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Review

Assessing the emotions of laboratory rats

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ABSTRACT

Rats are one of the most commonly used species in research, and decades of testing have yielded a large amount of information pertaining to their experience of emotion. The aim of this review is to bring together information on rat emotion from across a number of disciplines and over several decades, making this information easily accessible to those working with rats. Knowledge of rats' ability to experience emotions including pain is important as this helps to inform and motivate concerns for welfare. Rodents are used in greater numbers than other mammals, in more invasive research and are less likely to receive post-operative analgesia; this treatment likely reflects the perception that rats are somehow less able to experience the range of emotions that would result in suffering associated with these procedures. This paper reviews the range of scientific methods used to infer emotional states in animals including rats; evidence resulting from the application of these methods, as well as descriptions of spontaneous behavioural changes, provides evidence that rats likely experience a range of positive and negative emotions. Given these abilities we conclude that current standards of housing and care are likely to profoundly affect their welfare.

Contents

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1. Introduction

Rats are one of the most commonly used species in research, currently third behind mice and fish (CCAC, 2012; Home Office, 2012). Because rats are a better model than mice for many complex disorders common in humans, it is anticipated that the number of rats used in research will catch up and even surpass the number of mice currently used due to recent advances in rat genomics (Abbott, 2009; 'Rats!', 2010 [Editorial])

Rats have been used extensively in the field of psychology as a model organism to study the basis of several mental processes including emotion (Carmichael, 1950). While the focus has been on how results from rats apply to humans, the process has yielded a large amount of information on rats themselves. Knowledge of rats' ability to experience emotions including pain is important as this helps to inform and motivate concerns for welfare (Broida et al., 1993; Knight et al., 2009). The aim of this paper is to bring together information on rat emotion from across a number of disciplines and over several decades, making this information easily accessible to those working with rats. First we set the stage by reviewing people's beliefs about rat emotional abilities and how these beliefs may be affecting the way rats are used and treated; we then go on to describe and critique the methods used to infer existence of different emotional states in rats; we end by summarizing the evidence that rats experience specific negative and positive affective states.

2. The importance of rat emotions

2.1. Attitudes towards animal research

Research on people's attitudes towards animal use has shown that support for animal experimentation is greater when experiments are performed on rats and mice than on other mammals (Knight et al., 2003; Phillips et al., 2012). Even identical experiments are judged as more acceptable if they are to be performed on rats, mice, or non-mammalian species than if they are to be performed on dogs, cats, or monkeys (Driscoll, 1992). This effect of species is likely due to differences in people's 'belief in animal mind' (BAM; Knight and Barnett, 2008; Knight et al., 2003) – namely, whether they attribute to the animals mental capabilities such as the ability to reason and the capacity to experience a range of feelings and emotions (Hills, 1995). Factors affecting BAM include gender and age of the person (females and older people report higher levels of BAM than males and younger people), but the strongest determinant appears to be the species of animal (Morris et al., 2012). University students and scientists who conduct research on animals are less likely to attribute the capacity to experience at least moderate levels of pain, emotions, and suffering to rats, mice and non-mammalian species than to other mammals such as dolphins, chimpanzees, dogs, and cats (Herzog and Galvin, 1997; Knight et al., 2009; Phillips et al., 2012). People attribute higher capacity to experience all of the above to mice than they do to rats, and generally view research on mice as less acceptable than research on rats (Driscoll, 1992; Herzog and Galvin, 1997).

Several factors may explain why rats are routinely ascribed lower ability to experience pain and a range of emotions. Since the late 1800s rats have been blamed for the spread of the plague, and in most parts of the world they are viewed as vermin and carriers of disease (Edelman, 2002). This view is compounded by the world of films, books and cartoons which often demonize the rat and have made it the symbol of malevolence (Edelman, 2002). Research has shown that attitudes towards animals are also influenced by familiarity with the animal as well as aesthetic appeal (Knight and Barnett, 2008; Morris et al., 2012), both of which may be low due to rats' poor reputation. Animals that are perceived to be physically or mentally less similar to humans can be subjected to ingroup–outgroup biases (Plous, 1993) which lead to an under-estimation of their mental capacities and an over-estimation of between-group differences (Tajfel, 1970). Arguably, viewing rats as pest exacerbates people's tendency to see them as the outgroup and attribute to them lower mental and emotional abilities.

2.2. Trends in the use of rodents in research

Rodents are used in research in far greater proportions than other mammals, in large part due to their small size, lower purchase and maintenance costs, and high reproductive rate. Personal experience and anecdotal evidence suggest that researchers are also encouraged to perform experiments on rodents because of a perception that this will result in less harm – this is a common interpretation of the Three Rs concept of *refinement*, which refers to the modification of husbandry or experimental procedures to minimize pain and distress and improve welfare (Russel and Burch, 1959).

