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A portable, automated apparatus for testing cognitive bias in dogs

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Animal welfare science focuses on the assessment and the potential optimisation of the quality of life of animals. Animal welfare studies have traditionally focused on identifying negative states tied to stressors such as those causing pain, fear, anxiety and frustration (Duncan 2006; Boissy et al. 2007), as it was assumed that they reflect poor welfare and that therefore good welfare would be an absence of these states (Duncan 2006). However, there are problems with this approach. For example, negative states are adaptive and consequences of a stress response may be protective (Korte et al. 2007). It has been suggested that assessments of animal welfare should not focus purely on avoiding pain and suffering, but should also provide positive, pleasurable activities and resources (Seligman & Csikszentmihalyi 2000). It is therefore of growing importance to identify accurate indicators of positive and negative affective state in animals.

One potential method of identifying positive and negative affective states in animals is cognitive bias. Cognitive bias is a term that has been used in the human literature to describe the effects of emotional state on information processing and decision-making (Hinde 1985; see Paul et al. 2005 for review). It is now being put to similar use in non-human animals, referring to the phenomenon of affective state influencing cognitive processes (Mendl et al. 2009). In animals, the cognitive process under investigation so far has been judgement bias. A judgement bias refers to how animals interpret ambiguous signals and whether they seem to expect more positive or negative outcomes. A negative affective state leads to an expectation of negative outcomes and a negative bias in the interpretation of ambiguous signals. This is being referred to in the animal cognitive bias literature as pessimism [e.g. Bateson & Matheson 2007; Burman et al. 2009]. A positive affective state leads to an expectation of positive outcomes and positive biases in signal interpretation, which is being referred to as optimism [e.g. Matheson et al. 2008; Brydges et al. 2011]. Environmental conditions

that induce either a state of positive or negative affect can be used to test this concept in animals by changing environmental conditions to induce positive or negative affect and then testing whether cognitive bias changes correspondingly. This approach has been reported in rats (Harding et al. 2004; Burman et al. 2008a), starlings (Bateson & Matheson 2007; Matheson et al. 2008; Brilot et al. 2010; Brydges et al. 2011; Douglas et al. 2012), sheep (Doyle et al. 2010b; 2011; Destrez et al. 2012), chickens (Lindström 2010; Salmeto et al. 2010), cats (Tami et al. 2011), macaques (Bethell et al. 2012), pigs (Douglas et al. 2012), dogs (Mendl et al. 2010) Burman et al. 2011) and even honeybees (Bateson et al. 2011). In the species studied to date, negative judgement biases tend to positively correlate with conditions known to induce negative affect, and positive judgement biases positively correlate with conditions known to induce positive affect. These results support the use of cognitive bias in animals as a potential indicator of both positive and negative affective state.

Some studies have found that individuals that display more stereotypic behaviour than their conspecifics are also more likely to be more pessimistic (Brilot et al. 2010; Bethell et al. 2012). If all individuals within a given population are assumed to be equally prone to optimism or pessimism, using cognitive bias as a welfare assessment tool would be relatively straightforward. However, if individuals differ in their inherent tendencies towards optimism or pessimism, any assessment of welfare would need to take this inherent tendency into account.

This study reports on the trial of a portable, automated apparatus to train an operant task and then discrimination between auditory cues of different tones (low and high) to reveal dogs' expectations and therefore their cognitive bias. The device was designed to collect data on cognitive bias in a range of dogs from different environments, investigate population levels of optimism and pessimism and explore factors that may affect its expression.

Methods

SUBJECTS

Dogs older than eight years were excluded to avoid recruiting dogs that may be affected by canine cognitive dysfunction. Dogs younger than one year were excluded to exclude the possible influence of social immaturity on cognitive bias. The included subjects were 23 dogs of various breeds. Seventeen of the dogs were recruited via a positive training and pet boarding company based in the North Shore suburbs of Sydney, Australia. These dogs belonged to members of the public and thus had variable housing, feeding and exercise arrangements. The remaining six dogs were sourced from Assistance Dogs Australia's (Heathcote, NSW, Australia) advanced training facility. These dogs were 1-2 years old. Details of the dogs in the study are shown in **Table 1**.

