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Corrigendum

Corrigendum to "Effects of stereotypic behaviour and chronic mild stress on judgement bias in laboratory mice" [Appl. Anim. Behav. Sci. 174 (2016) 162–172]

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The authors regret that in the name of one of co-authors, Thomas S. Reichlin, the middle initial was missed to be added in the original publication. The authors would like to apologise for any inconvenience caused.

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Effects of stereotypic behaviour and chronic mild stress on judgement bias in laboratory mice

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ABSTRACT

Cognitive processes are influenced by underlying affective states, and tests of cognitive bias have recently been developed to assess the valence of affective states in animals. These tests are based on the fact that individuals in a negative affective state interpret ambiguous stimuli more pessimistically than individuals in a more positive state. Using two strains of mice we explored whether unpredictable chronic mild stress (UCMS) can induce a negative judgement bias and whether variation in the expression of stereotypic behaviour is associated with variation in judgement bias. Sixteen female CD-1 and 16 female C57BL/6 mice were trained on a tactile conditional discrimination test with grade of sandpaper as a cue for differential food rewards. Once they had learned the discrimination, half of the mice were subjected to UCMS for three weeks to induce a negative affective state. Although UCMS induced a reduced preference for the higher value reward in the judgement bias test, it did not affect saccharine preference or hypothalamic–pituitary–adrenal (HPA) activity. However, UCMS affected responses to ambiguous (intermediate) cues in the judgement bias test. While control mice showed a graded response to ambiguous cues, UCMS mice of both strains did not discriminate between ambiguous cues and tended to show shorter latencies to the ambiguous cues and the negative reference cue. UCMS also increased bar-mouthing in CD-1, but not in C57BL/6 mice. Furthermore, mice with higher levels of stereotypic behaviour made more optimistic choices in the judgement bias test. However, no such relationship was found for stereotypic bar-mouthing, highlighting the importance of investigating different types of stereotypic behaviour separately.

1. Introduction

The assessment of animal welfare has mostly focused on physiological and behavioural indicators of well-being (Abou-Ismail et al., 2007; Broom, 1991; Hurst et al., 1999). Such indicators, however, can be affected by arousal and do not provide reliable measures of affective valence (Mendl et al., 2009; Paul et al., 2005). In human research, influences of affective state on cognitive processes, such as attention, memory and judgement, have been well documented (Eysenck and Byrne, 1994; Eysenck et al., 1991). Thus, individuals in a negative affective state are more likely to interpret ambiguous information in a negative way, displaying a negative cognitive bias, than individuals in a positive affective state who exhibit more positive judgements of ambiguity (Eysenck et al., 1991; MacLeod and Byrne, 1996). Based on these findings, cognitive biases have been proposed as potential measures of affective valence in animals (Paul et al., 2005), and various tests applied in several animal species have provided promising results (Mendl et al., 2009).

The most commonly used test paradigm in rodents so far has been judgement bias, where an animal is trained to discriminate between two reference cues of the same stimulus modality associated with either reward or punishment or rewards of differential value. Using a go/no-go test, Harding et al. (2004) trained rats to respond to one tone by pressing a lever to obtain food, and to refrain from pressing the lever to avoid a burst of white noise when hearing a different tone. Judgement bias was then measured by testing the animal's response to ambiguous intermediate tones. Rats exposed to unpredictable chronic mild stress (UCMS), a paradigm known to induce depression-like states in rodents (Willner, 1997), made fewer go-responses to ambiguous tones. This negative judgement bias was interpreted as reflecting a more negative affective state (Harding et al., 2004).

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While go/no-go tests such as that used by Harding et al. (2004) are relatively simple and quick to learn, one of the disadvantages is that responses to ambiguous cues can be influenced by motivation and general activity, making it difficult to distinguish between a no-go response and a response omission due to lack of motivation for the reward (Mendl et al., 2009). One way to avoid these possible confounding factors are active choice tests, in which the animals have to make active responses to both trained cues (for review see (Mendl et al., 2009)). These tests require the use of two reward based outcomes, thereby avoiding aversive outcomes during training, which are likely perceived as stressful and may influence the animals' affective state. While active choice tests have been used in rats (Brydges et al., 2012, 2011; Chaby et al., 2013), they have as yet not been used in mice.

