Making Decisions under Ambiguity: Judgment Bias Tasks for Assessing Emotional State in Animals

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Judgment bias tasks (JBTs) are considered as a family of promising tools in the assessment of emotional states of animals. JBTs provide a cognitive measure of optimism and/or pessimism by recording behavioral responses to ambiguous stimuli. For instance, a negative emotional state is expected to produce a negative or pessimistic judgment of an ambiguous stimulus, whereas a positive emotional state produces a positive or optimistic judgment of the same ambiguous stimulus. Measuring an animal's emotional state or mood is relevant in both animal welfare research and biomedical research. This is reflected in the increasing use of JBTs in both research areas. We discuss the different implementations of JBTs with animals, with a focus on their potential as an accurate measure of emotional state. JBTs have been successfully applied to a very broad range of species, using many different types of testing equipment and experimental protocols. However, further validation of this test is deemed necessary. For example, the often extensive training period required for successful judgment bias testing remains a possible factor confounding results. Also, the issue of ambiguous stimuli losing their ambiguity with repeated testing requires additional attention. Possible improvements are suggested to further develop the JBTs in both animal welfare and biomedical research.

Keywords: cognitive bias, emotion, trait, state, cognition, discrimination learning, go/no-go task, go/go task

INTRODUCTION

Within the framework of animal welfare studies as well as in biomedical studies, assessment of the emotional state of an animal can yield highly relevant information. The majority of studies on animal emotions (most of them using rodent species) address anxiety, which is assessed with classical tests such as the open field test (OF), the light-dark (LD) test and the plus-maze (PM) test (for a recent critique of these tests see Ennaceur, 2014). These tests measure the unconditioned response of an animal to an unfamiliar situation (the testing environment) that contains elements which the animal perceives as adverse/threatening (such as open space and/or high light intensities). They may be less suited for assessing emotion in non-rodent species such as pigs (see, e.g., Murphy et al., 2014). Instead of looking at the animal’s response to unconditioned stimuli, one may use cognitive tests to assess emotion in animals, such as judgment bias tasks (JBTs; Harding et al., 2004; Paul et al., 2005; Murphy et al., 2013), or (variants of) decision...
making tasks (Murphy et al., 2015). Affective reactions may provide useful feedback, both explicitly and implicitly, from emotional appraisal processes (Storbeck and Clore, 2007). According to Marchant-Forde (2015), the most influential recent studies measuring emotional state as an index of animal welfare are those assessing judgment (cognitive) bias. Bateson and Nettle (2015) consider JBTs as the “gold standard” for measuring the mood of animals. In the area of biomedical research, cognitive bias research is still in its infancy, although the number of studies using this type of task is growing. Besides its value for the purpose of welfare assessment, within the area of biomedical research, the affective state of an animal may be a confound for other behavioral tests and a source of uncontrolled variation (Bateson and Nettle, 2015). Knowledge of the animals’ emotional state may contribute to understanding test results.

Emotion, Cognition and Judgment Bias

Emotions are adaptive processes that help individuals react adequately to internal or external stimuli, thereby avoiding harm and seeking valuable resources, while cognition can be described as information processing mechanisms. Emotions cannot be regarded separately from cognition. Emotional states affect cognitive processes and conversely cognitive processes are often the initiators of emotions (Lazarus, 1982; Dolcos, 2015). The interdependence of emotion and cognition is reflected in the definition by Kleinginna and Kleinginna, (1981 p. 355):

- “Emotion is a complex set of interactions among subjective and objective factors, mediated by neural–hormonal systems, which can
- (a) give rise to affective experiences such as feelings of arousal, pleasure/displeasure;
- (b) generate cognitive processes such as emotionally relevant perceptual effects, appraisals, labeling processes;
- (c) activate widespread physiological adjustments to the arousing conditions; and
- (d) lead to behavior that is often, but not always, expressive, goal-directed, and adaptive.”

The brain cannot be divided in cognitive and affective regions, since “affective” brain regions are also involved in cognition and brain regions that are viewed as cognitive are also involved in emotions. Cognition and emotion are integrated in the brain (Pessoa, 2008). Brain structures at the heart of the neural circuitry for emotion (e.g., the amygdala) impact cognitive processing from early attention allocation (Holland and Gallagher, 1999) through perceptual processing to memory (Phelps, 2006). Similarly, brain structures involved in the neural circuitry for cognition, such as dorsomedial and ventrolateral prefrontal cortex (DMPFC and VLPFC), have an intrinsic role in the experience of emotion (Barrett et al., 2007).

If one regards emotion as a result of an anticipated, experienced, or imagined outcome of an adaptationally relevant transaction between organism and environment, cognitive processes are always crucial in the elicitation of an emotion (Lazarus, 1982). Cognitive processes are closely linked to emotional states as they are, for example, necessary for the appraisal of environmental cues and for the “production” of emotions (Lazarus, 1982; Mathews and MacLeod, 1994). On the other hand, emotional states influence information processing in the brain, which helps individuals to react appropriately within a certain context (Mathews et al., 1997). Emotional influences on cognition are defined as cognitive biases, of which three types can be distinguished: attention biases, memory biases, and interpretation or judgment biases (see Paul et al., 2005). However, ascribing a reaction to a cue or stimulus to a cognitive bias implies that there is an unbiased, verifiable truth. It is, therefore, better to consider this phenomenon as result of “decision under ambiguity”, i.e., “judgment bias” instead of “cognitive bias”. Attention bias occurs in threatening situations as a result of an anxious emotional state and is characterized by an increased attention to negative and threatening cues (Mathews and MacLeod, 1994; Mogg and Bradley, 1998). Memory bias refers to the fact that events associated with positive or negative emotions are more readily remembered than neutral events, and includes memory storage, consolidation and retrieval processes (Cahill and McGaugh, 1996; Hamann et al., 1999). It is likely though, that the effects on memory are caused by high arousal and not by the valence of the emotion (Bradley et al., 1992). Judgment bias or interpretation bias (from now on referred to as judgment bias) refers to the influence of emotions on the interpretation of ambiguous information (Mathews et al., 1989; Eysenck et al., 1991; Richards and French, 1992).

There are numerous operational definitions of judgment bias. Combining definitions of Boleij et al. (2012) and Bateson and Nettle (2015),

A judgment bias is a relative reaction to an ambiguous stimulus, expressing an “interpretation” of this stimulus and an “expectation” about the consequences of the reaction (Boleij et al., 2012). In JBTs (“…) animals that respond to the ambiguous stimuli similarly to the positive stimulus are interpreted as displaying a high expectation of reward in the presence of ambiguous information, and hence an “optimistic” cognitive style indicative of a positive affective state. In contrast, animals that respond to the ambiguous stimuli similarly to the negative stimulus are interpreted as displaying a higher expectation of punishment or lower expectation of reward, and hence a more “pessimistic” cognitive style indicative of a more negative affective state”.

—(Bateson and Nettle, 2015, p. 3).

The processing of current information and the resulting behavioral choices are affected by optimism and pessimism (Dember et al., 1989). In JBTs, optimism is operationally defined “as a higher proportion of responses to an ambiguous cue as if it were the cue predicting the positive outcome, and pessimism as a higher proportion of responses to an ambiguous cue as if it were the cue predicting the negative outcome” (Douglas et al., 2012, p. 66). JBTs thus are believed to provide a cognitive measure of mood (Bateson et al., 2015; Mellor, 2015).

Aim of this Review

Since its introduction as a test for use in animals just a decade ago (Harding et al., 2004), a considerable number of JBTs
has been developed and applied in scientific studies, using a broad range of procedures and test equipment, in a large number of species (see “Supplementary Material, Table 1”, summarizing the publications about JBTs with animals as subjects). For continued (translational) research on animal emotions using these tasks, JBTs have to be validated and adapted to the abilities of each of these species (Anderson et al., 2012). Here, we review the different implementations of this task, and a number of questions to be solved, such as the cues and test arena’s used, measuring response latencies vs. categorizing responses as Go/No-go, and species-specific modifications. We discuss the potential of JBTs as a tool for measuring animals’ emotional state and to assess the effects of experimental manipulations of the emotional state. Outstanding questions for future research on measuring judgment bias in animals relevant for both animal welfare research and biomedical research are discussed.

**DISCRIMINATION LEARNING**

To successfully perform in a JBT, an animal has to first learn to discriminate between a stimulus (or set of stimuli) that predicts a positive consequence (S+) and a stimulus (or set of stimuli) that predicts a negative consequence (S−; see Figure 1). Once the animal has mastered this discrimination, at least one ambiguous stimulus is introduced that lies somewhere between the original stimuli, i.e., judgment bias is tested in situations where animals make decisions under ambiguity (Mendl et al., 2011; for an example, see Figure 2).

Two classes of JBTs can be distinguished: Go/Go and Go/No-go tasks. Go/No-go requires suppression of response at S−, whereas in Go/Go tasks the animal responds to both types of stimuli with an active response (Murphy et al., 2013).

In both Go/Go and Go/No-go tasks, the animal learns to discriminate between:

1. A favorable reward (large reward, immediate reward) and a less favorable reward (small reward, delayed reward), or
2. A positively valenced outcome (e.g., large food reward) and a negatively valenced outcome (e.g., small, less palatable food reward, or food with a bitter taste, no reward). In some cases, the negative outcomes consists of exposure to aversive noise or a frightening stimulus, such as a mild electric foot shock (e.g., Harding et al., 2004), a blower (e.g., Destrez et al., 2013), a dog (e.g., Doyle et al., 2011a), swaying a plastic bag in front of the animal (e.g., Douglas et al., 2012), i.e., consequences on a different modality than the consequences associated with the S+.

**JUDGMENT BIAS TASKS**

Cues used in JBTs are spatial (e.g., Briefer and McElligott, 2013; Destrez et al., 2013; Kis et al., 2015); visual (e.g., Salmeto et al., 2011); auditory (e.g., Murphy et al., 2013), olfactory (e.g., Boleij et al., 2012; see Figure 1), or a combination of different stimulus classes (Douglas et al., 2012). In the latter case it may be difficult to define ambiguous cues and their scaling on the continuum from S+ to S−. A large variety of testing equipment is used for judgment bias testing such as the home cage (e.g., Boleij et al., 2012), runways (e.g., Salmeto et al., 2011), open fields (e.g., Destrez et al., 2012), or mazes with arms radiating from a start box (e.g., Briefer and McElligott, 2013; see “Supplementary Figure 1”). Owing to the large range of animal species that has been tested in JBTs, species-specific modifications are necessary, concerning the size and layout of the testing arena (if any; e.g., dogs have been tested in their owner’s home: Karagiannis et al., 2015), the stimuli (cues) used; the type of response required (Go/Go, Go/No-go); the type of experimental manipulation used to affect emotion, and the type of consequences as result of the response to a cue (Figure 2; for an example see also Murphy et al., 2013).

**PEAK SHIFT**

When considering the use and results of JBTs, it should also be taken into account that basic psychological mechanisms such as generalization gradients and peak shift may play a role in responses to ambiguous stimuli in judgment bias paradigms. Generally, judgment bias paradigms start with the acquisition of a simple discrimination task, in which one stimulus provides a desired outcome (S+) and another stimulus provides an undesired outcome (S−). Thus, in Go/No-go tasks, responding (in whatever form the task requires) to the S+ increases, while responding to the S− decreases. This does not apply to Go/Go tasks, where maintenance of active responding to both the S+ and the S− is required.

It has been shown in a number of species, including humans, that when animals are trained using one S+ and then tested using stimuli similar to but not exactly the same as the S+, responses will be highest to the stimuli nearest to the original S+. This is called a generalization gradient (see Figure 3; Cheng et al., 1997). The response rate to intermediate stimuli found between an S+ and S− is thought to be predicted by the interaction between the two generalization gradients (Hanson, 1957; Kalish and Guttmann, 1957, 1959).

The distribution of responses in a generalization gradient around the S+ are usually symmetrical if only one stimulus is used. However, if a second stimulus is used (as in most JBTs), the peak of responses to the S+ may shift to a cue further from the S− (Ghirlanda and Enquist, 2003), a process termed peak shift. This particularly occurs if the S+ and S− are relatively similar to start with. A complicating factor for Go/No-go tasks is that it is difficult to assess whether there is a shift in the generalization gradient surrounding the S−, as there is generally a low response rate or no response at all to the S−, which predicts an undesired outcome (such as no reward). Results from studies specifically analyzing the responses to the S− seem to indicate that there is also a peak-shift in S− responses (Hanson, 1959), though it is not clear whether this is to the same degree as the influence on S+ responding. If peak shift differentially affects generalization...
FIGURE 1 | Schematic representation of judgment bias training and testing using visual, olfactory, spatial, or auditory cues, or a combination of cues from different stimulus dimensions (inspired by Bateson et al., 2011; Mendl et al., 2011). The experimental manipulation that is believed to affect emotion precedes the training phase (A; Scenario 1) or the testing phase (B; Scenario 2). Refreshment of the discrimination acquired during the training phase may be necessary, if the experimental manipulation preceding phase (B) lasts for a longer time period. An example of scenario 1 is studying the effects of growing up in different housing systems, whereas scenario 2 may be applied in a study assessing the effects of shorter lasting experimental manipulations, such as confinement, on emotion. Phase (B) may be repeated multiple times (e.g., Douglas et al., 2012) to test the effects of different experimental manipulations in the same animal. Specific challenges and limitations may be connected to the different phases. See Figure 2 for an example of the specific contingencies connected with responding to $S^+$, $S^-$ and ambiguous cues.

gradients surrounding the $S^+$ and $S^-$, then responding to ambiguous stimuli surrounding the $S^+$ and $S^-$ may also be differently affected.

For a better understanding of the processes underlying judgment bias, it may be necessary to address the generalization gradients around the discriminative stimuli used. This may include presenting ambiguous stimuli that are outside the values range between $S^+$ and $S^-$ (see Figure 3) to determine the role that peak shift may play in responding in JBTs (Ghirlanda and Enquist, 2003, p. 20).

STATE VS. TRAIT

Faustino et al. (2015) suggest that judgment bias may reflect either a state or a trait. However, JBTs have commonly been used to measure the affective state of an animal. Modulation of judgment bias through situational or contextual factors which can be observed as within-individual variability (e.g., by providing an enriched living environment, stress, or mood-enhancing drugs) is characteristic of a state. Emotional trait can be considered as a constant that is a permanent feature.
of the individual (Ramos and Mormède, 1998) i.e., may be the expression of a specific phenotype of an individual (Faustino et al., 2015). Similarly, Strelau (2001, p. 311) defined trait as a relatively stable and individual-specific generalized tendency to behave or react in a certain way expressed in a variety of situations (see also Figure 4). In order to assess a trait (which is stable over time), the test(s) used must yield highly replicable results (Carter et al., 2013). A trait thus is considered a permanent characteristic, whereas a state is considered as a transient condition that is only observable at particular moments (see also Fridhandler, 1986; Koski, 2011; Carter et al., 2013).

Anxiety, for example, can be seen as a trait, or a state. Trait anxiety is defined as the intrinsic basal anxiety characteristic of an individual, which does not vary from moment to moment, while state anxiety is defined as the anxiety that an individual experiences at a particular moment in time (Lister, 1990). Trait anxiety is determined by genetic factors, environmental...
influences and gene by environment interactions. Theoretically animals that have a higher trait anxiety respond to dangerous situations more frequently and with a greater intensity than individuals lower in trait anxiety (Spielberger et al., 1984). More anxious individuals thus have a higher level of trait anxiety and in threatening situations probably also higher levels of state anxiety. The difficulty with state anxiety is that the level of anxiety that is measured depends on both the situation and the level of trait anxiety of the individual. The most reliable measure of the anxiety characteristic of an animal or human thus would be a measure of trait anxiety, and not of state anxiety. In animals it remains to be investigated how to make a distinction between state and trait anxiety. Human studies revealed that judgment bias is influenced by trait anxiety (as measured by questionnaires; see for example Eysenck et al., 1991; Mathews and MacLeod, 1994) as well as state anxiety (Mathews and MacLeod, 1994; Anderson et al., 2012). However, traits are not static; they can change gradually over time under the influence of environmental factors (Strelau, 2001).

For further validation of JBTs, the animal’s behavior during the testing phase should be correlated with behavior in other tasks that are believed to assess emotional states and/or traits. For rodents, these may be tests such as the OF test, the LD test, the elevated PM, the novel object test, and/or the modified hole board, to name a few (e.g., van der Staay et al., 1990; Duncan and Keller, 2011; see Figure 4). In non-rodent species, these tasks may be less adequate and other tasks validated for those particular species must be applied. Some studies have compared JBT performance to another test of emotionality. Judgment bias has been shown to correlate with anxiety in pigs as measured by a novel object test (Carreras et al., 2016). Pessimistic judgment bias was positively correlated with a more fearful response during the novel object test. Rats which laugh when tickled (a confirmed behavioral signal of positive emotional state) have a more positive judgment bias than rats which don’t (Rygula et al., 2012). Destrez et al. (2012) found that lambs treated with an anxiolytic showed a positive judgment bias and were less fearful during isolation and suddenness tests. When studying the responses of laying hens in different tests of emotionality, some correlations were found between measured parameters during a JBT, a novel object test and an anticipation test. However, no clear relationship between the tests was found (Wichman et al., 2012). To test specifically for the effects of emotional traits on judgment bias, examining possible correlations with tasks that measure personality traits.

