

**A “How-To” Guide for Designing Judgement Bias Studies to Assess Captive Animal  
Welfare**

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Robust methods to assess animal emotion are essential for ensuring good welfare in captivity. Cognitive bias measures such as the judgement bias task have recently emerged as promising tools to assess animal emotion. The simple design and objective response measures make judgement bias tasks suitable for use across species and contexts. In reviewing 64 studies published to date, it emerged that (a) judgement biases have been measured in a number of mammals and birds, and an invertebrate; (b) no study has tested judgement bias in any species of fish, amphibian, or reptile; and (c) no study has yet investigated judgement bias in a zoo or aquarium. I propose that judgement bias measures are highly suitable for use with these understudied taxa and can provide new insight into welfare in endangered species housed in zoos and aquariums, where poor welfare impacts breeding success and, ultimately, species survival. I present a “how-to” guide to designing judgement bias tests with recommendations for working with currently neglected “exotics” including fishes, amphibians, and reptiles.

**Keywords:** animal welfare, cognitive bias, judgement bias, emotion, psychological wellbeing

## INTRODUCTION

### What Is Judgement Bias?

You wake up feeling happy (see Table 1 for glossary terms). You go to the kitchen and step on the mouse head your cat has left for you. The milk in the fridge is off. It is raining outside. Had you woken up in a bad mood, or if you suffer from anxiety or depression, any one of these events might have sent you back to bed. But you woke up happy, so you thank the cat for the present, have a rejuvenating juice, and appreciate how lush and green the garden is, before heading out. How you interpret day-to-day events, therefore, depends largely on your mood.

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Table 1 about here

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Empirical data from humans show that happy, content, people make more positive judgements about ambiguous information, recall more positive information, expect more positive events to occur in the future, and attend more readily to positive stimuli (Standage, Harris, & Fox, 2014). Having a tendency to make positive judgements about ambiguous or neutral stimuli increases the chances of positive biases in the other domains, leading to an upward spiral, or maintenance, of positive affective state (Seligmann, 1991).

People who are anxious or depressed, on the other hand, make more negative judgements about ambiguous information, recall more negative information, expect more negative events to occur in the future, and attend more readily to negative information (Bradley, Mogg, Millar, & White, 1995; MacLeod & Byrne, 1996; Mogg, Bradbury, & Bradley 2006; Richards et al., 2002; Richards, Holmes, Pell, & Bethell, 2013). Bad feelings

can have adaptive emotional underpinnings in a threatening environment: increased arousal in anxiety speeds responses to threat (Mogg, Bradley, & Williams, 1995); social withdrawal in depression conserves energy and may keep you away from harm (Nettle & Bateson, 2011). The heightened emotional response and accompanying feelings subside once the threat has passed, allowing time to attend to the more rewarding aspects of life (LeDoux, 1996). Anxiety and depression are “negative” affective states because, by definition, they make us “feel” bad, but their short-term survival value is high. Having a tendency to make negative judgements about ambiguous or neutral stimuli increases the chances of negative biases in the other domains leading to a “downward spiral” toward poor mental wellbeing, reduced quality of life, and ultimately clinical levels of anxiety or depression (Eysenck, Payne, & Santos, 2006).

### **Judgement Bias in Animals**

The first study to explore judgement bias in animals was conducted by Harding, Paul, and Mendl (2004, Figure 1a). The researchers trained rats on a discrimination task in which they learned to press a lever when they heard a tone that signaled reward (conditioned stimulus CS+), and not to press the lever when they heard a tone that signaled non-reward (conditioned stimulus CS-). Rats were then housed in either normal housing (and were therefore in a putatively more positive affective state) or poor housing (and were therefore in a putatively more negative affective state). After nine days, rats were played intermediate tones to those they had heard previously (ambiguous “probes”). Rats in the normal housing made more frequent and faster lever presses (“Go”) to intermediate tones than rats in the poorer quality housing: in other words, rats in the better housing made more “optimistic” judgements about the ambiguous tones than their more “pessimistic” counterparts.

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Fig 1 about here  
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### **Why Is Judgement Bias Important?**

Judgement bias is the most widely explored of a number of cognitive bias measures that present new methods to study animal welfare (see also Bethell, Holmes, MacLarnon, & Semple, 2012b; Burman, Parker, Paul, & Mendl, 2008b). This work builds on a long history of discussion about animal emotions (e.g., Darwin, 1872) and earlier work developing cognitive theories of emotion in animals (e.g., Dawkins, 1990; Desiré, Boissy, & Veissier, 2002; Duncan & Petherick, 1991; Spruijt, van den Bos, & Pijlman, 2000; Wemelsfelder, 1997).

Harding et al. (2004) used the term “cognitive bias” to describe the effect of emotion on judgements about ambiguous cues. Over the last decade, the paradigm has been reframed as the “judgement bias task” to reflect its presumed sensitivity to evaluative and expectancy processes (i.e., judgements about the reward value of ambiguous cues). The task may also be sensitive to other cognitive processes such as attention and memory (reviewed in Mendl, Burman, Parker, & Paul, 2009), and the term “judgement bias” may reflect as much the focus of researchers as the processes being measured. Herein I follow the literature and use the term judgement bias task to refer to variants of the original task developed by Harding et al. (2004). I use the term cognitive bias when discussing cognition-emotion interaction generally.

Arguments for the utility of cognitive bias over other (physiological and behavioral) measures are diverse (Paul, Harding, & Mendl 2005; Otovic & Hutchinson, 2015; cf. Wemelsfelder, 1997). First, cognitive bias measurements provide a metric for assessing

positive emotions in animals. There are few, if any, objective measures of positive emotions in animals despite widespread physiological and behavioral measures of negative emotions (Mellor, 2015; Paul et al., 2005). Good welfare requires the presence of positive states and a low occurrence of negative states (Boissy et al., 2007; Dawkins, 1990; Fraser & Duncan, 1998). In humans positive emotions are associated with positive judgement biases; therefore, in animals it is possible that positive judgement biases reflect positive emotions.

Moreover, the measurements may identify discrete emotions. In humans anxiety is associated with increased expectation of negative events, and depression is associated with both increased expectation of negative events and decreased expectation of positive events (Eysenck et al., 2006). Changes in response to ambiguous cues to reward and ambiguous cues to non-reward may allow us to distinguish different emotion states in animals (Bethell & Koyama, in press; Mendl et al., 2009).

The measurements also provide an inroad to assessing subjective feeling states in animals. Judgement — and other cognitive — biases provide an objective measure of emotion that is associated with subjective human experience (Fox, Cahill, & Zougkou, 2010; Mogg et al., 1995). The basic emotions are evolutionarily old survival mechanisms that arise from highly conserved brain structures that drove survival and reproduction throughout species' evolution (fear facilitates predator avoidance, attraction facilitates mating: Darwin, 1872; LeDoux, 1996). In the absence of evidence to the contrary, it is parsimonious to consider species which exhibit precursor processes that underlie subjective experience in humans may possibly experience subjective feeling states too (Paul et al., 2005).

In addition, they distinguish emotion from arousal. Physiological measures such as corticosteroids measure arousal, but they do not distinguish arousal associated with positive emotions (e.g., excitement and exploratory behavior associated with foraging and mating activity, or an enriched environment: Mendl, Burman, & Paul, 2010a) from arousal

associated with negative emotions (e.g., distress in fight or flight: Hemsworth, Mellor, Cronin, & Tilbrook, 2015; Selye, 1976). Cognitive bias tasks, by design, illicit responses that can be categorized as positive and negative, irrespective of level of arousal.

