it feels a noise or a movement
- it is smelling the environment while continuing to look around with a stiff posture, you are able to notice the white part of the eye, pupils are dilated
- It has a low posture (shows for example some of these behaviors: lowered head, ears back, lowered center of gravity, low tail or between paws)
- It remains firm and rigid, does not want to explore the new environment
- It stays close to the owner

31. How often does the dog show these behaviors when in a new situation/new environment?

- Always or almost every time
- Often
- Sometimes
- Seldom

32. How long has the dog been aroused/worried since leaving the new environment? The dog returns to normality:

- Straight after
- In a few minutes
- In 15 minutes - 1 hour
- In 1-5 hours

5. NOISE SENSITIVITY

33. Does your dog have hearing issues? **

- Yes
- No

34. If you answered "YES" to the above question, specify what hearing issues your dog has:**

THUNDERSTORM

35. How often does your dog feel a thunderstorm? *

- Never
- Sometimes a year
- About once a month
- Several times a month

If your dog hears the storm at least a few times a year, answer these questions, otherwise go to the fireworks section

36. Does your dog react to the sound of thunderstorm? Answer "NO" if your dog is surprised when only at the first thunder, but it relaxes soon after.

- Yes
- No
- I don't know (if you're not sure of the answer take a look at behaviors suggested in the next question)

37. If you replied "NO", go to the section on fireworks. If you replied "YES", indicate one or more behaviors that describe your dog's reaction. (Multiple options can be selected)

- It drools
- It defecates
- It urinates
- It destroys or chews objects
- It licks or scratches excessively
- It tries to escape or run away
- It pants
- It hides (e.g., under the bed)
- It trembles
- It barks/howls/cries
- It walks back and forth without a goal, often following the same path
- It immobilizes, it's rigid
- Low tail / between paws
- The dog gets excited when it hears thunder, the tail is raised, it can bark

Other:

38. How often does the dog show these behaviors when it feels the storm?

- Always or almost every time
- Often
- Sometimes
- Seldom

39. How long has the dog been aroused/worried since the noise starts? The dog returns to normality:

- A few minutes after the noise started, even if it is not ended
- 15 minutes - 1 hour after the noise started, even if it is not ended
- Right after the noise ends
- A few minutes after the noise ends
- 15 minutes - 1 hour after the noise ends
- 1 - 5 hours after the noise ends
- After more than 5 hours
- I do not know

FIREWORKS

40. How often does your dog hear fireworks? *

- Never
- Sometimes a year
- About once a month
- Several times a month

If your dog hears fireworks at least a few times a year, answer these questions, otherwise go to the gunshot section

41. Does your dog react to the sound of fireworks? Answer "NO" if your dog is surprised when hearing the first firework, but it relaxes soon after.

- Yes
- No
- I don't know (if you're not sure of the answer take a look at the behaviors suggested in the next question)

42. If you replied "NO" go to the section on the shots. If you replied "YES", indicate one or more behaviors that describe your dog's reaction. (Multiple options can be selected)

- It drools
- It defecates
- It urinates

- It destroys or chews objects
- It licks or scratches excessively
- It tries to escape or run away
- It pants
- It hides (e.g., under the bed)
- It trembles
- It barks/howls/cries
- It walks back and forth without a goal, often following the same path
- It immobilizes, it's rigid
- Low tail / between paws
- The dog gets excited when it hears fireworks, the tail is raised, it can bark
- Other:

43. How often does the dog show these behaviors when it hears fireworks?

- Always or almost every time
- Often
- Sometimes
- Seldom

44.

How long has the dog been aroused/worried since the noise starts? The dog returns to normality:

- A few minutes after the noise started, even if it is not ended
- 15 minutes - 1 hour after the noise started, even if it is not ended
- Right after the noise ends
- A few minutes after the noise ends
- 15 minutes - 1 hour after the noise ends
- 1 - 5 hours after the noise ends
- After more than 5 hours
- I do not know

GUNSHOTS

45. How often does your dog hear gunshots? *

- Never
- Sometimes a year
- About once a month
- Several times a month

If your dog hears gunshots at least a few times a year, answer these questions, otherwise move on to the other noises section

46. Does dog react to the sound of gunshots? Answer "NO" if your dog is surprised when hearing the first shot, but relax immediately afterwards.

- Yes
- No
- I don't know (if you're not sure of the answer take a look at the behaviors suggested in the next question)

47. If you replied "NO" go to the section on other noises. If you replied "YES", indicate one or more behaviors that describe your dog's reaction. (Multiple options can be selected)

- It drools
- It defecates
- It urinates
- It destroys or chews objects
- It licks or scratches excessively
- It tries to escape or run away
- It pants
- It hides (e.g., under the bed)
- It trembles
- It barks/howls/cries
- It walks back and forth without a goal, often following the same path
- It immobilizes, it's rigid
- Low tail / between paws
- The dog gets excited when it hears gunshots, the tail is raised, it can bark
- Other:

48. How often does the dog show these behaviors when it hears gunshots?

- Always or almost every time
- Often
- Sometimes
- Seldom

49. How long has the dog been aroused/worried since gunshots start? The dog returns to normality:

- A few minutes after the noise started, even if it is not ended
- 15 minutes - 1 hour after the noise started, even if it is not ended
- Right after the noise ends
- A few minutes after the noise ends

- 15 minutes - 1 hour after the noise ends
- 1 - 5 hours after the noise ends
- After more than 5 hours
- I do not know

OTHER NOISES

50. Does your dog react to other noises (vacuum cleaners, sirens, ambulances, alarm systems, etc.)?

- Yes
- No
- I don't know (if you're not sure of the answer take a look at the behaviors suggested in the next question)

51. If you replied "YES", indicate one or more behaviors that describe your dog's reaction. If you answered "NO", go to the last question in this section. (Multiple options can be selected)

- It drools
- It defecates
- It urinates
- It destroys or chews objects
- It licks or scratches excessively
- It tries to escape or run away
- It pants
- It hides (e.g., under the bed)
- It trembles
- It barks/howls/cries
- It walks back and forth without a goal, often following the same path
- It immobilizes, it's rigid
- Low tail / between paws
- The dog gets excited when it hears the noise, the tail is raised, it can bark
- Other:

52. How often does the dog show these behaviors when it hears the noise?

- Always or almost every time
- Often
- Sometimes
- Seldom

53. How long has the dog been aroused/worried since the noise starts? The dog returns to normality:

- A few minutes after the noise started, even if it is not ended

- 15 minutes - 1 hour after the noise started, even if it is not ended
- Right after the noise ends
- A few minutes after the noise ends
- 15 minutes - 1 hour after the noise ends
- 1 - 5 hours after the noise ends
- After more than 5 hours
- I do not know

54. If your dog is sensitive and reacts to noises (thunderstorms, fireworks, gunshots and/or other noises), have you tried to help him? If so, by what method?

