

# Trait Pessimism Predicts Vulnerability to Stress-Induced Anhedonia in Rats

Rafal Rygula<sup>\*1</sup>, Justyna Papciak<sup>1</sup> and Piotr Popik<sup>1,2</sup>

<sup>1</sup>Department of Behavioral Neuroscience and Drug Development, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland;

<sup>2</sup>Faculty of Health Sciences, Collegium Medicum, Jagiellonian University, Krakow, Poland

Depressive disorder is often associated with cognitive biases. In this study, we took a unique opportunity to investigate whether trait pessimism could predict vulnerability to stress-induced anhedonia in an animal model of depression. In a series of ambiguous-cue interpretation (ACI) tests, we identified animals displaying 'pessimistic' and 'optimistic' traits. Subsequently, the rats were subjected to chronic restraint, and the trait differences in response to stress were investigated using sucrose preference and ACI tests before, during and after the stress regime. Although stress resulted in anhedonia in both subgroups, it occurred faster and lasted longer in the 'pessimistic' compared with the 'optimistic' animals. Chronic stress exposure also increased the negative judgment bias in rats, although this effect was not dependent on the 'pessimistic' trait. For the first time, we demonstrated a link between cognitive judgment bias and vulnerability to stress-induced anhedonia in an animal model. We also introduced a cognitive biomarker, which may be of value for etiological depression studies.

*Neuropsychopharmacology* (2013) **38**, 2188–2196; doi:10.1038/npp.2013.116; published online 5 June 2013

**Keywords:** judgment bias; stress; ambiguous-cue; anhedonia; trait pessimism; depression

## INTRODUCTION

Several cognitive models have proposed that depression is associated with biases in cognitive processing—in particular, in the attention to, interpretation of, and recall of positive and negative stimuli (Beck, 2008; Kovacs and Beck, 1978). Although there is now relatively consistent evidence that cognitive biases co-occur with depressive episodes (Erickson *et al*, 2005; Hollon *et al*, 1986; Krantz and Rude, 1984; Murphy *et al*, 1999; Rubinow and Post, 1992), whether these biases predict the course of depression is still unclear. According to a cognitive model proposed by Aaron Beck (Beck, 2008; Kovacs and Beck, 1978), depressed and depression-prone individuals possess assumptive structures (depressive schemas and dysfunctional pessimistic beliefs) that result in consciously accessible depressive thinking. These depressive schemas may be dormant or inactive, but when activated by stressful life events, these schemas may result in depression. Although there have been several cross-sectional studies that indirectly support this proposal (Alloy *et al*, 1999; Johnson *et al*, 2007; Lewinsohn *et al*, 1999; Rude *et al*, 2002), no study thus far has directly shown, using a case-controlled design, that biased cognitive

processing induces substantial effects on depression via interaction with stressful life events. This lack is due to the difficulty in obtaining information regarding past cognitive biases of patients who experience their first depressive episode.

A previous seminal study conducted by Harding *et al* (2004) showed that cognitive judgment bias could be induced and measured in rats. This study opened a new and fascinating avenue of pre-clinical research that provided the opportunity to investigate cognitive–emotional interplay in animal depression models. Over the past decade, a number of studies have reported that cognitive biases can be induced in animals following different behavioral and pharmacological manipulations (Bateson *et al*, 2011; Bethell *et al*, 2007; Brilot *et al*, 2010; Doyle *et al*, 2011; Enkel *et al*, 2010; Harding *et al*, 2004; Mendl *et al*, 2010; Rygula *et al*, 2012). However, none of these studies have investigated cognitive bias as a stable behavioral trait, which could be used to study depression vulnerability.

In this study, we investigated the theoretical claim made by cognitive models of depression (Beck, 2008; Kovacs and Beck, 1978) that negative processing biases, and in this case, trait 'pessimism', can predict subsequent symptoms of depression, such as the intensity and duration of stress-induced anhedonia. According to the model proposed by Beck, biased acquisition and processing of information has a primary role in the development and maintenance of depression (Beck, 1967, 1987, 2008). In this model, negative and pessimistic processing of one's self and context

\*Correspondence: Dr R Rygula, Department of Behavioral Neuroscience and Drug Development, Institute of Pharmacology Polish Academy of Sciences, 12 Smetna Street, Krakow 31-343, Poland, Tel: +48 (12) 6623374, Fax: +48 (12) 6374500, E-mail: rygula@gmail.com  
Received 22 February 2013; revised 25 April 2013; accepted 30 April 2013; accepted article preview online 10 May 2013

become pervasive, including interpretation and judgment of environmental stimuli (Clark *et al*, 1999).

The natural propensity of rats to interpret environmental stimuli in a positive or negative manner has been established using multiple ambiguous-cue interpretation (ACI) tests (Enkel *et al*, 2010). On the basis of these screening results, the animals were classified into one of two groups: displaying positive, cognitive bias toward 'optimistic' judgments, called further 'optimistic' and displaying negative cognitive bias toward 'pessimistic' judgments, called further 'pessimistic'. Subsequently, the animals were subjected to chronic restraint stress, a treatment paradigm that has been reported to induce depressive symptoms in rats (Nikiforuk and Popik, 2011; Plaznik *et al*, 1989; Uchida *et al*, 2010; Zurita *et al*, 1996). Differences in response to stress between the 'optimistic' and 'pessimistic' animals have been investigated using a sucrose preference test (Papp *et al*, 1991; Willner *et al*, 1992). This task has been broadly used to assess the stress-induced loss of the ability to experience pleasure (anhedonia) in rodents, which is also one of the core symptoms of depression in humans. In addition, the effects of chronic stress on cognitive processing bias in 'pessimistic' and 'optimistic' rats were compared using ACI tests, which were performed before, during and after the stress regime.

## MATERIALS AND METHODS

### Ethics Statement

These experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee for Animal Experiments at the Institute of Pharmacology Polish Academy of Sciences.

### Subjects and Housing

Thirty-two male Sprague-Dawley rats (Charles River, Germany) weighing between 175 and 200 g on arrival were used in this study. The rats were group-housed (four rats per cage) in a temperature-controlled room ( $21 \pm 1^\circ\text{C}$ ) with 40–50% humidity under a 12/12-h light-dark cycle (lights on at 0600 hours). In all of the experiments, the rats were mildly food restricted to approximately 85% of their free feeding weights. This was achieved by providing 15–20 g of food per rat per day (standard laboratory chow). The food restriction started 1 week before training. Water was freely available, except during the test sessions. The behavioral procedures and testings were performed during the light phase of the light-dark cycle.