APPARATUS

The apparatus used in this study was designed to be portable and easy to set up and operate. It consists of three major external components: an interactive target that detects movement through the use of an infrared photointerruptor, and two feed trays assigned to either lactose-free milk or water. As a diet high in lactose is associated with diarrhoea in some dogs (Bennett and Coon 1966), lactose-free milk was chosen as a liquid reward to avoid causing digestive upsets. Throughout training and testing, dogs received a set amount of lactose-free milk and water ranging from 1-5mL, depending on their weight.

The apparatus prototype was constructed around an Arduino Uno micro-controller board (SmartProjects, Italy). The Arduino Uno controlled an LCD screen (V1.2 and V1.2: DFRobot, Beijing, China; V2.1: FORDATA ELECTRONIC Co. LTD, China), two peristaltic pumps (SmallPumps, Arlington, Texas, USA; part # SP200 517), six pin buttons (generic manufacturer, part# SP0710) used to set the training program variables, a power switch (generic manufacturer, part #:SK0960), and an infrared photointerruptor. The photointerruptor consisted of an infrared LED (Osram, Malaysia) and a phototransistor (Vishay, Germany). The flow rate on the pumps was approximately 100 mL/minute. Peristaltic pumps deliver small amounts of liquid by compressing a silicone delivery tube, thus ensuring the tubes were primed to deliver liquid the moment the pump was activated. The pumps were connected via plastic and silicone tubing to reservoirs in the form of 500mL intravenous transfusion bags and plastic tubing also delivered liquid from reservoirs to two feed trays fixed in front of the target. Each

delivery tube was dedicated to delivering either milk or water, and could be configured to deliver fluid into either the left hand tray or the right hand tray, thus allowing milk to be delivered to either side and controlling for any side preference shown by the dogs.

Four buttons provided a means to select options displayed on the LCD screen. This interface allowed the operator to select the weight class of the dog (0-7kg, 8-27kg, 28-47kg, 48kg+) the protocol (whether the milk tone was the highest tone or the lowest tone), the training phase, and to start the training session. The remaining two buttons activated the two pumps outside of the training program. This was essential for cleaning the tubes and pumps and priming the tubes before the training program began. A speaker volume control dial allowed adjustment of the volume of the tones emitted.

Training and cognitive bias testing

Dogs were trained in a go/no-go discrimination task where they were required to touch a target with their nose after a tone in order to trigger the delivery of a lactose-free milk reward or water. The tone informed the dog which outcome would be delivered, and thus whether they should go ahead and touch the target or avoid touching. When dogs showed a significant difference in their response to the two tones, the dog's judgement bias was probed by presenting 9 new, ambiguous tones that fell between the milk and water tones.

Three training phases were used to train the dogs in the discrimination task. These are summarised in **Table 1**. The testing phase was the cognitive bias test itself and was the only phase that included ambiguous signals. Training and test sessions were no more than 30-minutes long and consisted of four 5-minute training blocks and a 3-minute rest period between each training block. If dogs had not met success criteria within 30-minutes, they were given a subsequent training session within 24 hours.

Each dog was randomly assigned before being exposed to the apparatus to receive milk from either the left or right tray, and to protocol A, which had the highest tone as the milk tone and the lowest as the water tone, or protocol B, which was the reverse of protocol A. These were randomly allocated based on a coin toss. These measures were implemented to control for selective attention to one cue over the other and side preference.

Habituation

Dogs were habituated to the apparatus through a brief habituation program that involved placing a set number ($n=14$) of small liver treats around the apparatus for the dogs to find and consume. Dogs were then exposed to the apparatus tones. The volume was adjusted from the minimum starting point and set when the dog's ears came forward indicating they had noticed the tone. If the dog's ears did not come forward, the volume was set at maximum. The milk pump was then run manually until dogs licked the milk out of the milk tray without showing a response to the sound of the pump.

Training phases

Dogs were given at least one full session in each training phase, after which the criterion in Table 1. was implemented if it had not already been met. The only cue given in Training Phase 1 (TP1), Training Phase 2 (TP2) and Training Phase 2A (TP2A) was the tone associated with a lactose-free milk reward, henceforth, "milk tone". The tone associated with water delivery, henceforth, "water tone", was introduced in Training Phase 3 (TP3). Milk and water tones were played pseudo-randomly in TP3, with no more than two of the same tone being played in succession. This was in alignment with other similar cognitive bias studies in animals (Brilot et al. 2010; Doyle et al. 2010b).