While some of the reasons why more studies use rodents compared to other mammals are clear, it is less obvious why studies using rodents also use more animals *per study* than studies using other mammals. Structured

literature reviews of articles involving experimental surgical procedures in 2005–2006 reported that the median study size for studies using rats and mice was 40 compared to 15 for studies using other mammals (non-human primates, dogs, pigs, rabbits and sheep); the maximum number of animals used in a single study was 300 for studies using rats and mice, and 123 for studies using other mammals (Coulter et al., 2009; Stokes et al., 2009). Larger study sizes may be due to the different types of studies conducted with rodents – for example, rodents may be selected for studies that compare more treatments, or studies that require larger sample sizes due to the nature of the treatments. However, research has shown that scientists and animal welfarists alike believe that animals they perceive as having less ability to experience pain, emotions and suffering deserve less moral consideration (Herzog and Galvin, 1997; Knight et al., 2009). It is therefore possible that the oversight committees that review and approve research protocols are less demanding in the justification of animal numbers for studies involving rodents than for studies involving other mammals.

In addition to being used in greater numbers, rodents are also likely to be subjected to more invasive procedures. In Canada, experiments involving vertebrates are rated according to four 'categories of invasiveness' ranging from B to E. Studies rated as category of invasiveness E cause "severe pain near, at, or above the pain tolerance threshold of unanaesthetized conscious animals" (CCAC, 1991). In 2010, 3.7% of rats and mice were used in category E experiments, compared to 0.3% of non-human primates, dogs and cats (CCAC, 2012). In New Zealand, the most invasive procedures are rated as Grade E – "Very High Impact" and include major surgery without anaesthesia, exposure to extremely noxious stimuli from which escape is impossible, and experiments with death as an endpoint (MAF, 2010). In 2010, 18.7% of rats and mice were subjected to Grade E manipulations; the only other species in this category were guinea pigs and unspecified 'pest' species (NAEAC, 2011).

In the United States, the most invasive experiments are classified as belonging to column E, which includes studies where "pain or distress [was] not alleviated because pain-relieving drugs would have interfered with the research" (USDA, 1993). Statistics on the use of rats and mice in research are not collected in the United States, because these two species – along with birds, fish, reptiles and amphibians – do not fall under the Animal Welfare Act's definition of 'animals' (USDA, 1970). Other small rodents, such as hamsters and guinea pigs, are included. Statistics for rodent species for which data is collected reveal that in 2010, 22.8% of hamsters and guinea pigs were used in experiments classified as column E, compared to 1.4% of non-human primates, dogs and cats (USDA, 2011).

Rodents are also less likely than other mammals to receive analgesics after painful surgical procedures (Coulter et al., 2009; Stokes et al., 2009). Structured literature reviews that assessed the trends in the administration of analgesics to laboratory rats and mice revealed that in 1990–1992, only 3% of studies published in peer-reviewed journals reported analgesic administration to these rodents after a surgical procedure (Richardson and Flecknell, 2005). Subsequent follow-up with researchers who did not administer analgesics revealed that in 71% of cases, analgesic administration was not reported because it was in fact not administered. Reasons given for withholding analgesics included no signs of pain observed (35%) and a feeling that analgesics were unnecessary (35%). The proportion of studies that reported administration of analgesics to rats and mice increased to 10% by 2000–2001, and 20% by 2005–2006 (Stokes et al., 2009). In contrast, the reported administration of analgesics to other laboratory mammals (non-human primates, dogs, pigs, rabbits and sheep) following a surgical procedure was 50% in 2000–2001 and 63% in 2005–2006 (Coulter et al., 2009).

In summary, rodents are more likely to be used in research than other mammals; and when used, they are used in greater numbers, in more invasive research, and when they are harmed they are less likely to receive pain relieving drugs. We suggest that this treatment reflects the common perception that these laboratory rodents are somehow less able to experience the range of emotions that would result in suffering associated with laboratory research procedures.

3. What are emotions?

There is no clear agreement on how to define emotion (one survey describes 92 different definitions; Kleinginna and Kleinginna, 1981), but the general consensus is that emotion is a construct referring to four different, imperfectly related phenomena: (1) a change in brain activity to select stimuli, (2) a change in cognitive processes, (3) a preparedness for, or display of, a behavioural response, and (4) a consciously detected change in feeling that has sensory qualities (Kagan, 2007).

The first three components (changes in brain activity, in cognitive processing, and in behaviour) can be recorded, but the fourth component (the conscious experience of a change in feeling) is not amenable to direct assessment (e.g. Cohen and Dennett, 2011; Dehaene and Changeux, 2011). Although we consider it likely, we cannot definitively know whether animals 'feel' emotions even if they display changes in brain activity, cognitive processing and behaviour. In this paper we will discuss emotions without making conclusions as to whether they experience those emotions consciously.

The study of emotion has taken two approaches: the 'discrete emotions' approach that is focused on studying discrete emotions such as anger or fear (e.g. Panksepp, 1998; Plutchik, 1982), and the 'dimensional' approach that plots emotions in two-dimensional space along an 'affective valence' axis and an 'arousal' axis (Fig. 1; e.g. Russell, 1980; Stanley and Meyer, 2009). Valence refers to the positive or negative aspect of emotion and ranges from pleasant to unpleasant, while arousal refers to the activation or intensity of an emotion and ranges from high to low. Mendl et al. (2010) proposed a framework that integrates these two approaches and allows for the representation in two-dimensional space of affective states resulting from the interaction between discrete emotions, which they define are short-term responses to specific stimuli, and longer-term background mood.