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**FIGURE 4 | Measuring trait vs. state.** Repeatedly testing emotional state (e.g., across the lifespan of an animal) may yield information about its emotional trait, i.e., the behavior indicative for a particular trait needs to be repeatable (Carter et al., 2015); In this hypothetical example, the JBT and some other tasks that are believed to assess emotional state are applied multiple times (for an example, see Bethell and Koyama, 2015). Possible consequences of repeated testing are summarized. The description of the concepts trait and state are from Strelau (2001 p. 317, Table 2).
is necessary. For example, repeated comparisons of baseline judgment bias of individuals with high vs. low trait anxiety would be valuable. Individual differences in baseline judgment bias have been reported (e.g., Starling et al., 2014). Repeated judgment bias testing has been applied to a small sample of chimpanzees \( (n = 3) \). Individual differences in judgment bias were found, which remained stable across five test sessions (spanning a time period of 1–2 weeks; Bateson and Nettle, 2015). When a similar study was performed with pigs, no consistent results were found between two test sessions (with a 5 week intermittent period; Carreras et al., 2015). In rats, repeated testing of baseline judgment bias has produced stable results, which correlate with traits such as motivation and sensitivity to stress (Rygula et al., 2013, 2015b).

Though replicability and stability of result is a basic requirement of a trait, this may be difficult to demonstrate empirically. Unfortunately, the order of testing may affect behavior in subsequent tests (e.g., Mcllwain et al., 2001; Blokland et al., 2012). This is an observation that may also complicate a correlational approach (e.g., factor analysis) to validating JBTs. To the best of our knowledge, neither the correlations between different tests that are believed to assess emotion with JBTs, nor the effects of repeated (or longitudinal) assessment has yet been studied systematically.

**CUES ON ONE SINGLE STIMULUS DIMENSION OR ON DIFFERENT STIMULUS DIMENSIONS?**

There are several potential concerns related to the choice of stimulus dimension(s) when preparing a JBT design. There may be variation between animals in their capabilities to differentiate between cues. For example, when using auditory cues, the accuracy of perceiving differences between tones may be different for good and poor listeners in learning the original tone discrimination (see, e.g., Amitay et al., 2005). For olfactory stimuli, it needs to be ensured that mixtures of the \( S^+ \) and \( S^- \) odors are distinguishable as such, i.e., are not simply regarded as a novel odor, but as intermediates between \( S^+ \) and \( S^- \) (Dreumont-Boudreau et al., 2006). Differences in ability to discriminate between learned and ambiguous stimuli may similarly affect studies using visual or tactile cues. Additionally, there may be a non-linear relationship between the perception of the originally acquired \( S^+/S^- \) and the intermediate stimuli, i.e., due to the sensory capabilities of the species studied, the scaling of cues may not be perceived as intended. For example, what is intended to be an intermediate ambiguous cue may be perceived as having a higher similarity to the \( S^+ \) than to \( S^- \). The dimension and scaling of the cues used thus may affect performance in JBTs by affecting ambiguity. Therefore, it is important to adjust the dimension and scaling of cues to the species studied (e.g., auditory cues used in several rat studies are adapted to the species’ audiogram (e.g., Enkel et al., 2010; Rygula et al., 2013)).

A number of studies used cues from different dimensions. Although such a methodology might make discrimination between cues easier, they limit the interpretation of JBT results (Nogueira et al., 2015). Graded stimuli on a unidimensional scale allow for the prediction of response patterns (see Figure 5). When different dimensions are used for \( S^+ \) and \( S^- \), ambiguous cues can no longer be considered as intermediate. For example, Salmeto et al. (2011) used a series of chicken to owl morphs, where the \( S^+ \) was the mirror image of the tested chicken, whereas the \( S^- \) and the different morphs were silhouettes, printed on cards (the mirror image added an extra dimension to the \( S^+ \), namely movement). When comparing responses to a previous experiment using a chicken silhouette as \( S^+ \), it became clear that the chicks responded differently to the mirror than to printed stimuli, with decreased latencies to respond to the moving mirror images. When the ambiguous stimuli are unrelated to the trained reference cues, there is a risk of measuring response to novelty instead of ambiguity. For example, using wild peccary as subjects, Nogueira et al. (2015) used categorically different auditory stimuli (whistle and horn as \( S^+ \) and \( S^- \), bell as ambiguous). Ambiguity is characterized by the possibility to interpret a situation or stimulus in two (or more) distinct ways, i.e., in the case of JBTs ambiguous cues can be interpreted as predicting a similar outcome to either \( S^+ \) or \( S^- \). In order to obtain results which are interpretable as responses to ambiguity, it is suggested to only use cues on a single dimension.

**LOSS OF AMBIGUITY WITHIN A SMALL NUMBER OF TESTING TRIALS**

It is common practice in JBTs to leave test trials (i.e., presentations of ambiguous stimuli) unrewarded (see Figure 2). Such a lack of reward will stand out after extensive training where rewards were always present. This will facilitate learning about unrewarded ambiguous trials (Jamieson et al., 2012). As a result, repeated testing in JBTs could lead to a loss of ambiguity, as the animals will learn to associate the ambiguous stimuli with a specific outcome. This could influence the animals' subsequent choices during test trials and thereby lead to false conclusions of measured judgment bias (Doyle et al., 2010b). Such possible confounding effects of unrewarded testing trials have been recognized in numerous studies. Brilot et al. (2010) found that their study subjects (starlings) increased the response latencies as testing progressed, while failing to detect a cognitive bias. They concluded that their birds quickly learned that the ambiguous trials were never rewarded and therefore became slower to respond to ambiguous cues. Multiple other studies report a loss of ambiguity as a possible cause for increased response latencies of their study subjects (Doyle et al., 2011b; Sanger et al., 2011; Starling, 2012; Destrez et al., 2014; Starling et al., 2014; Verbeek et al., 2014; Karagiannis et al., 2015). In addition to an increased response latency, Murphy et al. (2013) found that their pigs also decrease the number of optimistic responses with repeated testing. Doyle et al. (2010a) suggested that a loss of ambiguity could even explain why stressed sheep responded more pessimistically than their non-stressed controls.
As mild stress may enhance learning (Mendl et al., 2009), the stressed animals could simply have learned about the lack of rewards during ambiguous trials faster than the control group. Similar conclusions have been drawn in other studies (Destrez et al., 2012, 2013; Scollo et al., 2014). A study dedicated solely to the effect of repeated testing in absence of any experimental manipulations or changes in environment found that sheep develop a reluctance to respond during ambiguous test trials (Doyle et al., 2010b). As there was no explanation for this change in behavior related to a change in their emotional state, an increase in pessimism seems unlikely. Rather, this study supports the notion that animals may learn about the outcome of ambiguous trials with repeated testing and change their responses accordingly.

Several possible solutions to the problem of loss of ambiguity have been suggested. Use of a secondary reinforcer during training and testing was successfully applied in a study by Keen et al. (2014). In addition to a high and low food reward, their bears were also reinforced with a clicker to maintain responsiveness. During ambiguous testing trials, no food rewards were given, but reinforcement with the clicker continued. A secondary reinforcing audio cue has also been used in a study with Rhesus macaques (Bethell et al., 2012). Another measure to reduce learning about the outcome of test trials is a partial reinforcement ratio schedule for training and control trials. For example, Neave et al. (2013) used partial reinforcement of positive trials during training. Although the punishment rate for negative trials remained 100%, they reduced the reward rate for positive trials to 50%. Using this training procedure, their calves learned to have lower expectations of reward during ambiguous trials. Partial reinforcement of training and control trials has also been successfully applied in various other studies (Bateson and Matheson, 2007; Matheson et al., 2008; Bethell et al., 2012; Richter et al., 2012; Neave et al., 2013; Daros et al., 2014; Bateson et al., 2015; Bethell and Koyama, 2015).

The number of learning opportunities about the outcomes of trials during judgment bias testing can also be reduced by minimizing the number of ambiguous test trials. In a study by Vögel et al. (2014), sheep were subjected to three test sessions of five trials, with each session containing only one single ambiguous trial. No reduction in visits to unrewarded ambiguous probes was reported. Similarly, studies by Rygula et al. (2013, 2015b) report stable judgment bias for their rats by using a relatively small number of ambiguous trials in comparison to positive/negative trials. Although these studies support the notion that limiting the exposure to unrewarded ambiguous probes can prevent loss of ambiguity, this measure would also reduce the number of trials that can be used to estimate effects of experimental manipulations on judgment bias. A reduced number of ambiguous trials may make the JBT results prone to chance findings. Using a between subjects design would at least minimize the number of exposures to ambiguous stimuli per animal (Brilot et al., 2010).

A final suggestion has been to reward ambiguous trials (Murphy, 2015, pp. 185–187; see also Carreras et al., 2015). This was shown to lead to a maintenance of optimistic choosing throughout test sessions, whereas unrewarded test trials lead to a decrease in optimistic choice. However, such a design may still lead to associative learning concerning the outcomes of ambiguous trials, rendering them no longer ambiguous.

Surprisingly, Bateson and Nettle (2015) used no specific measures to avoid loss of ambiguity, yet found no effects.
of repeated testing in three chimpanzees with respect to the latencies to react to intermediate stimuli. Consequently, they conclude that their JBT is suited for longitudinal assessment of welfare in this species. The authors ascribe their apparent maintenance of ambiguity to the difficulty of their discrimination task (paper cones of 20% vs. 60% gray, with intermediate shades as ambiguous stimuli). However, their very small sample size (n = 3) increases the chance of false positive findings (Tversky and Kahneman, 1971). Therefore, repeated testing with a larger sample size would be beneficial to further support their method of preventing loss of ambiguity.

Loss of ambiguity can become a considerable limitation to JBTs, as it renders them ineffective for the detection of changes in affective state (Brilot et al., 2010). Rather, it may cause animals to base their performance on associative learning as testing progresses. This could lead to incorrect conclusions about the effects of experimental manipulations on an animal’s affective state. Therefore, it is recommended to implement precautions against loss of ambiguity, such as the use of a partial reinforcement schedule during training and testing (e.g., Bateson et al., 2015). The specific design of a study will determine which precautions are the most suitable. Also, it is important to exclude loss of ambiguity as a possible explanation for results. This can be done by testing for changes in response to ambiguous stimuli in the absence of experimental manipulation (Neave et al., 2013; Daros et al., 2014).

GO/NO-GO VS. GO/GO TASKS (ACTIVE CHOICE TASKS)

In an article by Baciadonna and McElligott (2015), of the judgment bias publications reviewed, approximately 70% (22 of the 32 publications) were designed as a Go/No-go task. Of the studies summarized in the Table 1 of “Supplementary Material”, approximately 50% was of the Go/No-go type. Matheson et al. (2008) developed an active choice task for starlings in which the subject must respond to the S+ and S− with the same operant behavior (e.g., pecking a key (S+) associated with immediate reward, or a key (S−) associated with delayed reward). Other variants have since been developed such as an active choice task for pigs, in which responding in the goal box associated with the S+ yields a large reward, and responding to the goal box associated with the S−, yields a small reward (Murphy et al., 2013).

Theoretically, the main difference between Go/Go tasks and Go/No-go tasks is that in Go/Go or active choice tasks the animal is required to make an active response to both the S+ and S−, whereas in Go/No-go tasks, the animal is required to suppress a response to the S−. In Go/No-go tasks, a cut off criterion is defined to distinguish between the two response classes. Usually, trials in which an animal did not respond within a pre-determined cut-off time are scored as No-go (see Figure 5).

Alternatively, the median response time—an approach that determines the cut-off within a preset period empirically—serves to distinguish between Go (latency to respond below median latency) and No-go (latency to respond above median) responses (e.g., Wichman et al., 2012). In both instances, the proportion of animals responding in one of the two classes (Go, No-go) is analyzed. The selected cut-off time may determine the discriminating ability of the test.

The response suppression required for Go/No-go tasks may influence JBT results, as behavioral inhibition is thought to be influenced by emotion (Cyders and Smith, 2007). Moreover, in Go/No-go tasks, No-go responses could be considered an omission to react, rather than a pessimistic response (Guldemann et al., 2015). Active choice tasks circumvent this possible confounding factor of motivation by requiring active responses for both optimistic and pessimistic choices (Hales et al., 2014). As active choice tasks do not require behavioral inhibition and allow for omissions to be measured separately from optimistic/pessimistic responses, they may be more suited for measuring judgment bias than Go/No-go tasks (Murphy et al., 2013). It should be noted that, in practice, a Go/No-go and a Go/Go task are not necessarily mutually exclusive (see Figure 5). If the trial doesn’t stop when reaching the criterion of No-go, but lasts e.g., for two times the criterion duration, then the data can also be analyzed as reflecting active choice responses (see Douglas et al., 2012; Carreras et al., 2015; Gordon and Rogers, 2015).

Many different criteria have been used for mastering the basic discrimination task preceding judgment bias testing. In both Go/Go and Go/No-go tasks similar criteria are used, usually based on accuracy (60 to 90% correct responding to S+ and S−, Anderson et al., 2013; Keen et al., 2014; Rygula et al., 2015a), latency (shorter latencies to respond to S+ than to S−, Briefer Fremond et al., 2014; Kis et al., 2015), or running speed (faster to S+ than S−, Karagiannis et al., 2015). Specific to No-go trials, a predetermined number of no-approaches of S− (Sanger et al., 2011) has been used for determining when animals had learned the basic discrimination. The learning criterion must be reached over a predetermined number of training days, trials, or trials within a number of days. All animals that did not reach the criterion within this maximum are excluded from testing with ambiguous cues (e.g., Müller et al., 2012). Sometimes additional criteria, such as that the animal makes no omissions in a fixed number of trials (Anderson et al., 2013), are used.

In some studies, differences between the response to the S+ and S−, confirmed statistically by Wilcoxon test (e.g., Kis et al., 2015) or Mann-Whitney U-test (e.g., Starling, 2012) were used as criterion. It has not yet been investigated how the learning criterion, i.e., the level of mastering the original S+/S− discrimination, affects the sensitivity of subsequent testing with ambiguous cues. It is conceivable that a weak criterion decreases the likelihood to detect a judgment bias.

TRAINING AND TESTING IN ISOLATION VS. TESTING IN THE SOCIAL GROUP

Though not unique for JBTs, testing social animals individually, without direct contact to the group, may increase the stress level and/or decrease the willingness of an animal to learn the task and/or perform the required responses. Extensive habituation and pre-training may be necessary before judgment bias can be individually assessed (see Krasheninnikova and Schneider, 2014). For example, pigs need extensive habituation before they can...
be trained and tested individually in JBTs (Murphy et al., 2013, 2015).

Training and testing in a group setting is another solution to problems associated with individual testing of social animals. However, group testing is likely also accompanied by methodological issues. To date, only one study has used group training for a JBT. Training white-lipped peccary in isolation was methodological issues. To date, only one study has used group testing. However, group testing is likely also accompanied by problems associated with individual testing of social animals. Training and testing in isolation and in social groups have been reported so far. Due to the many potential limitations of training and testing in a group setting, it does not seem likely to be advantageous over individual habitation of social animals.

The effects of (short-term) isolation of social animals, applied as an experimental manipulation of the emotional state preceding judgment bias testing, have been explored in several species. Social isolation affected judgment bias in chicks, with duration of the isolation period having specific effects on JBT performance. A pre-testing isolation period of 5 min induced increased pessimistic responses, while an isolation period of 60 min also decreased optimistic responding (Salmeto et al., 2011; Hymel and Suška, 2012). For pigs and laying hens, no effect of short-term social isolation on JBT performance was found (Düpjan et al., 2013; Murphy et al., 2013; Hernandez et al., 2015). Only the study by Murphy et al. (2013) mentions habitation of the animals prior to training and testing, possibly explaining why no effect of social isolation was found. When male rats are moved from group housing to individual cages, their rate of optimistic responding decreased (no effect was found for female rats). However, as enrichment and available shelter were also removed when moving the rats, these could have been confounding factors in this study (Barker et al., 2016). Together, these studies suggest that habitation of social animals to the training and testing conditions is sufficient to avoid a confounding influence of stress during testing.

