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A spatial judgement task to determine background emotional state in laboratory rats, *Rattus norvegicus*

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Humans experiencing different background emotional states display contrasting cognitive (e.g. judgement) biases when responding to ambiguous stimuli. We have proposed that such biases may be used as indicators of animal emotional state. Here, we used a spatial judgement task, in which animals were trained to expect food in one location and not another, to determine whether rats in relatively positive or negative emotional states respond differently to ambiguous stimuli of intermediate spatial location. We housed 24 rats with environmental enrichment for 7 weeks. We removed the enrichment from half the animals prior to the start of training to induce a relatively negative emotional state, whereas we left it in place for the remaining rats. After 6 training days, the rats successfully discriminated between the rewarded and the unrewarded locations in terms of an increased latency to arrive at the unrewarded location, with no housing treatment difference. The subjects then underwent 3 days of testing in which three ambiguous 'probe' locations, intermediate between the rewarded and the unrewarded locations, were introduced. There was no difference between the treatments in the rats' judgement of two of the three probe locations, the exception being when the ambiguous probe was positioned closest to the unrewarded location. This result suggests that rats housed without enrichment, and in an assumed relatively negative emotional state, respond differently to an ambiguous stimulus compared to rats housed with enrichment, providing evidence that cognitive biases may be used to assess animal emotional state in a spatial judgement task.

Keywords: animal welfare; cognition; emotion; laboratory rat; *Rattus norvegicus*

The study of animal emotions is gaining increasing credence within the research community including psychology, neuroscience and behaviour (e.g. Rolls 2000; LeDoux 2003; Paul et al. 2005). Furthermore, the assumption that animals experience emotional states is likely to underpin public concern about animal welfare, and investigations of such states are thus of central importance in animal welfare science (e.g. Dawkins 1990, 2006; Mendl & Paul 2004). Emotional states are widely regarded by contemporary emotion researchers as comprising subjective, behavioural, physiological and cognitive components (e.g. Winkielman et al. 1997; Bradley & Lang 2000; Paul et al. 2005). It is not currently possible to obtain direct measures of the subjective component of emotional experience. Therefore, when we refer to animal emotion in this paper we cannot assume an accompanying conscious experience, even if other components of the emotional response are present.

Current methodologies for investigating emotions include the measurement of physiological and behavioural 'indicators' of stress and welfare (e.g. Broom 1991; Hurst et al. 1999; Abou-Ismail et al. 2007; Burman et al. 2007)—measures that are associated with putative aversive experiences. There are also many behavioural tests of fear and anxiety developed in neuroscience and psychopharmacology research (e.g. Ramos & Mormède 1998; File & Seth 2003; Paul et al. 2005) and tests that allow us to 'ask' an animal what it wants (preference tests; e.g. Sherwin 1996; Dawkins 2003; Merrill et al. 2006) or how much it wants it (consumer demand; e.g. Dawkins 1983; Warburton & Mason 2003; Sherwin 2007), and

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hence may indicate emotional states (e.g. ‘suffering’) in animals that are denied highly valued resources (Dawkins 1990).

There are, however, problems with the existing techniques. For many physiological and behavioural indicators, interpretation is complicated by the fact that the correspondence between a particular measure (e.g. heart rate/locomotory behaviour) and the valence (i.e. positive/negative) of a corresponding emotional state may be unclear. For example, increased heart rate or locomotory behaviour may be recorded during aversive (e.g. predator avoidance) or pleasurable (e.g. sex) activities. Related to this, there is a lack of clear a priori predictions for how responses in some tests (e.g. tests of spontaneous behaviour such as the open field) reflect emotional state (e.g. is activity in the open field an indicator of curiosity-motivated exploration or fear-motivated escape?), making implementation and interpretation of such tests in species other than the ones for which they were developed necessarily post hoc. A third issue is that there tends to be a bias towards the study of negative emotions (e.g. Paul et al. 2005; Boissy et al. 2007), with positive emotions receiving far less research attention. The development of further methodologies for assessing positive as well as negative affective states would therefore be advantageous.