1.1. Stereotypic behaviour and affective state

Judgement biases are sensitive both to short and long term changes in affective state (Bar-Haim et al., 2007; Brilot et al., 2010; Keen et al., 2013; Mendl et al., 2010; Pomerantz et al., 2012). While most studies so far have tested animals following a relatively short exposure to experimental treatments to alter affective state (Brydges et al., 2011; Burman et al., 2009, 2008; Harding et al., 2004), there has been less focus on mood states, which can arise from long term environmental conditions (Eysenck et al., 1991). For example, stereotypic behaviour usually develops in response to inadequate housing conditions, and is generally associated with other measures of impaired welfare (Mason and Latham, 2004; Rushen, 1993; Würbel and Stauffacher, 1998, 1997). The relationship between the expression of stereotypic behaviour and well-being is not clear, however, and within the same environment, individuals displaying stereotypic behaviour sometimes show signs of better welfare (Mason and Latham, 2004), indicating that for some animals, stereotypies might have rewarding properties, for example a stress reducing effect. Such conflicting results were also found in studies investigating the relationship between stereotypic behaviour and affective valence. For example, stereotypic somersaulting in starlings was associated with a negative judgement bias (Brilot et al., 2010), while stereotypic pacing in grizzly bears correlated positively with an optimistic judgement bias (Keen et al., 2013). Furthermore, capuchin monkeys showing stereotypic head twirls displayed negative judgement bias, while no bias was found in individuals showing stereotypic pacing (Pomerantz et al., 2012), indicating that some types of stereotypic behaviour may be dissociated from well-being.

Laboratory mice generally exhibit large individual differences in the expression of stereotypic behaviour in response to captive conditions (Engel et al., 2011; Gross et al., 2012, 2011; Latham and Mason, 2010; Würbel et al., 1996). If stereotypies in mice are an indicator of affective state, individual differences in the level of expression should reflect individual differences in affective state. We therefore assessed whether the level of stereotypic behaviour in the home cage predicts judgement bias in an active choice test. Furthermore, we explored whether judgement bias in mice is affected by short term changes in affective state. We thus manipulated affective state by exposing mice to UCMS and hypothesised that UCMS will induce a more negative judgement bias.

2. Methods

2.1. Subjects and housing

Sixteen female CD-1 and 16 female C57BL/6/JRcc mice (Harlan Laboratories GmbH c/o, The Netherlands) were purchased at three weeks of age and housed in pairs in Makrolon Type II cages (22 cm × 16 cm × 14 cm, Techniplast). The mice were kept on a 12:12 h light:dark cycle, with temperature maintained at 21 ± 1 °C. Cages were changed once per week by the experimenter (J.N.) and provided with fresh sawdust (Lignocel select, Rettenmaier & Söhne GmbH, Germany) and two Kleenex® tissue sheets as nesting material. All mice were four months old at the time of testing.

Food (Kliba Nafag, Provimi Kliba AG, Switzerland) and water were provided *ad libitum* until three days before the onset of training, when mice were fed a reduced amount of food once a day (3–4 g of food per day/per cage) and weighed daily to ensure that their body weight was maintained between 85% and 90% of their body weight when fed *ad libitum*. After ten days of training they were put back on *ad libitum* feeding but the food was removed from the food hopper 2 h prior to the training session. We avoided more severe food deprivation because during pilot studies we noticed that this would reduce the difference in palatability between the two rewards used in the judgement bias test.

2.2. Judgement bias test apparatus

Two identical testing apparatuses were set up in a room adjacent to the housing room. These consisted of a box measuring 20 cm × 50 cm, containing a start box (10 cm × 10 cm) in the middle of one end and two compartments (10 cm × 20 cm each) on the other end, each containing a goal pot. During training, the floor of one compartment and the outside surface of the goal pot in that compartment were covered with either coarse (P40) or fine sandpaper (P1200), whereas the other compartment had a grid floor and a goal pot with no sandpaper. The side of the apparatus containing the sandpaper, as well as the coarseness of the sandpaper, changed pseudorandomly from trial to trial. Both compartments had sliding doors at the entrance (Fig. 1).