EXCLUDING ANIMALS THAT DID NOT PASS THE TRAINING PRECEDING JUDGMENT BIAS TESTING

Many judgment bias studies report the exclusion of animals that failed to reach a required criterion during training (e.g., Starling, 2012; Starling et al., 2014; Verbeek et al., 2014; Bethell and Koyama, 2015; Hernandez et al., 2015). For example, Wichman et al. (2012) reported 10 out of 38 chickens were unable to acquire the discrimination between rewarded and unrewarded trials, in spite of a long training period. These animals could therefore not be subjected to judgment bias testing. Similarly, in group of 18 white-lipped peccary, four adult individuals did not learn the basic discrimination in a Go/No-go auditory discrimination task, and were consequently not tested in the subsequent JBT (Nogueira et al., 2015). In a study by Brajon et al. (2015), only 59% of their 54 pigs completed the training preceding judgment bias testing. Consequently, all results and conclusions from JBTs are based on the study subjects that were capable of learning the discrimination task. If not all animals are able to reach the preset learning criterion, the samples are biased toward “learners”. The larger the proportion of excluded “non-learners” is, the more biased a study is, and consequently, the less the results can be generalized. Development of tests that need less pre-training, e.g., by ensuring the discrimination training is better suited to the natural abilities/behaviors of the studied species, may allow for more animals to participate in subsequent judgment bias testing. Developing discrimination tasks which the studied animals are able to master fairly easily may also prevent selective loss of animals in experimentally manipulated groups. For example, possible effects of stress on learning could lead to animals undergoing a particular treatment (e.g., induced anxiety) being more likely to fail to pass the training phase (Mendl et al., 2009; Conrad, 2010). Increasing the difficulty of discrimination training may increase the likelihood of a larger proportion of non-learners in a specific treatment group.

USABILITY FOR ASSESSING ANIMAL WELFARE

In animal welfare research, JBTs have been applied to a wide range of species that are commonly kept in captivity for a variety of reasons (e.g., production animals such as pigs, Brajon et al., 2015; laboratory animals such as rats, Burman et al., 2008; zoo animals such as Grizzly bears, Keen et al., 2014; companion animals such as dogs, Titulaer et al., 2013). Most of these judgment bias studies have been aimed at investigating the effects of common conditions inherent to life in captivity. For example, the effects of providing environmental enrichment have been studied extensively (e.g., Douglas et al., 2012; Bethell and Koyama, 2015). Also, the effects of common handling procedures have been frequently tested using JBTs, such as dehorning procedures in cattle (e.g., Neave et al., 2013).

According to Bateson and Matheson, (2007 p. 36), “to be practically useful as a measure of how animals feel,
cognitive bias needs to be easy to measure in applied settings”. However, many studies needed extensive training on the basic discrimination task before judgment bias could be assessed, decreasing the practical applicability of JBTs as a form of welfare assessment. Additionally, an extensive training period could mask potential detrimental effects of experimental manipulation and is considered one of the most confounding factors in judgment bias test paradigms (Novak et al., 2015). Acting as cognitive enrichment, training could improve the welfare/affective state of the study subjects (Carlstead and Shepherdson, 2000; Puppe et al., 2007; Pomerantz and Terkel, 2009; Zebunke et al., 2011; Guldemann et al., 2015). In spite of negative affect manipulations, this could lead to optimistic responses from subjects (Düppjan et al., 2013). In line with this expectation, in preparation of judgment bias testing, Svendsen (2012) trained farmed mink categorized as fearful or as explorative, to induce a positive affective state. Whereas the fearful mink behaved more explorative at the end of training, an opposite effect of training was found in the mink categorized as explorative: these animals were rated as less positive post-training, possibly due to frustration about the absence of expected rewards during later training sessions. Svendsen et al. (2012, p. 366) cautions that “(...) studies that involve induced affective states and a lot of training of the animals to assess their welfare, such as the cognitive bias method, need to be interpreted carefully as the handling and training has a different effect on animals in different affective states”. Consequently, future research should focus on the question of whether training for a JBT itself modulates the animal’s emotional state.

The sensitivity of judgment bias to detect effects of experimental manipulations on emotions has not yet unequivocally been established. For example, in a study by Keen et al. (2014) the JBT was unable to detect differential effects of environmental enrichment methods in bears. Although behavioral observations showed that the different types of enrichment were valued differently by the bears (some items were interacted with more than others), this did not result in differences in measured judgment bias. It is possible that providing enrichment did not produce a measurable increase in judgment bias because the JBT was not sensitive enough to detect this change in affective state. However, lack of effect to be detected is another possibility, as the bears were already housed in enriched environments. The addition of an extra enrichment item may not have produced a measurable improvement in affective state to begin with. Similarly, another judgment bias study did not discriminate between the welfare of short and long term kenneled dogs (Titulaer et al., 2013). These similar results between animals which are assumed to be experiencing different levels of welfare, could have been considered a result of the lack of sensitivity of JBTs. However, additional measures of welfare (such as behavioral observations and stress hormone levels) did not differ between the groups either. This study shows the importance of validating judgment bias results by comparing them with other measures of welfare.

Reviewing JBTs as tools to assess welfare in farm animals, Baciadonna and McElligott (2015) conclude that these tasks are sensitive to manipulations that induce negative emotions, whereas experimental evidence for sensitivity to manipulations that induce positive emotions is yet weak. This lack of evidence for sensitivity to positive judgment bias could be due to a lack of scientific attention. The majority of judgment bias studies measure the effects of manipulations which are expected to produce a negative affective state. Studies investigating optimistic judgment bias are much less common. For example, Rygula et al. (2012) have shown that laughing rats (displaying a clear behavioral indication of positive affective state) have a more positive judgment bias than rats which don’t laugh when tickled. As improvement of animal welfare relies on both the reduction of negative emotions and the promotion of positive emotions (Boissy et al., 2007), studies aimed at the sensitivity of JBTs to positive emotions are important.

USABILITY FOR BIOMEDICAL RESEARCH

JBTs have been used as tools for affective state assessment in biomedical research. The majority of these studies have used rodents as their subjects (e.g., mice, Boleij et al., 2012; rats, Kregiel et al., 2016), reflecting the common use of rodents as animal models in biomedical research. In most of the biomedical studies, experimental manipulations were performed prior to testing (Scenario 2 in Figure 1). In such experiments, all study subjects experience similar conditions during training. This is in contrast to many welfare studies, which alter housing conditions, etc., prior to training. Studies which have different conditions for experimental groups prior to or during training, apply experimental manipulations that could affect both the discrimination training preceding judgment bias testing and responding in the JBT proper.

The main aim of judgment bias studies in biomedical research has been to investigate effects of experimental manipulations expected to affect mood in animal models of mood disorders such as depression and/or anxiety. Most of these studies have tested the effects of anxiolytics and/or anti-depressants on judgment bias performance (e.g., Doyle et al., 2011a; Destrez et al., 2012; Hymel and Sulka, 2012; Anderson et al., 2013; Rygula et al., 2014a,b, 2015c).

It is important that results of JBTs are generalizable to other species (e.g., results should simulate the clinical condition of depression/anxiety in humans and inform about the effects of therapeutics believed to modulate these clinical conditions; van der Staay, 2006). If this is not the case, the translational value of judgment bias measurements in non-human animals may be limited. JBTs appear to be a useful tool for studying animal models of depression and anxiety. Multiple studies have found responses comparable to those found in human studies of judgment bias (Einkel et al., 2010; Salmeto et al., 2011; Hymel and Sulka, 2012; Richter et al., 2012; Papiak et al., 2013; Klöke et al., 2014; Rygula et al., 2014a, 2015a; Kregiel et al., 2016).

JBTs appear to have particular potential to differentiate between anxiety disorders and depression. Although both mood
disorders result in a negative affective state, they produce different response profiles in the JBT. In humans, depression is expressed by a decrease in optimistic responses combined with an increase in pessimistic responses. Individuals suffering from anxiety only display increased pessimistic responding (MacLeod and Byrne, 1996; Miranda and Mennin, 2007). These findings have been replicated in judgment bias studies using a chick model of anxiety and depression (Salmeto et al., 2011; Hymel and Sufka, 2012). Chicks in an anxiety-like state displayed more pessimistic behaviors in response to ambiguous aversive cues (i.e., ambiguous cue near $S^-$) and to intermediate ambiguous cues. Chicks in a depression-like state behaved similarly, but in addition displayed less optimistic behaviors in response to ambiguous cues near the $S^+$. These results highlight the importance of using a spectrum of ambiguous stimuli, ranging from near-negative to near-positive. Different same-valence affective states (such as depression and anxiety) may produce different responses to these different forms of ambiguous cues (Kloke et al., 2014).

The translational value of biomedical judgment bias studies is of particular importance, as results are used for comparison with humans and/or other model species. Therefore, differences between species in baseline responding during a JBT require attention (e.g., is a pessimistic response caused by induction of a negative affective state or by a trait of the studied species, see “State vs. Trait” Section). Several studies have reported a baseline judgment bias of their study subjects. Using test designs with reward and punishment, both rats and BALB/c mice displayed a baseline negative judgment bias. They showed punishment avoidance during ambiguous cue presentation (Boleij et al., 2012; Anderson et al., 2013; Rygula et al., 2015c). These findings could have been a direct result of the test design and comparisons to studies using a discrimination task based on favorable/less favorable reward would be valuable. One study found a baseline positive judgment bias in rats and ascribed this optimism to the favorable testing conditions, i.e., the possibility of food reward and exploration of novel environment, further indicating effects of test design on baseline judgment bias performance (McGuire et al., 2015). Rygula et al. (2014b) reported a difference in baseline judgment bias between groups of rats used for separate experiments, citing this as a possible reason for differences in results found after experimental manipulation. This finding implies that even within-species/strain differences in baseline judgment bias are a possibility that needs to be taken into account. In support of this argument, several studies mention individual variation in judgment bias as a possible influence on their results (Verbeek et al., 2014; Kis et al., 2015).

Biomedical studies have commonly used JBTs to assess effects of drug treatments. When tested drugs have side effects, this could influence behavior in JBTs. One common pharmacological side effect is a reduction or increase of appetite. Most JBTs use food as a reward for correct responses to $S^+$, with numerous studies also using (less palatable) food as punishment predicted by $S^-$. When treated study subjects experience a decrease in appetite, their performance of optimistic behaviors (i.e., collecting a food reward) may be reduced independent of their affective state. Two studies have mentioned a decrease in appetite as a possible side effect of drug treatments and both used food reinforcers as part of their experimental design (Anderson et al., 2013; Rygula et al., 2014b). An increase in appetite after treatment with the anxiolytic diazepam was discussed as a possible explanation for the observed negative judgment bias (pessimistic responses required the intake of food items with low palatability, see Boleij, 2013). Observing animals’ responses to $S^+$ and $S^-$ could provide an indication of appetite-related side effects affecting treated subjects. For instance, when responding during food-rewarded positive trials remains high, it is unlikely that a change in appetite is responsible for a change in responding during ambiguous trials. Using an alternative to food reinforcers will rule out treatment-induced differences in food motivation altogether (e.g., Kloke et al., 2014). When food reinforcers have been used, the possibility of side effects of treatment on the consummatory behavior of study subjects should be ruled out (Mendl et al., 2009). There are other common side effects of pharmacological manipulation to reckon with that potentially influence behavior in JBTs (e.g., locomotor activity, lethargy). Doyle et al. (2011a), for example, examined possible side effects affecting the results of dose response trials in a JBT by examining the behavior and physiology of their animals in simple tests of food motivation, reactivity and locomotion.

**CONCLUSIONS AND FUTURE DIRECTIONS**

JBTs may be suited to assess the emotional state of an animal. Provided that judgment bias can be repeatedly tested in the same animal over a longer time period (Q.E.D., see “Discussion” above, “Loss of Ambiguity Within a Small Number of Testing Trials” Section), it may also be suited to assess emotional trait in animals.

Judgment bias can be tested in a very broad range of species, from insects to humans, i.e., may allow comparison between species, and may be suited for translational research. There is a large variation in test equipment and testing procedures between and within species (for a recent review see Bethell, 2015; see also “Supplementary Material, Table 1”). A huge variation in criteria is applied for mastering the basic discrimination task. Also, a large range of computational and statistical methods to analyze the judgment bias data has been used. Recently, Gygax (2014) reviewed these methods and gave recommendations, which already have been critically commented upon (Bateson et al., 2015). This gamut of statistical analyses hampers comparisons within and between studies and species.

JBTs have been used in the field of animal welfare research and in biomedical research. These tasks need to be further developed and adapted to the species of animals used and the research questions to be addressed. In welfare assessment studies, modifications may include the applicability under non-laboratory conditions, testing of social animals in groups and increase of the efficacy to train animals on the basic discrimination task. Owing to the extensive training preceding
judgment bias testing, this task appears to be less suited for routine monitoring of animal welfare.

In biomedical research, a lengthy training period, preceding testing with ambiguous stimuli, may be less of a concern, as drug treatments usually start after completion of learning the basic discrimination between $S^+$/$S^-$. However, the problem that ambiguous stimuli may lose their ambiguity very quickly, enabling collection of data in a few trials only, needs to be solved. Also, we need to assess whether the task is suited for repeated testing in a longitudinal design (see also Figure 4).

Many open questions, addressed in the present article, still need to be answered before JBTs may be considered as a validated, useful tool in the toolbox of researchers interested in measuring animal emotions in both the context of animal welfare studies and biomedical studies.

**AUTHOR CONTRIBUTIONS**

All authors contributed to writing the manuscript. All authors have approved the final version of the manuscript.

**SUPPLEMENTARY MATERIAL**

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Making decisions under ambiguity: judgment bias tests for assessing emotional state in animals

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Table 1. Summary of judgment bias experiments using animals as subjects. Note that, if a study encompassed different behavioral tests, only the judgment bias tasks are included in this table.

Abbreviations:
Sex:  f, female; m, male; When (1, 2) refer to when experimental manipulations were performed that are believed to affect emotion (see scenario 1 and scenario 2 in Figure 1); n.a.: not applicable; Test arena: A – H refer to arenas depicted in Supplementary Figure 1; S/S⁺, conditioned stimuli used: a, acoustic; o, olfactory; s, spatial; t, tactile; v, visual; d, different dimensions; Ambiguous stimuli: a, acoustic, o, olfactory, s, spatial, v, visual, d, different dimensions. In addition, number of different ambiguous stimuli is shown between parentheses; Go/No-go: Go/No-go task; Go/Go: active choice task; Welfare: indicates whether animal welfare was explicitly addressed.

| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Test arena | S/S⁺ | Ambiguous stimuli (nr.) | Training | Testing | Go/No-go | Go/Go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|-----|------------------------------|------|------------|------|-------------------------|----------|---------|---------|-------|---------|----------------------------------------|-----------|
| Rats, Lister-hooded    | m   | Pharmacology (experiment 1): acute diazepam, reboxetine and fluoxetine, 3 doses per drug | 2    | 1          | a    | a(3); tones: 4, 5 and 6 kHz | correct left or right lever press following S+/S- results in reward (food pellet)/avoidance of punishment (foot shock). Learning criterion: accuracy>60% and no omissions in 10 consecutive trials for 2 days | Randomized Latin square design, all animals received all treatments on separate days. Exposure 40 reference tones (negative and positive) and 20 mid-point probe tones (4, 5 and 6 kHz) | ✓       | Reboxetine reduced probability of reward response (negative bias) and increased omissions. Fluoxetine and diazepam had no effects. | (Anderson et al., 2013) |