### Apparatus

The behavioral tasks were performed in eight computer-controlled Skinner boxes (MedAssociates, St Albans, VT), where each box was equipped with light, a speaker, a liquid dispenser (set to deliver 0.1 ml of 20% sucrose solution), a grid floor through which scrambled electric shocks (0.5 mA) could be delivered, and two retractable levers. The levers were located at opposite sides of the feeder. All of the

behavioral protocols, including the data acquisition and recordings, were programmed in Med State notation code (Med Associates). The experimental procedures for the ACI test used in this study were modified versions of the procedures previously described by Enkel *et al* (2010) and have been described elsewhere (Rygula *et al*, 2012).

### Behavioral Training

**Positive tone training.** During this phase, the rats were trained to press the lever located on the left side of the feeder to receive the sucrose solution when a tone (50 s, 2000 Hz at 75 dB sound pressure level (SPL) or 9000 Hz at 75 dB SPL (counterbalanced)) signaled reward availability. Owing to its association with a palatable reward, this tone acquired a positive valence and was referred to as the 'positive tone,' and the associated lever was referred to as the 'positive lever.' A reliable active lever pressing for the reward was achieved in three training steps: (a) presentation of the positive tone (lasting 50 s) co-occurred with a constant delivery of the sucrose solution and was followed by a 10-s intertrial interval (ITI); (b) presentation of the positive tone co-occurred with a left lever extension and was followed by a 10-s ITI (each lever press during the tone was continuously rewarded by sucrose solution delivery); and (c) was similar to (b) with the exception that after the first lever press and reward delivery, the tone was terminated and followed by a 10-s ITI. Each training session lasted for 30 min, and the training sessions continued until the animals attained a stable performance on each of the training steps (>200 responses maintained over three consecutive training sessions during step b; and minimum 90% of responses to the positive lever following positive tone presentation maintained over three consecutive sessions during step c). Positive tone training was followed by negative tone training.

**Negative tone training.** During this stage, the rats were trained to press the lever located on the right side of the feeder to avoid an electric shock (0.5 mA, 10 s) when another tone (9000 Hz at 75 dB SPL or 2000 Hz at 75 dB SPL (counterbalanced)) signaled a forthcoming punishment. Owing to its association with a concomitant punishment, this tone acquired a negative valence and was referred to as the 'negative tone.' The associated lever was referred to as the 'negative lever.' A reliable active lever press avoidance response was achieved in two training steps: (a) the presentation of the negative tone was accompanied by the occurrence of electric shocks unless the rat pressed the right (negative) lever, which terminated the shock and tone presentation, and (b) the presentation of the negative tone preceded the occurrence of the electric shocks. The delay from the tone onset to the electric shock occurrence was progressively increased from 1 to 40 s. Pressing the negative lever before the shock onset terminated the tone and began a 10-s ITI (prevention response). Pressing the negative lever after the shock onset terminated the tone and shock and was referred to as the 'escape response.' The maximum duration of the tone/shock-application was 50 s (ie, 40 s of tone presentation followed by 10 s of a tone/shock co-occurrence), and the tone presentations were separated

by 10-s ITIs. Daily training sessions consisted of 40 tone presentations. The animals had to accomplish at least 60% correct prevention responses maintained over three consecutive training sessions, before proceeding to the discrimination training.

**Discrimination training.** During this phase, the rats were trained to discriminate between positive and negative tones by responding to the appropriate levers (as learned in previous training stages) to maximize reward and minimize punishment delivery. The tones, which consisted of 20 positive and 20 negative tones, were presented pseudo-randomly and separated by 10-s ITIs. Pressing the positive lever during the positive tone presentation resulted in an instant reward delivery and initiated the ITI. Pressing the negative lever during the negative tone presentation resulted in a negative tone termination and initiated the ITI. Pressing the wrong lever (eg, pressing the left lever instead of the right lever in response to a negative tone presentation) as well as escape responses or response omissions were considered failed trials. Animals had to minimally achieve 70% correct responses with each lever, maintained over three consecutive discrimination sessions to proceed to the ACI test.

**Ambiguous-cue testing.** The ACI testing session consisted of 20 positive, 20 negative, and 10 intermediate (ambiguous) tone presentations. The frequency of the intermediate tones was set to 5000 Hz at 75 dB. This frequency was selected on the basis of the protocol described by Enkel *et al* (2010) and was confirmed to be intermediate in terms of the response pattern in a pilot experiment (data not shown). The tones were presented in a pseudo-randomized order and separated by 10-s ITIs. Any lever press during the ambiguous tone presentation terminated the tone but had no consequences. If the rat did not respond within 50 s of the ambiguous tone presentation, the tone was terminated and a response omission was scored.

During ACI testing, the responses to each tone (positive, ambiguous, and negative) were scored and analyzed as the proportion of the overall number of responses to a given tone. The proportion of omissions was separately analyzed. To calculate the cognitive bias index, the proportion of negative responses to the ambiguous cues were subtracted from the proportion of positive responses, resulting in values ranging between  $-1$  and  $1$ , where values above  $0$  indicate an overall positive judgment and 'optimistic' interpretation of the ambiguous cue.

### Cognitive Bias Screening

To assess the cognitive judgment bias as a trait, individual rats were examined in a series of 10 consecutive ACI tests, which were performed in 1-week intervals. On the basis of the average cognitive bias index obtained from these 10 ACI tests, the rats were divided into two subgroups of 'optimistic' and 'pessimistic' animals. The animals that were classified as 'optimistic' had an average cognitive bias index above  $0$  whereas the cognitive bias index in the 'pessimistic' group was below  $0$ .