Before the training started, the mice were habituated to the food rewards in their home cage, once daily for seven days prior to the start of the training period. During habituation to food, each mouse received a high and low value food reward. Half of an almond flake (A) was used as a high value reward, and an oat flake (O) as a low value reward. During pilot studies, mice showed a strong preference for almond over oat flake, but ate both types of food (data not shown).

To habituate the mice to the apparatus, on day one, the mice were placed in the apparatus for 10 min of free exploration with both compartments open and three almond pieces and three oat flakes scattered across the central arena. On day two, the same procedure was repeated, with the goal pots but no sandpaper present in both compartments.

After initial habituation, the mice were trained to dig in the goal pots for rewards. Training started 1 h after the onset of the dark phase. The mice were transferred to the test room in their home cages, and both mice in a cage were trained at the same time, by two experimenters (J.N. and K.S.). The mice were assigned to experimenters randomly and each mouse was tested by the same experimenter in the same apparatus throughout the experiment. Half of the animals had a higher value reward associated with coarse sandpaper, the other half with smooth sandpaper, and the pairing of sandpaper grade with the higher value reward was counterbalanced per cage. Each mouse underwent one training session per day on alternate days. Sessions consisted of 16 trials per day; eight positive trials with almond as a reward and eight negative trials with an oat flake as a reward. In positive trials, the mouse had to choose between a compartment and goal pot covered in one grade of sandpaper (*e.g.* coarse) which contained half of an almond (correct choice) and a compartment and goal pot not covered in sandpaper which contained an oat flake (incorrect choice). In negative trials, the mouse had to choose between a compartment and goal pot not covered in sandpaper which contained an

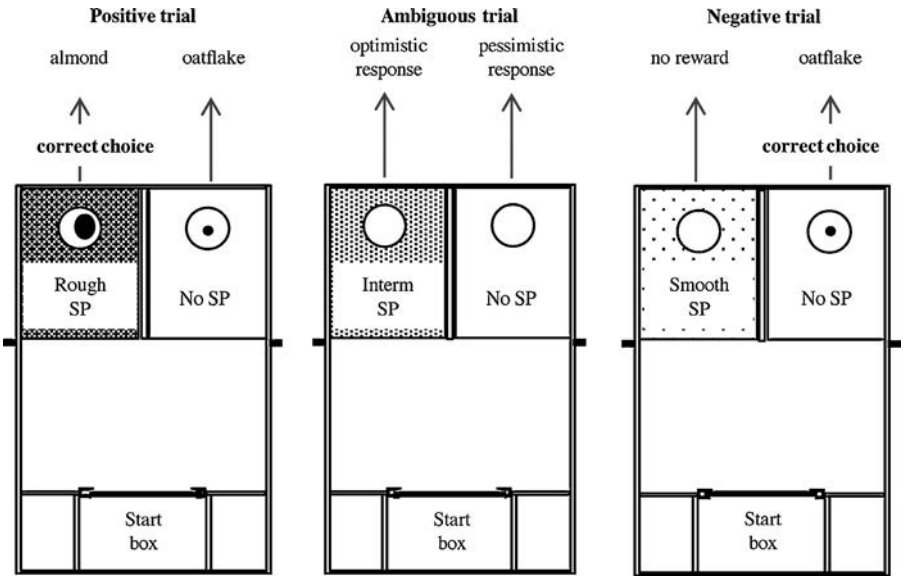

Fig. 1. Test apparatus and contingencies in the active choice judgement bias test. SP: sand paper.

oat flake (correct choice) and a compartment and an empty goal pot covered in the other grade of sandpaper (*e.g.* smooth; incorrect choice). Once the animal made a choice, the other compartment was closed. A choice was recorded whenever the mouse manipulated the digging material with its paws or nose. The sequence of trials (positive, negative) and the side of the sandpaper (left, right compartment) was changed daily, using a pseudorandom sequence so that no more than two consecutive presentations of the same cue would occur (*e.g.* OAOOAAOOAAOAOAA).