1 Tone discrimination task in a Skinnerbox
### Table: Visual discrimination using different sizes of wooden tubes

| Species (strain/breed) | ± | n | Experimental manipulation(s) | When | Test arena | S/S* | Ambiguous stimuli (nr.) | Training | Testing | Go/No-go Go/Go | Wolfing | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|---|---|-------------------------------|------|------------|------|-------------------------|----------|---------|-------------|---------|------------------------------------------|-----------|
| Rats, Lister-hooded    | m | 22 | Pharmacology (experiment 2); Two baseline training weeks (after acute drug testing, exp. 1). Then 2 groups: chronic fluoxetine and saline for 3 weeks. | 22 | 1 | a; tones; S'/S counterbalanced 2 or 8 kHz | a(1); tone of 5 kHz | Re-baselined with training after experiment 1. correct left or right lever press following S'/S results in reward (food pellet)/avoidance of punishment (foot shock). Learning criterion: accuracy>60% and no omissions in 10 consecutive trials for 2 days. Then training on Mon, Wed and Thu and exposure to ambiguous tone in trials on Tue and Fri. | Testing with mid-point probe on Tue and Fri. 35 reference tones (positive and negative) and 30 mid-point probe tones. Testing took place 5 weeks (1 week pre-treatment, during 3 weeks chronic treatment and 1 week post testing) | ✔ | Fluoxetine treatment increased probability of reward response over time (positive bias although also the control group shows this effect to a lesser extend | (Anderson et al., 2013) |
| Common marmosets (Callithrix jacchus) | f, m | 1 | Rearing conditions; Condition 1: family-reared twins; Condition 2: family-reared animals from triplet litters, where only two animals remained; Condition 3: family-reared triplets that received supplementary food | 1 | 3 | v; wooden tubes; S': 2 cm high; S: 15 cm high, or vice versa; A piece of rusk, hidden under the S' tube, served as reward | v(3); 5.5, 8.5, and 11.5 cm high tubes | S' and S were presented one a time. A go response to the S' was rewarded, a no-go response to the S was unrewarded, a go response to the S' was punished with a 2-second time out; Criterion: 80% correct responses to S' and S over 3 successive days, with S' and S presented in random order | Three test sessions with the S: S', and with the three ambiguous stimuli. Number of no-go responses to the ambiguous stimulus was recorded. Sessions in which the marmoset made < 80% correct responses to S' and S were omitted. | ✔ | No effects of rearing condition and gender on acquiring the discrimination task preceding judgment bias testing; no effects of rearing conditions on responding to the ambiguous stimuli. Triplets that had received supplementary food showed less go responses the intermediate ambiguous and the S' stimulus than marmosets from the other two conditions. | (Ash and Buchanan-Smith, 2016) |
| Sprague Dawley rats | f, m | 2 | Rats from standard group housing in open top cages were moved to individual metabolic cage housing; controls stayed in their open-top cages | 2 | J | f; rough (P80) versus smooth sandpaper (P220) | f(1); sandpaper of intermediate grade (P180). | One stimulus, rough sandpaper, was associated with a high-positive reward (chocolate), whilst the other, smooth sandpaper, was associated with a low-positive reward (cereal), or vice versa. One of the two food bowls in the goal box was associated with chocolate, the other with cereal reward. Rats were trained until they had learned the association between sandpaper, food bowl and reward | Rats were moved to the individual metabolic cages or stayed in their open-top cage, were trained 3 more days, and were then tested for 5 days with intermediate (sandpaper P180), unrewarded trials and with the originally learned associations. | ✔ | Initiating foraging behavior was longer in trials with the less preferred than with the preferred reward. The number of optimistic decisions over 5 days was larger in males that remained in standard housing than moved to the metabolic cages. This was not observed in females. Male rats moved to metabolic cages had a significantly longer time to initiate foraging for each probe trial than female rats. This difference was not observed between male and female rats that stayed in standard housing. | (Barker et al., in press) |
| European starlings (Sturnus vulgaris) | f, m | 1 | Housing enrichment | 1 | A | v; lid of petri dish; S'/S: counterbalanced white or dark grey | v(3); 20, 40 and 60% grey | S'/S is associated with palatable/unpalatable mealworm. Response: Flip lid of petri dish. Learning criterion: significant difference between white and dark lids flipped over 3 consecutive sessions | Test session with 4 reference cues (positive and negative) and 20 probe cues | ✔ | Startlings that experienced enrichment before standard housing conditions had a shift towards a more negative bias, while judgement biases were not different the other way around | (Bateson and Matheson, 2007) |

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2 Multiple testing during chronic treatment  
3 Visual testing during different sizes of wooden tubes
| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Test-arena | S/S* | Ambiguous stimuli (nr.) | Training | Testing | Go No-go | Go Go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|-----|-------------------------------|------|------------|------|------------------------|----------|---------|----------|-------|---------|------------------------------------------|-----------|
| Chimpanzees (f, m)     |     | Individual differences between chimpanzees (2 males, 1 female) | 1    | 4          | v; paper cone; S*: 20% grey, S: 80% grey | Pale grey cones concealed a peanut reward (go-response); dark grey paper cones concealed no reward (no-go); 107-240 acquisition trials. | 5 test phases, each preceded by a choice phase. In choice phases, both the S* and S were presented, with the position randomized (left/right). Progression to test phase if 18 or more choices for POS from a total of 24 trials Test consisted of 3 times 3 ambiguous and 8 S* and 8 S. Latency to touch the cone was recorded. The go response was never rewarded. | ✓ | ✓ | Chimpanzees showed differences in speed to touch the cones. In the testing phase, this latency served as covariate. The chimpanzees differed on the latency to touch ambiguous stimuli. Repeated testing did not affect the response to the ambiguous stimuli. Correlation with rank: highest rank: least pessimistic, lower ranks: most pessimistic. | (Bateson and Nettle, 2015) |
| Worker honeybees (Apis mellifera carnica) | f | 80 seconds shaking | 2    | 5          | o; S'/S : 2 different odors counter-balanced 1:9 or 9:1 (1-hexanol and 2-octanone) | 2 different odors were conditioned with palatable (sucrose) or unpalatable solution (quinine). One session of 12 trials, pseudorandom presentation. | On the same day as training, unreinforced test trial with 5 odors; the 2 reference odors and 3 ambiguous odors A binary response was measured as outcome variable: whether or not the honeybee extended its proboscis | ✓ | ✓ | Agitated honeybees are more likely to classify ambiguous cues with punishment (negative bias), as they extended their proboscis less towards the ambiguous and negative stimulus | (Bateson et al., 2011) |
| European Starlings (Sturnus vulgaris) | f, m | Developmental telomere attrition (possible candidate indicator of somatic state), high vs low competition nests, and number of heavier competitors in the nests | 1    | A          | v; lid of petri dish; S'/S : 2 different odors counter-balanced light (20%) or dark grey (60%) | The S* revealed a palatable mealworm, the S revealed an unpalatable mealworm injected with quinine. Criterion: S* latency > S latency per starling Mann-Whitney U-test. One 16-trials session on a partial reinforcement schedule to slow down extinction during judgment bias testing. | Four daily sessions of 18 trials, with no reinforcement in ambiguous-cue trials, and partial reinforcement in S* and S trials when the starlings were on average 96 days old | ✓ | ✓ | Starling from high competition nests with heavier competitors chose ambiguous stimuli slower. Birds with greater developmental telomere attrition showed lower telomere length. Males discuss whether increased reward expectancy reflects a more positive affective state. | (Bateson et al., 2015) |
| Rhesus macaques (Macaca mulatta) | m | Environmental enrichment vs. post-health check | 2    | 6          | v; line on screen; S'/S : 2 different odors counter-balanced short and long line | S* associated with 40% probability of reward (2 food pellets and tone) if screen was touched and S' with a burst of white noise if touching screen. Training criterion: ≥60% correct responses (>75% correct for both S* and S) | 6 test sessions. Three blocks per test session: 75% correct responses to reference cues means progression to the second block (24 S*, 24 S and 18 ambiguous cues), third block control (10 S* and 10 S). Latency and frequency of responses recorded. | ✓ | ✓ | More reward related responses towards cues closer to the positive cue in enriched animals in comparison with after the health check. | (Bethell et al., 2012) |

4 Visual discrimination using paper cones of different shades of grey
5 Proboscis extension towards odor
6 Discrimination task on touchscreen
| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Test-arena | S/S’ | Ambiguous stimuli (nr.) | Training | Testing | Go/No-Go | Go/Go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|-----|-------------------------------|------|------------|------|-------------------------|----------|---------|----------|-------|---------|-------------------------------------------|-----------|
| Hamster (Mesocricetus Auratus) | m   | Effects of adding or removing enrichment items from an enriched environment. Both orders of environmental conditions were presented | 2    | D          | S; S/S’ drinker positions (left or right) in a testing arena | S(3); drinker positions in between location S’ and S | The drinker positions were conditioned with palatable (sucrose or unpalatable solution (quinine), using a variable reinforcement schedule. Criterion of learning: mean latency to approach S’ < latency to approach S | During stay in environment with added or removed enrichment items, 3 sessions with S+, S- and 3 ambiguous locations. Drinkers were empty. | ✓ | ✓ | No effects of the treatments on latency to approach S’ and S were found. Adding enrichment induced a positive shift, removal induced a negative shift in proportion of responses towards the near-positive and near-negative cues. No effects were found on responses to the middle probe. No relationship with standard tests of emotionality (open field, light-dark test, approach to novel object) that were performed after judgment bias tests) | (Bethell and Koyama, 2015) |
| Mice, 129P3/J and BALB/cJ | m   | Strain comparison: no manipulation (experiment 1) | —    | B          | o; S/S’: counter-balanced vanilla or apple odor placed in odor cup | o(3): 15/85, 50/50 and 85/15% odor solutions | Odor cup presented with a palatable (positive) or unpalatable (negative, quinine flavored) almond piece. Short training 3 positive and 1 negative trial. | Different groups tested on positive, negative and probe cues; all cues presented with a normal almond piece, approach times measured. | ✓ | | Strain difference in performance of the odors conditioning task. BALB/c mice discriminate between the odors and seem to show intermediate reactions towards the ambiguous cues. | (Boleij et al., 2012) |
| Mice, BALB/cJ | m   | White light vs. dark light testing (experiment 2) | 2    | B          | o; S/S’: counter-balanced vanilla or apple odor placed in odor cup | o(1): 50/50% odor solution | Odor cup presented with a palatable (positive) or unpalatable (negative, quinine flavored) almond piece. Short training 5 positive and 3 negative trials. | Different groups tested on positive, negative and the probe cue. One group tested under a white lamp. All cues presented with a normal almond piece, approach times measured | ✓ | | Latencies to approach the ambiguous cue were comparable to that towards the negative cue, white light caused a general increase on latency times to approach all cues | (Boleij et al., 2012) |
| Mice, BALB/cJ | m   | Diazepam effects (0, 1 and 3 mg/kg) | 2    | B          | o; S/S’: counter-balanced vanilla or apple odor placed in odor cup | o(1): 50/50% odor solution | Odor cup presented with a palatable (positive) or unpalatable (negative, quinine flavored) almond piece. Short training 13 positive and 12 negative trials. | Three groups (0, 1, or 3mg/kg diazepam) tested on positive, negative and the probe cue (2 of each) in one test session. | ✓ | | A subgroup of animals tended to show a negative judgement bias. However, diazepam increased the tendency of mice to eat the bitter tasting almond piece, suggesting that reactions might have been influenced by the effect of diazepam on taste and palatability. | (Boleij, 2013) |
| Species (strain/breed) | S | Experimental manipulation(s) | When | Test-arena | S/S* | Ambiguous stimuli (nr.) | Training | Testing | Go, No-Go | Go, Go | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|---|-------------------------------|------|------------|------|-------------------------|----------|---------|-----------|-------|----------------------------------------|-----------|
| Pigs, Yorkshire x Landrace x Duroc | f, m | Gentle, rough, or minimal handling starting after weaning until the end of cognitive bias testing | 1-7 | 7 | a; low and high pitched tones (2093 Hz and 32.7 Hz) predicted a reward (P, S1) or punishment (N, S2). | Both US were given in a chamber which opened after playing the tone. Responding to S1 yielded different aversive stimuli such as water spray, air puff, falling tennis ball; response to S2 yielded two pieces of cereals. Training to the criterion of 5 out of 6 trials correct (at presentation of 3 rewarded (positive, approach) and 3 punished (negative, trials, avoid); only 32 of the 54 piglets reached criterion | Testing was performed in the presence or absence of a human observer -- the handler in the gentle and rough handling group, an unfamiliar person in the minimal handling group | Piglets in the gentle handling group showed a larger percent approaches at presentation of the ambiguous stimulus between S1 and S2; Presence of a human observer reduced the time in contact with the trough, irrespective of the experienced handling (gentle, rough, minimal)... but did not affect judgment bias per se. | (Brajon et al., 2015) |
| Goats, different breeds | f, m | No manipulation, groups based on history of the goats in the sanctuary (poor welfare or control) | 1 | E | s; S/S: Left or right arm in a radial arm maze, counterbalanced | Left or right arms of radial arm maze, associated with food or no reward. 3 positive and negative stimuli per day for 3 days. | Two test days, exposure to 2 times the reference cues and one time each ambiguous cue | Sex differences in judgement bias. Females rescued from poor welfare showed more optimistic and less pessimistic responses while there were no differences found between the male groups. | (Briefier and McElligott, 2013) |
| Horses, three Franches-Montagnes, three Trotters and six Swiss half-bred | f | Training using positive reinforcement (PR, food reward after correct behavior) or negative reinforcement (NR; cessation of uncomfortable stimulus after correct behavior) | 2 | D | s; S: bucket with food on one side of paddock, S2: empty bucket on opposite side of paddock | Training (3 days) to associate one location with food reward and other location with no reward. 6 (of 3) trials/day. Training criterion: significant difference in latency to approach between both. | One session of 7 trials/day, 2 testing days. 2 S1, 2 S2 and 3 ambiguous trials (1 for each ambiguous location), ambiguous trials in random order with S1 and S2 interspersed. Ambiguous trials unrewarded. | PR mares went slower to the negative location and adjacent ambiguous location, indicating more pessimistic responding. No differences were found for any of the other locations. | (Briefier and Freymont et al., 2014) |
| European starlings (Sturnus vulgaris) | f, m | Cage enrichment and removal of enrichment | 2 | A | v; lid of petri dish; S: dark grey (60%), S': white (0%) | If birds made the correct response, it was rewarded with three mealworms (S1) or one mealworm (S2). The incorrect response yielded not reward. Learning criterion: significant difference from chance level over three consecutive days (binomial test). | One session of 15 trials per day. 5 times each reference cue reinforced, two times unreinforced and three times ambiguous probe cues | Change in environmental conditions caused no differences in responses towards the ambiguous cues. However, stereotyping starlings showed more pessimistic responses. | (Briol et al., 2010) |
| European starlings (Sturnus vulgaris) | f, m | Auditory threat/alarm or sparrow hawk calls during testing | 2 | A | v; aversive stimulus: eyespots, positive stimulus: no eyespots | No training involved, natural aversive cues | Eight sessions per bird with 3 trials, each stimulus used. Exposure 2 times to the different auditory cues while tested | Alarm calls and white noise induced higher freezing and also the eyespots were aversive, since latency times towards the food were increased. No differences towards the ambiguous cues found. | (Briol et al., 2008) |

Note: Box with one food trough, equipped with air spray gun, water spray gun and a release mechanism to drop a tennis ball in the back of the piglet.
| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Test-arena | S/S* | Ambiguous stimuli (nr.) | Training | Testing | Go/No-go | Goal | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|-----------------------|-----|-------------------------------|------|------------|------|-------------------------|----------|---------|----------|------|---------|-----------------------------------------|-----------|
| Rats, Lister Hooded   | m   | Cage enrichment (1 week after training) | 2    | 8          | t; S/S; fine or course sandpaper in tunnel | t(1): intermediate grade of sandpaper | Rats move through a tunnel covered with S+ or S-; S+ results in a high value reward (1/2 chocolate drop) if correct choice is made to go to scented cup on left/right. S- results in a lower value reward (cheerio) if correct choice is made in a scented cup on the right/left. Learning criteria: ¾ trials correct over 5 days | Testing in one week, 5 days of 4 trials. One probe trial: presentation of intermediate sandpaper with no rewards present | ✓   | ✓       | Moving to an enriched environment induced a more optimistic choice bias | (Brydges et al., 2011) |
| Rats, Lister Hooded   | f, m | Juvenile stress               | 1    | 4          | t; S/S; fine or course sandpaper in tunnel | t(1): intermediate grade of sandpaper | Rats move through a tunnel covered with S+ or S-; S+ results in a high value reward (1/2 chocolate drop) if correct choice is made to go to scented cup on left/right. S- results in a lower value reward (cheerio) if correct choice is made in a scented cup on the right/left. Learning criteria: ¾ trials correct over 5 days | Testing in one week, 5 days of 4 trials. One probe trial: presentation of intermediate sandpaper with no rewards present | ✓   | ✓       | Control animals displayed a pessimistic choice bias, while animals that received juvenile stress were more optimistic in their choices | (Brydges et al., 2012) |
| Rats, Lister Hooded   | m   | Removal of enrichment in one group during whole experiment, other group remained enriched | 1    | F          | 6; S/S; place of goal pot presentation, one side of arena/other side of arena | s(3): three locations in between S+ and S- | S+ goal pot contains 2 food pellets, presentation of S- is unrewarded, contains 2 inaccessible food pellets. 12 (6 of each) trials/day. Training criterion: significant difference in latency between both. | 3 days of testing. Each ambiguous cue (3 in total) presented once each day in between the conditioned cues. 13 trials/day | ✓   | ✓       | Unenriched rats approached the probe cue nearest to the negative cue more slowly than enriched rats indicating a negative judgement bias. | (Burman et al., 2008) |
| Rats, Lister Hooded   | m   | high (H) or low light (L) training and/or subsequent testing (four conditions: HH, HL, LH, LL) | 2    | E          | 6; S/S; opposite locations in a radial arm maze | s(3): three arms in between S+ and S- | Left or right arms of radial arm maze, associated with goal pot with food pellet (S+) or quinine food pellet (S-). Learning criterion: significant difference in latency between both. | 3 days of testing. Each ambiguous cue (3 in total) presented once each day in between the conditioned cues. 13 trials/day | ✓   | ✓       | Only a change in light conditions had effects on latencies in the probe trials. HL rats ran faster in the probe trials than LH rats. LH rats a more negative judgement bias than HL rats. | (Burman et al., 2009) |
| Dogs, Beagle          | f   | 'Post-consumption' treatment (a rewarding event prior to testing) vs. 'neutral' treatment (no treatment prior to testing) | 2    | A          | 4; S/S; dark and light shade of grey | v(3); intermediate, shades of grey | Approach goal box at S+ (rewarded with food!) avoid approach. S- (unrewarded); criterion: approach S+ faster than S- for six consecutive trials | Three test days, test sequence of 15 trials with ambiguous stimuli interspersed between S+/S-; ambiguous stimuli unrewarded | ✓   | ✓       | 'Post-consumption' group responded slower to middle ambiguous stimulus | (Burman et al., 2011) |