For these reasons, consideration has been given to alternative methods of measuring emotional state that may avoid some of these technical or interpretative issues. One such alternative is the study of cognitive bias (Paul et al. 2005). There is a large body of evidence in the human psychology literature that background emotional state can influence the cognitive processes of individuals, resulting in biases in processes including judgement, attention and memory (Paul et al. 2005). For example, anxious individuals bias their attention to threatening stimuli (Mogg & Bradley 1998) and make more negative interpretations of ambiguous stimuli (e.g. Eysenck et al. 1991). The benefits of using cognitive bias as an indicator of emotional state include the ability to discriminate between emotional states of different valence (e.g. depression, pleasure), and potentially even between emotional states of the same valence (e.g. anxiety, depression), and the presence of clear and generalizable a priori predictions for how response and emotional state are related (Paul et al. 2005).

In a previous study (Harding et al. 2004), the authors developed a test of judgement bias, one category of cognitive bias (Paul et al. 2005), in which rats were trained to press a lever to gain a food reward after a particular tone had been played (e.g. 2 kHz), but to refrain from pressing the lever when a different tone (e.g. 4 kHz) was played to avoid a burst of white noise. Having learned to discriminate between these two ‘reference’ tones, half the rats were subjected to an unpredictable housing treatment (e.g. Harkin et al. 2002) before all the rats were tested, and their responses to the playback of various ambiguous ‘probe’ stimuli of tonal frequencies intermediate to the two reference tones (i.e. 2.5, 3, 3.5 kHz) were recorded. The prediction was that those rats that had experienced the unpredictable housing treatment would consequently be in a relatively negative emotional state and so would be more likely than control animals to respond to the ambiguous tones as though they predicted the negative rather than the positive outcome (operationally defined as a ‘pessimistic’ response). This was borne out by the results (Harding et al. 2004).

A novel finding of this nature requires replication and investigation of its generality, as well as further study due to its potential not only for practical uses in the assessment of animal emotion, but also for elucidating the processes involved in the interactions between cognition and emotion. There is also a need to develop other means of testing judgement bias in nonhuman animals that are quicker to implement and require less specialized technology and skill/knowledge (Bateson & Matheson 2007). In this study we therefore decided to investigate this promising approach further using location as the cue instead of auditory tones, as spatial location has a strong salience in cognitive tasks for many animals, including laboratory rats, because of its ecological relevance to contexts such as foraging behaviour (e.g. Olton & Samuelson 1976; Wood et al. 1999; Thorpe et al. 2002). To manipulate background emotional state we decided to use the presence or absence of environmental enrichment, as there is plentiful evidence that the presence of environmental enrichment can result in an improvement in welfare and therefore an associated positive emotional state (and vice versa for the absence of enrichment). For instance, previous research has indicated that the presence of environmental enrichment can reduce stress for many species, as determined by behavioural, physiological and pathological indicators (e.g. Van Loo et al. 2002; Hansen et al. 2007; Burman et al. 2006) and can also result in decreased levels of indexes of negative emotional state such as fearfulness and anxiety (i.e. ‘anxiolytic’ effects of enrichment; e.g. Fernandez-Teruel et al. 2002; Fox et al. 2006).

Our aim was therefore to determine the generality of the cognitive bias approach using a novel, ecologically based, location judgement bias task in laboratory rats. We predicted that animals in an assumed negative emotional state (i.e. experiencing absence/removal of enrichment) would be more likely to show a pessimistic-like bias in their judgement of ambiguous locations (i.e. responding to ambiguous locations as if they were unrewarded rather than rewarded), whereas animals in an assumed positive emotional state (i.e. in the presence of enrichment) would be more likely to show an optimistic-like bias (i.e. responding to ambiguous locations as if they were rewarded rather than unrewarded).

METHODS

Subjects

We used 24 male Lister-hooded rats (Harlan, U.K.), approximately 6 months of age at the start of testing. We randomly allocated the rats to groups of three and housed them in standard cages ($33 \times 50 \times 21$ cm) on a 12-h reversed-light cycle, lights off from 0800 to 2000 hours, with food (Harlan Teklad Laboratory Diet) and