In the first training session (shaping phase), all trials were forced trials with only one compartment being open. Initially, rewards were placed on the surface of the digging material (sawdust) in the corresponding goal pot. Throughout the session, the reward was buried progressively deeper in the digging material until it was placed at the bottom of the goal pot.

Once the animals had learned to obtain the reward from the bottom of the goal pot, they entered the "free-choice" phase of training. The first two trials of each session were always forced trials (one positive and one negative), with only the correct compartment open. The remaining trials were free choice trials, where the mouse was left to explore both compartments and goal pots freely until it had made a choice.

A trial was finished when the mouse dug out the reward. The mouse was then immediately put back into the start box and the next trial started. If the animal failed to make a choice within 120 s, the trial was terminated and recorded as an omission trial. Type of choice (correct, incorrect) and latencies to make a choice were recorded with a stopwatch. Latency to make a choice was defined as the time between a mouse touching the floor of any of the two compartments and making a choice, and has been used as a reliable indicator of food preference in rodents (Brydges et al., 2012, 2011; Burman et al., 2009, 2008).

The animals were trained to a learning criterion of at least 10 correct choices (five positive and five negative) out of the 14 free-choice trials per session for two consecutive days (binomial probability $P < 0.05$). Once a mouse had reached this criterion, it received intermittent training (once weekly) until all animals had reached the learning criterion. Sandpaper was not changed between sessions with different mice. However, in order to avoid odour cues from repeated use, the sandpaper was changed every two to three days during training. This also ensured that mice were responding to sandpaper grade rather than thickness or any inconsistencies in the shape of sandpaper sheets. The apparatus was cleaned with 70% ethanol solution between each mouse. To avoid odour cues from the rewards, oat flakes and almonds were placed underneath a mesh inside the goal pots so that they were inaccessible to the mice. The reward was put on top of the mesh and the pots filled with clean sawdust bedding.

2.3. Unpredictable chronic mild stress (UCMS)

When all mice had reached the learning criterion, cages were randomly assigned to either control treatment (CON) or unpredictable chronic mild stress (UCMS) for three weeks. During this time, the UCMS mice were housed in a room adjacent to the test room, while the control mice remained in the housing room. The UCMS treatment was adapted from Mineur et al. (2006), and five different stressors were randomly applied during the dark phase throughout the three week period, with different durations in the following order:

- Week 1: Lights on for 11 h, soiled rat bedding in cage and left there for 3 h, tilted cages (45°) for 9 h, wet bedding (1.5 dcl of water in the cage) for 6 h, pulsing light (2 × 1 h), tilted cages for 7 h and lights on for 8 h.
- Week 2: Wet bedding for 4 h, cages put in housing room with rats for 1 h, pulsing light for 5 h, lights on for 10 h, wet bedding for 12 h, tilted cages for 5 h, pulsing light for 7 h.
- Week 3: Lights on for 11 h, soiled rat bedding in cage for 3 h, tilted cages for 9 h, wet bedding for 6 h, pulsing light (2 × 1 h), cages put in rat room for 1 h, lights on for 7 h.

During the three week treatment period, the mice were trained by partial reinforcement once per week, with the number of unreinforced trials gradually increasing. In week one, mice underwent 16 trials, two forced and 14 free choice trials with all 16 trials being rewarded. In week two, two free choice trials were unrewarded (one positive and one negative trial) and in week three, the number of unrewarded trials increased to four (two positive and two negative trials).

After the three week treatment period, all mice underwent additional training for two consecutive days on the reference cue discrimination, with partially reinforced trials, before being tested with ambiguous cues. Mice were then tested using three different

intermediate grades of sandpaper (P80, P120 and P180) replacing unrewarded trials. Following the same procedure as during training, the mice were presented with 15 trials per session: six positive, six negative, and the three intermediate unrewarded trials with each intermediate cue being presented once per session. Testing consisted of three sessions over three days. Experimenters were not blind to the treatment at the time of testing.