8 Tactile stimuli task, tunnel with course or fine sandpaper
| Species                              | Sex | Experimental manipulation(s)                                                                 | When | Test-arena | S/S* | Ambiguous stimuli (nr.)                                                                 | Training                                                                                      | Testing | Go No-go | Go/Go | Welfare | Effect(s) of experimental manipulation(s) | Reference                  |
|-------------------------------------|-----|-----------------------------------------------------------------------------------------------|------|------------|------|----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|---------|----------|-------|---------|--------------------------------------------|----------------------------|
| Pigs, Large White Landrace halotane  | m   | Testing of cognitive bias twice, with an inter-test interval of 5 weeks; categorization of pigs as having a positive, neutral, or negative cognitive bias, based on first cognitive bias testing | 1    | D          | s;   | S-bucket location with inaccessible reward; S’-bucket location with accessible reward    | Approach bucket S’ (rewarded with chopped apple), avoid approach S bucket (inaccessible chopped apple); criterion: approach S’ faster than S for six consecutive trials | After 14 training sessions and 4 reminder sessions, pigs were tested in one cognitive bias session. After a 5-week interval, pigs received 4 training, 4 reminder sessions, and one cognitive bias session | ✔       | ✔        | ✔     | ✔       | Nearly all pigs learned the discrimination between S and S’ location (33 of 36 pigs). In the test session, most pigs showed positive cognitive bias. Test for repeatability revealed no correlation between time to contact the bucket during the first(original) and second (5 weeks later) cognitive bias testing indicating a learning effect from the first test session, | (Carreras et al., 2015) |
| Gene–RYR1−fr free sows with Pietrain heterozygous boars | f, m | Testing sex differences and the effects of the halothane (HAL) gene, referred to as the porcine stress syndrome gene | 1    | D          | s;   | S-bucket location with inaccessible reward; S’-bucket location with accessible reward    | Nineteen-week-old pigs were trained individually for the CB according to the methodology described by Carreras et al. (2015): Approach bucket S’ (rewarded with chopped apple), avoid approach S bucket (inaccessible chopped apple); criterion: approach S’ faster than S for six consecutive trials. Nine pigs did not discriminate between S+ and S during the reminder sessions and were not tested in the cognitive bias task | After 12 training sessions and 2 reminder sessions, pigs were tested in one cognitive bias session. | ✔       | ✔        | ✔     | ✔       | Neither gender differences nor effects of the HAL gene, not of their interaction were found on the latency to contact the bucket during the training and reminder sessions. The animal was classified as showing a positive, neutral, or negative cognitive bias. During cognitive bias testing, no effects of the HAL gene, of gender, and their interactions were found on latency to contact the bucket | (Carreras et al., in press) |
| Sheep, Romane (Lambs born from HR and LR ewes) | f, m | Prenatal chronic mild stress (aversive events such as social isolation, mixing, handling, transport, delayed feeding) in 10 highest responsive (HR) and 10 lowest responsive (LR), selected from flock of 120 ewes, during the third trimester of pregnancy. Prenataly stressed (PS) lambs were compared with control lambs | 1    | H          | s;   | S/S’ locations on left or right side of testing arena | Criterion learning basic discrimination task: approaching S+ (3 companion pen mate lambs) within 11s, Go-response and not approaching S (a blower) within 25 s (No-go response) in 2 sessions of 10 trials each. | Presentation of S+, S-, and ambiguous stimuli; ambiguous stimuli unrewarded | ✔       | ✔        | ✔     | ✔       | No differences between prenatally stressed and control lambs for S+/S- latencies, but PS lambs had longer latency to approach the ambiguous near S’ stimulus, i.e. a ‘pessimistic’-like bias. | (Coulon et al., 2015) |
| Cattle, Holstein                   | m   | Separation from dam                                                                         | 2    | A          | v;   | (white screen as S vs red screen as S) | Trained to approach screen when S’ is shown (food reward) and not to approach screen when S is shown (punished with time-out). Trained to criterion: 85% correct responses for S’ and S over 3 consecutive sessions | 2 sessions before separation, 3 sessions after separation. 20 screens per session, with 5 trials for each of the 3 ambiguous stimuli, interspersed between S’S+ ambiguous stimuli unrewarded | ✔       | ✔        | ✔     | ✔       | Less responses to ambiguous cues after separation, labelled pessimistic response bias | (Daros et al., 2014) |
| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Test-arena | S/S* | Ambiguous stimuli (nr.) | Training | Testing | Go/No-go | Go-Go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|-----|--------------------------------|------|------------|------|-------------------------|----------|---------|---------|-------|---------|----------------------------------------|-----------|
| Cattle, Holstein       | m   | Hot-iron dehorning             | 2    | A          | √(3); inter- | Trained to approach screen when S* is shown (food reward) and not to approach screen when S is shown (punished with time-out). Trained to criterion: 85% correct responses for S* and S over 3 consecutive sessions | 2 sessions before separation, 3 sessions after separation. 60 screens per session, with 5 trials for each of the 3 ambiguous stimuli interspersed between S*/S, ambiguous stimuli unrewarded | ✓       | ✓       | Less responses to ambiguous cues after dehorning, labelled pessimistic response bias | (Daros et al., 2014) |
| Sheep, Romane          | f   | Diazepam-induced reduction of fearfulness | 2    | D          | s(3); inter- | Approach bucket at S* (rewarded with food)/avoid approach S (punished by revealing blower); criterion: correct responses for two consecutive sessions of five trials | One test day, test sequence of five trials with ambiguous stimuli presented after S* and S, ambiguous stimuli unrewarded | ✓       | ✓       | No overall effect of treatment on approaches to all stimuli; control group slower to approach near-positive ambiguous stimulus over test sessions | (Destrez et al., 2012) |
| Sheep, Romane          | f   | Daily exposure to positive events during the final four weeks of a seven-week chronic stress treatment (exposure to unpredictable and aversive events) | 2    | D          | s(3); inter- | Approach bucket at S* (rewarded with food)/avoid approach S (punished by revealing blower); criterion: correct responses for two consecutive sessions of five trials | One test day, test sequence of five trials with ambiguous stimuli presented after S* and S, ambiguous stimuli unrewarded | ✓       | ✓       | Exposure to positive events induced positive bias to near-positive ambiguous stimulus (faster approach time). | (Destrez et al., 2014) |
| Sheep, Romane          | f   | Chronic stress induced by 6-week exposure to unpredictable and aversive events | 1    | D          | s(3); inter- | Approach bucket at S* (rewarded with food)/avoid approach S (punished by revealing blower); criterion: correct responses for two consecutive sessions of five trials | Two test days, test sequence of five trials with ambiguous stimuli presented after S* and S, ambiguous stimuli unrewarded | ✓       | ✓       | No effect of treatment during training. Treated animals took longer to approach ambiguous stimuli during testing (negative bias) and increased approach time to near-positive stimulus over test sessions. Control animals increased approach time to near-negative ambiguous stimulus/decreased approach time to ambiguous stimulus over test sessions. | (Destrez et al., 2013) |
| Pigs, Large White + Landrace gilts | f   | Enriched versus barren housing | 1    | A | a; S click of a dog-training clicker; S+ note on a glockenspiel | Approach hatch at sound of S* (rewarded with apple)/avoid approach S (punished with plastic bag waved in the face); criterion: correct response on at least 16 out of 20 trials on one day | Five test days (test 1 after training in initial environment, test 2 and 3 after moving to opposite environment., test 4 and 5 after moving back again), two test sequences of 12 trials with ambiguous stimuli interspersed between S*/S, ambiguous stimuli unrewarded | ✓       | ✓       | No effect of treatment during training. Enriched housing group more optimistic (higher nr. ‘go’ responses, faster approach time to ambiguous stimulus) during testing. | (Douglas et al., 2012) |
| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Test-arena | S/S* | Ambiguous stimuli (nr.) | Training | Testing | Go/No-Go | Go/Go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|-----|-------------------------------|------|------------|------|-------------------------|----------|---------|---------|-------|---------|----------------------------------------|-----------|
| Sheep, Merino f        | 2   | Restrained and isolation stress (non-visual contact with conspecifics) for three consecutive days | D    | D          | S/S  | Approach bucket at S* (rewarded with food) / avoid approach S' (punished by presenting dog behind sliding panel); criterion: respond correctly to all ambiguous stimuli | Three test days per week for three weeks (Tue., Wed., Fri.), sequence of five trials with ambiguous stimuli interspersed between S/S; ambiguous stimuli unrewarded | ✓       | ✓       |         |       | ✓       | Treatment group (restraint and isolation stress) more optimistic (higher nr. of 'go' responses to all ambiguous stimuli). | (Doyle et al., 2010a) |
| Sheep, Merino f        | 2   | None, examining effects of repeated testing | D    | D          | S/S  | Approach bucket at S* (rewarded with food) / avoid approach S' (punished by presenting dog behind sliding panel); criterion: respond correctly to all ambiguous stimuli | Three test days per week for three weeks (Tue., Wed., Fri.), sequence of five trials with ambiguous stimuli interspersed between S/S; ambiguous stimuli unrewarded | ✓       | ✓       |         |       | ✓       | Decreasing nr. of 'go' responses to middle ambiguous stimuli with repeated testing. | (Doyle et al., 2010b) |
| Sheep, Merino f        | 2   | Serotonin inhibitor (pCPA) treatment | D    | D          | S/S  | Approach bucket at S* (rewarded with food) / avoid approach S' (punished by presenting dog behind sliding panel); criterion: respond correctly to all ambiguous stimuli | Three test days after three and five days of treatment and five days post-treatment, test sequence of five trials with ambiguous stimuli presented prior to S/S; ambiguous stimuli unrewarded | ✓       | ✓       |         |       | ✓       | Treatment group (pCPA) more pessimistic (lower nr. 'go' responses to middle and near-positive ambiguous stimuli). | (Doyle et al., 2011a) |
| Sheep, Romane f        | 2   | Long-term exposure to unpredictable/aversive events (four weeks during training) | D    | D          | S/S  | Approach bucket at S* (rewarded with food) / avoid approach S' (punished by presenting dog behind sliding panel); criterion: respond correctly to all ambiguous stimuli | Three test days after three and five days of treatment, test sequence of five trials with ambiguous stimuli presented before S/S; ambiguous stimuli unrewarded | ✓       | ✓       |         |       | ✓       | Treatment group (exposed to unpredictable events) more pessimistic (lower nr. 'go' responses to near-positive ambiguous stimuli). | (Doyle et al., 2011b) |
| Pigs, German landrace | f   | Chronic intermittent isolation paradigm (2.5 h of social isolation twice daily for three days, then once daily for four days) | F    | F          | S/S  | Approach goal box at S' (rewarded with food) / avoid approach goal box at S* (unable to reach food by covering bowl with perforated plate) | Three test days post-treatment, test sequence of six trials with ambiguous stimuli at third trial, ambiguous stimuli unrewarded | ✓       | ✓       |         |       | ✓       | No effect of treatment found, all animals displayed positive bias to all ambiguous stimuli (approach latencies similar to rewarded position). | (Dipjan et al., 2013) |
| Rats, cLH and CNLH (bred from Sprague Dawley) | m   | Strain comparison – learned helplessness (cLH) rats (animal model of depression) versus non-learned helplessness (cNLH) rats | 9    | F          | a/3  | Press lever of left wall at S* (rewarded with sweetened milk), press other lever at S (avoid punishment with foot shock); criterion: respond correctly on at least 70% of trials | Six test days, test sequence of 15 trials with ambiguous stimuli interspersed between S/S; ambiguous stimuli unrewarded | ✓       | ✓       |         |       | ✓       | cLH rats showed a lower number of positive responses for middle and near-negative ambiguous cues and a higher number of negative responses for the middle ambiguous cue. | (Enkel et al., 2010) |

9 Skinner box equipped with two retractable levers on opposite walls
| Species (strain/breed) | Sex | Experimental manipulation(s)                                                                 | When | Text-arena | S/S* | Ambiguous stimuli (nr.)                                                                 | Training | Testing | Go/No-go | Go/Go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|-----|-----------------------------------------------------------------------------------------------|------|-------------|------|-----------------------------------------------------------------------------------------|----------|---------|----------|-------|---------|-------------------------------------------|-----------|
| Rats, cLH and CNLH (bred from Sprague Dawley) | m   | Noradrenaline reuptake inhibitor and corticosterone co-treatment (neurobiological stress induce ment) injected before testing | 2    | a;        | a;   | S/S: tones of different frequencies and sound pressure level (2 kHz, 75 dB and 9 kHz, 63 dB). | S/S: tones of different frequencies and sound pressure level. Press lever of left wall at S* (rewarded with sweetened milk), press other lever at S (avoid punishment with foot shock); criterion: respond correctly on at least 70% of trials | Six test days, test sequence of 15 trials with ambiguous stimuli interspersed between S'/S, ambiguous stimuli unrewarded | ✓         | Treated group (“Rbx + cort”) fewer positive responses for all ambiguous cues, higher number of omissions for middle and near-pos. ambiguous cues. | (Enkel et al., 2010) |
| Dogs, various breeds  | F, m | None, testing effects of presented stimuli with valence (picture of happy or angry human face) | 2    | C         | v;   | S: picture of angry human face; S*: picture of happy human face | v(3); mor phed combinations of S'/S, containing 25/50/75% happy elements | Touch specific symbol (circle or triangle) at S'/S (both rewarded when chosen correctly); criterion: 12 out of 15 trials correct for both happy and angry face | Six test blocks of five trials each, with ambiguous, stimuli interspersed between S'/S, ambiguous stimuli unrewarded | ✓         | None of the subjects learned to discriminate between S'/S | (Fernandes, 2012) |
| Common marmosets (Callithrix jacchus) | f, m | Handedness of marmosets: left-handed (LH), right-handed (RH) | —    | 10        | v;   | S: black bowl; S*: white bowl | v(2); near white and near black probe | Response to S* yielded food reward; Response to S* yielded no reward; | 20 probe trials (8 S+, 8S^- and 4 ambiguous, grey bowl) across 5 days; Ambiguous bowls were unrewarded; Second test series with 20 trials per ambiguous stimulus over 10 days, using the 2 ambiguous probes S^+ rewarded | ✓         | No differences between LH and RH marmosets for inspecting the S+ and S^-; LH treated ambiguous stimulus as negative; In second test series, LH marmosets reached for the ambiguous stimulus near S^- slower, indicating ‘pessimism’ | (Gordon and Rogers, 2015) |
| Sheep, Lacaune       | f   | Unpredictable, stimulus-poor environment vs. predictable, stimulus rich environment; measurement of hemodynamic, frontal brain reactions during cognitive bias testing | 2    | D         | S;   | S/S: goal boxes containing food bowls located in opposite corners of room | s(3); goal boxes in between S and S | Sheep had been used in a judgment bias experiment before. Re-training until they made a correct a Go response to the S^+ goal box (feed and salt) No-go response to the S^- goal box (draw and aversive LED light) in all 5 trials on each of 3 successive days. Enriched sheep learned faster | One session of 5 trials: S^+ S^- and three ambiguous goal box positions, randomized per sheep. In parallel, hemodynamic, frontal brain reactions were measured | ✓         | Unexpectedly, sheep from the predictable, stimulus rich environment appeared to make more pessimistic choices. The visual assessment of the choice area in the testing apparatus lead to a general frontal cortical deactivation. | (Guldimann et al., 2015) |
| Rats, Lister hooded  | m   | Housing in unpredictable conditions (negative interventions made at random times)                | 2    | 11        | a;   | S/S: tones of different frequencies (2 and 4 kHz) | a(3); tones of intermediate frequencies between S'/S (2.5, 3 and 3.5 kHz), Press lever at S^- (rewarded with food), do not press lever at S (avoid punishment with white noise); criterion: correct response to each tone more than 50% of the time during three sessions | 10 test sessions, ambiguous stimuli interspersed between S'/S^- ambiguous stimuli unrewarded | ✓         | Animals from unpredictable housing were slower to respond to near-positive ambiguous stimulus, also tended to show fewer responses, indicating a negative bias | (Harding et al., 2004) |