The measurements are sensitive to emotions not detected by behavioral measures. Behaviors may become dissociated from the underlying affective state (e.g., Higham, MacLarnon, Heistermann, Ross, & Semple 2009), be hard to distinguish (e.g., few overt behaviors distinguish a contented resting animal from one who is depressed or has learned helplessness), or only reflect extreme levels of suffering (e.g., stereotypes). Judgement bias may detect more subtle shifts in emotions that lack distinguishable behavioral indices.

The measurements are also advantageous because they allow a priori hypotheses and predictions. Judgement bias tasks provide opportunity to make a priori predictions about responses following affect manipulations. This could provide a useful means of assessing the effectiveness of husbandry interventions to improve mood state. (For example, improved housing, social introductions, and therapeutic drugs should all lead to more optimistic judgement bias.) A positive shift in judgement bias (i.e., more positive responses to one or more ambiguous probes) would allow staff to monitor improvements (or, by contrast, deterioration) in emotional or psychological state.

Cognitive bias measurements should be adaptable for all species. There is an adaptive benefit for all organisms to distinguish rewarding stimuli from dangerous stimuli (Nettle & Bateson, 2011). With careful design, the task should be adaptable to any animal species. The test has particular value as a tool to assess emotion in taxa for which measures of emotion do not currently exist, are limited, are considered too difficult to study, or are simply ignored due to our anthropocentric approach to the study and attribution of animal emotions (Morris, Knight, & Lesley, 2012) and cognitive abilities (Eddy, Gallup, & Povinelli, 1993).

The measurements also provide a generalizable measure across species and contexts. Outcome measures from the judgement bias task may be generalizable across species and settings. This would allow comparison of emotion states of the same species across different captive environments and between different species in similar settings, as well as improve our understanding of species similarities and differences in emotion and cognition.

There are clear theoretical arguments for developing cognitive bias measures such as the judgement bias task. These measures should improve our understanding of animal emotions within and across taxa.

## **METHODS TO MEASURE JUDGEMENT BIAS**

Well-designed operant tasks — such as those developed by Harding et al. (2004) — can be used to measure an animal's judgement bias by “asking” them whether they expect positive or negative outcomes following certain behaviors. Three types of judgement bias task have been developed.

### **Go-NoGo Task (+, -)**

The Go-NoGo task, using tone cues, has been adapted for use with dogs (Starling, Branson, Cody, Starling, & McGreevy, 2014) and pigs (Douglas, Bateson, Walsh, Bédoué, & Edwards, 2012: Table 2). Starling et al. (2014) developed an automated test and found individual differences in rates of discrimination training (9–33 sessions) and judgement bias in dogs who were categorized on a scale from “optimistic” to “pessimistic”. Douglas et al. (2012) developed a judgement bias test using tones for pigs and validated it using environmental enrichment. Enrichment led to a positive shift in judgement bias suggesting enriched pigs were more optimistic.

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Table 2 here

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The Go-NoGo task using spatial cues (Burman, Parker, Paul, & Mendl, 2008a: Figure 1b) has been more widely adopted. Animals are trained to approach one location for food and to avoid approaching another location that has no food, unpalatable food, or some other mildly aversive reinforcer. Tendency and speed to approach the intermediate probe locations are then tested. The Go-NoGo task with spatial cues has been developed for use with goats, pigs, sheep, horses, dogs, cats, mice, hamsters, rats, and chickens (Table 2). For each of these animal groups, there is at least one study reporting judgement bias effects.

In two studies with sheep, location cues were combined with color cues (Verbeek, Ferguson, & Lee, 2014a; Verbeek, Ferguson, de Monjour, & Lee, 2014b). In both studies, mood was manipulated with food restriction and so the CS+ and the CS- were social companions and a dog, respectively, avoiding confounding effects of hunger on the reward value of the CS+. In a third study with sheep, Vogeli, Lutz, Wolf, Wechsler, and Gyga (2014) positioned an air blower behind the “negative” location. It was left on through all trials, providing an additional cue to the location of the CS-. In most studies, however, spatial location is used without any additional cues.

The Go-NoGo task has also been developed with visual cues — usually lids that cover a container concealing either food or an unpalatable item — and lines of different length (primates: Pomerantz, Terkel, Suomi, & Paukner, 2012) and predator shapes (chicks: Hymel & Sufka, 2012) have also been used. This version of the Go-NoGo task has been developed with cattle, dogs, primates, chicks, and starlings (Table 2), and all these studies showed evidence for judgement bias in the species tested.

Despite its wide use, a criticism of the Go-NoGo task is that a “NoGo” may reflect any one of a number of different underlying processes including arousal, distraction, confusion, or lack of motivation from reduced hunger or thirst (Mendl et al., 2009).

### **Active Choice Task with Positive Reinforcement (+, +)**

A second type of judgement bias task, the active choice task, was developed to address problems of interpretation in the Go-NoGo task. Animals are trained to discriminate cues signaling high reward (CS+) or low reward (CS-) and then tested on their responses to intermediate cues. The active choice task is more robust since the two responses (Go) are equivalent and should not be differentially affected by changes in arousal, which is a problem for Go-NoGo tasks. The active choice task has been developed for use with pigs (tone cues: Murphy, Kraak, van den Broek, Nordquist, & van der Staay, 2015), grizzly bears (colored lid cues: Keen et al., 2013), capuchin monkeys (visual length cues: Pomerantz et al., 2012), rats (tone cues: Parker, Paul, Burman, Browne, & Mendl, 2014; sandpaper cues: Brydges, Hall, Nicolson, Holmes, & Hall, 2012; Chaby, Cavigelli, White, Wang, & Braithwaite, 2013), starlings (flashing light cues: Matheson, Asher, & Bateson, 2008), and laying hens (colored lid cues: Hernandez Hinch, Lea, Ferguson, & Lee, 2015). Evidence for an effect of emotion manipulation on judgement bias was reported in all studies except Hernandez et al. (2015).

Potential problems with the active choice task with positive reinforcement are that it may be more difficult to train animals to discriminate between the CS+ and CS- (both are rewards), and while the test may be suitable for detecting shifts in judgements about possible future rewards, it may not be suitable for detecting shifts in judgements about possible future neutral or negative events (Mendl et al., 2009).

### **Active Choice Task with Negative Reinforcement (+ Z )**

A third type of judgement bias task is the active choice task with negative reinforcement (e.g., electric shock: Rygula, Papciak, & Popik 2014). Animals are rewarded for responses to the CS+, but responses to the CS- are now negatively reinforced so that animals Go to the CS- in order to stop the onset of a negative reinforcer. Negative reinforcement is generally not suitable for welfare studies, but the results from this third approach, so far conducted with rats undergoing pharmacological manipulations (Table 2) concur with data from the Go-NoGo and active choice (reward-reward) tasks. Pharmacological studies using negative reinforcement are included in Table 2 for reference, but are not discussed further for designing welfare studies.