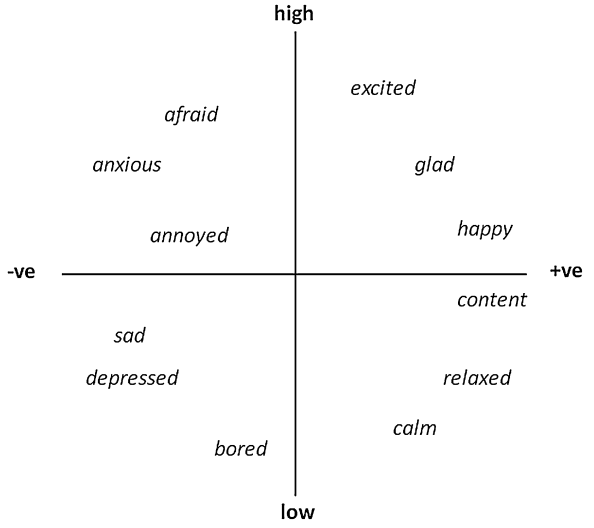

Fig. 1. Bi-dimensional representation of emotion. Possible locations of discrete emotions along the valence (−ve to +ve) and arousal (high to low) scales.
Adapted from Mendl et al. (2010) and Russell (1980).

4. Scientific methods of assessing emotions in animals

Traditionally, scientists often relied upon physiological measures (e.g. hormone levels, heart rate, etc.) to draw inferences about emotional states in animals, but these measures are more related to arousal than affective valence. For example, elevated cortisol levels are associated with both negative stimuli such as restraint, and positive stimuli such as mating (Moberg, 2000). Behavioural measures and changes in cognitive processing appear to be more related to affective valence. For example, a negative judgement bias is indicative of depression (a low arousal negative affective state) or anxiety (a high arousal negative affective state; Mendl et al., 2010).

Although both arousal and valence ultimately contribute to the overall emotion, valence may be of more practical use to animal welfare science. Knowing that an animal is in a negative affective state without knowing whether the state is high arousal (e.g. fearful) or low arousal (e.g. depressed) may matter more than knowing that an animal is highly aroused without knowing whether the arousal has positive valence (e.g. excited) or negative valence (e.g. fearful).

We therefore focus below on methods used to measure changes in behaviour and cognitive processing rather than changes in physiology, distinguishing between methods more suitable for assessing shorter-term discrete emotions associated with a specific stimulus ('discrete emotions') versus those for the assessment of more pervasive mood states ('mood').

4.1. Discrete emotions

4.1.1. Place conditioning

Conditioned place preference and conditioned place aversion, collectively known as 'place conditioning', are used to determine whether stimuli are rewarding or aversive to animals. In this paradigm, an environment is paired with a stimulus, and later the animal's willingness to re-enter or avoid that particular environment is used to infer whether the animal found the stimulus rewarding or aversive (Carlezon, 2003). The amount of time animals spend in the environment compared to baseline and compared to occasions when other stimuli are offered are taken as indications of the strength of the affect induced by the stimulus (i.e. arousal).

Place conditioning has mostly been used to assess the rewarding or aversive properties of drugs (e.g. amphetamines) but is now increasingly used in animal welfare science to examine the affective state induced by various stimuli. For example, in recent years scientists have started using this method to assess the emotional component of pain (Tzschentke, 2007).

One advantage of place conditioning is that it tests animals in a 'sober' state; when tested, animals are not affected by the stimulus under study (Bardo and Bevins, 2000). This is important because some stimuli may have direct effects on behaviour including locomotion (e.g. induction with inhaled anaesthetics causes ataxia) and cognitive processing (e.g. pain impairs rat cognitive function: Moriarty et al., 2011), thereby influencing the choice irrespective of the rewarding properties of the stimuli. One drawback of place conditioning is that motivation to explore novel environments may influence and confound behaviour on testing day (Bardo and Bevins, 2000). During conditioning, one environment is paired with a stimulus but it is possible that the stimulus is impairing familiarization with that environment (e.g. inhaled anaesthetics impair rat memory: Alkire and Gorski, 2004). If this happens, the environment that had been paired with the stimulus during conditioning may seem relatively more novel for reasons other than positive or negative associations with that environment.

4.1.2. Motivation tests with an operant

In operant tests, animals are trained to work (i.e. perform a specific behavioural response) to access a resource; the more work they are willing to perform the higher their motivation to access that resource is inferred to be. Motivation tests with an operant differ from simpler preference tests because they allow us to quantify the strength of motivation for a resource, instead of merely showing which of a set of resources is preferred.

Motivation testing with an operant has been used extensively in both farm and laboratory animal welfare science (see Patterson-Kane et al., 2008 for a review). In rats, the work to be performed often consists of pressing a lever, turning a wheel, or pushing or lifting a weighted barrier (Manser et al., 1996).