10 Visual discrimination using black or white lid covering food bowl
11 Skinner box equipped with lever
| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Test-arena | S/S* | Ambiguous stimuli (nr.) | Training | Testing | Go/No-Go | Go Go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|-----------------------|-----|--------------------------------|------|------------|------|------------------------|----------|---------|----------|-------|---------|----------------------------------------|-----------|
| Chickens, Brown laying hens | f | Exposure to social isolation for 5 minutes in an unfamiliar environment (stressed), or left undisturbed in their group (control). | 2 | C | v; S white card; S* black card, or vice versa | v(3), intermediate shades of grey | Training on a two-choice task, where the left side (at presentation S*) yielded a small reward (1 mealworm), and the right side (at presentation S) yielded a large reward (4 mealworms) or vice versa; latency to approach was recorded; criterion: 9 of 10 choices correct on two successive days of training; 20 out of 30 birds learned the task | Nine runs to S*; S- and intermediate, ambiguous stimuli (cards with intermediate shades of grey); ambiguous cues yielded no reward | ✓ | ✓ | Response to the cue near S- and the intermediate cue was more optimistic in stressed than in control chickens; This effect was larger when the previous trial showed the S* | (Hernandez et al., 2015) |
| Chickens, breed unknown | m | Pharmacological reversal of isolation-induced anxiety (5 min isolation stressor) and depression (80 min isolation stressor) through treatment with clonidine and imipramine, respectively | 2 | A | v; Intra-aversive affective stimuli used (mirror and owl image). | v(2); 75% chick/25% owl morphed silhouette and 25% chick/75% owl morphed silhouette | One trial to measure start and goal latency and farthest distance travelled in anxiety/depression condition towards S* | One trial to measure start latency and farthest distance travelled towards all stimuli after pharmacological reversal of condition | ✓ | Anxiety group displayed longer start latencies to ambiguous owl (near-negative) stimulus compared to control, this was not reversed by pharmacological treatment. Depression group displayed longer start latencies and shorter distance travelled towards ambiguous chick (near-positive) stimulus and ambiguous owl stimulus, this was reversed by pharmacological treatment. | (Hymel and Suflka, 2012) |
| Dogs of different breeds and age | f, m | 5 control dogs, 5 dogs with diagnosed 'separation anxiety'. Dogs with separation anxiety were treated with fluoxetine in combination with a behavior modification plan starting after baseline cognitive bias testing | 1, 2 | F | S; S' locations counterbalanced left/right side of the arena | s(3); locations in between S, and S* | Training to discriminate between the S* location (yielding food reward), and the S- location (no reward) until the adjusted speed (m/s) to approach the S* location exceeded the adjusted speed to approach the S | First testing (baseline), re-testing 2, 4, and 6 weeks later (separation anxiety group; controls were not tested 4 weeks after baseline). Test: Ambiguous probes interspersed between S' and S locations, 40 trials (9 ambiguous) | ✓ | Baseline measurement: Dogs with separation anxiety approached the ambiguous stimulus near S- slower than the control dogs (pessimist). Treatment normalized their behavior which became similar to that of the control dogs | (Karagiannis et al., 2015) |
| Grizzly bears (Ursus arctos horribilis) | f, m | Pilot study reward contrast - positive reward of three versus six apple slices (Experiment 1) | 2 | 1-4 | v; S*/S dark and light shade of grey presented on cardboard squares | v(3); intermediate shades of grey | Touch S* with nose (rewarded with apple slices), touch S with paw (rewarded with single apple slice) and vice versa; criterion: five successive training sessions (30 trials) at 90% accuracy | Four test sessions, test sequence of 30 trials with one ambiguous stimulus interjected between S*/S once every 10 trials, ambiguous stimuli rewarded with secondary reinforcer (clicker) | ✓ | No difference found between groups receiving three or six slices as high reward; no bias in response toward ambiguous stimuli found. | (Keen et al., 2014) |

12 Some neutered
13 Similar to F with respect to the start and goal positions; distances varied, and the tests were not performed in an enclosed arena, but at the owner’s home
14 Stimuli were presented outside animal enclosure, with animals responding through the fence
| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Test-arena | S/S* | Ambiguous stimuli (nr.) | Training | Testing | Go/No-Go | Go/Go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|-----|-------------------------------|------|------------|------|--------------------------|----------|---------|---------|------|---------|------------------------------------------|-----------|
| Grizzly bears (Ursus arctos horribilis) | f, m | Enrichment session pre-testing with three types of enrichment item (low, medium and high interest item) (Experiment 2) | 2 | 14 | v, S/S-dark and light shade of grey presented on card-board squares | v(3): inter-mEDIATE shades of grey | Touch S+ with nose (rewarded with four apple slices), touch S- with paw (rewarded with single apple slice) and vice versa; criterion: five successive training sessions (30 trials) at 90% accuracy | 18 test sessions, test sequence of 30 trials with one ambiguous stimulus interjected between S+/S-: once every 10 trials, ambiguous stimuli rewarded with secondary reinforce (clicker) | ✓ | ✓ | No effect found of enrichment condition, no bias in response toward ambiguous stimuli found. | (Keen et al., 2014) |
| Dogs, different breeds and age | f, m | Responses to ambiguous stimuli in the cognitive bias task after intranasal administration of oxytocin or placebo, in a communicative or non-communicative condition | 2 | F15 | S-, S+/S- left or right side of the test arena | s(1): intermediate between S+ and S- | Experimenter stood between S+ (bowl with food reward) and S+ (empty bowl), experimenter called dog with name ‘look’, and put the food bowl on the floor; owner released dog to start trial; Criterion: latency to reach negative location longer than reaching positive location (calculated by Wilcoxon test). | Re-training on S+, S in the presence (communicative context) or absence (non-communicative condition) of the experimenter, followed by testing response to the ambiguous food bowl location, (order of trials: negative, positive, ambiguous trial) in the presence or absence of the experimenter | | ✓ | Oxytocin-treated dogs had higher positive expectancy than control dog when presented the ambiguous bowl position; the communicative condition (experimenter present) increased the positive expectancy | (Kis et al., 2015) |
| Mice, C57BL/6N | f | Exp. 1: Validating assessment of differing behavioral outcomes in response to an ambiguous stimulus between mice anticipat-ing a positive or negative event (exp. 1) | — | E | S- air-puff upon reaching hole at end of maze arm; S+ hole at end of maze arm was exit of maze | s(1): maze arm in between S+ and S- arms | Optimistically trained mice could use either of 2 maze arms to exit maze, pessimistically trained mice received air-puff upon reaching a hole at the end of either of 2 available maze arms, 13 trials over 3 training days | Response to unfamiliar maze arm, located in between S+/S- arms, single testing trial per mouse | ✓ | Optimistically trained mice had decreased latencies to reach the hole in the unfamiliar maze arm | (Köke et al., 2014) |
| Mice, C57BL/6J | f | Exp. 2: Validating applicability of a spatial location as a discriminatory stimulus for mice (exp. 2) | — | E | s- air-puff upon reaching hole at end of maze arm; S+ hole at end of maze arm was exit of maze | s(3): maze arms in between S+ and S- arms | Mice were trained to discriminate between positive (exit) and negative (air-puff) arm on either side of maze, 21 trials over 5 training days, criterion: shorter latency to reach positive arm than negative arm | Response to ambiguous arms (unfamiliar central arm, near-negative arm or near-positive arm), single testing trial per mouse | ✓ | Mice were faster to reach the near-positive arm and slower to reach the hole in the near-negative arm | (Köke et al., 2014) |
| Mice, 5-HTT +/-, +/- and +/- (C57BL/J background) | f | Pilot study with 5-HTT knockout mice (+/- with anxiety- and depression-like phenotype) | 1 | E | s- air-puff upon reaching hole at end of maze arm; S+ hole at end of maze arm was exit of maze | s(1): maze arm in between S+ and S- arms | Mice were trained to discriminate between positive (exit) and negative (air-puff) arm on either side of maze, 25 trials over 5 training days, criterion: shorter latency to reach positive arm than negative arm | Response to unfamiliar maze arm, located in between S+/S- arms, single testing trial per mouse | ✓ | Non-significant trend for 5-HTT +/- mice to have highest latency and +/- mice to have lowest latency to reach the hole in the central arm | (Köke et al., 2014) |

15 Similar to F with respect to the start and goal positions; the tests were not performed in an arena, and only one ambiguous location (intermediate between S+ and S-) was presented during testing.
| Species (strain/breed) | M | Experimental manipulation(s) | When | Test-arena | S/S* | Ambiguous stimuli (nr.) | Training | Testing | Go/No-Go | Go/Go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|-----------------------|---|-------------------------------|------|------------|------|-------------------------|----------|---------|----------|--------|---------|-----------------------------|-----------|
| Rats, Sprague Dawley | m | Single i.p injections of dopaminergic precursor L-DOPA (2, 4, 8 mg.kg⁻¹), D2 receptor antagonist haloperidol (0.01, 0.02, 0.05 mg.kg⁻¹), or serotonin reuptake inhibitor escitalopram (0.5, 1, 2 mg.kg⁻¹) (randomized Latin square design with saline as vehicle control); one-week wash-out intervals between doses (Experiment 1) | 2    | 16        | a; S: 9000 Hz tone: right lever to avoid delayed foot-shock; S⁺: 2000 Hz tone: left lever to gain sucrose reward, or vice versa. | Training on a operant task with one auditory stimulus that predicts reward, the other that predicts punishment. Introduction of an intermediate ambiguous stimulus that lead to no consequences. After separate e training on S and S⁺: pseudo-random presentation of S and S⁺ (20:20). Training to criterion of 70% correct discrimination performance. | Pseudorandom presentation of S, S⁺, and ambiguous stimulus (20:20:10) during single testing session per dose | ✓       | No effect: L-DOPA, escitalopram; Effect: haloperidol deceased positive and negative lever presses to the ambiguous tone; also increased omissions were measured | (Kregiel et al., 2016a) |
| Rats, Sprague Dawley | m | daily injection of L-DOPA (8 mg.kg⁻¹), haloperidol (0.05 mg.kg⁻¹), escitalopram (2 mg.kg⁻¹) for 2 weeks; the tryptophan hydroxylase inhibitor 4-cloro-DL-phenylalanine methyl ester (PCPA, 400 mg.kg⁻¹) was administered daily once on the first two days of the first and second week (Experiment 2) | 2    | 16        | a; S: 9000 Hz tone: right lever to avoid delayed foot-shock; S⁺: 2000 Hz tone: left lever to gain sucrose reward, or vice versa. | Trained rats from exp. 1 were re-used two weeks after the end of exp. 1; they were assigned randomly to five groups (4 groups received a drug, one group served as saline control) | After chronic administration of test compounds (or saline) the animals were tested in one session | ✓       | No effect: haloperidol, escitalopram, PCPA; Effect: L-DOPA increased positive lever presses in response to ambiguous tone | (Kregiel et al., 2016a) |
| Rats, Sprague Dawley | m | Treatment with the irreversible anandamide hydrolysis inhibitor URB597 in three doses (experiment 1) | 2    | 16        | a; S: 9000 Hz tone: right lever to avoid delayed foot-shock S⁺: 2000 Hz tone: left lever to gain sucrose reward, or vice versa. | Training on a operant task with one auditory stimulus that predicts reward, the other predicts punishment. Introduction of an intermediate ambiguous stimulus that lead to no consequences. After separate e training on S and S⁺: pseudo-random presentation of S and S⁺ (20:20). Training to criterion of 70% correct discrimination performance. | Pseudorandom presentation of S, S⁺, and ambiguous stimulus (20:20:10) during single testing session, in baseline session and 30 or 60 minutes after drug administration | ✓       | Experiment 1: URB597 increased “optimistic” choices to the ambiguous tone at the highest dose tested (1 mg.kg⁻¹). | (Kregiel et al., 2016b) |

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16 Two-lever Skinnerbox with sucrose reward and footshock punishment
| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Text-arena | S/S* | Ambiguous stimuli (nr.) | Training | Testing | Go/No-go | Go/Go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|-----------------------|-----|-------------------------------|------|------------|------|--------------------------|----------|---------|----------|-------|---------|------------------------------------------|-----------|
| Dawley Rats, Sprague-Dawley | m   | Treatment with URB597, the cannabinoid receptor type 1 (CB1) inverse agonist AM251, the CB2 inverse agonist AM630, combination URB597-AM251, and combination URB597-AM630 (Experiment 2) | 2     | 16        | a; S: 9000 Hz tone: right lever to avoid delayed foot-shock S': 2000 Hz tone: left lever to gain sucrose reward, or vice versa. | a(1); 5000 Hz tone | Training on a operant task with one auditory stimulus that predicts reward, the other predicts punishment. Introduction of an intermediate ambiguous stimulus that lead to no consequences. After separate training on S and S': pseudo-random presentation of S and S': (20:20). Training to criterion of 70% correct discrimination performance. | Pseudorandom presentation of S; S': and ambiguous stimulus (20:20:10) during single testing session, in baseline session and 30 or 60 minutes after drug administration | ✔ | Experiment 2: AM251 and AM630 alone had no effect, but both antagonized the effect of URB597 (see result experiment 1) | (Kregiel et al., 2016b) |
| Domestic chickens (Sturnus vulgaris) | f   | Housing in battery cage until 67 weeks of age; testing 2 and 4 months after rehousing to ground housing with litter, laying nest and perch | 2     | 1           | F | s; s-empty bowl; S' bowl containing the food reward | s(3); bowl in between S' and S' location | Training until approach to bowl with food in less than 2 min for 3 consecutive trials. Eight trials per training session for 12 or more sessions | 3 runs with ambiguous probe positions mixed with 10 runs with unambiguous positions at 2 months after rehousing. This was repeated at 4 months after rehousing. | ✔✔ | Hens took longer to reach center ambiguous position four months after rehousing compared to two months after rehousing. No differences at other positions. | (Lindström, 2010) |
| European starling (Sturnus vulgaris) | f, m | Housing in enriched or unenriched cages | 2     | A | d (visuotemporal): S: light on 2 s; S': light on 10 s | d(8) intermitent duration stimulus | Training to peak one colored key for immediate reward (after S) and a different colored key for a delayed reward (after S'). Maximum 54 trials or 2.5 h per session. Criterion: 65% correct for 3 consecutive sessions. | 360 ambiguous probe trials (light on durations in between S and S') across 10 days, each session had 18 reinforced trials and 36 probe trials | ✔✔ | Subset of animals was significantly more likely to choose the S' associated key when housed in enriched environment; overall no effects of enrichment were found. | (Matheson et al., 2008) |
| Dawley Rats, Sprague-Dawley | m   | Subcutaneous treatment with 0.001 mg.kg⁻¹ oxytocin, 5 minutes before testing trials | 2     | 17        | d (Visuospatial); S: white compartment with smooth floor; S': black compartment with textured floor, and vice versa | d (2) Visuospatial; white compartment with textured floor and black compartment with smooth floor | After habituation to the place preference apparatus, rats received 2 to 5 training sessions with 6 aversive trials (compartment with quinine soaked food) and 6 reward trials (compartment with palatable food), presented in a randomized order. Criterion of learning was that latency to approach food bowl in rewarded compartment was at least 5 seconds shorter that approach to aversive compartment. | One testing session on each of two successive days. Ambiguous trials were not rewarded, whereas the food bowls contained food as during training in the S' and S' trials | ✔ | Oxytocin treatment had no effect on the latency to approach the food bowl during ambiguous trials. However, rats behaved in ambiguous trials with short latencies, suggesting that they showed a positive cognitive bias. The dose of oxytocin administered may have been ineffective. | (McGuire et al., 2015) |