Cognitive Bias Test (CBT)

Cognitive bias testing involved the presentation of auditory probes. The latency of the dog to respond to probe tones by touching the target was logged. The probes were interspersed throughout a regular training session. No more than two tones of the same type were played in a row, with the exception of probe tones, which were played randomly. Each of the 9 probes were presented twice in a cognitive bias test, and each dog was given 3 cognitive bias tests over the space of 2 weeks. A session of TP3 was run in the next session after each cognitive bias test to ensure responses to milk and water tones remained strong and dogs were given little chance to learn not to respond to any probe tones.

Statistics

All statistical analyses were carried out in R, version 2.15. A one-tailed Mann-Whitney U-test was used to test whether dogs were significantly faster to touch the target after milk tones than water tones. The 'survival' package was used to analyse cognitive bias tests using a Cox Proportional Hazards regression model. This model was chosen as the data was censored at 10 seconds. If dogs had not touched the target within 10 seconds of the tone, their latency was recorded as 10 seconds and marked as censored. This kind of analysis is called a survival

TABLE 1 Summary of training phases and cognitive bias testing phase.

Phase	Training Objective	Structure	Max. sessions allowed	Criteria
TP1	Dogs to pass nose through photointerruptor towards visual target.	8s block after reward triggered.	5	Reward trigger rate of at least 8 in 2 of 3 training blocks
TP2	Dogs to move their nose to the target on cue.	Milk tone played, 10s window to respond, 20s ITI.	3	80% successful trigger after tone for 2 of 3 training blocks
TP2A	Reduce reinforcement rate	Milk tone played, 10s window to respond, 30s ITI.	3	80% successful trigger after tone for 2 of 3 training blocks
TP3	Dogs to discriminate between 2 tones.	Milk or water tone played pseudo-randomly, 10s window to respond, 20s ITI.	25	Milk latency significantly shorter than water latency (Mann-Whitney U-test)
CBT	Test cognitive bias	2x9 probes, 15 water, 15 milk presented pseudo-randomly, 20s ITI.	N/A	N/A

analysis, and the dependent variable has two parts: the event indicator and the latency to the event. In this case, the event indicator is touching the target (or reaching the end of the 10-second window without touching the target), and critical latency is to touch the target after a tone. The regression model was built using the step-wise method and the final model was chosen using the Akaike Information Criterion (AIC).

Results

The fate of all dogs in the study is shown in Table 2. Fifteen of the 23 dogs included in the study completed all three cognitive bias tests. The exclusion rate was much higher in pet dogs (47%, $n=17$) than in Assistance Dogs Australia advanced training dogs (0%, $n=6$). Reasons for exclusion of dogs during the training program included inconsistent or low rates of targeting resulting in a failure to meet the criterion for TP1, extinguishing of targeting in later training phases when reinforcement rates decreased, and two dogs appeared to dislike the lactose-free milk, avoiding the milk tray and ignoring coaxing towards it from the experimenter. Dogs that completed training took 12-32 training sessions (Mean=19 \pm S.D=5.768) from TP1 to reaching the criterion at the end of TP3. The fifteen dogs that completed cognitive bias tests gave 144 responses to various cues each over the three cognitive bias tests.

Latency to touch the target during cognitive bias tests differed significantly between dogs ($N=15$, $DF=14.7$, $p<0.001$). The likelihood of dogs touching the target before the 10-second window closed was significantly less than that for the milk tone in all other cues ($N=2097$ responses, Coef. ranged from -0.79 to -1.851, $DF=1$, $p<0.001$ for all cues, SE ranged between 0.073 to 0.186). The test number (1-3) also had a significant effect, with a decrease in the likelihood of touching the target within the 10-second window with each successive test ($N=3$, Coef.=-0.082, s.e=0.033, $p=0.014$). The likelihood of dogs touching the target after each cue are shown in Figure 1. Figure 2 shows how the likelihood of touching the target for all cues differs between dogs.