This type of testing assesses motivation rather than affect, and evidence suggests that the neural substrates of 'wanting' (motivation) and 'liking' (affect) are different (Berridge, 1996; Berridge and Robinson, 2003). 'Wanting' involves mesotelencephalic dopamine systems, while 'liking' involves neurotransmitter systems such as opioid and GABA systems. However, several emotional theorists suggest that affect motivates most behaviour with the goal

of maximizing pleasure or minimizing displeasure (e.g. Cabanac, 1992; Fraser and Duncan, 1998; Spruijt et al., 2001), and as such, these operant tests could be good indicators of conditions associated with an overall increase in affective valence. However, these tests cannot differentiate between resources that animals 'want' because they induce positive affect versus those that decrease negative affect. For example, if animals work to access an enriched cage, is it because the enriched cage induces positive affect (e.g. from playing with toys) or reduces negative affect (e.g. alleviate boredom) or both? The resource (e.g. enriched cage) being a positive affect-inducer or negative affect-reducer is also not fixed but will depend on the context (e.g. animal's existing housing conditions). In humans, there is evidence that enjoyment and preference for a resource tends to grow with familiarity (e.g. Zajonc, 1971). If this was shown also to be true in other animals, then recording the amount of work performed over time could help differentiate between resources animals 'want' because they induce positive affect versus those they 'want' because they alleviate negative affect. The rationale is that work for a resource that induces positive affect will increase over time as familiarity and enjoyment increase, while work for a resource that strictly decreases negative affect will be more stable over time. Such an experiment would have to include adequate controls for the effects of habituation.

A final drawback of operant testing is that operant tasks themselves can be rewarding. Contrafreeloading is the phenomenon in which animals choose to work for a resource that is freely available (e.g. Inglis et al., 1997), perhaps because of long-term fitness advantages associated with investing some effort into exploration in addition to exploitation of existing resources. One way to control for the inherently rewarding properties of performing an operant is to establish the amount of work animals are willing to perform to access a resource they can also access freely; for example, rats may be asked to press a lever to access rat chow that is also freely available in their cage. This would provide a baseline against which other resources can be judged.

4.1.3. Embodied emotion

In humans, many discrete emotions are associated with distinct, universal facial expressions that vary little across cultures (Darwin, 1872; Ekman and Oster, 1979). Until recently rodents were generally believed to lack facial expressions, but more recent studies suggest otherwise. For example, rats display highly consistent facial expressions in response to flavours that they 'like' and those they 'dislike', and these expressions are homologous in a variety of mammalian species including humans (Berridge and Robinson, 2003; Grill and Norgren, 1978). Sotocinal et al. (2011) found that rats display facial expressions of pain, and that these are broadly similar to those expressed by humans and mice in pain (Langford et al., 2010). This 'Rat Grimace Scale' was validated by verifying that expressions identified as being indicative of pain were attenuated by the administration of morphine in a dose-dependent manner.

The Rat Grimace Scale is said to be usable in real time on freely moving animals, but because this scale was developed and validated using still photographs captured from video, the validity and accuracy of real time assessment has yet to be determined. Capturing frames is labour intensive, but the development of the Rodent Face Finder® software may aid in the process (Sotocinal et al., 2011). As a prey species rats are likely motivated to avoid displays of weakness, so overt behavioural signs including facial expressions may only emerge when pain is severe. We suggest that the study of facial expressions in rats (and indeed other species) is still in its infancy and that more homologies useful in the assessment of emotions will be uncovered in the years to come.

4.1.4. Vocalizations

Vocalizations have been linked to the expression of affective state in several species including rats (e.g. Knutson et al., 2002; Manteuffel et al., 2004). Rats emit a variety of ultrasonic vocalizations that mainly fall in the range of 20–32 kHz and 35–70 kHz, and these calls have been termed collectively as '22-kHz calls' and '50-kHz calls'. Fifty-kHz calls are believed to indicate positive affect akin to the excitement seen during high arousal and anticipation of a reward, while 22-kHz calls are believed to indicate negative affect associated with anticipation of punishment (Knutson et al., 1999)

Fifty-kHz calls are emitted in and are predictive of naturalistic contexts that elicit approach behaviours; emission of these calls has been behaviourally associated with locomotor activity, rearing and exploration (Fu and Brudzynski, 1994). Twenty-two-kHz calls are emitted during and are predictive of the onset of avoidance behaviours; emission of these calls has been behaviourally associated with tense, motionless crouching, freezing and flight (Brudzynski et al., 1993). Twenty-two-kHz calls may be more indicative of anxiety rather than fear and can be attenuated by the administration of an anxiolytic (Jelen et al., 2003; Nobre and Brandão, 2004).

These two types of calls are for the most part accepted as indicating positive and negative affect, but some discrepancies remain. For example, 50-kHz calls generally associated with positive affect have also been recorded during presumably negative events, such as carbon dioxide euthanasia (Niel and Weary, 2006) and agonistic interactions (Takahashi et al., 1983). Some evidence suggests that 50-kHz calls may be used as signals to establish or keep contact with other rats (Wöhr et al., 2008; Wöhr and Schwarting, 2007), and are not necessarily indicative of affective valence. Calls labelled as '22-kHz' or '50-kHz' actually vary considerably in frequency, length, and shape, and may consist of several subpopulations (e.g. Brudzynski et al., 1993). Closer study may help differentiate between the different types of calls, rendering them a more reliable indicator of affect.