17 Modified conditioned place preference apparatus (grey start box, with a larger black and a larger white box on either side of the start box)
| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Text-arena | S/S* | Ambiguous stimuli (nr.) | Training | Testing | Go/No-go | Go/Go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|-----|--------------------------------|------|------------|------|-------------------------|----------|---------|---------|------|---------|-------------------------------------------|-----------|
| Dogs various breeds    | f, m | Animals which perform destructive separation-related behavior | 1    | G          | s; S-bowl on one side; S' bowl on the other side of the arena | s(3); bowl in between S- and S' location | Training to approach the S+ (bowl containing food) and to suppress approaching the S- (empty bowl), for at least 15 trials. S+ and S- were presented in pseudorandom order. Learning criterion: when from the preceding three positive trials and negative trials, the longest latency to reach the S+ was shorter than any of the latencies to reach the S-. 3 probe trials, ambiguous locations between S+ and S-. Each probe separated by 4 trials with S-/S+. Measure: latency to approach bowl. | ✓     | ✓      |         |         | | Dogs with higher destructive separation-related behavior showed higher latencies at mid position, interpreted as more pessimistic response. | (Mendl et al., 2010) |
| Dogs, various breeds   | f, m | Separation from owner vs owner present (within subjects design) | 2    | C          | a; S-; S; 2 tones (either 200 or 1000Hz) | a (3); intermediate tones | Blocks of 10 trials, with 5 S- (empty bowl) and 5 S+ (bowl containing food reward Criterio: statistically significant difference in latency to approach S and S' or maximum of 120 trials | ✓     |         |         |         | ✓      | No effect of separation from owner on cognitive bias. | (Müller et al., 2012) |
| Pigs (Duroc x Yorkshire and Duroc x Danish Landrace versus Göttingen minipig) | f | Genetic background and restraint (1-5 minutes) | 1, 2 | A          | v; S-red video screen; S' white screen | v;3 intermediate colors between red and white | Trained to perform operant response in one location following S+, and another location following S-. Trained to criterion of three consecutive sessions with at least 4 out of 5 correct choices for both S+ and S-. Two phases Phase 1 testing: four sessions of 16 trials, of which 3 forced and 10 free trials with S+ and S-, and 3 trials with ambiguous tones. Phase 2: isolation for 5 minutes before and 15 minutes after testing, testing as in Phase 1. | ✓     |         |         |         | ✓     | No effect of breed or isolation on judgment bias. | (Murphy et al., 2013) |
| Dairy calves (Holstein) | m   | Hot iron de-horning           | 2    | A          | v; S-red video screen; S' white screen | v;3 intermediate colors between red and white | Trained to nose touch a screen when S+ is shown to receive milk and not to touch the screen when S is shown to prevent a time out. Trained to criterion: 90% correct responses over 3 consecutive sessions in experiment 1, 85% correct responses over 3 consecutive sessions in experiment 2. 3 sessions before disbudding, 2 sessions after disbudding. 60 screens per session, with 8% of trials for each of the 3 ambiguous stimuli. | ✓     |         |         |         | ✓     | Fewer responses to the intermediate and near-negative stimuli after de-horning. | (Neave et al., 2013) |
| Species (strain/breed)          | Sex | Experimental manipulation(s)                                      | When | Test arena | S/S* | Ambiguous stimuli (nr.) | Training                                                                 | Testing                                                                 | Go No-go | Go/No-go | Welfare | Effect(s) of experimental manipulation(s) | Reference                                                                 |
|--------------------------------|-----|------------------------------------------------------------------|------|------------|------|------------------------|-------------------------------------------------------------------------|----------------------------------------------------------------------|----------|----------|---------|-------------------------------------------|---------------------------------------------------------------------------|
| White-lipped peccary (Tayassu pecari) | ?   | Trapping before the second judgment bias test                   | 2    | I          | a; S- horn; S+ whistle | a(1) bell                  | Go response to S+, i.e. approach to a food dispenser; No-go response to S-, staying away from food dispenser (cut-off: 60 seconds) | 30 trials per test over 2 days, 10 ambiguous trials (bell, unrewarded). Animals were exposed to three tests one basic (T1), 7 days later T2 (30 minutes after trapping) and T3 (7 days after T2) speed (m.s⁻¹) to get to the feed dispenser was registered. | ✔        | ✔        | ✔       | The peccary discriminated between S+ and S-: Whereas proportion of go-responses was intermediate between S+ and S- in testing periods 1 and 3 (no trapping), it was near the S- after trapping in testing period 2, suggesting a pessimistic judgment of this cues | (Nogueira et al., 2015) |
| Mice, CD1                        | f   | Daily handling with two different methods, tail and cup (week 3-18), home cage recording (week 19-25). Judgment bias training and test in week 26 & 27, animals were also handled in training and testing weeks. | 2, 18 | E¹⁹       | s    | S(4)                   | Running towards the positive arms (S⁺) turns off the overhead lights (400 lux) and delivers a food pellet. Running towards the negative arms (S⁻) turns on overhead lights and white noise. 6 days of training were performed, no learning criterion, but data shows increased discrimination between positive and negative arms | One test session of one minute in which all arms were open. The four arms in between the S- and S+ were the ambiguous cues. Exploration time of each arm was examined | ✔        | ✔        | ✔       | The different handling methods had no effects on duration and frequency of exploration of ambiguous arms in the test. Test shows potential since mice explored near positive arms more than the near-negative arms. | (Novak et al., 2015) |
| Mice, CD-1 and C57BL/6J(Rcc)     | f   | Unpredictable chronic mild stress (UCMS): after reaching criterion during the training phase, all mice underwent unpredictable chronic mild stress during a 3-week period. During stress treatment, mice were trained on a partial reinforcement schedule, i.e. a proportion of the trials was unrewarded. Half of the mice served as controls and were not subjected to UCMS. | 2    | I          | t; S/S- coarse or fine sandpaper associate the a high or low value reward | (3) intermediate grades of sandpaper) | Training positive trials: compartment and goal pot covered with fine or coarse sandpaper with a hidden almond flake, a high value reward vs. compartment and goal pot without sandpaper (i.e. incorrect choice); negative trial: compartment and goal pot without sandpaper with a hidden oat flake, a low value reward, vs. compartment with the other grade of sandpaper (i.e. the incorrect choice). Criterion: series of 10 correct choices in a series of 14 trials. | Three judgment bias sessions with 15 trials each (six positive, six negative and 3 intermediate stimulus presentations); optimistic choices were trials in which the mice dig in the goal pot and compartment covered with sandpaper | ✔        | ✔        | ✔       | Non-learners (2 CD-1, 4 C57BL mice) were excluded. Overall, CD-1 mice were faster learners. UCMS tended to decrease responding to the positive (almond) trials. The control mice of both made a graded response to the intermediate (ambiguous) cues, and made more optimistic responses to the near positive cue, and less optimistic responses to the near negative cue, whereas the UCMS mice made similar responses to all intermediate cues. Also, UCMS responded faster to the intermediate cues. | (Novak et al., 2016) |
| Rats, Sprague Dawley             | f   | Social stress (resident-intruder paradigm)                      | 2    | C          | a; S/S- two tones of 9000 or 2000 Hz | a(1); intermediate tone of 5000 Hz | Trained to press a lever at S+ to gain sucrose solution and to press a second lever at S- to avoid a shock. Trained to criterion of 70% correct responses for each lever for 3 consecutive sessions. | One session of 20 S+; 20 S- and 10 ambiguous stimuli before social stress, and one session of 20 S+; 20 S- and 10 ambiguous stimuli after social stress. | ✔        | ✔        | ✔       | More responses on S+ lever after social stress (pessimistic response). | (Papciak et al., 2013) |

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18 Part of the enclosure where the peccary were kept
19 The whole radial arm was used
| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Test-arena | S/S* | Ambiguous stimuli (nr.) | Training | Testing | Go/No-Go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|-----|-------------------------------|------|------------|------|--------------------------|----------|--------|----------|---------|----------------------------------------|-----------|
| Rats, Lister hooded    | m   | Unpredictable housing (negative interventions made at random times) | 2    | 20         | a; S'/S : two tones with different frequencies (either 2 or 4 kHz), counter-balanced | a(14); single-frequency tones (1.6-4.4 kHz with 200 Hz increments) or dual-frequency tone consisting of combined S- and S+ | Trained to press a lever at S- to gain 2 food pellets and to press a second lever at S+ to gain 1 food pellet. Criterion: 3 consecutive sessions of performance which was significantly greater than chance-level for each trial type. 6 single-frequency tone testing sessions (3 before and 3 after treatment). 156 trials or 60 minutes with 50% S- and 2 dual-frequency tone sessions (1 before and 1 after treatment). 64 trials or 30 minutes with 50% S+, ambiguous trials were not rewarded. | ✓ ✓ | Control group decreased responses to ambiguous tones over time, treated group did not. No other differences between groups. | (Parker et al., 2014) |
| Capuchin monkeys       | f, m| None, correlation of judgement bias with stereotypical behaviors | 1    | C          | v; S'/S : large or small striped panel, counter-balanced | v(1); striped panel of intermediate size | Monkeys were trained to respond to S' by retrieving preferred reward from one location, and S by retrieving non-preferred reward from another location. Five 20-trial sessions on consecutive days with 9 S-, 9 S+, and 2 ambiguous trials per session. | ✓ ✓ | Negative correlation between probability to choose the positive reward and amount of stereotypy displayed. | (Pomerantz et al., 2012) |
| Sprague Dawley rats    | m   | None, classification of rats as optimistic or pessimistic before testing them in a rat slot machine task | 1    | C          | a; S'/S+ : two tones of 9000 or 2000 Hz | a(1); intermediate tone of 5000 Hz | The rats were trained to press one lever when a ‘positive’ tone (2000 Hz at 75 dB) signaled a reward (5% sucrose solution) and to press second lever when another ‘negative’ tone (9000 Hz at 75 dB) signaled punishment (0.5 mA foot shock, duration: 10 s). Criterion: 70% correct responses on each lever, over three consecutive discrimination sessions. Cognitive judgment bias as a trait was assessed across a series of 10 consecutive tests at one-week intervals. Based on the average cognitive bias index obtained across the 10 tests, rats were classified as optimistic or pessimistic. | ✓ ✓ | No experimental manipulations | (Rafa et al., 2016) |
| Rats, 71st and 72nd generation of selection (cLH and CNLH) lines originating from Sprague-Dawley | m   | Selection lines for high or low learned helplessness tested before and after 4 weeks of environmental enrichment | 1, 2 | E          | s; 2 arms at opposite location in a radial arm maze | s(3); arms at intermediate positions | Training to retrieve “fruit loops” cereal (US+) or avoid “fruit loops” cereal soaked in quinine (US-) from reference locations. 12 trials per session, 6 with US+ and 6 with US-, of which 1 trial had no reward at S- location (partial reinforcement). Rats were trained to individual criterion: significant difference in latencies to approach the S- and S+ goal pots on two consecutive days (Mann-Whitney U Test, one-tailed, p ≤ .05). Training for a minimum of 3 days but for no more than 7 days. 3 testing sessions with 13 trials per session, of which 3 ambiguous (1 trial per ambiguous location per session, totaling 3 trials per ambiguous location). Higher latencies to reach all three ambiguous goal-pots in selection line for high learned helplessness compared to low learned helplessness line (more negative bias); both groups showed reduced latency time to dip nose into any goal pot following enriched housing. | ✓ | | (Richter et al., 2012) |