- No
- Yes, with medication
- Yes, with natural medication
- Yes, with desensitization, e.g., with reproduction of sound recordings the ones that causes fear
- Other:

6. SURFACES AND HEIGHTS

55. Does your dog have issues/is afraid to walk on a metal grid?

- Yes, always
- Sometimes, it depends on the environment (for example if the dogs is on a metal grid and sees the void below)
- Seldom
- Never
- I do not know

56. Does your dog have issues/is afraid to walk on shiny floors (such as in shopping malls)?

- Yes, always
- Sometimes, it depends on the environment
- Seldom
- Never
- I do not know

57. Does your dog have issues/is afraid to climb the stairs if it sees the void between the steps?

- Yes, always
- Sometimes, it depends on the environment
- Seldom
- Never
- I do not know

58. Does your dog have issues/is afraid to climb the stairs if it does not see the void between the steps?

- Yes, always
- Sometimes, it depends on the environment
- Seldom
- Never
- I do not know

59. Does your dog have issues/is afraid to walk near glass railings, for example on the second floor of a shopping mall?

- Yes, always
- Sometimes, it depends on the environment
- Seldom
- Never
- I do not know

60. Does your dog have problems on other surfaces or in high places? Describe them below.

7. DOG REACTIONS WHEN AT HOME ALONE

61. Has your dog ever been at home alone? *

- Yes
- No

If you replied "NO" go to the next section

62. How do you know what your dog does when it is at home alone?

- I have a camera in my house
- My neighbors told me
- I can feel my dog's mood when I get home
- The dog destroyed objects
- Other:

63. Have you ever observed one or more of these behaviors when your dog stays home alone? (You can select multiple options.) If your dog has never behaved in this way, move on to the next section

- It drools
- It defecates/ urinates
- It licks or scratches excessively
- It tries to escape or run away (e.g., it scratches the door)
- It pants
- It hides (e.g., under the bed)
- It trembles

- It barks/howls/cries
 - It walks back and forth without a goal, often following the same path
 - It yawns
 - it loses its appetite (e.g., it do not eat the food you leave for it)
 - Other gastrointestinal symptoms (vomiting, diarrhea)
64. How often does the dog show these behaviors when it is home alone?
- Always or almost every time
 - Often
 - Sometimes
 - Seldom

8. STEREOTYPICAL BEHAVIOR

65. Does your dog try to catch his tail and/or turn on himself? The dog can stop and stare at its tail.

- I have never noticed this behavior
- It happened a few times
- It happens about every month
- It happens every week
- It happens almost every day

66. Does your dog stare/chase reflections or shadows or does it look like he's trying to catch invisible things?

- I have never noticed this behavior
- It happened a few times
- It happens about every month
- It happens every week
- It happens almost every day

67. Does your dog walk aimlessly following a fixed path?

- I have never noticed this behavior
- It happened a few times
- It happens about every month
- It happens every week
- It happens almost every day

68. Does your dog bite or lick excessively, and for an extended time, its paws, its side or other part of its body?

- I have never noticed this behavior
- It happened a few times

- It happens about every month
- It happens every week
- It happens almost every day

69. Does your dog exhibit other "strange" behaviors? Describe them below:

70. Help us to improve! Are there any questions in the questionnaire that you did not understand or that were not clear? If so, which ones?

* A comprehensive examination of responses was done: in case the owner answered that his/her dog had never experienced a certain situation but he/she contextually affirmed that the dog showed in that situation behaviours potentially related to stress, the score of that category was transformed in a missing value

** Regarding the "sound sensibility" category, a further adjustment was done: if the owner declared that the dog had a pathology that compromises the hearing, the score of that category was transformed in a missing value

Appendix 1. Questionnaire – Italian version

1. Ho letto l'informativa

Presa visione

2. CONSENSO AL TRATTAMENTO DEI DATI PERSONALI REGOLAMENTO (UE)2016/679 E CONSENSO ALLA RICERCA

Accetto

1. INFORMAZIONI GENERALI

3. Nome e Cognome del proprietario del cane:

4. Numero di telefono:

5. Indirizzo e-mail:

6. Nome del cane:

7. Razza del cane:

8. Data di nascita del cane:

9. Sesso del cane:

Maschio

Femmina

10. Il cane è stato sterilizzato/ castrato?

Sì

No

11. Da quanto tempo il cane vive con lei?

meno di 6 mesi

6 mesi - 1 anno

1 anno o più

12. Dove vive il suo cane?

Sempre in casa

Sempre fuori

Un po' in casa, un po' fuori

Altro:

13. Quanto tempo il suo cane fa esercizio (giochi, passeggiate, attività sportive e di educazione) in una giornata tipo?

3 ore o più

2-3 ore

- 1-2 ore
- 30 minuti- 1 ora
- meno di 30 minuti

14.Svolge alcune attività con il suo cane (anche a casa)? Che cosa? (È possibile selezionare più opzioni)

- Obedience
- Agility
- Utilità e difesa
- Ricerca persone e salvataggio
- Addestramento per la caccia
- Sheepdog
- Mobility
- Dog dance
- Educazione di base e/o tricks
- Attività di fiuto
- Attività di attivazione mentale
- Altro:

15. Se ha selezionato almeno un'opzione delle precedenti, con quale frequenza svolge queste attività con il suo cane?

- Una volta all'anno
- 1-2 volte al mese
- 1-2 volte a settimana
- 2-4 volte a settimana
- Quasi ogni giorno
- Più volte al giorno

16.È disposto a venire all'Università con il suo cane per eseguire un test comportamentale (durata di circa 1 ora)?