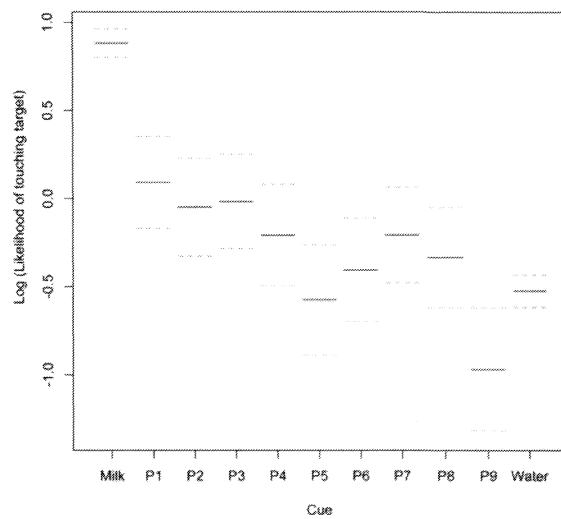


FIGURE 1 The likelihood of all dogs ($n=15$) touching the target before the 10-second time out for each cue shown on a log scale on the y-axis with the cues on the x-axis. Likelihood is high for the milk tone, showing all dogs were highly likely to touch the target after the milk tone. Standard errors are shown with the broken orange lines. The lowest likelihood was for P9, the probe most like water. This shows dogs were unlikely to touch the target after the P9 probe cue. P5, the most ambiguous cue, also showed a low likelihood of dogs touching the target after this cue. Ambiguity in this case may represent a response to the novelty of the most clearly unfamiliar signal.

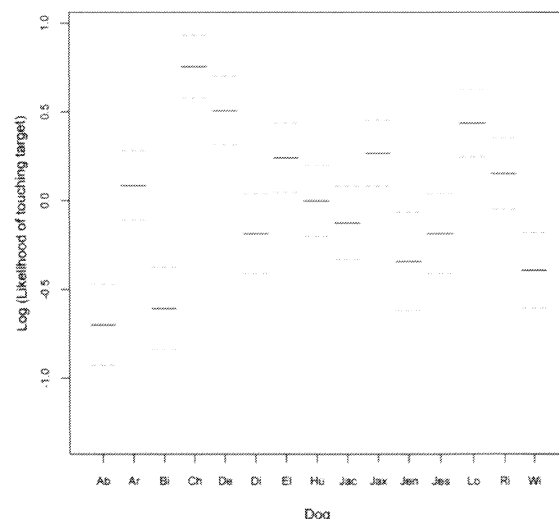


FIGURE 2 The likelihood of touching the target before the 10-second time out for all cues is shown on the y-axis in a log scale, and individual dogs are shown on the x-axis. Standard errors are shown with broken orange lines. Some dogs are much more likely to touch the target than others, for example, dogs "Ab" and "Bi" have a low likelihood of touching the target regardless of cue, and dogs "Ch" and "De" have a high likelihood of touching the target regardless of cue.



TABLE 2 A history of dogs in the study, showing where they were sourced from (ADA=Assistance Dogs Australia), their breed, sex (M=male, F=female) and reproductive status (N=neutered, E=entire), the protocol they were assigned to (A=milk tone lowest, B=milk tone highest), the side the milk was dispensed to, the training phase reached before the dog was excluded, and the reason for exclusion. Dogs that reached CBT (cognitive bias tests) were not excluded.

Dog	Source	Breed	Sex/ Reproductive status	Protocol	Milk tray side	Phase reached	Reason for exclusion
Jazz	Public	Spoodle	F/N	A	R	TP1	Inconsistency in targeting rate, ear interference
Murphy	Public	Whippet x Border collie	M/N	A	R	TP1	Rate of targeting too low
Declan	Public	Labrador	M/N	A	L	CBT	
Ellie	Public	Labrador	M/N	A	R	CBT	
Oscar	Public	Schnauzer	M/N (implant)	B	R	TP1	Avoided lactose-free milk
Jack	Public	Australian cattle dog	M/N	B	R	CBT	
Zack	Public	Maltese cross	M/N	A	R	TP2A	Targeting extinguished
Apollo	Public	German shepherd dog	M/N	A	L	Habituation	Avoided lactose-free milk
Ellie U	Public	Groodle	F/N	A	R	TP3	Targeting extinguished
Abbie	Public	Golden retriever	F/N	B	L	CBT	
Diesel	Public	Groodle	M/N	A	L	TP3	Targeting extinguished
Sinbad	Public	Border collie	M/N	A	R	TP3	Targeting extinguished
Jenna	Public	Border collie	F/N	A	L	CBT	
Jesse	Public	Border collie	F/N	A	R	CBT	
Lola	Public	Labrador	F/N	B	L	CBT	
Diesel T	Public	Rhodesian ridgeback	M/N	B	R	CBT	
Archie	Public	Pug x Schnauzer	M/N	B	R	CBT	
Chance	ADA	Labrador mix	M/N	A	R	CBT	
Hudson	ADA	Labrador	M/N	B	L	CBT	
Jaxon	ADA	Labrador	M/N	B	R	CBT	
Biscuit	ADA	Labrador	M/E	B	R	CBT	
Risky	ADA	Labrador mix	M/N	B	L	CBT	
Willow	ADA	Golden retriever	F/N	B	R	CBT	