### Experimental Design and Behavioral Measures

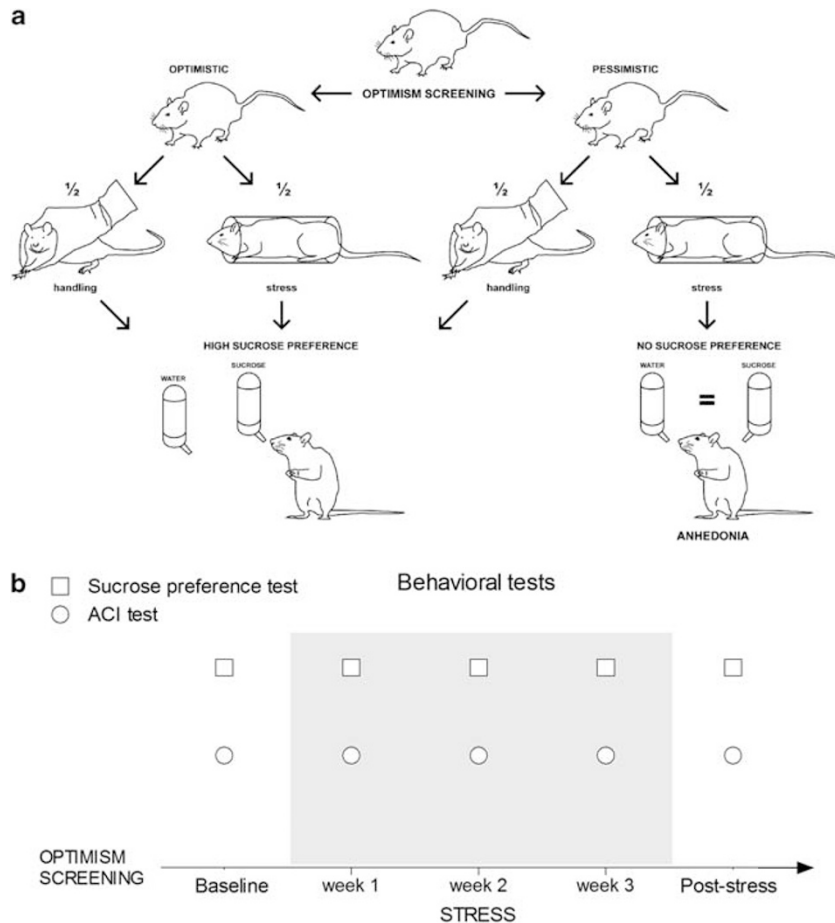
The experimental design is schematically presented in Figure 1b. After attaining a stable discrimination performance ( $>70\%$  correct responses to each tone over 3 consecutive days), each rat was subjected to the cognitive bias screening procedure as previously described. After establishing the 'optimistic' and 'pessimistic' traits in the individual animals, the rats were divided into four experimental groups: 'control-optimistic', 'stressed-optimistic', 'control-pessimistic', and 'stressed-pessimistic'. 'Stressed-optimistic' and 'stressed-pessimistic' groups of animals were subjected to chronic restraint stress. The stress paradigm consisted of 1-h daily immobilization sessions that were performed over 3 consecutive weeks. Rats were transferred from a housing facility to the stress-room and then separated into the test room. The animals were placed into perforated plastic tubes (6.5 cm inner diameter) of an adjustable length. The restraint enabled normal breathing and limited movements of the head and limbs. Control animals were handled daily throughout the experiment.

To assess the effects of chronic stress on the development of behavioral correlates of depression in 'optimistic' and 'pessimistic' animals, the rats were subjected to sucrose preference tests performed 24 h after the last baseline ACI test and 24 h after each ACI test during stress and post-stress stages of the experiment. Decreased preference to the sweet sucrose solution has been previously postulated to be an indicator of anhedonia in rodents (Papp *et al*, 1991; Willner *et al*, 1992). During these tests, which were performed in 1-week intervals, the rats were separated into single cages and were offered a voluntary choice for 1 h between two bottles, where one bottle contained a 0.8% sucrose solution and the other bottle contained tap water. To prevent potential effects of side preference in drinking, the position of the bottles was switched after 1/2 h. The consumption of water and sucrose solution was measured by weighing the bottles. The preference for sucrose was calculated from the amount of sucrose solution consumed, and expressed as a percentage of the total amount of liquid that was consumed.

To investigate the effects of the chronic stress regime on cognitive judgment bias in rats, the animals were subjected to individual ACI tests administered in 1-week intervals starting from the baseline. The ACI tests were performed as previously described.

### Statistics

The data were analyzed using SPSS (version 20.0, SPSS, Chicago, IL). Distribution of the cognitive bias index data was tested using Kolmogorov-Smirnov test. Differences in the processing of the ambiguous cue and the positive and negative tones between the 'optimistic' and 'pessimistic' animals were investigated using four-way analysis of variance (ANOVA) with the between-subject factor of cognitive bias (2 levels: optimistic and pessimistic) and the within-subject factors of test (10 levels: baseline test 1-10), lever (2 levels: positive and negative), and tone (3 levels: positive, ambiguous, and negative). The differences in the preference to the sweet sucrose solution and in the cognitive bias index between the 'control-optimistic',



**Figure 1** Graphical abstract of the main result (a) and schematic representation of the experimental schedule (b).

'control-pessimistic', 'stressed-optimistic', and 'stressed-pessimistic' animal groups were investigated using three-way repeated-measures ANOVA with the between-subject factors of stress (two levels: control and stressed), cognitive bias (optimistic and pessimistic), and the within-subject factor of stage (five levels: baseline, stress-week 1, stress-week 2, stress-week 3, and post-stress). The effects of stress on the processing of the ambiguous cue and reference tones in the 'optimistic' and 'pessimistic' animals were investigated using five-way repeated-measures ANOVA with between-subject factors of stress (two levels: control and stressed) and cognitive bias (optimistic and pessimistic) and the within-subject factors of stage (five levels: baseline, stress-week 1, stress-week 2, stress-week 3, and post-stress), lever (two levels: positive and negative), and tone (three levels: positive, ambiguous, and negative). Finally, in secondary analyses, Pearson correlations between baseline cognitive bias index and sucrose preference at different stages of experiment were run. For pair-wise comparisons, the values were adjusted using Sidak's correction factor for multiple comparisons (Howell, 1997). All of the tests of significance were performed at  $\alpha=0.05$ . Homogeneity of variance was confirmed using Levene's test. For repeated-measures analyses, the sphericity was also verified using Mauchly's test. The data were presented as the mean  $\pm$  SEM.

## RESULTS

Rats that showed lack of progress during training sessions ( $N=8$ ) were excluded from the analysis. Twenty-four rats reached the criterion of at least 70% correct responses with each lever, maintained over three consecutive discrimination sessions and qualified for cognitive bias screening. The animals that were classified as 'optimistic' reached the criteria of positive tone, negative tone, and discrimination trainings after  $17 \pm 1$ ,  $26 \pm 1$ , and  $9 \pm 1$  days, respectively, whereas the 'pessimistic' group reached the criteria after  $14 \pm 1$ ,  $25 \pm 1$ , and  $8 \pm 1$  days, respectively. No significant differences were observed in the total duration of the training between the 'optimistic' and 'pessimistic' animals ( $t(22) = 1.278$ , NS).

The average cognitive bias index of all of the experimental animals established on basis of the cognitive bias screening was  $-0.047 \pm 0.045$ . The distribution of the cognitive bias index data at baseline (Figure 3b) was normal ( $Z = 0.149$ ,  $N = 24$ , Kolmogorov-Smirnov test).