- Sì
- No

17.Ha già partecipato con il suo cane a qualcuno dei nostri studi?

- Sì
- No

18.Il suo cane ha qualche problema di salute? Se sì, quali?

19.Il suo cane assume farmaci? Se sì quali e perché?

2. INCONTRO CON PERSONE SCONOSCIUTE

20. Il suo cane abbaia eccessivamente o è estremamente eccitato quando qualcuno suona il campanello o bussa alla porta?

- Sempre o quasi sempre
- Spesso
- A volte
- Raramente
- Non lo so (se ad esempio il cane vive in giardino e non sente mai il campanello)

21. Il suo cane incontra mai persone sconosciute?*

- Sì
- No

Se ha risposto "NO" passi alla sezione successiva

22. Ha mai osservato uno o più di questi comportamenti quando il suo cane incontra una persona sconosciuta, indipendentemente dal comportamento di questa persona? (È possibile selezionare più opzioni). Se il suo cane non si è mai comportato in questo modo, passi alla sezione successiva

- Si allontana
- Abbaia (non va verso la persona)
- Ringhia (non va verso la persona)
- Abbaia e/o ringhia e va verso la persona
- Coda bassa/tra le zampe
- Sta vicino al proprietario
- Evita il contatto visivo con la persona conosciuta (ad esempio annusando a terra)
- Ha il pelo dritto sulla schiena, sul collo o sulla coda

23. Quanto spesso il cane mostra questi comportamenti quando incontra una persona sconosciuta?

- Sempre o quasi sempre
- Spesso
- A volte
- Raramente

24. Per quanto tempo il cane è agitato/preoccupato dopo che lo sconosciuto è andato via? Il cane torna alla normalità:

- Subito dopo
- In pochi minuti
- In 15 minuti -1 ora
- In 1-5 ore

3. INCONTRO CON CANI SCONOSCIUTI

25. Il suo cane incontra mai cani sconosciuti?*

- Sì
- No

Se ha risposto "NO" passi alla sezione successiva

26. Ha mai osservato uno o più di questi comportamenti quando il suo cane incontra un cane sconosciuto, indipendentemente dal comportamento di questo cane? (È possibile selezionare più opzioni). Se il suo cane non si è mai comportato in questo modo, passi alla sezione successiva

- Si allontana
- Abbaia (non va verso il cane)
- Ringhia (non va verso il cane)
- Abbaia e/o ringhia e va verso il cane
- Coda bassa/tra le gambe
- Evita il contatto visivo con il cane sconosciuto (ad esempio annusando a terra)
- Rimane vicino al proprietario
- Ha il pelo dritto sulla schiena, sul collo o sulla coda
- È di solito amichevole, ma risponde in modo aggressivo se un altro cane mostra un comportamento aggressivo
- È di solito amichevole, ma a volte inizia una lotta con l'altro cane

27. Quanto spesso il cane mostra questi comportamenti quando incontra un cane sconosciuto?

- Sempre o quasi sempre
- Spesso
- A volte
- Raramente

28. Per quanto tempo il cane è agitato/preoccupato dopo che il cane sconosciuto è andato via? Il cane torna alla normalità:

- Subito dopo
- In pochi minuti
- In 15 minuti -1 ora
- In 1-5 ore

4. SITUAZIONI E AMBIENTI NUOVI

29. Il suo cane si trova mai in nuove situazioni o nuovi ambienti?*

- Sì
- No

Se ha risposto "NO" passi alla sezione successiva

30. Ha mai osservato uno o più di questi comportamenti quando il suo cane è in una nuova situazione o in un nuovo ambiente? Non consideri la reazione del suo cane dal veterinario. (È possibile selezionare più opzioni). Se il suo cane non si è mai comportato in questo modo, passi alla sezione successiva

- Cerca di fuggire dal nuovo ambiente
- Abbaia
- Ha la coda bassa o tra le zampe
- Ansima
- Trema
- Se gli viene offerto del cibo non lo accetta
- Sobbalza se percepisce un rumore o un movimento
- Annusa l'ambiente continuando a guardarsi intorno con corpo rigido, si nota la parte bianca dell'occhio, le pupille sono dilatate
- Assume una postura bassa (mostra ad esempio alcuni di questi comportamenti: testa abbassata, orecchie indietro, baricentro abbassato, coda bassa o tra le zampe)
- Rimane fermo e rigido, non vuole esplorare il nuovo ambiente
- Rimane vicino al proprietario

31. Quanto spesso il cane mostra questi comportamenti quando è in una nuova situazione/nuovo ambiente?

- Sempre o quasi sempre
- Spesso
- A volte
- Raramente

32. Per quanto tempo il cane è agitato/preoccupato da quando lascia il nuovo ambiente? Il cane torna alla normalità:

- Subito dopo
- In pochi minuti
- In 15 minuti -1 ora
- In 1-5 ore

5. SENSIBILITÀ AL RUMORE

33. Il suo cane ha problemi di udito?*

- Sì
- No

34. Se ha risposto "Sì" alla precedente domanda specifichi quali problemi di udito ha il suo cane:*

TEMPORALE

35. Quanto spesso il suo cane sente un temporale?*

- Mai
- Alcune volte all'anno
- Circa una volta al mese
- Più volte al mese

Se il suo cane sente il temporale almeno alcune volte l'anno risponda a queste domande, altrimenti passi alla sezione relativa ai fuochi d'artificio

36. Il suo cane reagisce al suono del temporale? Risponda "NO" se il suo cane è sorpreso quando sente solo il primo tuono, ma si rilassa subito dopo.