Choices made to sandpaper cues are presented as the proportion of optimistic choices. A choice was categorized as optimistic if the mouse chose to dig in the goal pot with the sandpaper. In positive trials this was the goal pot associated with the higher value reward and in negative trials this was the empty goal pot. Responses to intermediate cues were always unrewarded.

2.4. Saccharin preference and corticosterone metabolite measures

To assess the effectiveness of the UCMS, we measured saccharin preference on a weekly basis. Mice were habituated to saccharin by placing a bottle with 0.2% saccharin solution next to the water bottle in the food hopper of the home cage for three days. One week before the start of training, during training and during the UCMS, a bottle of 0.2% saccharin solution and a bottle of water were placed in the food hopper of the cage for the first 4 h of the dark period for four consecutive days every week. Positions of water and saccharin bottles changed daily, to control for potential side biases. Saccharin intake was measured by weighing water and saccharin bottles before and after the four day period each week, throughout the training and UCMS period. Saccharin preference was then calculated as proportion of saccharin consumed relative to all liquid consumed. Saccharin was chosen over sucrose, to avoid a caloric effect on the UCMS effects (Schweizer et al., 2009).

Faeces were sampled from each pair of cage mates once per week throughout the study by collecting all faecal boli in the cages 24 h after the cage changes. Samples were frozen at −20 °C until processed (for details see (Touma et al., 2003)).

2.5. Home cage behaviour

To assess stereotypy performance, home cage behaviour was recorded before and after treatment (UCMS or CON). Each recording period lasted for two consecutive days, starting when lights were off (09:00 am–09:00 pm) using infrared sensitive cameras. Activity levels and stereotypic behaviour were scored using one-zero sampling at 15 s intervals, for 2 h each day, spread across 4 h (the first half hour of each hour from 11 am to 3 pm). In total, 240 data points were analysed per mouse per hour, resulting in 960 data points per mouse before the stress treatment and 960 data points after the stress treatment. The following activities were recorded:

2.5.1. General activities

Active. All behaviour performed except inactive behaviour.

Inactive. Sitting or lying motionless throughout the recording interval; occasionally interrupted by brief single twitches lasting no longer than 5 seconds.

2.5.2. Stereotypic activities

Bar-mouthing. Hanging on the cage lid from all paws or fore paws only, or standing on the hind legs, while chewing at the bar for at least 3 seconds, with the bar held in the gap between the incisors and molars; may be performed on the spot or by moving along the bar while chewing.

Cage-top twirling. Spinning around while hanging on the cage lid from the fore paws; at least three repetitions.

Circling. Running around the cage in circles; at least three repetitions.

Patterned climbing. Climbing at the cage lid along fixed routes; at least three repetitions.

Cages were relabeled before the start of recording, so that the observer (J.N.) was blind to the treatment. Since UCMS mice were housed in a separate room, however, complete blinding was impossible for video observations after UCMS because the rooms in which animals were housed were different.

2.6. Ethical approval

This study was carried out in strict accordance with the regulations in the Swiss Animal Welfare Act (TSchG 455) and the Swiss Animal Welfare Ordinance (TSchV 455.1). It was approved by the Cantonal Veterinary Office in Bern, Switzerland (Permit Number: BE12/12).

3. Statistical analyses

All statistical analyses were performed with R (R Core Team 2013) and R Studio (2013). *P*-values below 0.05 were considered significant for all analyses and the function glmer in the R package lmer4 (Bates et al., 2014) with a logit link function was used to fit generalized linear mixed effects models to analyse choices made to ambiguous cues and linear mixed effects models (function lmer of the above mentioned package) to analyse all other measures. The assumptions of normally distributed residuals and homogeneity of variance were examined graphically with the use of QQ plots and Tukey–Anscombe plots. Corticosterone metabolites were square root transformed, choices made to reference cues, stereotypy and bar-mouthing levels were arcsine-squareroot transformed and latencies to make a choice were ln transformed to meet the test assumptions. Treatment, strain and cue were included in the models as fixed effects, test session was used as a repeated factor and cage was included as a random effect, with mouse nested within cage, to control for possible dependencies between cage mates. Where strain differences were detected, data were analysed for each strain separately. *P*-values in glmer models were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question. Data are presented as means with standard error. When analysing latencies to make a choice to ambiguous cues, we used mean latency in positive and negative trials for each mouse as an additional covariate. Activity is presented as proportion of active time relative to total observation time, and stereotypic behaviour as proportion of stereotypic behaviour occurring during active time. The relationship between optimistic choices and home cage behaviour was analysed using partial correlation, controlling for cage.