Table 2 lists the 64 peer-reviewed studies of judgement bias that were available for download in April 2015, ordered by animal group, type of task, and cues used. These studies show that (a) animals in a putatively more positive emotion state display a more positive judgement bias for ambiguous cues than animals in a putatively more negative emotion state (55 studies reported finding some evidence for judgement bias); (b) judgement biases may be reliably measured using well-designed studies with species-specific protocols; (c) these biases exist in a range of animal taxa including mammals (54/64 studies), birds (nine studies) and an invertebrate (one study); (d) only rats and sheep have been extensively studied (17 and 12 studies, respectively); (e) sample sizes are often modest (on average ~26; range 6 – 147); (f) amount of discrimination training needed and training accuracy vary greatly between species and research groups (range: 1-62 days of training; 50%–80% accuracy); (g) judgement bias has not been tested in any species of fish, amphibian, or reptile; (h) few studies have examined “exotics” (one study with grizzly bears; three with non-human primates); and (i) no study has been conducted in a zoo or aquarium.

## **PROBLEMS AND PITFALLS**

As the number of studies applying the judgement bias task has increased, issues in terminology, design, and interpretation have arisen. First, researchers use terms such as “optimistic bias” (Matheson et al., 2008) and “pessimism” (Bateson & Matheson, 2007) to describe changes in responses to the probes. However, the judgement bias task does not yet include a baseline against which to assess whether responses reflect categorically positive or categorically negative states. As a result, the task may therefore be less robust for measuring shorter-term emotions than longer-term moods.

In addition, experiments using longer-term affect manipulations showed mood-congruent shifts in judgement bias (e.g., Harding et al., 2004). Studies using shorter-term, acute stressors showed varying patterns. Doyle, Fisher, Hinch, Boissy, and Lee C (2010) and Sanger Doyle, Hinch, and Lee (2011) tested sheep after restraint for sheering and found a positive shift in judgement bias, suggesting the task captured animals’ relief at the termination of the stressor.

Moreover, the order in which affect manipulations are conducted can influence results. Bateson and Matheson (2007) and Douglas et al. (2012) found that when animals were moved from enriched to standard housing, there was a pronounced negative shift in judgement bias. When animals were moved from standard to enriched housing there was no, or a much reduced, shift in the other direction in starlings and pigs, respectively. Recent experience may therefore influence current performance.

Another issue that animals learn is that ambiguous probes are not rewarded over time. Doyle et al. (2010b), Starling et al. (2014), and Scollo, Gottardo, Contiero, and Edwards (2014) found that sheep, dogs and pigs, respectively, approached ambiguous probes less over repeated testing sessions. The task may therefore not be suitable for frequent or sustained testing.

A change in responses to the conditioned stimuli creates problems for interpreting responses to the probes. In Harding et al. (2004), rats in poor housing responded less to the CS+ and the probes closest to it. Bees who had undergone a simulated predatory attack showed a reduction in responses to the CS- as well as the probe next to it (Bateson, Desire, Gartside, & Wright, 2011). In both cases, alternative explanations such as changes in food motivation, arousal, learning, memory or risk-taking behavior cannot be ruled out.

Finally, early life experience and individual differences influence judgement bias. Brydges et al. (2012) found stress during the juvenile phase influences judgement bias in adult rats. Rats were trained on an active choice task with positive reinforcement. Females were faster to learn the discrimination task than males. Rats of both sexes who had experienced early life stress showed an (unpredicted) optimistic bias, suggesting sex and early life experiences can impact judgement bias as can confounding effects of impulsivity and risk-taking behavior by lighter-bodied animals in a foraging context. Briefer and McElligott (2013) found positive judgement bias in female, but not male, goats who had experienced former neglect. Starling et al. (2014) found variations in optimism and pessimism between dogs of different breeds. Gordon and Rogers (2014) found left-handed marmosets had a more negative judgement bias than right-handed marmosets.

The picture emerging is complex, but with further development the judgement bias method should provide insight and streamlined tools for assessing animal welfare. Probably the greatest value of cognitive bias measures for welfare will be in developing an individual animal's profile and, using infrequent tests, assessing changes in judgement bias over time. While we cannot yet identify categorically positive or negative emotion states, we can, at least, track shift in bias over time — identifying animals in downward or upward spirals. In the following sections, I apply the current state of knowledge to designing controlled judgement bias tasks for use with species that have yet to be tested.

## **HOW TO DESIGN A JUDGEMENT BIAS STUDY: A STEP-BY-STEP GUIDE**

### **APPLIED TO FISH, AMPHIBIANS, AND REPTILES**

Figure 2 presents a roadmap for designing judgement bias tasks (left column) with a worked example of the decision process applied to hamsters (middle column) and recommendations for designing studies with fish, amphibians, and reptiles (right column). For researchers designing studies with mammals, birds, or invertebrates — especially exotics in these categories — Table 2 provides a breakdown of test design, cues, and response modalities tested to date, with information on training duration and training criteria that may be useful for planning.

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Fig 2. here

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#### **Purpose**

Is your purpose to (a) develop a protocol and validate the judgement bias task for use with your chosen species or (b) refine your protocol to assess changes in emotion? The former requires an emotion manipulation. Include additional tests to assess alternative explanations and identify confounds. Manipulations may include testing before and after disruptive handling procedures (examples for fish: Ashley, 2007; Brydges, Boulcott, Ellis, & Braithwaite, 2009) or enrichment (fish: Schroeder, Jones, Young, & Sneddon, 2014; reptiles and amphibians: Burghardt, 2013). Behavioral indicators of affect may include aggression, redirected activities, stereotypies, displacement behaviors, weight change, and change in skin color (fish: Ashley, 2007; reptiles: Silvestre, 2014). Consider the possible influence of sex,

age, weight, and early life experience. Use a cross-over design to control for order of testing (e.g., Bateson & Matheson, 2007).

Assess the speed at which animals learn that probes are never rewarded with repeated trials over days or weeks (e.g., Doyle et al., 2010). Changes in motivation can be assessed by looking for reduced responding to the CS+, or consumption of reinforcers. A change in responses to the CS+ or CS- may indicate a change in memory, motivation, arousal, or impulsivity, rather than changes in judgements about ambiguity. Additional indicators of arousal may include glucocorticoid levels and locomotion (fish: Ashley, 2007; reptiles: Silvestre, 2014).

Assess response to novelty: Brydges et al. (2012) measured neophobia in sticklebacks, bishops, and trout by placing fish in a tank with a bright plastic toy and recording the distance of the fish to the toy every 20s for 10 min. Fish that spent more time away from the novel toy were considered more fearful. Because probes are novel stimuli, it is important to understand how your species responds to novelty. Finally, include at least three probes. Studies showed changes in responses at the different probes, and this may reflect different emotions associated with reward and non-reward (e.g., Bethell & Koyama, in press).

## **Type of Task**

The majority of published studies used the Go-NoGo task (45/64: Table 2), indicating this may be the easiest to develop initially. Natural preferences for certain foods and situations can be co-opted for these tasks (e.g., zebrafish exhibit light and substrate color preferences, possibly shaped by early life experience: Blaser & Vira, 2014; fire-bellied toads prefer lepidopteran larvae over other foods, depending on the season: Jenkin & Laberge, 2010). Evidence for rudimentary numerical abilities in fish (e.g., Agrillo & Bizassa, 2014),

amphibians (e.g., Krusche, Uller, & Dicke, 2010), and reptiles (e.g. Burghardt, 2013) suggest active choice tasks with positive reinforcement could be developed with these taxa.