- Sì
- No
- Non lo so (se non è sicuro della risposta dia un'occhiata ai comportamenti suggeriti nella domanda successiva)

37. Se ha risposto "NO" passi alla sezione sui fuochi d'artificio. Se ha risposto "Sì", indichi uno o più comportamenti che descrivono la reazione del suo cane. (È possibile selezionare più opzioni)

- Saliva, sbava
- Defeca
- Urina
- Distrugge o mastica oggetti
- Si lecca o si gratta eccessivamente
- Cerca di scappare o scappa
- Ansima
- Si nasconde (ad esempio sotto al letto)
- Trema
- Abbaia/ulula/piange
- Cammina avanti e indietro senza una meta, spesso seguendo sempre lo stesso percorso
- Si immobilizza, è rigido
- Coda bassa / tra le zampe
- Il cane si agita quando sente un tuono, la coda è alzata, può abbaiare
- Altro:

38. Quanto spesso il cane mostra questi comportamenti quando sente il temporale?

- Sempre o quasi sempre
- Spesso

A volte

Raramente

39. Per quanto tempo il cane è agitato/preoccupato da quando il rumore inizia? Il cane torna alla normalità:

Dopo pochi minuti dall'inizio del rumore, anche se il suono non è terminato

Dopo 15 minuti- 1 ora dall'inizio del rumore, anche se il suono non è terminato

Subito dopo che il suono è terminato

Dopo pochi minuti da quando il suono è terminato

Dopo 15 minuti-1 ora da quando il suono è terminato

Dopo 1-5 ore da quando il suono è terminato

Dopo più di 5 ore

Non lo so

FUOCHI D'ARTIFICIO

40. Quanto spesso il suo cane sente i fuochi d'artificio?*

Mai

Alcune volte all'anno

Circa una volta al mese

Più volte al mese

Se il suo cane sente i fuochi d'artificio almeno alcune volte l'anno risponda a queste domande, altrimenti passi alla sezione sugli spari

41. Il suo cane reagisce al suono dei fuochi d'artificio? Risponda "NO" se il suo cane è sorpreso quando sente solo il primo fuoco d'artificio, ma si rilassa subito dopo.

Sì

No

Non lo so (se non è sicuro della risposta dia un'occhiata ai comportamenti suggeriti nella domanda successiva)

42. Se ha risposto "NO" passi alla sezione sugli spari. Se ha risposto "Sì", indichi uno o più comportamenti che descrivono la reazione del suo cane. (È possibile selezionare più opzioni)

Saliva, sbava

Defeca

Urina

Distrugge o mastica oggetti

Si lecca o si gratta eccessivamente

Cerca di scappare o scappa

- Ansima
- Si nasconde (ad esempio sotto al letto)
- Trema
- Abbaia/ulula/piange
- Cammina avanti e indietro senza una meta, spesso seguendo sempre lo stesso percorso
- Si immobilizza, è rigido
- Coda bassa / tra le zampe
- Il cane si agita quando sente i fuochi, la coda è alzata, può abbaiare
- Altro:

43. Quanto spesso il cane mostra questi comportamenti quando sente i fuochi d'artificio?

- Sempre o quasi sempre
- Spesso
- A volte
- Raramente

44.

Per quanto tempo il cane è agitato/preoccupato da quando il rumore inizia? Il cane torna alla normalità:

- Dopo pochi minuti dall'inizio del rumore, anche se il suono non è terminato
- Dopo 15 minuti- 1 ora dall'inizio del rumore, anche se il suono non è terminato
- Subito dopo che il suono è terminato
- Dopo pochi minuti da quando il suono è terminato
- Dopo 15 minuti-1 ora da quando il suono è terminato
- Dopo 1-5 ore da quando il suono è terminato
- Dopo più di 5 ore
- Non lo so

SPARI

45. Quanto spesso il suo cane sente degli spari?*

- Mai
- Alcune volte all'anno
- Circa una volta al mese
- Più volte al mese

Se il suo cane sente gli spari almeno alcune volte l'anno risponda a queste domande, altrimenti [passi alla sezione sugli altri rumori](#)

46. Il suo cane reagisce al suono degli spari? Risponda "NO" se il suo cane è sorpreso quando sente solo il primo sparo, ma si rilassa subito dopo.

- Sì
- No
- Non lo so (se non è sicuro della risposta dia un'occhiata ai comportamenti suggeriti nella domanda successiva)

47. Se ha risposto "NO" passi alla sezione sugli altri rumori. Se ha risposto "Sì", indichi uno o più comportamenti che descrivono la reazione del suo cane. (È possibile selezionare più opzioni)

- Saliva, sbava
- Defeca
- Urina
- Distrugge o mastica oggetti
- Si lecca o si gratta eccessivamente
- Cerca di scappare o scappa
- Ansima
- Si nasconde (ad esempio sotto al letto)
- Trema
- Abbaia/ulula/piange
- Cammina avanti e indietro senza una meta, spesso seguendo sempre lo stesso percorso
- Si immobilizza, è rigido
- Coda bassa / tra le zampe
- Il cane si agita quando sente lo sparo, la coda è alzata, può abbaiare
- Altro:

48. Quanto spesso il cane mostra questi comportamenti quando sente gli spari?

- Sempre o quasi sempre
- Spesso
- A volte
- Raramente

49. Per quanto tempo il cane è agitato/preoccupato da quando il rumore inizia? Il cane torna alla normalità:

- Dopo pochi minuti dall'inizio del rumore, anche se il suono non è terminato
- Dopo 15 minuti- 1 ora dall'inizio del rumore, anche se il suono non è terminato
- Subito dopo che il suono è terminato
- Dopo pochi minuti da quando il suono è terminato
- Dopo 15 minuti-1 ora da quando il suono è terminato

- Dopo 1-5 ore da quando il suono è terminato
- Dopo più di 5 ore
- Non lo so

ALTRI RUMORI

50. Il suo cane reagisce ad altri rumori (aspirapolvere, sirene, ambulanze, sistemi di allarme, etc.)?

- Sì
- No
- Non lo so (se non è sicuro della risposta dia un'occhiata ai comportamenti suggeriti nella domanda successiva)

51. Se ha risposto "Sì", indichi uno o più comportamenti che descrivono la reazione del suo cane. Se ha risposto "NO" passi all'ultima domanda della sezione. (È possibile selezionare più opzioni)