4.2. Mood

4.2.1. Drug self-selection

In drug self-selection studies, animals are given the choice between consuming food or water that does or does not contain a specific drug. The inference is that a drug will be reinforcing only to animals that suffer from the

condition the drug is targeting, so only affected animals, but not healthy controls, will consume more of the drug. For example, rats in pain given the option between drinking water with or without an analgesic tend to drink more of the medicated water than healthy controls, and the amount of drug consumed mirrors the intensity of pain (e.g. Colpaert et al., 1980, 1982).

To the best of our knowledge, drug self-selection studies have mostly been performed with analgesics, but one study on mice found preferential intake of an anxiolytic (Sherwin and Olsson, 2004). We suggest that there is scope to extend this approach to other classes of drugs, including self-selection of antidepressants as a method of assessing whether animals in certain environments are depressed. One limitation of this approach is that the palatability of the drug may also affect intake. Animals must also be given sufficient forced exposure to each condition (e.g. water with and without the drug) to be able to form an association, and must be able to discriminate between the two conditions during the choice phase to be able to display their preference.

4.2.2. Anticipatory behaviour

Anticipatory behaviour was first described as "a state of agitation which continues so long as [a desired] stimulus is absent" (Craig, 1918, p. 91). In rats, anticipatory behaviour is characterized by an increased level of activity resulting from frequent and abrupt behavioural transitions between short fragments of behaviour (Spruijt et al., 2001; Van der Harst et al., 2003). Anticipatory behaviour can be observed in the interval between presentation of a conditioned stimulus (e.g. light or tone) and the arrival of the unconditioned stimulus (e.g. food reward). It has been argued that the hyperactivity in anticipation of the arrival of a reward reflects the activation of reward centres in the brain, and that the level of activation depends in part on the incentive value of the reward (Koob, 1996; Spruijt et al., 2001). Thus, the presence of anticipatory behaviour before the arrival of an unconditioned stimulus is used to infer that the stimulus is rewarding to the animal, and the magnitude of the response gives clues as to the strength of the reward. Rats display no hyperactivity (and sometimes, a decrease in activity; e.g. Carrive, 2000) before the presentation of an aversive stimulus (Van der Harst et al., 2003).

The display of anticipatory behaviour may also be used to draw inferences regarding an existing affective state. In general, negative experiences affect reward sensitivity. More specifically, it seems that most negative events increase sensitivity to rewards (Piazza et al., 1990), but negative events resulting in depression diminish sensitivity to rewards (Cabib and Puglisi-Allegra, 1996). As such, deprived rats tend to exhibit more anticipatory behaviour than controls before access to a reward, while depressed rats display less anticipatory behaviour than controls. Anticipatory behaviour in presumably depressed animals can be re-established by treatment with an antidepressant (Von Frijtag et al., 2002). These results suggest that anticipatory behaviour can be used to determine existing affective state, and even differentiate between animals in a strongly valenced negative state versus those in a milder negative state.

One limitation is that the 'reward' used to elicit anticipatory behaviour must be perceived as such; a lack of anticipatory behaviour could either indicate that animals are depressed or that the 'reward' is perceived as neutral or aversive (see above), irrespective of existing affective state. Furthermore, due to the curvilinear nature of the relationship between affective state and anticipatory behaviour, a lack of anticipatory behaviour could also be associated with a very positive state (Boissy et al., 2007). In practice in may be clear whether an animal failing to exhibit an anticipatory response is in a very positive versus anhedonic state, but testing after the administration of an antidepressant may help confirm this.

4.2.3. Cognitive bias

Cognitive bias may also be used to draw inferences regarding affective state in animals. This approach borrows from research in humans showing that people in a negative affective state tend to judge ambiguous stimuli negatively (e.g. MacLeod and Byrne, 1996). Animal welfare researchers have used this phenomenon to test whether particular manipulations induce positive or negative affective states in several animal species including rats (see Mendl et al., 2009 for a review). Manipulations are usually environmental (e.g. standard vs. enriched housing) but genetically 'helpless' rats used as a model of depression also judge ambiguous stimuli more negatively than genetically 'normal' rats (Enkel et al., 2010; Richter et al., 2012).

In cognitive bias testing, animals are trained to perform one response when exposed to a particular stimulus to obtain a positive outcome (e.g. press left lever after hearing a high frequency tone to receive a food reward) and to perform another response when exposed to a different stimulus to avoid a negative outcome (e.g. press right lever after hearing a low frequency tone to avoid being exposed to noise). Animals are then tested with ambiguous stimuli (e.g. intermediate frequency tone); those in a negative affective state are expected to interpret the stimuli negatively (e.g. by pressing the right lever, a pessimistic judgement bias) and those in a positive state are expected to interpret the stimuli positively (e.g. by pressing the left lever, an optimistic judgement bias).