## **Cues**

Most published studies used visual, location, and auditory cues (Table 2). Figure 2 summarizes a number of studies in which fish, amphibians, and reptiles have been trained to discriminate visual and auditory cues. Fire-bellied toads were trained to discriminate video footage of black prey (crickets) from video of white prey (Jenkin & Laberge, 2010). Toads were rewarded (with a live cricket presented at eye-level) for snapping at one video (CS+), but not for snapping at the other video (CS-). Toads took part in one 5-min trial per day, conducted at 3-to-4-day intervals, and learned the discrimination task within six (black cricket group) or nine (white cricket group) sessions. It would be small steps to include intermediate (grey) prey cues or to introduce unpalatable food for incorrect snaps to the CS-.

Touch and smell cues may also be appropriate for development. Fish (Marvit & Crawford, 2000; Zion, Karplus, & Barki, 2010) and frogs (Bee, 2015) have been successfully trained to discriminate sound-pressure cues. Zion et al. (2010) trained carp to discriminate between two acoustic stimuli that differed in frequency and temporal pattern. Fish were rewarded for approaching a feeder when one tone (CS+) was played, but not when the other (CS-) was played. Fish were played 36 trials per day (3 CS+ and 33 CS-) and learned the discrimination task in 21 days, but failed to learn on a schedule with fewer daily trials.

## **Response**

Fish, amphibians, and reptiles have a number of behaviors they exhibit when they anticipate food: toads snap (Jenkin & Laberge, 2010) and lizards tongue-flick (López, Ortega, & Martín, 2014); salamanders nose tap pellets they expect to contain useful chemosensory



information (e.g., food, conspecifics: Jaeger & Forester, 1993). Mueller-Paul et al. (2014) successfully trained two juvenile tortoises to use a touch screen, taking a minimum of 15 sessions to learn to touch a single stimulus, although two other tortoises failed to learn the basic task.

## **Resources and Reviews**

Reviews of cognitive theories of emotion present the rationale for using cognitive bias measures and discuss limitations and future directions (Mellor, 2015; Mendl & Paul, 2004; Mendl et al., 2009; Otovic & Hutchinson, 2015; Paul et al, 2005). For researchers designing studies, there are a number of taxa-specific reviews (pigs: Murphy, Nordquist, & van der Staay, 2014; rodents: Hales, Stuart, Anderson, & Robinson, 2014; dogs: Rayment et al 2015; dogs and horses: Starling, Branson, Cody, & McGreevy, 2013; farm livestock: Baciadonna & McElligott, 2015; insects: Mendl, Paul & Chittka, 2011). Papers that review cognitive bias in the context of animal models of human psychopathology discuss the effect of pharmacological manipulations on cognitive processes and may be useful for designing studies around veterinary interventions (Cocker & Winstanley, 2015; Hales et al., 2014). For designing studies with as-yet unstudied taxa, Burghardt (2013) provides a recent review of reptile and amphibian cognition; and Braithwaite, Huntingford, and van den Bos (2013), Broglio et al. (2003) and Brown, and Laland and Kruse (2008) provide good reviews of fish cognitive abilities.

## **Other Cognitive Bias Methods**

I have focused on the judgement bias task because of the breadth of data available. A number of other cognitive bias measure are worthy of further exploration: attention biases — innate biases in attention to biologically relevant stimuli (macaques' attention to facial expressions:

Bethell et al., 2012b; starlings' attention to eye-spots: Brilot, Normandale, Parkin, & Bateson, 2009; parrots' attention to a human observer: Cussen & Mench, 2014; sheep's attention to food: Verbeek et al., 2014; for earlier discussion see Mendl et al., 2009; Paul et al., 2005); expectancy biases — akin to optimism and pessimism regarding future events (rats: van der Harst, Baars & Spruijt, 2003), and reward sensitivity — susceptibility to despondency following loss or failure (rats: Burman et al., 2008b; Mendl et al., 2009). These processes likely feed into judgement biases and are worthy of more focused study in themselves.

## CONCLUSION

In summary, the judgement bias task has been adapted to study emotion in a number of mammals and birds, and an invertebrate, in domestic, research, and agricultural settings. Judgement bias has not yet been tested in many exotics, and in no species of fish, amphibians or reptiles; nor has it been used in a zoo. In the first decade of judgement bias studies, investigators focused on identifying whether manipulations presumed to be negative (e.g., restraint) or positive (e.g., enrichment) lead to a change in judgement bias, proving the concept and identifying appropriate cues and test designs for different species. Challenges for the next decade are to refine methods for working with small groups (e.g., high conservation status individuals), develop quicker and easier methods to assess welfare in real-world captive situations, and to validate the test for understudied groups such as the fishes, amphibians, and reptiles; data from these taxa may prove to be the most fruitful use of the judgement bias task yet.

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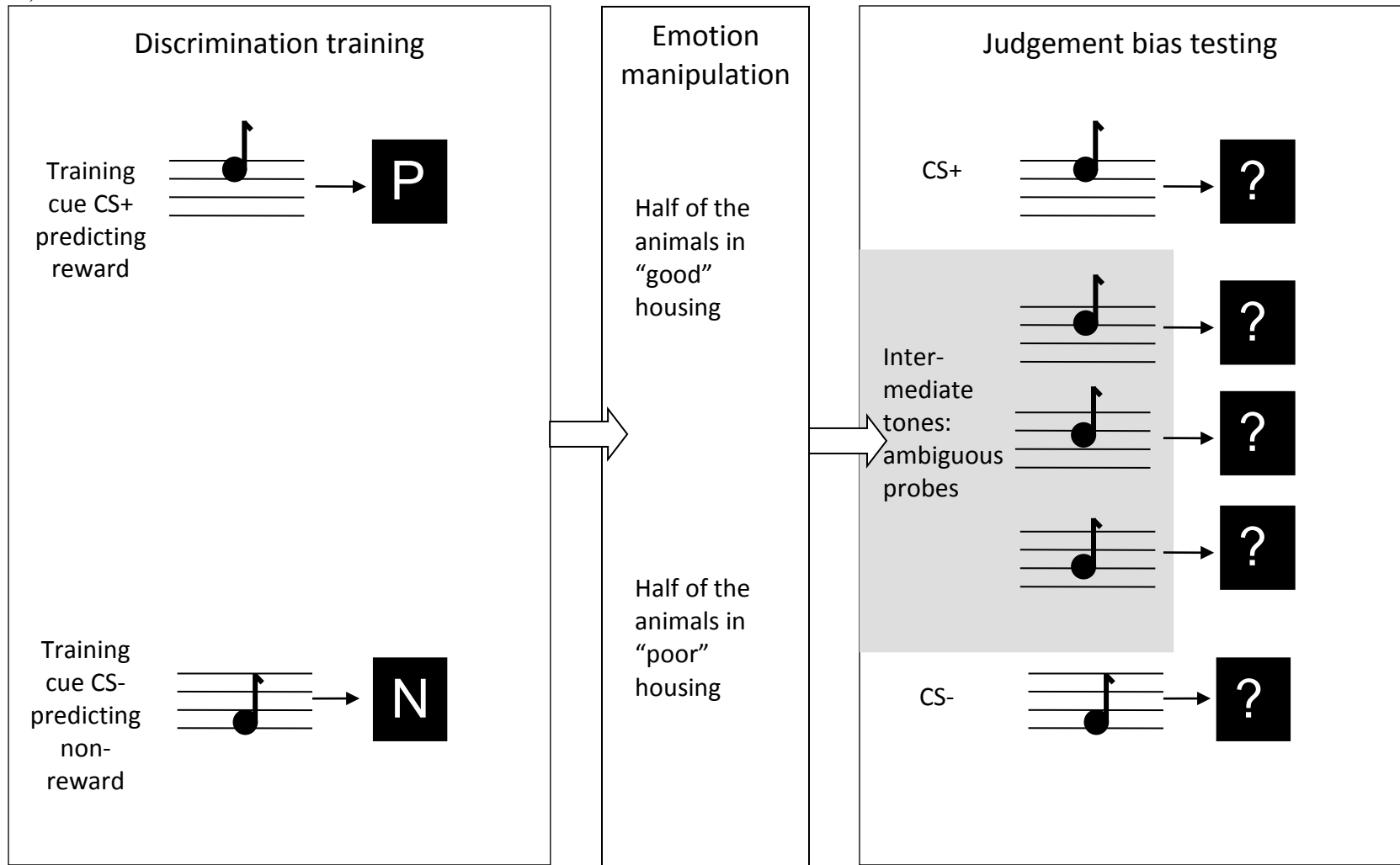
Animal silhouettes downloaded from [www.PublicDomainPictures.net](http://www.PublicDomainPictures.net)