- Saliva, sbava
- Defeca
- Urina
- Distrugge o mastica oggetti
- Si lecca o si gratta eccessivamente
- Cerca di scappare o scappa
- Ansima
- Si nasconde (ad esempio sotto al letto)
- Trema
- Abbaia/ulula/piange
- Cammina avanti e indietro senza una meta, spesso seguendo sempre lo stesso percorso
- Si immobilizza, è rigido
- Coda bassa / tra le zampe
- Il cane si agita quando sente il rumore, la coda è alzata, può abbaiare
- Altro:

52. Quanto spesso il cane mostra questi comportamenti quando sente altri rumori?

- Sempre o quasi sempre
- Spesso
- A volte
- Raramente

53. Per quanto tempo il cane è agitato/preoccupato da quando il rumore inizia? Il cane torna alla normalità:

- Dopo pochi minuti dall'inizio del rumore, anche se il suono non è terminato
- Dopo 15 minuti- 1 ora dall'inizio del rumore, anche se il suono non è terminato
- Subito dopo che il suono è terminato
- Dopo pochi minuti da quando il suono è terminato
- Dopo 15 minuti-1 ora da quando il suono è terminato
- Dopo 1-5 ore da quando il suono è terminato
- Dopo più di 5 ore
- Non lo so

54. Se il suo cane è sensibile e reagisce ai rumori (temporale, fuochi d'artificio, spari e/o altri rumori), ha cercato di aiutarlo? Se sì, con che metodo?

- No
- Sì, con farmaci
- Sì, con prodotti naturali
- Sì, con desensibilizzazione, ad es. riproduzione di registrazioni del suono che provoca paura
- Altro:

6. SUPERFICI E ALTEZZE

55. Il suo cane ha difficoltà/paura a camminare su una griglia metallica?

- Sì, sempre
- A volte, dipende dal luogo (ad esempio se è su una scala di metallo e vede il vuoto sotto)
- Raramente
- Mai
- Non lo so

56. Il suo cane ha difficoltà/paura a camminare su pavimenti lucidi (come ad esempio nei centri commerciali)?

- Sì, sempre
- A volte, dipende dal luogo
- Raramente
- Mai
- Non lo so

57. Il suo cane ha difficoltà/paura a salire le scale se vede il vuoto tra i gradini?

- Sì, sempre
- A volte, dipende dal luogo

Raramente

Mai

Non lo so

58. Il suo cane ha difficoltà/paura a salire le scale se non vede il vuoto tra i gradini?

Sì, sempre

A volte, dipende dal luogo

Raramente

Mai

Non lo so

59. Il suo cane ha difficoltà/paura a camminare vicino alle ringhiere di vetro, ad esempio al secondo piano di un centro commerciale?

Sì, sempre

A volte, dipende dal luogo

Raramente

Mai

Non lo so

60. Il suo cane ha problemi su altre superfici o in luoghi alti? Li descriva di seguito.

7. REAZIONI DEL CANE QUANDO È A CASA DA SOLO

61. Il suo cane è mai rimasto a casa da solo?*

Sì

No

Se ha risposto "NO" passi alla sezione successiva

62. Come fa a sapere cosa fa il suo cane quando è a casa da solo?

Ho una telecamera in casa

Me lo hanno detto i miei vicini

Percepisco lo stato d'animo del mio cane quando torno a casa

Il cane ha distrutto oggetti

Altro:

63. Ha mai osservato uno o più di questi comportamenti quando il suo cane resta a casa da solo? (È possibile selezionare più opzioni). Se il suo cane non si è mai comportato in questo modo, passi alla sezione successiva

Saliva, sbava

Urina o defeca

Distrugge/mastica le cose

- Si lecca o si gratta eccessivamente
- Ansima
- Ulula, abbaia o piange
- Sbadiglia
- Si nasconde (es: sotto al letto)
- Trema
- Cerca di fuggire (ad es. gratta la porta)
- Cammina avanti e indietro senza una meta, spesso seguendo sempre lo stesso percorso
- Perde l'appetito (ad es. non mangia il cibo che gli lasci)
- Altri sintomi gastrointestinali (vomito, diarrea)

64. Quanto spesso il cane mostra questi comportamenti quando è da solo?

- Sempre o quasi sempre
- Spesso
- A volte
- Raramente

8. COMPORAMENTO STEREOTIPATO

65. Il suo cane cerca di catturare la sua coda e/o gira su sé stesso? Il cane può fermarsi e fissare la coda.

- Non ho mai notato questo comportamento
- È successo alcune volte
- Accade circa ogni mese
- Accade ogni settimana
- Accade tutti i giorni o quasi

66. Il suo cane fissa/insegue riflessi o ombre o sembra che stia cercando di catturare cose invisibili?

- Non ho mai notato questo comportamento
- È successo alcune volte
- Accade circa ogni mese
- Accade ogni settimana
- Accade tutti i giorni o quasi

67. Il suo cane cammina senza meta seguendo un percorso fisso?

- Non ho mai notato questo comportamento
- È successo alcune volte
- Accade circa ogni mese
- Accade ogni settimana

Accade tutti i giorni o quasi

68. Il suo cane si morde o si lecca eccessivamente e per un tempo prolungato zampe, fianco o altra parte del corpo?

Non ho mai notato questo comportamento

È successo alcune volte

Accade circa ogni mese

Accade ogni settimana

Accade tutti i giorni o quasi

69. Il suo cane ha altri comportamenti "strani"? Li descriva di seguito:

70. Ci aiuti a migliorare! Ci sono domande del questionario che non ha capito o non erano chiare? Se sì, quali?

* Complessivamente, è stata svolta un'approfondita analisi delle risposte: se il proprietario ha risposto che il suo cane non si è mai trovato in una data situazione, ma contestualmente ha anche affermato che il suo cane ha mostrato in quella situazione comportamenti riconducibili ad ansia e stress, il valore assegnato al cane nella relativa categoria è stato trasformato in un dato mancante.

** Per quanto riguarda in particolare la categoria "sensibilità al rumore", è stata applicata un'ulteriore correzione: se il proprietario ha dichiarato che il suo cane ha una patologia che ne compromette l'udito, il punteggio del cane in questa categoria è stato trasformato in un dato mancante.