Studies have reported different types of judgement biases in rats. One study reported bias only at probes nearest to the positive training cue (e.g. Harding et al., 2004), two only at probes nearest to the negative training cue (e.g. Burman et al., 2008; Enkel et al., 2010) and one at all probe locations (e.g. Richter et al., 2012). If we assume that an ambiguous cue is most strongly associated with the training cue it is closest to, then an ambiguous cue closest to the positive training cue should lead to a positive response and therefore a negative response would indicate a decreased expectation of positive events. Similarly, biases closest to the negative cue may reflect an increased expectation of negative events. In humans, the former is associated with depression while the latter is associated with anxiety (MacLeod and Salaminiou, 2001; MacLeod et al., 1997), so biases at different probe locations may reflect different emotional states (Burman et al., 2008).

4.2.4. Startle potentiation

The use of the startle reflex as a measure of affective state in animals is also borrowed from human psychology, where reflexes have been shown to vary according to the subject's emotional state (Bowditch and Warren, 1890; Ison and Hoffman, 1983; Sechenov, 1965). Specifically, defensive reflexes (e.g. startle following a sudden loud noise) are enhanced if the subject is already in a negative emotional state, and attenuated if the subject is in a positive emotional state. Similarly, appetitive reflexes (e.g. salivation following a sucrose probe) are enhanced if the subject is already in a positive emotional state, and attenuated if the subject is in a negative emotional state (Lang et al., 1990). A common use of this method has been to measure the magnitude of the startle reflex following a loud noise in fear-conditioned versus control rats.

The advantage of the startle reflex in drawing inferences regarding affective valence is that elicitation, recording and quantification are relatively simple. However, care must be taken to ensure that the magnitude of startle is not influenced by other extraneous factors. In rats and other species, presentation of a prestimulus just before the probe (e.g. Blumenthal and Gescheider, 1987; Hoffman and Ison, 1980) attenuates the magnitude of the startle response, as does habituation (e.g. Valsamis and Schmid, 2011). In humans, attenuation of the startle response when in a positive affective state is much less reliable than an exaggeration in the startle response when in a negative affective state (see Grillon and Baas, 2003), so this tool may be more useful in assessing negative rather than positive affective states.

5. Current knowledge of rat emotions

In the previous section we described methods that have been validated (to the extent that true validation of methods assessing subjective states is possible) for rats as indicating certain affective states. In this section we will give examples of how these methods have been applied to rats and what the results can tell us about their experience of various emotional states. In addition to the methods described in the previous section, we will also give examples of spontaneous behavioural responses, explaining how these may also be used to draw inferences regarding emotional states.

5.1. Negative emotions with high arousal

Fear and anxiety are considered to be high arousal negative states, and much psychological research has focused on understanding how they develop and respond to drugs. Fear and anxiety are often investigated together since they share common neural substrates (e.g. Bandler and Depaulis, 1992; Panksepp, 1998); because these neural substrates are similar across all mammals, much research on fear has been done on rats (Bandler and Depaulis, 1992; LeDoux, 2002).

Because fear and anxiety are believed to have evolved as a means to protect animals from danger (e.g. Blanchard and Blanchard, 1990), the underlying assumption is that behaviours associated with danger are driven by fear. Panksepp (1998) has shown that activation of brain regions located along specific pathways situated in deep, subcortical limbic regions common to all mammals elicits distinct emotional reactions. Among others, Panksepp identified a FEAR pathway (this is always capitalized to indicate that it refers to a specific emotional circuit in the brain) which causes humans and other animals including rats to freeze or flee. In humans, stimulation of this system also leads to verbal reports of intense anxiety. Rats show conditioned place aversion to the location where the stimulation took place, and when given the opportunity will turn off stimulation of this pathway (Panksepp, 2005; Sacks and Panksepp, 1987).

The bulk of fear research on rats has focused on characterizing behaviours elicited by natural predators (innate fear) or signals predicting electric shock (conditioned fear). When confronted with a natural predator such as a cat or a dog, or an object impregnated with their odour, rats typically react by fleeing if an escape route is available (Blanchard and Blanchard, 1971; Blanchard et al., 1975). If there is no escape route, rats react by freezing, orienting towards the threat, vocalizing in the 22-kHz range, and may bare teeth, bite, and attack if the predator is very close (Blanchard and Blanchard, 1990; Blanchard et al., 1990). For up to 24 h after the predator or the predator scent is removed (Blanchard and Blanchard, 1989), rats continue to show reduced mobility and exhibit so-called 'risk assessment' behaviours that are believed to indicate anxiety (Blanchard and Blanchard, 1990; Molewijk et al., 1995). These risk assessment behaviours consist of rats poking their head out into the area where they encountered the predator, approaching the area with a flattened back, and a stretched attention posture (Blanchard and Blanchard, 1990; Kaesermann, 1986; Van der Poel, 1979). Risk assessment behaviours are diminished by the administration of anxiolytic drugs (for a review, see Blanchard et al., 1993).