20 Retractable lever on either side of the food trough
| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Test-arena | S/S* | Ambiguous stimuli (nr.) | Training | Testing | Go/NoGo | Goal | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|-----|-------------------------------|------|------------|------|--------------------------|----------|---------|---------|------|---------|------------------------------------------|----------|
| Rats, Sprague–Dawley   | m   | Selection of rats, based on 1 weekly session of cognitive bias testing over 10 weeks as ‘optimistic’ or ‘pessimistic’ (baseline). Then, half of the optimistic and half of the pessimistic rats received daily 1-h immobilization sessions over a period of 3 weeks, whereas the other half (controls) was handled. Effects of immobilization stress were tested once per week during this period in the judgement bias task | 1, 2 | 16 | a; S: 9000 Hz tone: right lever to avoid delayed foot-shock; S': 2000 Hz tone: left lever to gain sucrose reward, or vice versa. a(1); 5000 Hz tone | Training on a operant task: S*: auditory stimulus predicted sucrose reward, the other S*: auditory stimulus predicted food shock, avoidance could be achieved when right lever was pressed. After separate training on S and S*: pseudorandom presentation of S and S* (20:20). Training to criterion of 70% correct discrimination performance. | Pseudorandom presentation of S, S*: and ambiguous stimuli (20:20:10). | ✓ | ✓ | The stability of the cognitive bias response during baseline measurement in the course of 10 weeks suggests that ‘pessimism’ and ‘optimism’ are behavioral traits. Rats that underwent repeated immobilization stress of both the ‘optimistic’ and the ‘pessimistic’ group (according to baseline testing) were more pessimistic than the handled control rats, compared with their baseline values. The two control groups did not change their bias during the immobilization period. | (Rygula et al., 2013) |
| Rats, Sprague–Dawley   | m   | Acute pharmacological stimulation of the serotonin (5-HT), noradrenaline (NA) and dopamine (DA) systems 5HT: selective serotonin reuptake inhibitor (SSRI) citalopram (1, 5 and 10 mg.kg^-1); NA: noradrenaline reuptake inhibitor desipramine (1, 2 and 5 mg.kg^-1); DA: DA (and to a lesser extent NA and 5-HT) releaser d-amphetamine (0.1, 0.5 and 1 mg.kg^-1) | 2    | 16 | a; S: 9000 Hz tone: right lever to avoid (delayed) foot-shock; S': 2000 Hz tone: left lever to gain sucrose reward, or vice versa a(1); 5000 Hz tone | Training on a operant task: S*: auditory stimulus predicted sucrose reward when pressing the left lever, the other S*: auditory stimulus predicted food shock, avoidance could be achieved when right lever was pressed. After separate training on S and S*: pseudorandom presentation of S and S* (20:20). Training to criterion of 70% correct discrimination performance. | Pseudorandom presentation of S, S*: and ambiguous stimuli (20:20:10) during single testing session | ✓ | ✓ | S-HT stimulation induced negative cognitive bias at 1 mg.kg^-1 citalopram (reduction of optimistic lever presses). At higher dosages (5 or 10 mg.kg^-1) positive cognitive bias was induced by reducing pessimistic lever presses. NA stimulation induced negative bias in all tested doses of desipramine by reducing optimistic lever presses and increasing pessimistic lever presses. DA stimulation at 1 mg.kg^-1 d-amphetamine induced positive bias by reducing pessimistic lever presses. No effects were found at lower doses (0.1 and 0.5 mg.kg^-1). | (Rygula et al., 2014a) |
| Rats, Sprague–Dawley   | m   | Chronic, daily administration of psychostimulants (amphetamine or cocaine) for a duration of 2 weeks | 2    | 16 | a; S: 9000 Hz tone: right lever to avoid (delayed) foot-shock; S': 2000 Hz tone: left lever to gain sucrose reward, or vice versa a(1); 5000 Hz tone | Training on a operant task: S*: auditory stimulus predicted sucrose reward when pressing the left lever, the other S*: auditory stimulus predicted food shock, avoidance could be achieved when right lever was pressed. After separate training on S and S*: pseudorandom presentation of S and S* (20:20). Training to criterion of 70% correct discrimination performance. | Pseudorandom presentation of S, S*: and ambiguous stimuli (20:20:10) during single testing session | ✓ | ✓ | Treatment impaired both groups of rats in their ability to discriminate between and/or react to the S*/S: neither drug resulted in a significant effect on the interpretation of the ambiguous stimulus. | (Rygula et al., 2015c) |
| Species (strain/breed) | m | Experimental manipulation(s)                                                                 | When | Test-arena | S/S* | Ambiguous stimuli (nr.) | Training                                                                 | Testing                                                                                                                                                                                                 | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|---|-----------------------------------------------------------------------------------------------|------|------------|------|-------------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|------------|
| Rats, Sprague-Dawley   | 2 | Acute treatment with valproic acid (100, 200, 400 mg.kg⁻¹), or lithium chloride (10, 50, 100 mg.kg⁻¹) (Latin square design), with 1-week washout period between drug administrations | 16   |            | a;  S* 9000 Hz tone: right lever to avoid (delayed) foot-shock; S: 2000 Hz tone: left lever to gain sucrose reward, or vice versa | a(1); 5000 Hz tone.                                                      | Training on a operant task: S* auditory stimulus predicted sucrose reward when pressing the left lever, the other S auditory stimulus predicted food shock, avoidance could be achieved when right lever was pressed. After separate training on S and S*: pseudo-random presentation of S and S* (20:20). Training to criterion of 70% correct discrimination performance over three consecutive days. | Pseudorandom presentation of S*, S*, and ambiguous stimuli (20:20:10) during single testing session. | Valproic acid had no effects in any dose tested; lithium at the dose of 50, but not 10 and 100 mg.kg⁻¹ affected responding to the ambiguous tone cue, indicating an optimistic bias. | (Rygula et al., 2015a) |
| Rats, Sprague-Dawley   | 2 | Ticking of the rats, vs. handling without tickling in a cross over schedule (within subjects comparisons). Subdivision of rats into “laughing when tickled” (emission of 50-kHz ultrasonic vocalizations) and “not laughing when tickled” group. | 16   |            | a;  S* 9000 Hz tone: right lever to avoid delayed foot-shock; S: 2000 Hz tone: left lever to gain sucrose reward, or vice versa. | a(1); 5000 Hz tone.                                                      | Training on a operant task: S* auditory stimulus predicted sucrose reward when pressing the left lever, the other S auditory stimulus predicted food shock, avoidance could be achieved when right lever was pressed. After separate training on S and S*: pseudo-random presentation of S and S* (20:20). Training to criterion of 70% correct discrimination performance. | Pseudorandom presentation of S*, S*, and ambiguous stimuli (20:20:10) during single testing session. | The laughing when tickled rats showed a positive bias toward the ambiguous tone; no effects of tickling were seen toward the positive and negative tones. Both subgroups showed slightly more response omissions to ambiguous tone | (Rygula et al., 2012) |
| Rats, Sprague-Dawley   | 2 | Acute pharmacological stimulation of the dopamine (DA) system by administration of either cocaine or mazindol | 16   |            | a;  S* 9000 Hz tone: right lever to avoid (delayed) foot-shock; S: 2000 Hz tone: left lever to gain sucrose reward | a(1); 5000 Hz tone.                                                      | Training on a operant task: S* auditory stimulus predicted sucrose reward when pressing the left lever, the other S auditory stimulus predicted food shock, avoidance could be achieved when right lever was pressed. After separate training on S and S*: pseudo-random presentation of S and S* (20:20). Training to criterion of 70% correct discrimination performance. | Pseudorandom presentation of S*, S*, and ambiguous stimuli (20:20:10) during single testing session. | Cocaine had no effect on the rats’ ambiguous cue interpretation. Administration of mazindol resulted in a negative bias by reducing optimistic lever presses and increasing pessimistic lever presses. | (Rygula et al., 2014b) |
| Rats, Sprague-Dawley   | 2 | Selection of “optimistic” and “pessimistic” rats, based on responding in Judgment bias task. Then: assessment of motivation to gain food reward and to avoid punishment using a progressive ration of reinforcement schedule | 16   |            | a;  S* 9000 Hz tone: right lever to avoid (delayed) foot-shock; S: 2000 Hz tone: left lever to gain sucrose reward | a(1); 5000 Hz tone.                                                      | Training on a operant task: S* auditory stimulus predicted sucrose reward when pressing the left lever, the other S auditory stimulus predicted food shock, avoidance could be achieved when right lever was pressed. After separate training on S and S*: pseudo-random presentation of S and S* (20:20). Training to criterion of 70% correct discrimination performance. | Pseudorandom presentation of S*, S*, and ambiguous stimuli (20:20:10) during single testing session; rats were selected for optimistic and pessimistic traits, motivation for food and avoidance of punishment was investigated. | The two groups did not differ for avoidance of punishment; the optimistic group showed a higher motivation to gain food reward. | (Rygula et al., 2015b) |
| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Test-area | S/S* | Ambiguous stimuli (nr.) | Training | Testing | Go/No-go | Go/Go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|-----|-------------------------------|------|-----------|------|-------------------------|----------|---------|----------|-------|---------|--------------------------------------------|-----------|
| Chickens (Gallus gallus) | f   | Testing twice (at 4 and 5 days of age), experiment 1 | 2    | A         | v; innate aversive and affective stimuli used (mirror and owl image). | One trial to measure the latencies to leave the start box and reach the goal box (S*: mirror image of the chick) on day 4 | One trial to measure the latencies to leave the start box and reach the goal box (S*: image of owl, S*: image of chick, ambiguous cues: three morphs between chick and owl) on day 5 | ✓ | ✓ | Latency to reach end of runway increased with degree of similarity of stimuli with owl silhouette. | (Salmeto et al., 2011) |
| Chickens (Gallus gallus) | f   | 3 groups of chickens: 5 minutes isolation, 60 minutes isolation, or no isolation (control), experiment 2 | 2    | A         | v; S*: mirror image of chick tested; S*: owl silhouette | One trial to measure the latencies to leave the start box and reach the goal box (S*: mirror image of the chick) on day 4 | One trial to measure the latencies to leave the start box and reach the goal box (S*: image of owl, S*: mirror image of chick, ambiguous cues: three morphs between chick and owl) on day 5 | ✓ | ✓ | Latency to reach end of runway increased with degree of similarity of stimuli with owl silhouette.60-minutes of isolation increased latencies more than 5 minutes isolation. (3-minutes isolation interpreted as anxiety-like phenotype, 60-minutes isolation interpreted as depression-like phenotype) | (Salmeto et al., 2011) |
| Sheep, Merino ewes    | f   | Sheering, vs. unshorn controls; Sheep were shorn immediately before entering the cognitive bias facility | 2    | D         | S: bucket on one side of the arena; S*: bucket on the other side | Discrimination between location of S (dog exposed behind sliding panel) and S* (bucket containing the food reward); criterion: no approach of S: bucket on 3 of 4 successive days | Sheep were tested in two cohorts, each consisting of 3 shorn sheep and 3 unshorn controls | ✓ | ✓ | | (Sanger et al., 2011) |
| Pigs, crossbred Large White X Landrace | f, m | Housing at two different stocking densities: low (conventional) vs. higher space allowance | 1    | F         | S: bucket on one side of the arena; S*: bucket on the other side | Discrimination between location of S (empty bucket) and S* (bucket containing pelleted weaner food); Criterion: statistically significant difference in latency to approach S, S* | 3 test days (each preceded by a day with the bucket in in the trained S, S* position) on test days, the bucket was located in a pseudorandom sequence in S*, S and each of the three ambiguous locations. | ✓ | ✓ | No effects of space allowance on learning the discrimination between S and S*. No effects on the latencies to approach the originally trained and the ambiguous bucket positions. No effects on physiological measures (salivary cortisol, α-amylase, but more sitting behavior and more skin lesions in pigs with low space allowance | (Scollo et al., 2014) |
| Chickens (Gallus gallus) | f   | Chicks were housed in groups of 8 in round pens divided in three-area’s: dark area, litter area (floor covered with saw dust), feed area from hatching until the end of cognitive bias testing at 8 weeks of age. Each of the three area’s was shut off for four days in following weeks. | 1    | F         | S: bucket on one side of the arena; S*: bucket on the other side | Discrimination between location of S (bowl with a piece of puffed rice soaked in quinine sulphate solution) and S* (mealworm); Criterion: 2 s mean difference in running speed to approach the S, S* location | 3 test series (with each treatment) in 3 successive week, each starting with the presentation of the bowl at S and S* position; The bowl was then located in S*, S and each of the three ambiguous locations. | ✓ | ✓ | Chicks appeared to continue learning during the 3 testing weeks. Shorter running speed to the near negative bowl position after shutting off the litter area may indicate that this manipulation affected the chicks less negatively than shutting off each of the other two areas. | (Seehuus et al., 2013) |
| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Text-arena | S/S* | Ambiguous stimuli (nr.) | Training | Testing | Go/No-go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|-----|-------------------------------|------|------------|------|-------------------------|----------|---------|---------|---------|--------------------------------------------|-----------|
| Dogs, various breeds   | f, m| Different breeds and ages. Individual differences between dogs. | 1    | 16        | a; S high or low tone; S' the other of the two tones, a (9); tones between the ones associated with S and S' | Discrimination between S (Signaling water) and S' (signaling cat milk): touching a target within 10 sec with the snout to receive the US; Criterion: S' latency > S' latency per dog (Mann-Whitney U-test). 15 of the 23 dogs successfully passed training | 15 S and S' trials, and 2 times the 9 ambiguous probes were presented pseudo-randomly. No reward was given; test was repeated twice over the course of 2 weeks | ✓ | ✓ | Large breed differences in the latencies (and likelihood) to touch a target. Shorter latencies (and higher likelihood to touch target) for S' and probes near S and longer latencies (and lower likelihood to touch target) for S' and probes near S. Dogs appeared to learn that responding during testing was unrewarded. | (Starling, 2012) |
| Dogs, various breeds   | f, m| Investigate baseline optimism in dogs from different environments: companion dogs, dogs in training for assistance roles and security/detection dogs | 1    | 21        | a; S high or low tone signaling water as reward S' the other of the two tones, signaling lactose free milk as reward | Discrimination between S (signaling water) and S' (signaling cat milk): touching a target within 10 sec with the snout to receive the US; Criterion: S' latency > S' latency per dog (Mann-Whitney U-test). 20 of the 40 dogs successfully passed training | 15 S and S' trials, and 2 times the 9 ambiguous probes (tones in between S' and S') were presented pseudo-randomly. No reward was given; test was repeated 3 times over the course of 2 weeks | ✓ | ✓ | Dogs were slower to touch the target as probes became more similar to S'. Inter-individual differences in responses to ambiguous probes, also between dogs from the same treatment group. | (Starling et al., 2014) |
| Cats, domestic shorthair | f, m, 22 | Individual differences between cats. | F    | F; s = S' bucket on one side of the arena); S bucket on the other side; s(3); buckets in between S and S' location | Discrimination between location of S (inaccessible food) and S' (bucket containing the food reward); Criterion: statistically significant difference in latency to approach S, S' on two consecutive days | 3 consecutive days, with 13 trials (5 rewarded, 5 unrewarded, 3 unrewarded ambiguous locations, in between S' and S') | ✓ | | Strong differences between cats to discriminate between rewarded and unrewarded locations. Shorter latencies for S' and probes near S' and longer latencies (and lower likelihood to touch target) for S' and probes near S | (Tami et al., 2011) |
| Dogs, various breeds   | f, m| Dogs kenneled for > 6 months 'long term' (LT) group, vs. dogs kenneled between approximate- ly 1 week and 3 months 'short term' (ST). Groups were matched for age, sex, breed and breeding status | 1    | 12        | s; S' bucket on one side of the arena); S bucket on the other side; s(3); buckets in between S and S' location | Discrimination between location of S (empty bucket) and S' (bucket containing the food reward); Criterion: shorter latency to approach the S' than the S' location in each trial of a series of 6 | 3 series of tests with ambiguous locations, followed by the originally trained S and S' locations twice, to re-establish the original discrimination | ✓ | ✓ | No effects of the LT and ST group on latencies to approach the different bucket locations. Shorter latencies for S and probes near S' and longer latencies (and lower likelihood to touch target) for S' and probes near S | (Tiltaer et al., 2013) |

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21 Operant apparatus, equipped with a touch area and a milk/lactose delivery system
22 All neutered
| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Test-arena | S/S⁺ | Ambiguous stimuli (nr.) | Training | Testing | Go No-go | Go/Go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|------------------------|-----|-------------------------------|------|------------|-----|--------------------------|----------|---------|---------|-------|---------|-------------------------------------------|-----------|
| Sheep, Merino ewes     | f   | Group 1: sheep fasted for 24 h before cognitive bias testing; Group 2: sheep treated with ghrelin (7µg.kg⁻¹ body weight); Group 3: untreated controls (Experiment 1) | 2     | D          | v⁻ | S⁺ green panel of very high or low brightness, S⁺ panel with brightness opposite to S⁻, v(3); green panels with brightness between S⁻ and S⁺ | Training to approach the S⁺ location (, exposure to 2 sheep when the panel is raised) and to avoid the S⁻ location (exposes the sheep to a dog when the panel is raised), where no-go was defined as not approaching the locations for 30 seconds. | Five consecutive trials with the five different cue locations: S⁻ and S⁺ location were reinforced, ambiguous cue locations had no consequences. | ✓       | ✓       | ✓       | ✓       | ✓       | 100% approaches to S⁺ location, very low proportion of approaches to S⁻ location. Tendency to more pessimistic bias in ghrelin treated sheep. | (Verbeek et al., 2014a) |
| Sheep, Merino ewes     | f   | Group 1: high feed (HF): increasing feed amount from 110 to 150% of required maintenance level (days 1-4), followed by supplying 170% (days 4-8). Group 2: low feed (LF): food deprivation (day 1), followed by supplying approx. 50% of maintenance level (day 2-6). (Experiment 2) | 2     | D          | v⁻ | S⁺ green panel of very high or low brightness, S⁺ panel with brightness opposite to S⁻, v(3); green panels with brightness between S⁻ and S⁺ | Exp. 2 followed exp.1 within one week Training to approach the S⁺ location (, exposure to 2 sheep when the panel is raised) and to avoid the S⁻ location (exposes the sheep to a dog when the panel is raised), where no-go was defined as not approaching the locations for 30 seconds. | Five consecutive trials with the five different cue locations: S⁻ and S⁺ location were reinforced, ambiguous cue locations had no consequences. | ✓       | ✓       | ✓       | ✓       | ✓       | The HF group tended to approach all locations less often than the LF group, i.e. LF sheep appeared to have a more optimistic judgement than the HF sheep. Group 1: Cognitive bias testing on day 7, 3 h after feeding. Group 2: Food deprivation on day 7, cognitive bias testing on day 7 | (Verbeek et al., 2014a) |
| Sheep, Merino ewes     | f   | Group 1: morphine (1 mg.kg⁻¹ body weight) i.v. Group 2: naloxone (2 mg.kg⁻¹ body weight) i.v. Group 3: controls, receiving 10 ml sterile water i.v. Injections 10 minutes before the start of cognitive bias testing. Sheep received either palatable food pellets (70 grams) or unpalatable food (wood chips) in the start box, before they were released into the testing arena, counterbalanced for half of the animals on day 1 and 2 of testing. | 2     | D          | v⁻ | S⁺ green panel of very high or low brightness, S⁺ panel with brightness opposite to S⁻, v(3); green panels with brightness between S⁻ and S⁺ | Training to approach the S⁺ location (green panel of very high or low brightness, exposure to 2 sheep when the panel is raised) and to avoid the S⁻ location (other brightness that exposes the sheep to a dog when the panel is raised), where no-go was defined as not approaching the locations for 30 seconds. | Two testing days, separated by one day rest. Per testing day: five consecutive trials with the five different cue locations in random order: S⁻ and S⁺ location was reinforced, approaching an ambiguous cue locations had no consequences. | ✓       | ✓       | ✓       | ✓       | ✓       | Strong carry over effects of cognitive bias testing on day 2 of the testing on day 1. Day 2 data therefore were not analyzed. Ambiguous cues were approached sooner when animals were exposed to palatable food in the startbox and this effect seemed to be strengthened by morphine, however no differences in pessimism were found between the control group and morphine treated animals indicating that the wood chips were not less aversive for morphine treated animals. Also no differences in optimism were found between the naloxone and control group. | (Verbeek et al., 2014b) |
| Species (strain/breed) | Sex | Experimental manipulation(s) | When | Text-arena | S:S* | Ambiguous stimuli (nr.) | Training | Testing | Go/No-Go | Go/Go | Welfare | Effect(s) of experimental manipulation(s) | Reference |
|-----------------------|-----|--------------------------------|------|------------|------|--------------------------|----------|---------|----------|-------|---------|------------------------------------------|-----------|
| Sheep, Laucune ewes   | f   | Housing in either unpredictable, stimulus-poor environment or predictable, stimulus-rich environment for a duration of several months. | 1    | D          | ≤6   | S'/S boxes presented in different locations s(3); boxes presented in intermediate positions between S'/S locations | Approach box at S* (one side of the room, rewarded with food)/avoid approach at S* (other side of the room, punished by presenting blower with cloth attached), training continued until a sheep performed 15 correct responses in a row (9 positive, 6 negative in semi-random order). Eleven of 12 sheep from predictable group and 7 of 12 sheep from unpredictable group reached criterion. | Three test days, test sequence of five trials containing 1 ambiguous stimulus presented between S'/S, each day a different ambiguous location. Ambiguous stimuli were unrewarded | ✓       | ✓       | ✓       | Sheep from predictable group needed fewer training sessions than sheep from unpredictable group. Sheep from unpredictable group were less likely to approach middle and near-positive ambiguous stimulus but more likely to approach near-negative ambiguous stimulus | (Vögeli et al., 2014) |
| Dogs, various breeds  | f, m | Removal of conspecific in pair-housed dogs. | 2    | F          | ≤6   | S' bucket on one side of the arena; S' bucket on the other side s(3); bowl in between S and S' location | Approach bowl at S* (bowl containing food reward)/refrain from approaching bowl at S* (empty bowl). Training continued for a minimum of 15 trials in randomized order until the longest latency to reach S* was shorter than any of the 3 preceding latencies to reach S. | Testing prior to and after separation. During a test session 3 trials were undertaken for each ambiguous location (total of 9 ambiguous trials). Prior to and between ambiguous trials, 2 S' and 2 S trials were performed. | ✓       | ✓       | ✓       | Latency to approach increased as bowl was placed nearer the S- location. No effect of separation found on latencies to reach ambiguous bowl locations, indicating no change in emotional state. | (Walker et al., 2014) |
| Chickens (gallus gallus) | f   | Housing in basic or enriched environment; housing in these environments started 3 days before testing (first subgroup) and 2 months before testing (second subgroup) | 1    | D          | ≤6   | S' bucket on one side of the arena; S' bucket on the other side s(3); buckets in between S' and S' location | Discrimination between location of S' (empty bowl) and S* (bowl containing the food reward); Criterion: Latency to approach the S' S s longer than approaching S* location (in at least 3 out of 4 times that the bowl was in the S* position, in the 8 trials of a training session) | 3 days each separated by one test free day, with 13 trials (5 rewarded, 5 unrewarded, 3 unrewarded ambiguous locations) | ✓       | ✓       | ✓       | Training on the cognitive bias task was time consuming (approx. 150 trial needed to reach training criterion). The housing conditions did not affect proportion of chicks (with cut off 20 sec), nor the latencies to approach the different bowl locations. | (Wichman et al., 2012) |
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Supplementary Material

Making decisions under ambiguity: judgment bias tests for assessing emotional state in animals

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Supplementary Figure 1. Simplified schematic representation of test arenas for assessing judgment bias in animals. (A): runway (e.g. Burman et al., 2011; Salmeto et al., 2011); (B): rodent home cage (Boleij et al., 2012); (C): two choice box (e.g. Murphy et al., 2013); (D), (F): arenas for presenting cues in spatially distinct places (e.g. Destrez et al., 2012; Doyle et al., 2010; Düpjan et al., 2013); (E): maze to present cues in spatially distinct places (e.g. Briefer and McElligott, 2013; Richter et al., 2012); note that in some studies, only three positions are used, namely the S* and S- position, and the position intermediate between S+ and S- (e.g. Carreras et al., 2015); (G): same as (D), but stimuli are equidistant from start area (Titulaer et al., 2013); (H): similar to (D), but guillotines control access to the food bowls, and guillotine doors in the rear control access to UCS+ (e.g. conspecifics) and UCS- (e.g. a dog) (Verbeek et al., 2014a, 2014b); (I): apparatus for testing mice using tactile stimuli (Novak et al., in press); (J): apparatus for testing rats using tactile stimuli (Barker et al., in press). Note: not drawn to same scale. Size of the testing equipment depends on size of the species tested. Judgment bias task in arenas as depicted in (A), (B) and (C) normally use non-spatial cues, although the basic discrimination training in arena (C) also involves a spatial component. In (D) – (H), only one location is presented per trial during testing.