Table A. Conversion Table – English version

Answers were converted in numerical values as follows:

Items 20, 23, 27, 31, 38, 43, 48, 52, 64

Always or almost every time = 4,

Often = 3,

Sometimes = 2,

Seldom = 1

I do not know = NA (not available)

Items 24, 28, 32

Straight after = 1

In a few minutes = 2

In 15 minutes - 1 hour = 3

In 1 - 5 hours = 4

Items 39, 44, 49, 53

A few minutes after the noise started, even if it is not ended = 1

15 minutes - 1 hour after the noise started, even if it is not ended = 2

Right after the noise ends = 3

A few minutes after the noise ends = 4

15 minutes - 1 hour after the noise ends = 5

1 - 5 hours after the noise ends = 6

After more than 5 hours = 7

I do not know = NA (not available)

Items 55, 56, 57, 58, 59

Yes, always = 3

Sometimes, it depends on the environment = 2

Seldom = 1

Never = 0

I do not know = NA (not available)

Items 65, 66, 67, 68

I have never noticed this behaviour = 0

It happened a few times = 1

It happens about every month = 2

It happens every week = 3

It happens almost every day = 4

Table A. Conversion Table – Italian version

Le risposte sono state trasformate in un punteggio numerico secondo il seguente criterio:

Domande 20, 23, 27, 31, 38, 43, 48, 52, 64

Sempre o quasi sempre = 4,

Spesso = 3,

A volte = 2,

Raramente = 1

Non lo so = NA (dato mancante)

Domande 24, 28, 32

Subito dopo = 1

In pochi minuti = 2

In 15 minuti - 1 ora = 3

In 1 - 5 ore = 4

Domande 39, 44, 49, 53

Dopo pochi minuti dall'inizio del rumore, anche se il suono non è terminato = 1

Dopo 15 minuti - 1 ora dall'inizio del rumore, anche se il suono non è terminato = 2

Subito dopo che il suono è terminato = 3

Dopo pochi minuti da quando il suono è terminato = 4

Dopo 15 minuti - 1 ora da quando il suono è terminato = 5

Dopo 1 - 5 ore da quando il suono è terminato = 6

Dopo più di 5 ore = 7

Non lo so = NA (dato mancante)

Domande 55, 56, 57, 58, 59

Si, sempre = 3

A volte, dipende dal luogo = 2

Raramente = 1

Mai = 0

Non lo so = NA (dato mancante)

Domande 65, 66, 67, 68

Non ho mai notato questo comportamento = 0

È successo alcune volte = 1

Accade circa ogni mese = 2

Accade ogni settimana = 3

Accade tutti i giorni o quasi = 4

Table B. Characteristics of dogs tested with the judgment bias test

Name	Breed	Age (years)	Sex	Reproductive status	Training level	Group
Abanera	Rottweiler	1,80	F	Entire	2	NA
Akim	English Setter	6,30	M	Entire	2	NA
Akira	Mixed breed	3,35	M	Neutered	1	NA
Arya	Labrador Retriever	1,60	F	Entire	2	NA
Asia	Labrador Retriever	7,32	F	Entire	0	NA
Birra	Mixed breed	5,16	F	Neutered	2	A
Boris	Australian Shepherd	1,49	M	Entire	2	A
Charlie	Mixed breed	8,16	M	Neutered	2	NA
Cipolla	Jack Russell Terrier	7,87	F	Entire	2	A
Daisy	Cairn Terrier	3,67	F	Neutered	2	NA
Diego	Mixed breed	1,55	M	Entire	1	A
Elvira	Mixed breed	10,15	F	Neutered	2	A
Eve	Labrador Retriever	6,33	F	Neutered	2	NA
Falcor	Golden Retriever	7,01	M	Entire	1	A
Flo2	Mixed breed	2,86	F	Neutered	0	A
Flora2	Golden Retriever	2,23	F	Neutered	1	NA
Hallie	Australian Shepherd	7,91	F	Entire	0	NA
Hansel	Mixed breed	6,88	M	Neutered	1	A
Iago	Golden Retriever	10,75	M	Entire	2	NA
Junior	Labrador Retriever	1,83	M	Entire	2	NA
Lady	English Setter	7,16	F	Neutered	1	NA
Marley	Mixed breed	6,50	M	Neutered	2	A
Marley2	Mixed breed	9,62	M	Neutered	0	A
Mia	Border collie	2,80	F	Entire	1	A
Mia2	Mixed breed	8,05	F	Neutered	0	A

Mirtilla	English Cocker Spaniel	2,01	F	Neutered	0	A
Niky	Mixed breed	5,74	F	Neutered	1	A
Oro	Flat Coated Retriever	4,43	M	Entire	2	NA
Paquito	Jack Russell Terrier	13,32	M	Neutered	2	NA
Pedro	Mixed breed	2,45	M	Neutered	2	A
Pimpa2	Mixed breed	9,72	F	Entire	0	NA
Pippo	Miniature Pinscher	6,84	M	Entire	2	A
PippoGoal	Mixed breed	10,80	M	Entire	0	A
PJ	Golden Retriever	3,66	M	Entire	1	NA
Scott	Hovawart	3,19	M	Entire	2	NA
Sirius	Mixed breed	10,94	M	Neutered	2	A
SpecialAgentCooper	Mixed breed	3,19	M	Neutered	1	A
Tabata	Cairn Terrier	5,97	F	Neutered	1	NA

M = male, F = female, A = Anxious, NA = Non-Anxious

Conclusions

Limitations of the classical protocol for measuring optimism and pessimism in dogs

The judgment bias test (JBT) is a promising tool to evaluate the optimistic/pessimistic attitude of animals towards ambiguity and to infer the animals' emotional state consequently. The classical spatial JBT applied to dogs [1] entails that dogs learn to discriminate between a positive location (named P), where a bowl with a treat was placed, and a negative location (named N, at the opposite side of the room), where an empty bowl was placed. After learning to discriminate between P and N, dogs were presented with an empty bowl positioned in three new and ambiguous locations (named NP – near positive, M – middle, and NN – near negative) situated between P and N. Latencies to reach these ambiguous locations are interpreted as indexes of dog's optimistic or pessimistic expectations. However, this protocol not always led to clear results [2–5], suggesting the need for a deeper investigation on methodological and statistical aspects [6,7].