An analysis of the response of animals to the positive and negative levers following reference and ambiguous tones across the baseline indicated no test-retest effects. Although the test  $\times$  lever  $\times$  tone interaction was significant ( $F(18, 414) = 3.73$ ,  $p < 0.001$ ), *post hoc* pair-wise comparisons revealed significant differences only in responding to

the positive lever, following ambiguous tone presentation between baseline tests 2 vs 4 ( $p < 0.05$ ), 2 vs 5 ( $p < 0.01$ ), and 2 vs 6 ( $p < 0.001$ ), which is also reflected in the change of the cognitive bias index shown on Figure 3a. The proportion of omissions remained unchanged over the baseline period.

There was no regularity in the distribution of the average baseline cognitive bias index within the cages. In two cages all the rats were 'pessimistic', in one cage all the rats were 'optimistic', in one cage three rats were 'optimistic' and one was 'pessimistic' and in two cages two rats were 'optimistic' and two were 'pessimistic'.

### 'Optimistic' Vs 'Pessimistic' Animals

Results of the cognitive bias screening enabled the separation of two groups of animals, which were clearly distinctive in the interpretation of the ambiguous cues over time: 'optimistic' ( $N = 11$ , AVG cognitive bias index  $> 0$ ) and 'pessimistic' ( $N = 13$ , AVG cognitive bias index  $< 0$ ). The animals that were classified as 'optimistic' had an average cognitive bias index ranging from 0.05 to 0.35, whereas the cognitive bias index in the 'pessimistic' group ranged from  $-0.04$  to  $-0.36$  (Figure 2a).

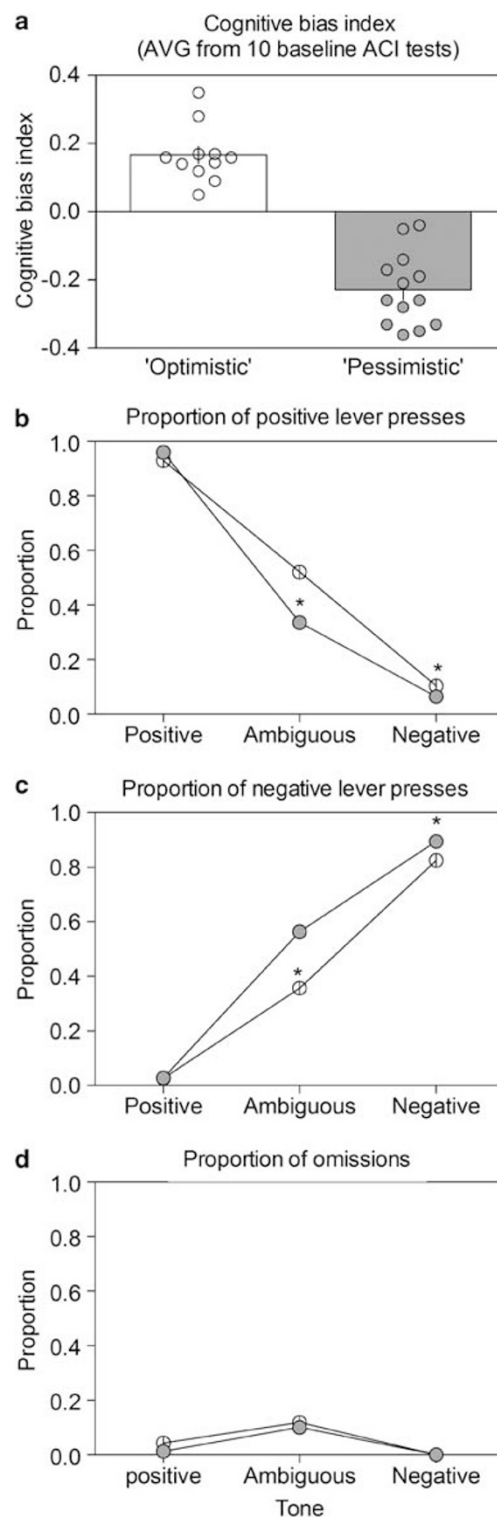
Further analysis revealed significant differences in the pattern of responding between 'optimistic' and 'pessimistic' groups (lever  $\times$  tone  $\times$  cognitive bias interaction ( $F(2, 44) = 31.19$ ,  $p < 0.001$ )). The 'optimistic' animals responded significantly less often to the negative lever in response to the ambiguous tone compared with their 'pessimistic' counterparts ( $p < 0.001$ , Figure 2c). In contrast, the animals classified as 'pessimistic' in response to the ambiguous tone responded significantly less to the positive lever ( $p < 0.001$ , Figure 2b). The 'optimistic' animals also responded more often to the positive lever in response to the negative tone ( $p < 0.05$ , Figure 2b) and less often to the negative lever in response to the negative tone ( $p < 0.01$ , Figure 2c).

'Optimistic' and 'pessimistic' rats did not differ in the number of omissions made (no significant effect of cognitive bias or cognitive bias  $\times$  tone interaction), however, all of the animals made more omissions ( $p < 0.001$ ) after the ambiguous tones compared with after the reference tones (significant effect of tone ( $F(2, 44) = 49.58$ ,  $p < 0.001$ , Figure 2d).

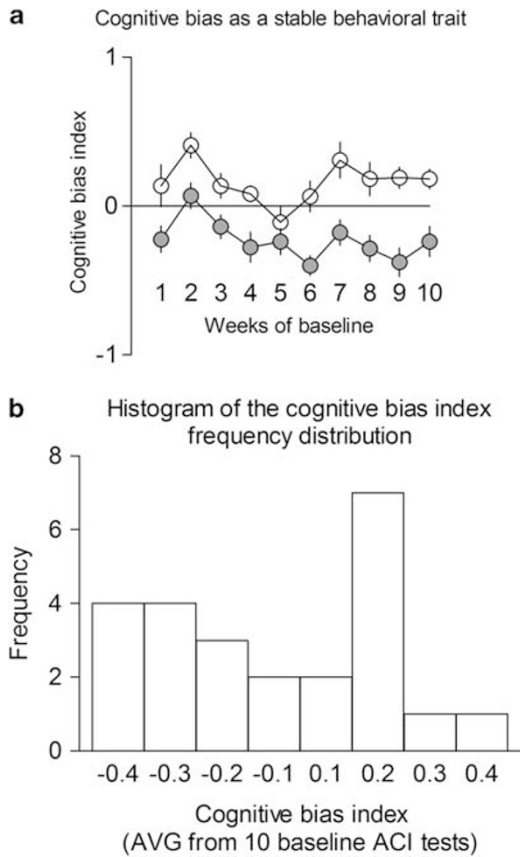
Although, as mentioned previously, the cognitive bias index fluctuated in both groups of animals (significant test  $\times$  lever  $\times$  tone interaction ( $F(18, 414) = 3.73$ ,  $p < 0.001$ ), the differences between 'optimistic' and 'pessimistic' groups did not significantly change across the screening period (no significant test  $\times$  cognitive bias interactions; highest  $F(9, 199) = 1.62$ ) indicating stability of the traits (Figure 3a).