## Figure captions

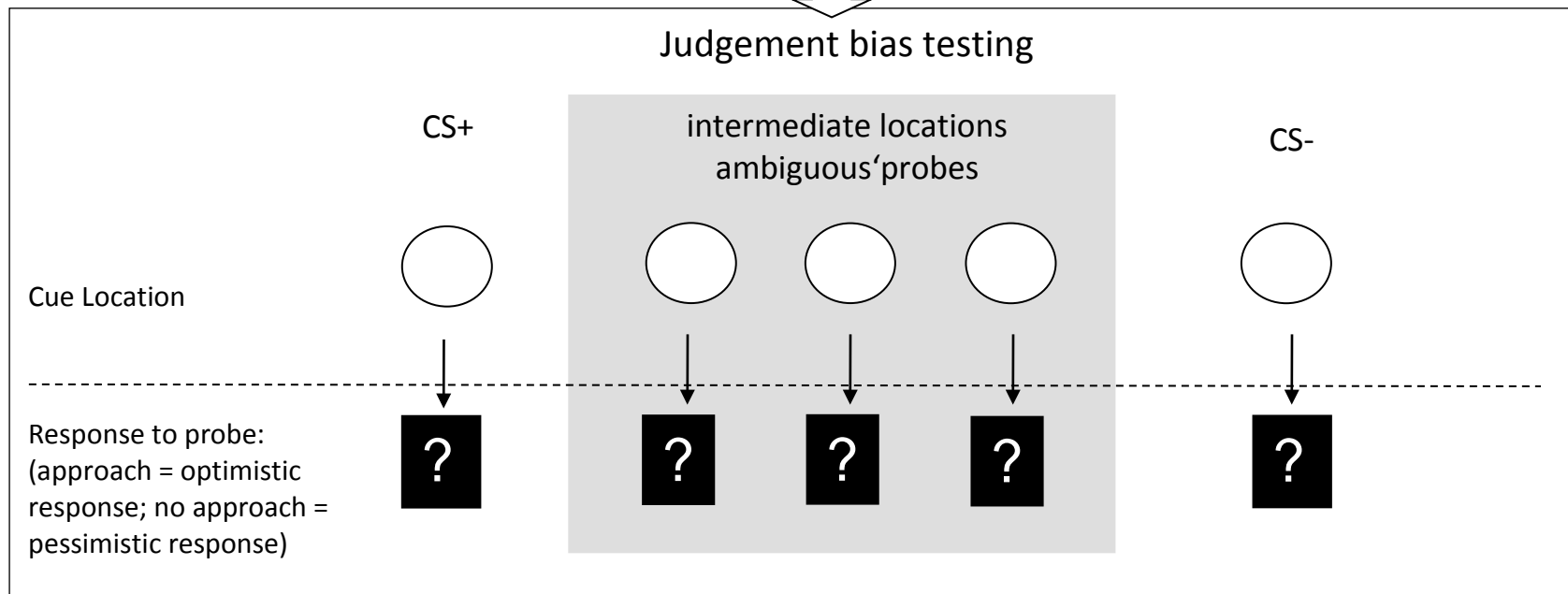
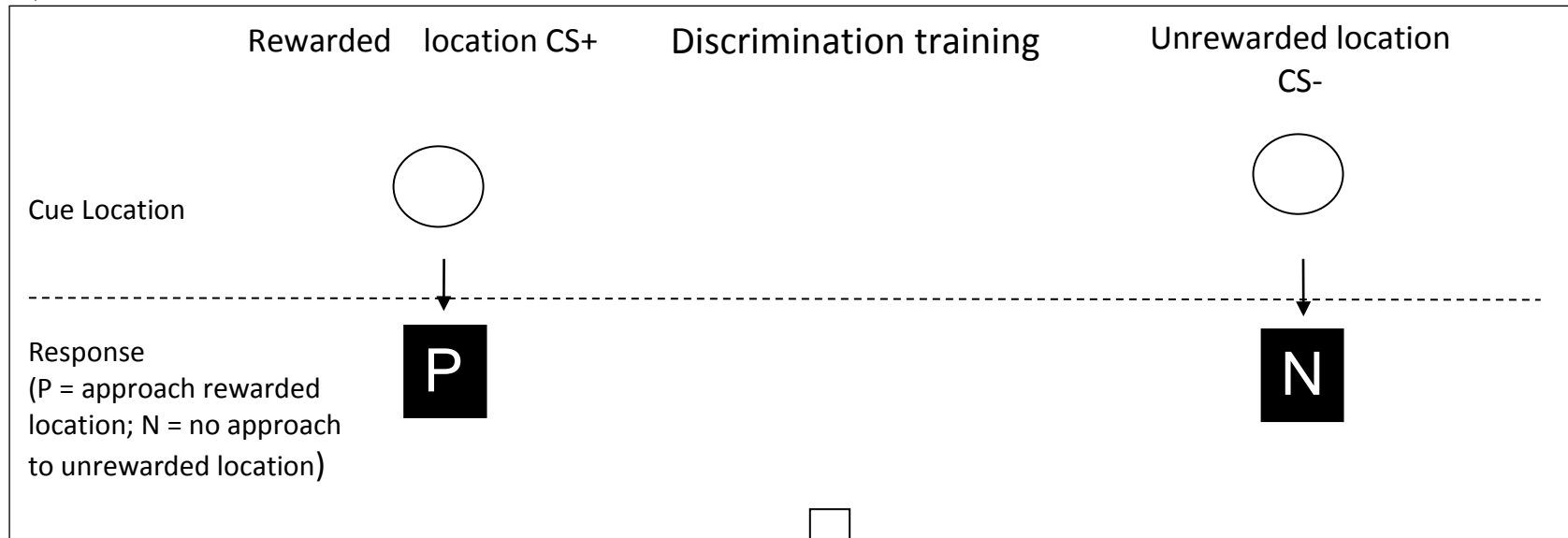
Figure 1. The Judgement bias task. (a) Go-NoGo task with tone cues used by Harding et al. (2004). Adapted with permission; Discrimination training: P = on hearing the CS+ tone, rats learned to press the lever for a reward; N = on hearing the CS- tone, rats learned not to press the lever to avoid a negative outcome; ? = the response variable – a higher proportion or speed of responses to ambiguous probes suggests a more optimistic judgement bias. (b) Go-NoGo task with location cues. P = approach the CS+ for reward; N = do not approach the CS-, to avoid non-reward.