4. Results

4.1. Judgement bias test

Two CD-1 and four C57BL/6 mice did not reach the learning criterion, therefore they were not tested with ambiguous cues and were excluded from further analyses. Overall, CD-1 mice learned the test quicker and reached the learning criterion in 14.14 ± 1.19 sessions, while C57BL/6 mice needed 19.67 ± 1.29 sessions. When looking at the number of sessions needed to learn positive and negative cues, we found a strong interaction between strain and cue ($F_{1,51} = 15.75$, $P = 0.0003$; Fig. 2). While CD-1 mice needed a similar number of sessions to reach the criterion for both positive and negative trials, C57BL/6 learned positive trials much quicker than negative trials (Fig. 2).

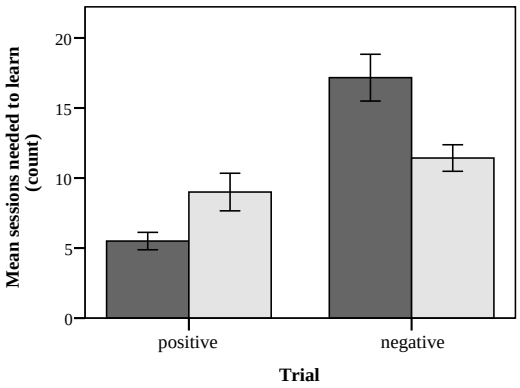

Fig. 2. Number of sessions needed to reach the criterion in positive and negative trials for C57BL/6 (dark grey) and CD-1 (light grey) mice. Data are presented as mean ± SEM.

4.1.1. Choices and latencies to reference cues

During testing, all mice made a choice in all trials and no omissions were recorded. Both strains clearly discriminated between the reference grades of sandpaper and preferred almond over oat flake ($F_{1,155} = 328.35$, $P < 0.001$), making more optimistic choices in positive trials than in negative trials (0.76 ± 0.04 compared to 0.18 ± 0.02). There was no significant effect of strain ($F_{1,155} = 1.32$, $P = 0.26$) on responses to reference cues. However, UCMS mice tended to show decreased responding in positive trials, when choosing almond over oat flake, while responses in negative trials were similar in both treatment groups (cue*treatment interaction, $F_{1,155} = 3.79$, $P = 0.053$; Fig. 3).

CD-1 mice were faster to make a choice to both reference cues than C57BL/6 mice ($F_{1,155} = 18.93$, $P = 0.001$), with a mean latency of 8.07 ± 2.60 s compared to 10.28 ± 1.78 s (Fig. 4). Moreover, UCMS mice were faster than CON mice in making a choice in negative trials, when choosing a less preferred reward, while latencies to make a choice in positive trials were similar between treatments (cue*treatment interaction, $F_{1,155} = 6.21$, $P = 0.005$).

4.1.2. Choices and latencies to ambiguous cues

While both strains made similar proportions of optimistic responses to ambiguous cues (χ^2 (2) = 1.68, $P = 0.26$), there was a significant interaction between cue and treatment (cue*treatment interaction, χ^2 (2) = 4.61, $P = 0.03$; $M_{diff} = -1.1 \pm 0.37$; Fig. 3). CON mice discriminated between ambiguous cues and displayed a graded response (χ^2 (2) = 8.84, $P = 0.003$), making most optimistic choices to the near positive cue and least optimistic choices to the near negative cue. By contrast, UCMS mice responded to all three ambiguous cues similarly (χ^2 (2) = 3.04, $P = 0.67$; Fig. 3). When looking at responses to each cue separately, we found no treatment differences in choices made to any of the ambiguous cues (χ^2 (1) = 3.59, $P = 0.16$; χ^2 (1) = 0.01, $P = 0.99$; χ^2 (1) = 1.82, $P = 0.20$ for near positive, middle and near negative ambiguous cue; using the Bonferroni correction for three comparisons, the P value has to be below 0.017 for an effect to be significant at the 0.05 level.).