### Stress-Induced Anhedonia in 'Optimistic' and 'Pessimistic' Animals

Initially, all of the animals showed a high ( $\sim 80\%$ ) preference to the sweet sucrose solution and no significant differences were observed in this preference between the 'optimistic' and 'pessimistic' animals ( $t(22) = -1.97$ , NS). The chronic restraint treatment significantly reduced sucrose preference in both groups of stressed rats (significant stage  $\times$  stress interaction ( $F(4, 80) = 9.38$ ,  $p < 0.001$ )) and there were significant differences between 'optimistic'



**Figure 2** 'Optimistic' vs 'pessimistic' animals; results of the cognitive bias screening. (a) The mean  $\pm$  SEM cognitive bias index of the animals classified (on basis of 10 ACI tests) as 'optimistic' (open bar,  $N = 11$ ) and 'pessimistic' (filled bar,  $N = 13$ ). An cognitive bias index above 0 indicates an overall positive judgment and 'optimistic' interpretation of the ambiguous cue. (b) The mean  $\pm$  SEM proportion of positive; (c) negative and (d) omitted responses to the trained and ambiguous tones in the 'optimistic' (open circles,  $N = 11$ ) and 'pessimistic' (filled circles,  $N = 13$ ) rat groups. \*Indicates significant ( $p < 0.05$ ) differences between the 'optimistic' and 'pessimistic' animals

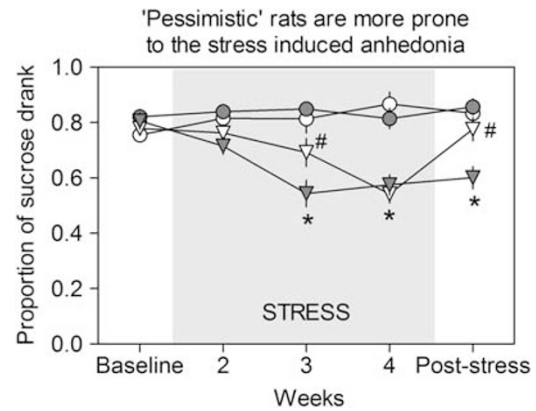


**Figure 3** Cognitive bias as a behavioral trait and histogram of the cognitive bias index data distribution at the baseline. (a) The mean  $\pm$  SEM cognitive bias index of the animals classified as 'optimistic' (open circles,  $N=11$ ) and 'pessimistic' (filled circles,  $N=13$ ) across all 10 baseline ACI tests. (b) Histogram of the average cognitive bias index frequency distribution in all ( $N=24$ ) animals at the baseline.

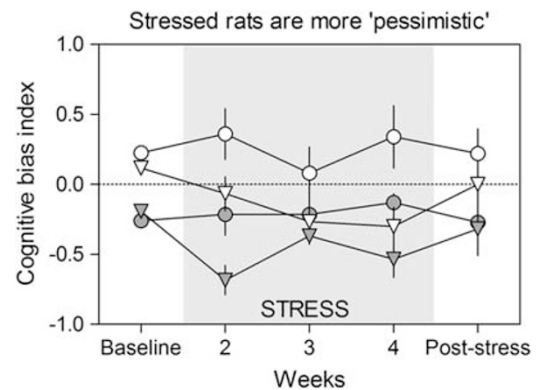
and 'pessimistic' groups of animals (stage  $\times$  stress  $\times$  cognitive bias interaction ( $F(4, 80) = 2.58$ ,  $p < 0.05$ )). The rats classified as 'pessimistic' demonstrated a significantly reduced preference to the palatable solution after 2 weeks of stress ( $p < 0.001$  compared with baseline), and this effect remained significant 1 week after the termination of the stress regime ( $p < 0.01$  compared with baseline). The rats classified as 'optimistic' showed a significantly decreased sucrose preference after 3 weeks of stress ( $p < 0.001$  compared with baseline), and this decrease was abolished with the termination of the stress regime (NS compared with baseline and  $p < 0.01$  compared with the stress at week 3). 'Optimistic' animals undergoing restraint stress displayed a significantly higher sucrose preference compared with their 'pessimistic' counterparts after 2 weeks of stress ( $p < 0.05$ ) and 1 week after the termination of the stress ( $p < 0.01$ ). The 'control-optimistic' and 'control-pessimistic' groups of animals did not significantly change their preference for the sweet sucrose solution throughout the entire experiment (Figure 4).

### Stress-Induced Changes in Cognitive Judgment Bias

The stressed animals significantly decreased their cognitive bias index during and after the stress regime (stress  $\times$  stage

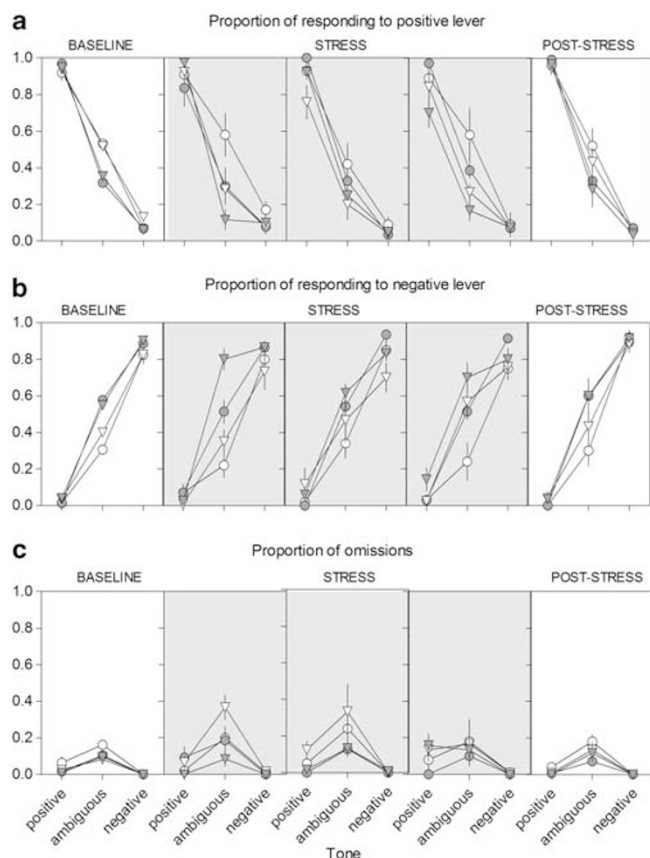


**Figure 4** 'Pessimistic' rats are more prone to stress-induced anhedonia. The mean  $\pm$  SEM sucrose preference of the 'control optimistic' (open circles,  $N=5$ ), 'control pessimistic' (filled circles,  $N=7$ ) 'stressed optimistic' (open triangles,  $N=6$ ), and 'stressed pessimistic' (filled triangles,  $N=6$ ) animals, as measured at baseline, during the stress and post-stress phases of the study. \*Indicates a significant ( $p < 0.05$ ) decrease in sucrose preference compared with baseline. #Indicates a significant ( $p < 0.05$ ) difference in sucrose preference between the 'optimistic' and 'pessimistic' animals. The gray area outlines the stress period.