In fear conditioning studies, an initially neutral stimulus (conditioned stimulus; usually a light or tone) is paired with an aversive stimulus (unconditioned stimulus; usually a mild electric shock). Animals quickly learn that the conditioned stimulus precedes the unconditioned stimulus, and react to the conditioned stimulus alone. In these studies, rats are usually confined to a cage, and just as they would react to a natural predator when no escape route is available, they exhibit freezing. Rats exhibit a stronger startle reflex if they are startled after the presentation of the conditioned stimulus (Steiner et al., 2011), and this reflex is attenuated after administration of an anxiolytic (Steiner et al., 2012). Rats receiving non-contingent foot shock (the unpredictability of which likely causes anxiety; Grillon et al., 2004) self-administer more cocaine (Goeders and Guerin, 1994) and anxiolytics (Cook and Davidson, 1973) than rats receiving no shock or rats that could control shock delivery. Rats also develop conditioned place aversion to locations where they recieve mild electric shock (Ferguson et al., 2004; Panksepp, 1996).

Housing rats individually in small, opaque cages also likely causes a negative affective state akin to anxiety. Alexander et al. (1978) have shown that rats housed in such conditions drank more water containing morphine than rats housed socially in a large, enriched enclosure; morphine is believed to have anxiolytic effects (see Gray, 1987).

5.2. Negative emotions with low arousal

Rats are inquisitive (Small, 1899), but standard laboratory conditions offer rats few stimuli to explore and few opportunities to perform behaviours other than sleeping and reaching for food and water. Several authors have argued that such environments may engender boredom or helplessness (Wemelsfelder, 1990; Van Rooijen, 1991). Boredom can be defined as "the unpleasantness of monotony, [. . .] and the seeking of stimulation" (White, 1959), while helplessness can result from the long-term experience of lack of control over the environment and the inability to change the aversive situation (Fox, 1986).

A multitude of studies have shown that rats will work to access cages that provide extra stimulation (e.g. Collier and Hirsh, 1971; Denny, 1975; Bradshaw and Poling, 1991; Iversen, 1998; Patterson-Kane et al., 2001). Rats reared in isolation display less anticipatory behaviour before access to sucrose than do pair-housed rats (Van den Berg et al., 1999; Von Frijtag et al., 2000), indicating that they are likely in a state of depression. Compared to controls, rats housed under conditions where aversive events occurred on an unpredictable schedule (e.g. reversal of the light/dark cycle, damp bedding, tilting of the cage) showed negative cognitive bias indicating a negative affective state. Because bias occurred at the ambiguous probe closest to the positive training cue, this negative state was likely depression (Harding et al., 2004).

5.3. Positive emotions

Positive states are largely under-studied in animals (Boissy et al., 2007) and specific discrete emotions are not well qualified; however, the high-arousal emotion experienced in anticipation of a reward can be referred to as excitement, while the emotion experienced during access to the reward may be referred to as joy. Lower arousal and often longer lasting mood states may be described as contentment or happiness.

Play has often been linked to the experience of positive emotions (Fraser and Duncan, 1998; Špinka et al., 2001) and is sometimes used to infer positive welfare (for a review, see Held and Špinka, 2011). Juvenile rats readily engage in rough-and-tumble play with other rats; rat play consists of 'pouncing', where one rat approaches the other and attempts to nose or rub the other's nape of the neck, and 'play pinning', where one rat lies on its back while the other stands over it (Vanderschuren et al., 1997).

Rats also seem to enjoy being 'tickled' in a manner similar to the way we would tickle a child (for a review, see Panksepp, 2007). Rats will seek out hands that have tickled them much more than hands that have petted them an equal amount of time (rats use their sense of smell to distinguish between individual humans; Panksepp, 2007), and will learn to press a lever for a tickling reward (Burgdorf and Panksepp, 2001). When being tickled and during social play, rats emit 50-kHz calls that may be indicative of positive affect (e.g. Burgdorf et al., 2008; Panksepp, 2007). Panksepp argues that 50-kHz vocalizations during play and tickling are analogous to laughter in human infants. In addition to neural and functional homologies, Panksepp reports that just as infants will not laugh unless they feel safe and comfortable, tickled rats will not emit 50-kHz vocalizations in the presence of predatory or stress odours (e.g. cats) or in laboratories where they are frequently punished (e.g. fear learning). This last finding also indicates that these calls are not simply an automatic response to physical stimulation but are dependent on affective state (Panksepp and Burgdorf, 1999).

Several contexts are believed to induce positive affect in rats, and these include nonagonistic encounters with conspecifics as well as engaging in sexual behaviour. Rats vocalize in the 50-kHz range in anticipation of as well as during social contact with other rats (Brudzynski and Pniak, 2002); they will work for contact with other rats (Patterson-Kane et al., 2002, 2004); and they show anticipatory behaviour before contact with other rats (Van den Berg et al., 1999). Rats also emit 50-kHz calls during appetitive aspects of sexual behaviour (Barfield et al., 1979; McGinnis and Vakulenko, 2003); they develop conditioned place preference for locations where they engaged in sexual behaviour (Hughes et al., 1990); they will work for the opportunity to engage in sexual behaviour (reviewed by Pfaus et al., 2001); and they show anticipatory behaviour before an opportunity to engage in sexual behaviour (Mendelson and Pfaus, 1989; Van der Harst et al., 2003).