Figure 2. Judgement bias decision tree. The left-hand column shows a decision tree for designing a judgement bias task. The central column shows a worked example of how decisions were made in a study with hamsters. The right-hand column contains suggestions for designing judgement bias tasks with fish, amphibians, and reptiles.

1a)



1b)
























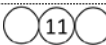





	Decision tree	Example decision process: hamsters <sup>1</sup>	‘How to’ suggestions: fish, amphibians and reptiles
Purpose?	<pre> graph TD     A{Is your purpose to develop a protocol to validate JB in your species?} -- Yes --&gt; B(Use robust manipulations of emotion state Run additional tests to explore alternative explanations for results Use at least 3 probes)     A -- No --&gt; C{Is your purpose to refine your JB protocol to assess emotion in your species?}     C -- Yes --&gt; D(Quick tests Few trials Minimal training Minimal number of probes)           </pre>	<p>Our purpose was to develop a JB test with hamsters. We manipulated emotion state by testing during a week of enrichment and during a week with none.</p> <p>Habituation and discrimination training lasted 4 weeks to maximise discrimination learning. JB testing lasted 2 weeks to investigate learning about probes over time.</p> <p>We included control trials to control for arousal effects and motivation. We ran additional tests (open field, light-dark emergence and neophobia) to explore whether the results could be explained by changes in exploration, risk-taking behaviour or novelty (since the probes were ‘novel’ locations).</p> <p>We used 3 intermediate probe locations to increase chances of finding effects at either end.</p> <p><i>Having proven the concept, we can simplify the design for future testing.</i></p>	<p>Initial development and validation of a protocol requires time and additional tests to explore alternative explanations and confounds.</p> <ul style="list-style-type: none"> <li>• Learning and memory (run repeated trials over time)</li> <li>• Motivation (do responses to CS+orCS- change during testing? Do animals consume less of the CS+?; run additional tests of attention biases)</li> <li>• Arousal and impulsivity (are there overall changes in response speed or accuracy at the CS+ and CS-?)</li> <li>• Response to novelty (run additional neophobia tests)</li> <li>• Change in responses to the probes closest to the CS+ may reflect different emotions to change in responses to probes closest to the CS- (use at least 3 probes).</li> </ul> <p><i>Considerations:</i> A future challenge is to refine tasks to be quick and easy to run. Streamlined designs using in-housing automated systems<sup>2</sup> and naturalistic stimuli<sup>3</sup> are worth further exploration</p>
Which type of task?	<pre> graph TD     A{Do animals learn to discriminate high v low reward?} -- Yes --&gt; B(++ )     A -- No --&gt; C{Do animals learn to discriminate reward v non-reward?}     C -- Yes --&gt; D(+- )     C -- No --&gt; E(+ )           </pre>	<p>A Go-NoGo test (+ - ) was used as we expected this to be the easiest discrimination task for hamsters initially.</p> <p>Review and pilot testing revealed hamsters will work for sugar water (CS+) while they find quinine aversive (CS-). we used an empty drinker on 50% of the CS+ and CS- trials to ensure hamsters were responding to location and not olfactory cues, and to reduce one-trial learning that probe trials were not rewarded.</p> <p><i>Considerations:</i> Pilot tests revealed hamsters chose sugar water over plain water, and chose plain water over quinine water. These could be used in future tests using the active choice test (++).</p> <p><i>Pilot tests familiarised hamsters with sugar and quinine water and drinkers prior to training so there were no ‘novelty’ effects during testing.</i></p>	<p>Fish, amphibians and reptiles exhibit preferences (and aversion) for a range of reinforcers.</p> <p><b>Fish</b> exhibit natural preferences for particular foods, social contact and substrates<sup>4-6</sup>, and show aversion to some tastes, falling stone, net trawl, colour, social and predatory cues<sup>4,7</sup>. Some ability to count<sup>8</sup>.</p> <p><b>Amphibians</b> exhibit natural preferences for some foods, social cues and environmental complexity<sup>9-11</sup>, and aversion to some social cues<sup>10</sup>. Some ability to count<sup>12-13</sup>.</p> <p><b>Reptiles</b> exhibit natural preferences for foods and social contact<sup>9,14</sup>, and show aversion to some social cues<sup>14</sup>, anecdotally able to count<sup>9</sup>.</p> <p><i>Considerations:</i> Active choice tasks (+ +) discriminating between signals of high versus low rewards may be possible utilising species’ innate preferences and rudimentary numerical abilities.</p> <p><i>Active choice requires training to discriminate secondary reinforcers* (e.g. tones), which may be more difficult.</i></p>
Which cues?	<pre> graph LR     A{Species' primary sensory modality?} --&gt; B[Visual]     A --&gt; C[Auditory]     A --&gt; D[Touch haptic and somatosensory]     A --&gt; E[Taste and olfactory chemosensory]     A --&gt; F[Multimodal]     B --&gt; B1((5))     B --&gt; B2((5))     B --&gt; B3(Predator shapes, line length, flashing lights)     C --&gt; C1(( ))     D --&gt; D1(Sandpaper of different grades)     E --&gt; E1(Odour)     F --&gt; F1((5))           </pre>	<p>Literature review revealed hamsters reliably learn to discriminate spatial locations. Design chosen:</p> <p>Reward, non-reward, and intermediate spatial locations were positioned to be perceptually distinguishable (distance between CS+ and CS- was ~1m in a 1m x 1m arena).</p> <p><i>Considerations:</i> Data were lacking for colour vision, audition, touch and olfaction in hamsters. Data from other rodent species suggest it may be worth exploring these cues in the future.</p> <p><i>Control for potential odour cues from reinforcers by including unreinforced control trials (here, we used an empty drinker at each of the 5 locations on test trials). Alternative solutions are to have food present at locations but inaccessible<sup>22</sup> or delivering rewards to a central feeder after the response has been made<sup>23</sup>.</i></p>	<p>Known to respond to a wide range of visual, auditory, tactile and olfactory cues (fish<sup>4,5</sup>, amphibians<sup>9-10</sup>; reptiles<sup>9,14,15</sup>). There are published protocols for training on discrimination tasks:</p> <p><b>Fish:</b> <b>Visual</b> stimuli (e.g. geometric shapes, line lengths, quantities and some colours: zebrafish<sup>4</sup>, sharks<sup>5</sup>, mosquitofish<sup>16</sup>) <b>Auditory</b> (sound-pressure) stimuli differing in frequency or tempo (elephantfish<sup>17</sup>, carp<sup>18</sup>).</p> <p><b>Amphibians:</b> <b>Visual</b> stimuli (e.g. black v white prey: toads<sup>11</sup>; quantity of prey: salamanders<sup>12</sup>). <b>Auditory</b> (sound-pressure) stimuli differing in pulse rate (frogs<sup>19</sup>).</p> <p><b>Reptiles:</b> <b>Visual</b> stimuli (shades and colours: lizards<sup>20</sup>; turtles<sup>21</sup>).</p> <p><i>Considerations:</i> Human bias to study visual and auditory senses.</p> <p><i>Somatosensory and Chemosensory senses worth considering.</i></p> <p><i>Closely related species may detect stimuli differently: e.g. ability to discriminate UV and near-infrared light, possibly related to species differences in colouration in lizards<sup>20</sup>.</i></p> <p><i>Natural preferences may be utilised in designing tests for discrimination training using naturalistic stimuli</i></p>
Which response?	<pre> graph TD     A{Do you have access to, or money to buy, operant training equipment?} -- Yes --&gt; B('Press lever'; 'touch screen')     A -- No --&gt; C('Approach'; 'touch target'; 'flip lid')           </pre>	<p>We collected data on frequency and latency to approach by observing and recording all trials.</p> <p>Criterion for an ‘approach’ was to move to within a few millimetres of the drinker (but not to drink from the drinker on probe or control trials). We intended this would reduce chances of learning that probe trials were unrewarded.</p> <p><i>Considerations:</i> Training animals to approach a location, touch a target or flip a lid to directly access a reward (the primary reinforcer*) may require fewer trials than training animals to associate rewards with other types of secondary reinforcer* (e.g. a tone).</p>	<p>Use natural responses to reward or threat to asses positive and negative expectations, such as approach and feeding gestures.</p> <p>Fish can be trained to swim to reinforced locations. This is quicker and just as accurate as training them to press a lever for food<sup>16</sup>.</p> <p>Amphibians may ‘snap mouth’ when a food reward is expected (toads<sup>11</sup>) and ‘nose tap’ pellets when social information is expected (salamanders<sup>10</sup>).</p> <p>Tortoises can be touch-screen trained<sup>24</sup>. ‘Tongue-flick rate’ may indicate expectation of food in lizards<sup>15</sup>.</p>
	<p>1. Bethell &amp; Koyama (2015); 2. M.Mendl, <i>Pers. comm.</i>; 3. Brilot et al (2009); 4. Blaser &amp; Vira (2014); 5. Fuss &amp; Schluessel, (2015); 6. Schroeder et al (2014); 7. Gerlai et al (2009); 8. Agrillo &amp; Bizassa (2014); 9. Burghardt (2013); 10. Jaeger &amp; Forester (1993); 11. Jenkin &amp; Laberge (2010); 12. Krusche et al (2010); 13. Stancher (2015); 14. Scott et al (2013); 15. Lopez et al (2014); 16. Agrillo et al (2012); 17. Marvit &amp; Crawford (2000); 18. Zion et al (2010); 19. Bee (2015); 20. Martin et al (2015); 21. Leighty et al (2013); 22. Wheeler et al (2014); 23. Bethell et al (2012); 24. Mueller-Paul et al (2014).</p>		