**Figure 5** Chronic stress makes rats more 'pessimistic' in the ACI test. The mean  $\pm$  SEM cognitive bias index of the 'control optimistic' (open circles,  $N=5$ ), 'control pessimistic' (filled circles,  $N=7$ ) 'stressed optimistic' (open triangles,  $N=6$ ), and 'stressed pessimistic' (filled triangles,  $N=6$ ) animals, as measured at baseline, during the stress and post-stress phases of the study. Exposure to stress caused a significant decrease in the cognitive bias index of both stressed groups of animals (significant stress  $\times$  stage interaction), but there were no significant differences between the 'optimistic' and 'pessimistic' animals (no significant interaction of stress or stage with cognitive bias). The gray area outlines the stress period.

interaction ( $F(4, 80) = 2.83$ ,  $p < 0.05$ ). *Post hoc* analyses revealed that stressed rats were more 'pessimistic' during weeks 2, 3, and 4 ( $p < 0.05$ , 0.05, and 0.01, respectively) compared with baseline (Figure 5). Stressed animals were also more 'pessimistic' compared with controls during stress weeks 2, 3, and 4 ( $p < 0.01$ , 0.072, and 0.001, respectively). This effect was abolished by the stress termination. Although it appears that the 'pessimistic' group was less sensitive to stress because the effect of stress was not present in week 3 in that group (Figure 5), the cognitive bias  $\times$  stress  $\times$  stage interaction was not significant



**Figure 6** Stress-induced negative judgment bias results in a reduced positive response. The mean  $\pm$  SEM proportion of positive (a), negative (b), and omitted (c) responses to the trained and ambiguous tones in the ‘control optimistic’ (open circles,  $N=5$ ), ‘control pessimistic’ (filled circles,  $N=7$ ) ‘stressed optimistic’ (open triangles,  $N=6$ ), and ‘stressed pessimistic’ (filled triangles,  $N=6$ ) animals. The effects of stress on tone processing were independent on the initial cognitive bias index. Stress reduced the response to the positive lever after ambiguous and positive-reference tone exposures in both groups of stressed animals (a) and increased the response to the negative lever after ambiguous tone presentation (b). All of the animals made more omissions after exposure to the ambiguous tone compared with after exposure to either of the reference tones (c).

( $F(4, 80) = 0.19$ ). The ‘control-optimistic’ and ‘control-pessimistic’ groups of animals did not significantly change their cognitive bias index throughout the entire experiment.

Further analysis of the effects of stress on responding to positive and negative levers following presentation of the experimental tones at different experimental stages (significant ( $F(8, 160) = 3.156$ ,  $p < 0.01$ ) stage  $\times$  lever  $\times$  tone  $\times$  stress interaction) revealed that stressed animals in response to the ambiguous tone responded significantly less to the positive lever after 1, 2, and 3 weeks of stress ( $p < 0.015$ , 0.055, and 0.01, respectively, Figure 6a) and significantly more to the negative lever after stress weeks 1 and 3 ( $p < 0.01$ , Figure 6b). Stressed animals also responded significantly less to the positive lever in response to the positive tone after stress week 2 and 3 ( $p < 0.05$ , Figure 6a). The effects of stress on tone processing were independent on the initial cognitive bias index (the cognitive bias  $\times$  stage  $\times$  lever  $\times$  tone  $\times$  stress interaction was not significant ( $F(8, 160) = 0.857$ )).

As shown in Figure 6c, all of the animals made significantly more omissions after the ambiguous tone compared with after the reference tones (main effect of tone ( $F(2, 40) = 66.25$ ,  $p < 0.001$ )), and the ‘optimistic’ animals made significantly more omissions after the ambiguous tone compared with the ‘pessimistic’ group (tone  $\times$  cognitive bias interaction ( $F(2, 40) = 4.78$ ,  $p < 0.05$ )).

Analysis of two significant interactions involving cognitive bias revealed only that ‘optimistic’ animals in response to the ambiguous tone responded significantly ( $p < 0.01$ ) more to the positive lever (Figure 6a) and significantly ( $p < 0.001$ ) less to the negative lever (Figure 6b) compared with ‘pessimistic’ group (lever  $\times$  tone  $\times$  cognitive bias interaction ( $F(2, 40) = 28.32$ ,  $p < 0.001$ )) and that ‘optimistic’ animals made significantly less responses after stress weeks 1 and 2 ( $p < 0.01$  and 0.05, respectively) compared with the ‘pessimistic’ group (stage  $\times$  stress  $\times$  cognitive bias interaction ( $F(4, 80) = 2.88$ ,  $p < 0.05$ )).

A correlation analysis performed on the baseline data revealed that the cognitive bias index and sucrose preference were significantly, negatively correlated ( $r = -0.57$ ,  $N = 24$ ,  $p < 0.01$ ). In the control group, the analysis of the relationship between cognitive bias index and sucrose preference revealed a significant, negative correlation only at the baseline ( $r = -0.66$ ,  $N = 12$ ,  $p < 0.05$ ). In the stressed group, cognitive bias index and sucrose preference were significantly, positively correlated after 1 week of stress ( $r = 0.603$ ,  $N = 12$ ,  $p < 0.05$ ) and 1 week after the termination of the stress procedure ( $r = 0.761$ ,  $N = 12$ ,  $p < 0.01$ ).

## DISCUSSION

In this study, we examined in an animal model of depression whether trait pessimism can interact with the development of stress-induced anhedonia. Our results indicated that rats displaying the ‘pessimistic’ trait were more prone to develop stress-induced anhedonia compared with their ‘optimistic’ conspecifics. Analysis of the changes in the cognitive bias index also revealed that chronic stress caused the animals to be more biased toward negative judgments- ‘pessimistic’ and that this effect was universal for both ‘pessimistic’ and ‘optimistic’ animals.