I applied the classical protocol to a population of shelter dogs and analyses revealed that this protocol has indeed many flaws [8]. First of all, dogs seemed to struggle to understand the association between the trained stimuli (P and N bowl location) and their outcomes (presence/absence of a treat in the bowl): approximately only three-quarters of all dogs managed to pass the training phase, in line with other studies [3]. This aspect must not be overlooked, as dogs that did not reach the learning criterion might be the ones who suffer from more negative mood: among other negative emotions, fear and anxiety could impair learning, concentration, working memory functioning and spatial navigation [9–14]. Therefore, this protocol carries a high risk of excluding dogs in a more negative welfare state from a study whose main focus is to investigate welfare status itself.

Even considering dogs who passed the training phase, some issues emerged: latencies to reach P and N were different, but the variabilities of these latencies were very high, especially regarding the intra-individual variability in latencies to reach N, suggesting a great inconsistency in responses which is not coherent with a stable learning [15–17]. The applied learning criterion (a difference of 0.5 seconds between the slowest approach to P and the fastest approach to N in the last six training trials) might have been insufficient to assess whether dogs indeed learned to discriminate between P and N.

These results might be ascribed to the very mild negative salience of the stimulus in N, i.e. an empty bowl [18], compared to the strong positive reinforcement of treats placed in P. Since responses to ambiguous and trained cues are compared in JBT to detect the expectation of the negative/positive outcome previously learned, an unstable learning makes assessing pessimistic/optimistic attitude unreliable and arduous.

The second critical aspect identified by my analyses regarded dog's failure in discriminating adjacent bowl locations (P and NP, in line with other studies [1,4,19], NP and M, M and NN). Given that emotions with same valence and different levels of arousal might cause different expectations about ambiguity (e.g., depression is characterised by low expectations of positive events – i.e. pessimistic bias towards NP - whereas a high expectation of negative events is typical of anxiety – i.e. pessimistic bias towards NN) [20–24], JBT requires subjects to be able to discriminate properly among all the cues (see for reviews [25,26])).

Finally, my analyses suggested that the position proposed for one of the two researchers might have a confounding influence on test outcomes, in line with other studies [3,6,27]: the researcher who placed the bowl on the ground was standing behind the M location throughout the whole test and latencies to reach that location were extremely short. Although this could be ascribed to a strong optimistic attitude, it is also possible that dogs could have reached this location so quickly because they were interested in the researcher, rather than the bowl.

A new protocol to assess judgment bias in dogs: improvements and achievements

Considering all the caveats of the classical protocol, I proposed a novel experimental set-up, to solve the observed issues and to obtain more reliable measures of canine optimism and pessimism [28].

In order to ease the learning process, the trained bowl locations (P and N) were replaced by two corridors (again named P and N): at the end of P dogs found their owner, while at the end of N (located at the opposite side of the arena) dogs found a blowing black cloth; the owner and the cloth were not visible from the corridors' entrance.

Is important to note that in this setting, the negative stimulus was represented by a proper negative outcome and not by the lack of a reward. The stronger difference between the payoff of the two trained stimuli helped dogs to understand the association between these stimuli and their outcomes: even if I applied a learning criterion that was more severe than the one used in the classical protocol, dogs learned faster (less training trials required) and almost all of them succeeded in the training phase. Behavioural analyses confirmed that the P corridor was perceived as a very pleasant location: the interest in P was maintained even when its door was closed and other corridors were accessible. Furthermore, dogs perceived the N corridor as an unpleasant stimulus: subjects exhibited more stress-related behaviours when N was accessible, than when P was accessible.

The shorter duration of the novel protocol (26 trials in the testing phase *versus* 42 trials in the classical protocol, maximum length of 15 s for each trial *versus* 30 s) may have contributed to resolve learning issues, facilitating dogs' concentration and preventing them to get tired or bored.

Learning was certainly more stable and solid: during the testing phase dogs chose to enter N only occasionally, latencies to enter P and N were different and their variabilities were extremely lower than the variabilities to enter ambiguous corridors. The latter were three empty corridors (named NP, M and NN) located between P and N, and behavioural analyses confirmed that they had an ambiguous

connotation from dogs' point of view: subjects were hesitant towards these stimuli, as they walked towards their open doors but they stopped before entering them more frequently than during P trials.

The ability to discriminate between adjacent bowl locations was fully achieved by dogs: this is an indicator that the novel protocol improved dogs' performance.

Finally, to prevent that the presence of researchers might influence test outcomes, the person who opened the corridors' door, whose function was analogous to the one of the researcher who placed the bowl on the ground in the classical protocol, stood near the open door and therefore she changed her position for every trial (e.g., she stood near the M door only during M trials). This way, even if her presence had a confounding influence on dogs' decisions, this effect would be the same towards each stimulus and it would not be restricted only towards one cue. Results confirmed that this adjustment solved the issue previously detected with the classic protocol: latencies to enter M were not so short anymore and they were instead intermediate between latencies to enter P and N.

Complexity of the evaluation of canine anxiety: is the judgment bias approach useful?

To evaluate the effectiveness of the new JBT protocol to assess canine optimism and pessimism, I compared performances of dogs in a putative negative affective state - i.e., subjects showing in everyday life behaviours suggesting an anxiety-like state - with the performance of dogs in a presumed positive emotional state [29]: the hypothesis was that the former would show a more pessimistic bias than the latter, especially towards the NN corridor, considering that anxiety entails high expectations of negative events.

To recruit the two samples of dogs (anxious dogs as cases, non-anxious dogs as control) I realized an online questionnaire, based on previously validated ones ([30–33]) and I asked dogs' owners if they had ever observed, in different situations, their dog showing one or more behaviours from a fixed list of behaviours potentially related to anxiety, and I also asked them to state the frequency of these behaviours and the time of recovery.