Discussion

Latency to touch the target differed significantly between probes, showing that dogs respond differently to probes, being on average quicker to touch the target after probes that were more similar to the milk tone than those similar to the water tone. This supports the expectation that dogs respond differentially to signals and that this may correspond to their expectations of positive and negative outcomes. The dogs in this study were not subjected to any manipulations expected to alter their affective state or expectations. Therefore, no conclusions can be drawn from this study about the efficacy of cognitive bias in measuring affective state in dogs. Nonetheless, the differing responses between dogs suggests dogs are interpreting probes differently to each other, which adds support to the use of cognitive bias as a surrogate measure of affect. It is also possible that the differences shown in this study between dogs and their responses to probes represents different degrees of optimism and pessimism relating to personality differences rather than differences in affect. The root cause of these differences in responses between dogs is unknown. Cognitive biases in humans are sensitive to both short-term changes in an individual's level of anxiety (state anxiety) and long-term, individual difference in an individual's tendency to experience anxiety (trait anxiety). There is some evidence in animals that some individuals may be inherently more pessimistic than others, for example, stereotyping starlings and macaques are more pessimistic than non-stereotyping or reduced stereotyping conspecifics (Brilot et al. 2010; Bethell et al. 2012), and dogs that show indications of separation-related distress are more pessimistic than dogs that do not (Mendl et al. 2010). It is possible, particularly with Assistance Dogs Australia dogs that are kept in the same environment, that the differences in responses between dogs represent a fundamental difference in how individual dogs cope with challenging environments, or perhaps an inherent tendency towards optimism or pessimism akin to the trait anxiety described above.

Latencies differed significantly between cognitive bias tests, suggesting that some dogs at least may respond to fewer probes over time as they learn that probes are not reinforced. This effect has been documented in sheep (Doyle et al. 2010a), but despite being searched for in dogs, was not identified (Mendl et al. 2010). It is possible this effect was not found before in dogs because the method used by Mendl et al. (2010) required fewer trials (21-61 as opposed to at least 12 session of 48 trials each in this study) with fewer probes (4 vs 9 in this study), thus not giving dogs ($n=24$) the opportunity to learn that probes are unreinforced. Nonetheless, the effect of test

number on latency in this study was significant, but small (Regression coeff=-0.082, S.E.=0.033, D.F.=1, p -value=0.014). A refinement of the methodology presented here by reducing the number of probes may aid in reducing the test effect. However, reducing the number of probes may also reduce the power of detecting fine scale differences in optimism and pessimism between dogs. It was beyond the scope of this study to test the optimum number of probes to present, and this is part of the cognitive bias methodology that has not been systematically investigated yet.

Further research into the personality of dogs excluded from the study may reveal patterns in personality traits that may explain why the dogs are not able to complete the training. It is likely a certain level of optimism is necessary for dogs to persist with the self-directed training when reinforcement rates drop. The reinforcement rate was stepped down over three phases during training, which was adequate for many dogs, but may have been too fast or large a drop between phases for other dogs. A study that found that rats were more sensitive to reward loss when their welfare was compromised (Burman et al. 2008b) may help to explain why dogs failed to meet criteria during training. Although it is difficult to draw parallels between reward loss and a reduction in reinforcement rate, further research into the personality of those dogs being excluded due to extinction of the targeting behaviour may prove critical.

Conclusions

This study provides proof of concept for the portable, automatic apparatus used to both train dogs and test their cognitive bias. It also lends support to the use of cognitive bias as a tool to objectively measure affective state in dogs. Further research into the ideal number of probes to use and steps that may reduce the test number effect may improve the methodology currently used in this approach. Further research into extinction curves and personality of dogs that were excluded from the study may reveal important information about the affective state of dogs that failed to respond appropriately to early training.

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