In cognitive theories, information processing biases were considered as a vulnerability factor for the etiology, maintenance and recurrence of depression (Beck, 1967, 1987, 2008; Clark *et al*, 1999). This vulnerability has been conceptualized as a trait-like latent endogenous process reactive to the effects of stress, which reside in genetic, biological, and psychological variables (De Raedt and Koster, 2010). However, to date, there have been no studies that directly support this theoretical claim. Recent development of the ACI test, a task that can be used to measure cognitive judgment bias (optimism and pessimism) in animals (Enkel *et al*, 2010; Harding *et al*, 2004; Rygula *et al*, 2012), enabled the investigation of cognitive vulnerability to depression in an animal model. We assumed that in animals, similar to humans, cognitive judgment bias has both enduring trait and transient state components (Kluemper *et al*, 2009). A trait represents a stable individual difference in the level of pessimism/optimism that is generally experienced, whereas state captures the

pessimism/optimism that may change on the basis of the situation or contextual factors. Following this assumption and on the basis of a number of ACI tests, we isolated two groups of animals that significantly and persistently differed in their cognitive bias index over time. These two groups of 'pessimistic' and 'optimistic' rats were subsequently subjected to the chronic restraint stress treatment and examined for their sensitivity to stress-induced anhedonia.

Rats that normally exhibit a high preference for palatable sweet solutions when these are paired with water, often significantly decrease this preference following exposure to chronic stress (Katz, 1981; Papp *et al*, 1991; Willner *et al*, 1992). It has been postulated that this change in preference represents an alteration in the ability to experience pleasure (Willner *et al*, 1992) that is analogous to human anhedonia—one of the core symptoms of depression according to DSM-IV (American Psychiatric Association, 1994). Importantly, in contrast to, for example, the forced swim test, measuring sucrose preference is relatively non-stressful and non-invasive, allowing multiple, consecutive tests without influencing the animal's behavior.

Also in our study, initially the animals showed strong preference to the palatable sucrose solution and in agreement with previous reports (Plaznik *et al*, 1989; Uchida *et al*, 2010; Zurita *et al*, 1996), chronic exposure to restraint stress significantly reduced this preference. However, the stress-induced anhedonia appeared earlier and lasted longer in rats displaying trait 'pessimism' (Figure 4). To the best of our knowledge, this was the first direct evidence that trait pessimism could be considered as a vulnerability factor predicting severity and longevity of stress-induced depressive symptoms. Although further studies are needed to directly pinpoint the neurobiological correlates of the observed interaction, our results may be, at least partially, explained using the framework of Becks developmental model of depression (Beck, 2008). In this model, genetic diathesis causes hyperreactivity of the amygdala to the external stimuli resulting in cognitive bias, and an exaggeration of stressful events and hyperactivity of the HPA axis. This, in turn, results in the dominance of limbic activity over prefrontal function and the development of depressive symptoms. Further studies are required to evaluate whether trait pessimism could interact with other behavioral correlates of depression in rats. Such a generalization is plausible since it has been suggested (Strekalova *et al*, 2004) that anhedonia, but not chronic stress per se is associated with other analogues of depressive symptoms, such as increased floating during the forced swimming test and exploration of novelty in rodents.

ACI tests performed during the stress period revealed that both groups of stressed animals showed a decrease in their cognitive bias index. This was consistent with the results of Harding *et al*, 2004, who reported a negative response bias in rats after chronic uncontrollable stress and with results obtained in a study conducted by Enkel *et al*, 2010 who reported a negative judgment bias in animals after pharmacological and genetic manipulations resembling depressive-like state. It is noteworthy that chronic uncontrollable stress paradigm described by Harding *et al* (2004) was similar to the 'chronic mild stress' procedure

that has been shown extensively to elicit symptoms of depression that are antidepressant-reversible (Moreau *et al*, 1992; Sanchez *et al*, 2003; Willner *et al*, 1987). Our results also paralleled human studies where depression was often associated with negative processing bias (Erickson *et al*, 2005; Hollon *et al*, 1986; Krantz and Rude, 1984; Murphy *et al*, 1999; Rubinow and Post, 1992). Interestingly, although vulnerability to stress-induced anhedonia was determined by the trait pessimism, the stress-induced shift in valence of the cognitive judgment bias was not. Both subgroups of stressed rats showed a substantial decrease in their cognitive bias index. A relatively milder decrease in the 'pessimistic' group may be explained by the floor effect, which reduced the effect of stress on this measure.

Analysis of the relation between the cognitive bias index and sucrose preference yielded equivocal results. The initial, negative correlation was not detected in control animals during later weeks of the experiment. The positive correlation revealed in the stressed group after 1 week of stress was not observed until the last week. Clearly, further studies are required to determine the exact nature of these relations.

The negative response bias observed after chronic stress exposure also resulted from an overall decreased positive response that was not limited to the ambiguous tone, but was generalized to the reference positive tone. Together with the reduced sucrose preference, this suggests that the applied stress protocol resulted in a functional deficit that involved both appetitive and consummatory behaviors and resulted in the lack of response to hedonic stimuli and lack of an appropriate affective response to the anticipation of pleasure. An association between anhedonia and reduced positive response resulting in 'pessimistic' judgment might suggest the possibility of a causal and bidirectional relationship between the two and their involvement in a pathogenic mechanism of depression. Future studies are required to address this association, in particular, if there may be a common pathway linking stressful stimuli to negative judgment bias and anhedonia.

Taken together, using multiple, consecutive ACI tests, we demonstrated that the valence of cognitive judgment bias in rats may be considered as an enduring behavioral trait. We also showed that this trait may determine the sensitivity of the animals to stress-induced anhedonia. Moreover, we confirmed that chronic stress increased the negative judgment bias in rats, although this effect was not dependent on the 'pessimistic' trait. Our results call for further investigation of the neurobiological mechanisms involved. Future studies should also determine whether the 'pessimistic' trait interacts via stress with other behavioral and physiological correlates of depression in rodents such as intracranial self-stimulation, swimming duration in the forced swim test, activity of the HPA axis or hippocampal neurogenesis, among others. Finally, it is possible that cognitive bias screening could be used to evaluate the individual differences in response to the therapeutic effects of antidepressant drugs.

## FUNDING AND DISCLOSURE

The authors declare no conflict of interest.



## ACKNOWLEDGEMENTS

This study was supported by the Foundation for Polish Science 'Homing Plus' Program, which was co-financed by the European Regional Development Fund (Innovative Economy Operational Program 2007–2013) and the statutory funds of the Institute of Pharmacology Polish Academy of Sciences. We thank Dr T Enkel for his help in establishing the ACI paradigm and A Leszczynska for graphical support.