I tested dogs from both groups (Anxious – A group and Non-anxious – NA group) with the novel JBT protocol. Almost all of them passed the training phase, but seven dogs from the A group (N = 19) were extremely stressed when separated from their owners, and a change to the standard procedure was necessary: as these dogs came with two owners, I positioned one of them at the starting position during all the test. None of the NA dogs (N= 19) needed this adjustment; this is a potential index of the effectiveness of the survey in detecting the most anxious and the most non-anxious dogs.

I verified that dogs had perfectly learned the association between the trained stimuli (P and N corridors) and their outcomes: dogs behaved differently towards the two corridors and their behaviour was consistent among trials. Furthermore, dogs were able to discriminate between each pair of adjacent corridors, with latency gradually increasing from P to N corridor.

Even if JBT results were robust and consistent and all test requirements were satisfied (i.e., stable learning of trained stimuli, full discrimination among all the stimuli, gradual increase in latencies from P to N), anxious dogs were not more pessimistic than non-anxious ones; even dogs tested with the modified procedure (potentially the most anxious ones) did not behave differently towards ambiguous corridors compared to dogs tested with the standard procedure.

A possible explanation of these results could be related to issues in detecting anxious dogs: the questionnaire might not have been sensitive enough to identify the most anxious and the most non-anxious dogs. Even if dogs from A group behaved differently from dogs in NA group (as stated above, seven of them did not bear staying without their owners for few seconds), surveys could be obviously influenced by owners' biases, emotions, expectations and misinterpretations [32].

Furthermore, canine anxiety is among the most difficult behavioural problems to diagnose [34] and even clinical monitoring of anxiety tend to depend on unvalidated methods, with no standard general scale to assess the severity of anxiety [35]. Anxiety-disorders are probably the most common class of disorders in pet animals [34]: in a recent study, Salonen and colleagues found that 73% of Finnish pet dogs have some kind of highly problematic behaviour related to anxiety [36]; Kurachi and colleagues found that 50% of dog owners feel that their dog has anxiety-related behaviours [37]. However, these issues are frequently underestimated or not even noticed by owners [38,39]: dogs that exhibit subtle and not obvious signs of distress (e.g., withdrawal, inactivity, salivation, whimpering and pacing) may not be identified as having anxiety issues [40].

In a recent study about canine anxiety, questionnaire results were compared with behavioural test outcomes: a strong relationship between the two was found regarding fear towards humans and situations, but survey outcomes were not confirmed by a novel-space-exploration test, which is a behavioural test similar to the open field [32]. Promising results have been found using the open field test with laboratory dogs, but only related to the assessment of sound sensibility [41,42] and of diazepam effects [43]. To date, nothing is known about open field results with pet dogs [44] and surveys remain the main and more applied tool to study canine anxiety [32,33,36,38,45–48].

An interesting and unexpected result of my study is that dogs from the “Anxious” group (A) entered the N corridor more frequently than dogs from the “Non-anxious” group (NA); however, this difference was not found between dogs tested with the modified procedure (i.e., potentially more anxious dogs, who needed their owner always by their side during the test) and dogs tested with the standard procedure.

The most likely explanation of these results is that the test itself generates anxiety in more sensitive subjects: dogs from A group were more anxious to find their owner than the ones from AN group, and so they took more chances on the N corridor; however, A dogs tested with the modified procedure, which were comfortable with their owner standing by them, did not feel the urgency to reach the other owner. This raises doubts about the adequacy of this JBT protocol to test anxious dogs, especially regarding dogs who are in discomfort without their owner and/or in presence of stranger people: the JBT purpose is to assess a potential judgment bias to infer the animal’s long-term emotional state and not to elicit discrete emotions, such as stress or anxiety state, that could bias test results itself. Therefore, some methodological adjustments could be proposed to make more sensitive dogs feel comfortable (e.g., applying the modified procedure to all subjects or let the owner stand at the starting position and employing only food reward as reinforcement in P).

Overall and contrary to expectations, from this pilot study I can’t infer that anxious dogs have a more pessimistic attitude than non-anxious ones. The lack of differences in the attitude towards ambiguity between A and AN dogs could also be ascribed to the inadequacy of the protocol, or judgment bias test in general, in detecting dogs’ affective state. It is worth remembering that, despite of the large number of studies that have used JBTs to evaluate the affective state in animals, results are not always in line with predictions, with some studies even leading to null results or to opposite findings to those expected [7,49–52], particularly in dogs [2–5,19,53]), raising some doubts about the real meaning of JBT results. Some authors propose that, other than affective state, personality might also influence the judgment bias, as found in humans [54–56]: neuroticism is linked to pessimism and extraversion is linked to optimism [57]. Even if this topic is largely unexplored in non-human animals, some evidences

exist [58,59]: in case of dogs, the ones that are more sociable, more excitable and more prone to non-social fear appear more optimistic in JBT [60].

Furthermore, optimism is also linked to motivation other than to affective state: optimistic people give up less often than pessimistic ones who are in turn less self-confident and persistent [61,62]. Anderson and colleagues found that in humans, judgment bias was indeed related to motivational measurements [63]; Rygula and colleagues found evidences also in non-human animals: more optimistic rats were more motivated to gain reward than more pessimistic ones [64].

Overall, scientific research on domestic dog could help investigating affective state, personality, cognition, motivation and, finally, psychological welfare. Dogs represent excellent research subjects as models for human conditions: they allow us to investigate the basic emotional systems shared by social mammals [26], they share our environment and are susceptible to psychological disorders similar to ours [38].

Scientific research on domestic dogs is important also for dogs' welfare (regarding pets, shelter, stray, laboratory and working dogs [65–71]): this kind of research could help to inform owners on how to care about their pets and how to address their behavioural problems; it could also help to enrich shelter and laboratory environments [72], to enhance rehoming possibility for kennel dogs [67,73–75], to improve canine training techniques [76–78] and to guide artificial selection of canine breeds [79].

Hopefully, advancements in canine welfare science, together with a large-scale dissemination of this knowledge towards whoever is responsible for dogs' care (not only scientists, but also shelter staff, dog trainers and dogs' owners), might truly reciprocate a thousand-year-long relationship and make humans dog's best friends.

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