Table 1: Glossary of terms

Term	Definition
<b>Affect</b>	A general term which covers short-lived emotions and longer lasting moods. Affect and mood are diffuse states. An emotion is elicited by a stimulus.
<b>Cognition/ Cognitive</b>	Information processing in the brain. In its broadest sense this includes attention, interpretation, action selection, and storage and retrieval (memory) processes.
<b>Cognitive bias</b>	In the field of animal cognition and welfare, this term describes the influence of affect on cognition. Positive emotions are coupled with positive cognitions, negative emotions with negative cognitions. In humans, emotion-cognition interaction leads to the conscious experience of feelings <del>which-that</del> underpin psychological wellbeing.
<b>Emotion</b>	An emotion is a short-lived response to a stimulus <del>which-that</del> guides animals towards rewards (e.g., food <del>and</del> , mates) and away from danger (e.g. freeze, flight, <del>and</del> fight). Emotions have behavioural, physiological, and neural components and can occur without awareness in humans and other animals.
<b>Feeling</b>	The subjective experience of underlying affective state, including (conscious) awareness of (preconscious) emotion.
<b>Judgement bias</b>	The influence of affect on <del>the</del> interpretation of, and response to, ambiguous stimuli. The model presumes that positive emotion or mood is reflected in more positive judgements about ambiguous stimuli (" <del>glass half full</del> "), whereas negative emotion or mood is reflected in more negative judgements about ambiguous stimuli (" <del>glass half empty</del> ").
<b>Judgement bias task</b>	A task in which an animal is trained to discriminate between two stimuli that differ along one dimension (e.g., size, colour, <del>and</del> location). One cue (e.g., " <del>left location</del> ") is rewarded and the other cue (e.g., " <del>right</del> " location) is not rewarded. During a judgement bias test, an ambiguous cue is presented (i.e., an intermediate location). Speed and frequency of response reflect whether the animal judges the intermediate cue to be more 'positive' or 'negative'.
<b>Operant task</b>	A task in which an animal learns to associate an action (e.g., flip lid, approach bowl, <del>and</del> touch target) with an outcome (e.g., get food).
<b>Parsimonious</b>	The law of parsimony states we should use the simplest explanation for a phenomenon <del>—</del> i.e., one that makes the fewest assumptions.
<b>Reinforcer</b>	A reinforcer is something an animal will work to gain (food is a positive reinforcer) or to avoid (electric shock is a negative reinforcer). Food and shock are known as primary reinforcers because they are intrinsically rewarding and aversive. Secondary reinforcers are cues <del>which-that</del> come to be associated with a primary reinforcer through learning <del>—</del> e.g., that a black lid signals food, and a white lid signals non-reward.
<b>Welfare</b>	The physical health and physiological functioning of an individual, the opportunity to express natural behaviours, and the balance of positive emotions <del>with</del> negative emotions. Definitions of welfare vary greatly between disciplines, often restricted by those components considered " <del>measurable</del> " using each discipline's methods. All acknowledge it may include a psychological or affective component, even if the means to measure these have been lacking.
<b>Wellbeing</b>	<del>"Welfare"</del> with particular emphasis on the psychological and affective components. <del>It may</del> include a conscious experience. I use the terms 'welfare' and 'well-being' interchangeably, as both definitions include psychological and affective components.




















Table 2: Published studies of the judgement bias task arranged by the six major animal classes (\*approx. number of orders within the class). Mammals, birds and invertebrates are represented; reptiles, amphibians and fishes have yet to be tested.

Animal group-/ Species	Test design	Cues	Resp.	Training sessions days ( <i>n</i> trials)	Training accuracy	Setting	References	<i>n</i>	
Mammals (26*)									
Artiodactyla (even toed ungulates)									
<i>Capra hircus</i> Goat	 		App.	3 (18)	Latency (+ < -)	D	Briefer et al., 2013	18	
<i>Bos taurus</i> Dairy cattle	 		App.	>16 (>368)	85% <del>never</del> 3 days	U	Daros et al., 2014	13	
			Nose	>50 (>850)	90%		Neave et al., 2013	8	
<i>Sus scrofa</i> pigs	 		App.	6 (66)	Latency (+ < -) <del>xx</del> 5 days	U	Dupjan et al., 2013 <sup>x</sup>	30	
				30 (105)	Latency (+ < -) <del>xx</del> 6 trials	F	Scollo et al., 2014	40	
	 			16 (380)	80% (16/20)		Douglas et al., 2012	10	
				30 (364)	80%		Murphy et al., 2013 <sup>x</sup> & 2015	15 16	
	<i>Ovis aries</i> sheep	 			App.	18 (76)	10/10 <del>x</del> 2 days	F	Destrez et al., 2013
>15 (>69)			Feed from positive bucket			Destrez et al., 2014	30		
<36 (<180)			Prop (binomial)			Doyle et al., 2011a	26		
>15 (>67)			App. 9/12 <del>'''</del> <del>'''</del> in <10 s; No <del>go</del> 6/8 <del>'''</del> <del>'''</del> buckets <del>xx</del> 4 days			Destrez et al., 2012	16		
 			App.	30 (45)	20/20 <del>never</del> 4 days	U	Guldimann, 2015	24	
				23 (>85)	25/25 <del>never</del> 5 days		Sanger et al., 2011	24	
				30 (>150)	15/15 <del>never</del> 3 days		Doyle et al., 2010a	20	
							Doyle et al., 2011b	36	
				30 (45)	20/20 <del>never</del> 4 days		Doyle et al., 2010b	17	
				23 (>85)	25/25 <del>never</del> 5 days	F	Verbeek et al., 2014a & 2014b	41 34	
			30 (>150)	15/15 <del>never</del> 3 days	Vogeli et al., 2014		24		
Perissodactyla (odd toed ungulates)									
<i>Equus caballus</i> horse – mares	 		App.	6 (24)	Latency (+ < -, $p < 0.05$ ) <del>x</del> 2 days	F	Freymond et al., 2014	12	