Similar to choices made to reference cues, CD-1 mice made choices to ambiguous cues faster than C57BL/6 mice, as indicated by shorter latencies ($F_{1,233} = 25.91$, $P = 0.0003$; 8.09 ± 2.88 s compared to 13.51 ± 1.56 s). Latency to make a choice was also affected by treatment, with UCMS mice being faster to respond compared to CON mice ($F_{1,233} = 4.22$, $P = 0.04$; 7.21 ± 1.07 s compared to 13.97 ± 3.22 s; Fig. 4). When looking at each ambiguous cue separately, we found no treatment effects ($F_{1,77} = 3.88$, $P = 0.04$; $F_{1,77} = 2.65$, $P = 0.09$ and $F_{1,77} = 0.28$, $P = 0.71$ for near positive, middle and near negative cue; using the Bonferroni correction for three comparisons, the P value has to be below 0.017 for an effect to be significant at the 0.05 level.).

4.2. Saccharin preference and corticosterone metabolites

Initial saccharin preference was higher in C57BL/6 compared to CD-1 mice (Mann–Whitney, $U(14) = 9$, $P = 0.001$). Therefore, to further analyse the effect of UCMS on saccharin preference, we analysed each strain separately. There was no treatment*phase effect in either strain ($F_{2,22} = 1.07$, $P = 0.53$ for CD-1 and $F_{2,16} = 1.80$, $P = 0.83$ for C57BL/6 mice). Saccharin preference decreased over time in both strains ($F_{2,22} = 23.98$, $P = 0.004$ for CD-1 and $F_{2,16} = 267.38$, $P = 0.001$ for C57BL/6; Fig. 5a).

While basal levels of corticosterone metabolites were similar in both strains and both treatment groups, they increased over time in C57BL/6 mice (strain*phase interaction; $F_{2,34} = 6.35$, $P = 0.01$). When looking at levels for each strain separately, we found no interaction between treatment*phase ($F_{2,22} = 1.92$, $P = 0.53$ for CD-1 and $F_{2,16} = 1.51$, $P = 0.25$ for C57BL/6). However, concentrations of corticosterone metabolites increased over time for both treatment groups in C57BL/6 mice ($F_{2,16} = 4.11$, $P = 0.001$). In contrast, there was no change in time in corticosterone metabolite concentrations in CD-1 mice ($F_{2,22} = 1.76$, $P = 0.06$; Fig. 5b).