## REFERENCES

- Alloy LB, Abramson LY, Whitehouse WG, Hogan ME, Tashman NA, Steinberg DL *et al* (1999). Depressogenic cognitive styles: predictive validity, information processing and personality characteristics, and developmental origins. *Behav Res Ther* **37**: 503–531.
- American Psychiatric Association (1994). *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)*, 4th edn. American Psychiatric Association: Washington, DC.
- Bateson M, Desire S, Gartside SE, Wright GA (2011). Agitated honeybees exhibit pessimistic cognitive biases. *Curr Biol* **21**: 1070–1073.
- Beck AT (1967). *Depression: Clinical, Experimental, and Theoretical Aspects*. Harper and Row: New York.
- Beck AT (1987). Cognitive models of depression. *J Cogn Psychother* **1**: 5–37.
- Beck AT (2008). The evolution of the cognitive model of depression and its neurobiological correlates. *Am J Psychiatry* **165**: 969–977.
- Bethell EJ, Holmes A, Maclarnon A, Semple S (2007). Evidence that emotion mediates social attention in rhesus macaques. *PLoS One* **7**: e44387.
- Brilot BO, Asher L, Bateson M (2010). Stereotyping starlings are more 'pessimistic'. *Anim Cogn* **13**: 721–731.
- Clark DA, Beck AT, Alford BA (1999). *Scientific Foundations of Cognitive Theory and Therapy of Depression*. John Wiley & Sons: New York.
- De Raedt R, Koster EH (2010). Understanding vulnerability for depression from a cognitive neuroscience perspective: a reappraisal of attentional factors and a new conceptual framework. *Cogn Affect Behav Neurosci* **10**: 50–70.
- Doyle RE, Lee C, Deiss V, Fisher AD, Hinch GN, Boissy A (2011). Measuring judgement bias and emotional reactivity in sheep following long-term exposure to unpredictable and aversive events. *Physiol Behav* **102**: 503–510.
- Enkel T, Gholizadeh D, von Bohlen Und Halbach O, Sanchis-Segura C, Hurlmann R, Spanagel R *et al* (2010). Ambiguous-cue interpretation is biased under stress- and depression-like states in rats. *Neuropsychopharmacology* **35**: 1008–1015.
- Erickson K, Drevets WC, Clark L, Cannon DM, Bain EE, Zarate CA Jr. *et al* (2005). Mood-congruent bias in affective go/no-go performance of unmedicated patients with major depressive disorder. *Am J Psychiatry* **162**: 2171–2173.
- Harding EJ, Paul ES, Mendl M (2004). Animal behaviour: cognitive bias and affective state. *Nature* **427**: 312.
- Hollon SD, Kendall PC, Lumry A (1986). Specificity of depressotypic cognitions in clinical depression. *J Abnorm Psychol* **95**: 52–59.
- Howell DC (1997). *Statistical Methods for Psychology*, 4th edn. Wadsworth: Belmont, CA.
- Johnson SL, Joormann J, Gotlib IH (2007). Does processing of emotional stimuli predict symptomatic improvement and diagnostic recovery from major depression? *Emotion* **7**: 201–206.
- Katz RJ (1981). Animal models and human depressive disorders. *Neurosci Biobehav Rev* **5**: 231–246.
- Kluemper D, Little LM, DeGroot T (2009). State or trait: effects of state optimism on job-related outcomes. *J Organizational Behav* **30**: 209–231.
- Kovacs M, Beck AT (1978). Maladaptive cognitive structures in depression. *Am J Psychiatry* **135**: 525–533.
- Krantz SE, Rude S (1984). Depressive attributions: selection of different causes or assignment of dimensional meanings? *J Pers Soc Psychol* **47**: 193–203.
- Lewinsohn PM, Allen NB, Seeley JR, Gotlib IH (1999). First onset versus recurrence of depression: differential processes of psychosocial risk. *J Abnorm Psychol* **108**: 483–489.
- Mendl M, Brooks J, Basse C, Burman O, Paul E, Blackwell E *et al* (2010). Dogs showing separation-related behaviour exhibit a 'pessimistic' cognitive bias. *Curr Biol* **20**: R839–R840.
- Moreau JL, Jenck F, Martin JR, Mortas P, Haefely WE (1992). Antidepressant treatment prevents chronic unpredictable mild stress-induced anhedonia as assessed by ventral tegmentum self-stimulation behavior in rats. *Eur Neuropsychopharmacol* **2**: 43–49.
- Murphy FC, Sahakian BJ, Rubinsztein JS, Michael A, Rogers RD, Robbins TW *et al* (1999). Emotional bias and inhibitory control processes in mania and depression. *Psychol Med* **29**: 1307–1321.
- Nikiforuk A, Popik P (2011). Long-lasting cognitive deficit induced by stress is alleviated by acute administration of antidepressants. *Psychoneuroendocrinology* **36**: 28–39.
- Papp M, Willner P, Muscat R (1991). An animal model of anhedonia: attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. *Psychopharmacology (Berl)* **104**: 255–259.
- Plaznik A, Stefanski R, Kostowski W (1989). Restraint stress-induced changes in saccharin preference: the effect of antidepressant treatment and diazepam. *Pharmacol Biochem Behav* **33**: 755–759.
- Rubinow DR, Post RM (1992). Impaired recognition of affect in facial expression in depressed patients. *Biol Psychiatry* **31**: 947–953.
- Rude S, Wenzlaff R, Gibbs B, Vane J, Whitney T (2002). Negative processing biases predict subsequent depressive symptoms. *Cognition Emotion* **16**: 423–440.
- Rygula R, Pluta H, Popik P (2012). Laughing rats are optimistic. *PLoS One* **7**: e51959.
- Sanchez C, Gruca P, Papp M (2003). R-citalopram counteracts the antidepressant-like effect of escitalopram in a rat chronic mild stress model. *Behav Pharmacol* **14**: 465–470.
- Strekalova T, Spanagel R, Bartsch D, Henn FA, Gass P (2004). Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. *Neuropsychopharmacology* **29**: 2007–2017.
- Uchida S, Hara K, Kobayashi A, Funato H, Hobara T, Otsuki K *et al* (2010). Early life stress enhances behavioral vulnerability to stress through the activation of REST4-mediated gene transcription in the medial prefrontal cortex of rodents. *J Neurosci* **30**: 15007–15018.
- Willner P, Muscat R, Papp M (1992). Chronic mild stress-induced anhedonia: a realistic animal model of depression. *Neurosci Biobehav Rev* **16**: 525–534.
- Willner P, Towell A, Sampson D, Sophokleous S, Muscat R (1987). Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology (Berl)* **93**: 358–364.
- Zurita A, Murua S, Molina V (1996). An endogenous opiate mechanism seems to be involved in stress-induced anhedonia. *Eur J Pharmacol* **299**: 1–7.