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Animal group/ Species	Test design	Cues	Resp.	Training days ( <i>n</i> trials)	Training accuracy	Setting	References	<i>n</i>
Carnivora								
<i>Ursos arctos</i> Grizzly bear	 		Nose vs. paw press	35-50 (>1530)	90%	U	Keen et al., 2013 <sup>x</sup>	8
<i>Canis familiaris</i> domestic dog	 		App.	1 (15-61)	All latencies to “+” < any latencies to “-” on last 6 trials (3+; 3-) or last 10 trials (5+; 5-)	D	Titulaer et al., 2013 <sup>x</sup> ;	53
				2 (<140)			Karaglannis et al., 2015; Walker et al., 2014 <sup>x</sup> ; Mendl et al., 2010 <sup>x</sup> ; Muller et al., 2012 <sup>x</sup>	12
				1 (21-45)			Kis et al., 2015	23-
							3 (90-220)	Burman et al., 2011
			Nose	9-33 (<1,986)	80%	D	Starling et al., 2014	64
<i>Felis sylvestris</i> Domestic cat	 		App.	6-12 (72-144)	Latency (+ < -, $p < 0.05$ ) $\times 2$ days	D	Tami et al., 2011	40
Primates								
<i>Callithrix jacchus</i> Marmoset	 		Flip lid	10-22 (200–440)	85% $\times 3$ days	U	Gordon & Rogers, 2015	12
<i>Macaca mulatta</i> macaque	 	Visual (line length)	Hand touch target	19-43 (1,678-2,666)	70% $\times 3$ days	U	Bethell et al., 2012	7
<i>Cebus apella</i> capuchin				14 (210)	14/18 $\times 3$ days	U	Pomerantz et al., 2012	12

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Animal group/ Species	Test design	Cues	Resp.	Training sessions (n trials + habituation)	Training accuracy	Setting	References	n
Rodentia								
<i>Mus</i> sp Laboratory Mouse	+ -	○5○	App.	3 (13)	Latency (+ < - $p < 0.05$ ) on all trials on day 3	U	Kloke et al. 2014	50
		○3○		4 (21)				
		○3○		5 (25)				
		Odour		8 (12)	Latency to eat (+ < - $p < 0.05$ ) (group level analysis)		Boileij et al. 2012	100
<i>Mesocricetus auratus</i> hamster	+ -	○5○	App.	64%–84%	Latency (+ < - ) $\times \times$ 3 days	U	Bethell & Koyama (in press)	24
<i>Rattus norvegicus</i> rat				6 (72)	Latency (+ < - $p < 0.05$ ) on day 6		Burman et al. 2008a	24
	+ -	○5○	App.	5 (25)	Latency (+ < - $p < 0.05$ ) on day 2		Burman et al. 2009	24
				6-10 (37-85) 17-28 (180-376)	Latency (+ < - $p < 0.05$ ) $\times$ 2 days		Richter et al. 2012 Wheeler et al. 2015	24 42
		Speaker (x5)	Press lever	>12 (?)	50% $\times \times$ 3 days		Harding et al. 2004	9
		Sandpaper of different grades	App.	>10 (40) >25 (>62)	3/4 trials/day $\times \times$ 4 days 3/4 trials/day $\times \times$ 5 days	U	Chaby et al. 2013	12
	+ +						Brydges et al. 2011; Brydges et al. 2012	12; 24
		Speaker (x15)		33 (>1,400)	Performance significantly greater than chance (binomial test) $\times$ 3 days		Parker et al. 2014	16
		Speaker (x5)	Lever press	>16 (315) <62 (<6,200)	70% 80% $\times \times$ 2 days		Enkel et al. 2010 Anderson et al. 2013 <sup>x</sup>	16 20

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





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					3-36 (120-1,440)	70% <del>xx</del> 3_days		Papciak et al., 2013; Rygula et al., 2012, 2013, 2014a, 2014b, 2015a, 2015b	26-80
Birds (23*)									
Passeriformes									
<i>Sturnus vulgaris</i> Starlings				Flip lid	>3 (>48)	90%		Bateson & Matheson, 2007	6
					>21 (>366)	Latency (+ < $p < 0.05$ )	U	Brilot et al., 2010	8
			Flashing colored lights	Peck light	33 (1,782)	68%--84%		Matheson et al., 2008	8
Galliformes (Fowl)									
<i>Gallus gallus</i>					>18 (>198)	5_s faster app. <del>“+”</del> than <del>“+”</del>		Wichman et al., 2012 <sup>x</sup>	25 <sup>z</sup>
					<26 (<123)	2_s faster app. <del>“+”</del> than <del>“+”</del>		Seehuus et al., 2013	24
			Predator shapes	App.	2 (2)	<del>na</del> NA	U	Salmeto, 2011	40
					2 (2)	NA <del>na</del>		Hymel & Sufka, 2012	10-99
					>24 (>82)	90%		Hernandez, 2015 <sup>x</sup>	20
Invertebrates (>40*)									
<i>Apis mellifera</i> bee			Odor	App.	1 (12)	<del>na</del> NA	U	Bateson, 2011	147
Reptiles (4*)						No published studies			
Amphibians (3*)						No published studies			
Fishes (~60*)						No published studies			

Note. <sup>x</sup>approximate number of orders within the class. Mammals, birds and invertebrates are represented; reptiles, amphibians and fishes have yet to be tested.

Test design:   Go-NoGo task;   Active choice test, with positive reinforcement;   Go-Go test, with negative reinforcement; Cues:  -spatial

location task ( $n$  locations indicated);  -lids of different shades ( $n$  shades indicated);  -spatial location combined with different shades;  tones of different frequency ( $n$  tones indicated); other cue types given as text.<sup>z</sup>

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**Response:** App.: approach to within a specified distance of the cue; Nose: poke target with nose; Nose vs. paw press: bears were trained to press one colour target with their nose and the other target with a paw; Flip lid: remove lid from container using any body part (beak, hand); hand touch target: touch target with hand or paw — typically on a touch-sensitive monitor; Lever press: press an automated lever; peck light: birds were trained to peck lights of different colours.

**Training days:** indicative number of daily training sessions required to reach criterion (*n* trials in brackets/parentheses).

**Training accuracy:** predetermined criterion for completing training as reported in method section, or training accuracy reached as reported in results section.

**Setting:** D = domestic settings and rescue shelters; F = farming and agricultural settings; U = University and other research laboratories. X = no reported influence of affect manipulation on judgement bias.

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