Electrical stimulation of rewarding brain centres as well as administration of drugs that lead to the release of dopamine (e.g. morphine and amphetamines) are also associated with positive affect in rats. Rats vocalize in the 50-kHz range during and in anticipation of electrical stimulation of rewarding brain centres (Burgdorf et al., 2000). Rats also emit 50-kHz calls during the unconditioned administration of amphetamine or morphine (Burgdorf et al., 2001; Thompson et al., 2006) and in locations where they have previously received morphine or amphetamine (Knutson et al., 1999); they show conditioned place preference for locations where they received amphetamine or morphine (Bardo et al., 1995); and they exhibit anticipatory behaviour before administration of morphine (Hinson and Siegel, 1983).

5.4. Pain

Pain is not usually considered to be an emotion, but because the emotional aspect of pain is suggested to be fundamental to the pain experience (Le Bars and Cadden, 2005) we have chosen to include it in this paper. Indeed, the International Association for the Study of Pain defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage".

Rats are the most common model for the study of pain in humans, and have been for more than four decades (Mogil, 2009). Studies observing involuntary reflex responses to pain are historically common, but these do not allow for strong inferences regarding the emotional components of pain. For pain perception to occur, signals must reach specific cerebral structures – Reflexes, however, are mediated by separate circuits and do not engage brain regions involved in the sensory aspects of pain. Behavioural responses to pain may be investigated, but the focus should

be on behaviours that are cortically mediated, as they are more likely to engage the structures involved in processing the emotional and motivational reactions to pain (Vierck et al., 2008). Indeed, while behavioural measurements of pain in animals may not be definitive indicators of the emotional experience of pain, they are becoming increasingly popular and are currently the prevailing method in the study of pain and analgesia (Mogil, 2009).

Bennett and Xie (1988) showed that rats with a ligature around one of their common sciatic nerves exhibited pain behaviour like that seen in humans. These rats walked with a limp suggesting a reluctance to place weight on the affected paw, they often raised the affected paw from the floor and held it in a protected position next to the flank ('guarding behaviour'), they slept or rested on the side opposite to the affected paw, with the affected paw in the guarded position, and developed overgrown claws on the affected side. Studies using brain imaging techniques such as PET scans or fMRI have shown that these behaviours are cortically mediated, and that key cortical regions involved in the perception of pain in humans are also activated in the rat. For example, Neto et al. (1999) have shown that rats afflicted with arthritis in one hind limb displayed 'guarding behaviour' of the inflamed paw and responded with agitation, struggle, and vocalization during the 20 min they were subjected to additional mechanical stimulation. These rats were shown to exhibit brain activity in many structures, including thalamic, limbic and cortical regions. Other studies have shown similar results (e.g. Mao et al., 1993; Paulson et al., 2000; Porro et al., 1999).

Although several key cortical regions are involved in the perception of pain in humans, each is believed to process a different component of the pain experience; for example, some regions are involved in the detection of pain and others in the encoding of pain intensity. The unpleasantness of pain in humans has been shown to be mediated by a region in the frontal cortex known as the anterior cingulate cortex (ACC; Rainville et al., 1997; Tölle et al., 1999). The ACC also appears to be involved in the affective component of pain in rats: studies using place conditioning have shown that rats developed conditioned place aversion for locations where they received a noxious stimulus (indicating a negative association with these locations; e.g. Lei et al., 2004) and that destruction of neurons originating from the ACC reduced this place aversion (Gao et al., 2004; Johansen et al., 2001).

Using the drug self-selection method, Colpaert et al. (1980, 1982) showed that arthritic rats consumed more water containing an analgesic (a nonsteroidal anti-inflammatory, i.e. NSAID in the first study, and an opioid in the second study) versus sweetened water than did healthy controls, and that the amount of drugged water consumed mirrored the inflammatory process as assessed by the diameter of paws and joints. They further confirmed that pain, and not the rewarding or addictive action of the drugs, was the stimulus for self-selection (Colpaert et al., 2001). This suggests that arthritic rats learn to choose water from one bottle over another, and that this choice is likely driven by the relief from pain afforded by the medicated water. Similarly, Mickley et al. (2006) showed that rats that had undergone surgery drank more water containing an analgesic than did control rats, and that animals that had experienced the surgery drank enough medicated water to raise pain thresholds on a hot-plate test.

In humans, chronic pain is associated with a wide range of conditions that affect quality of life, such as anxiety, depression, appetite suppression, attentional deficits and sleep disruption. All of these conditions have also been recorded in rats experiencing pain (see Mogil, 2009).

6. Implications and conclusion

Current methods of assessing emotions in animals provide compelling evidence that rats indeed experience a range of positive and negative emotions. These results suggest that any differential treatment afforded to rats compared to other laboratory mammals, on the basis that they have more limited abilities to experience emotions, is not justified. We conclude that the 'wants' and desires of rats should be taken into account when deciding how to house and care for them. Existing standards in many laboratories, where rats are housed singly without access to a shelter and without opportunities to play or socialize with conspecifics are likely to profoundly affect their welfare.

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