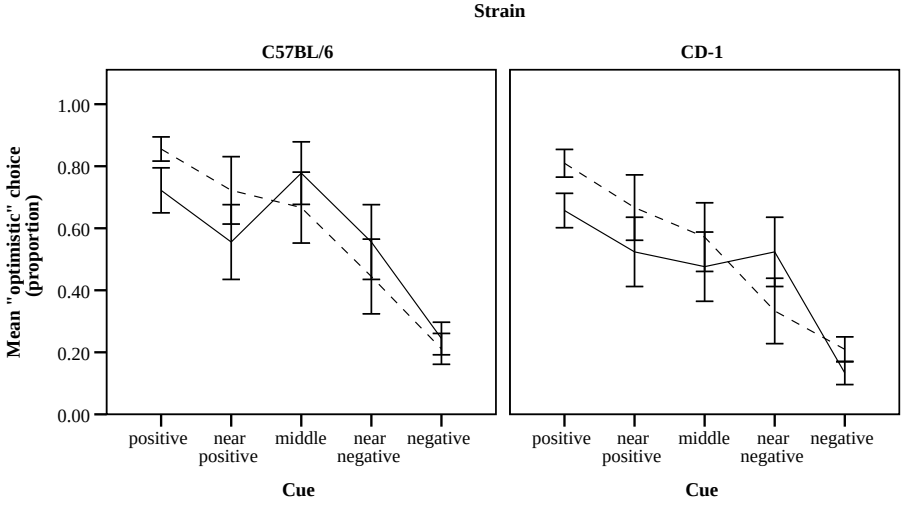

Fig. 3. Proportion of optimistic choices made to positive and negative reference cues and three intermediate ambiguous cues in CON (dashed line) and UCMS mice (solid line). Data are presented as mean ± SEM.

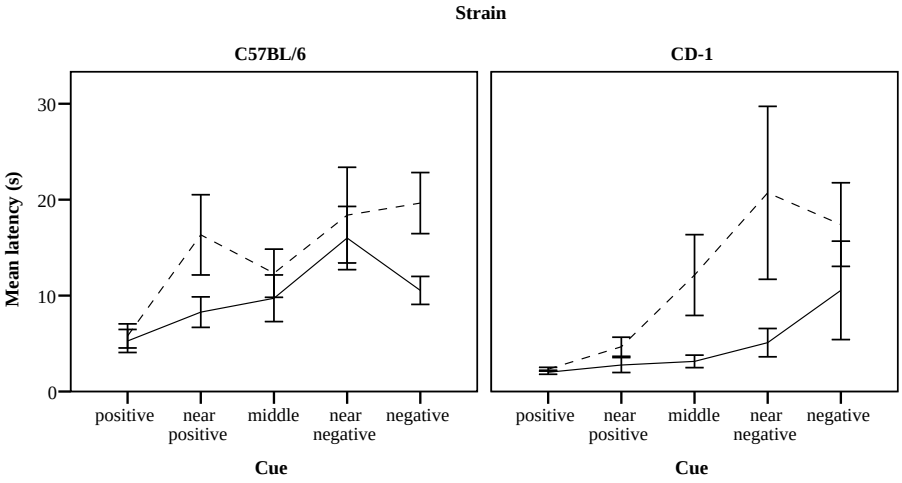

Fig. 4. Latency to make a choice to positive and negative reference cues and three intermediate ambiguous cues in CON (dashed line) and UCMS mice (solid line). Data are presented as mean ± SEM.

4.3. Home cage activity and stereotypic behaviour

Both strains displayed similar home cage activity levels ($F_{1,25} = 3.20$, $P = 0.09$; 39.21 ± 5.74% in CD-1 compared to 54.98 ± 6.56% of observed time in C57BL/6) and these remained unchanged after UCMS ($F_{1,25} = 1.60$, $P = 0.22$). Twelve CD-1 mice performed stereotypic bar-mouthing (14.19 ± 2.82% of all active time), with one mouse performing cage-top twirling (5.11%) and bar-mouthing (11.53%) combined and one mouse performing circling (62.86%). All C57BL/6 mice performed both bar-mouthing (8.35 ± 3.15%) and patterned climbing (7.09 ± 3.03%). Levels of total stereotypy were similar in both strains ($F_{1,25} = 0.29$, $P = 0.49$; 19.04 ± 4.14% in CD-1 compared to 15.44 ± 4.87% of active time in C57BL/6), and did not change after the UCMS treatment phase ($F_{1,25} = 0.56$, $P = 0.74$; Fig. 6a).

The most prevalent type of stereotypy was bar-mouthing, which was performed at higher levels in CD-1 mice compared to C57BL/6 mice ($F_{1,25} = 5.48$, $P = 0.03$; Fig. 6b). We therefore looked at the effect of UCMS on bar-mouthing for each strain separately. There was an interaction between treatment and phase in CD-1 mice ($F_{1,13} = 3.20$, $P = 0.03$), indicating that bar-mouthing increased in UCMS mice, but decreased in CON mice, while UCMS had no effect on bar-mouthing in C57BL/6 mice ($F_{1,11} = 0.14$, $P = 0.71$).

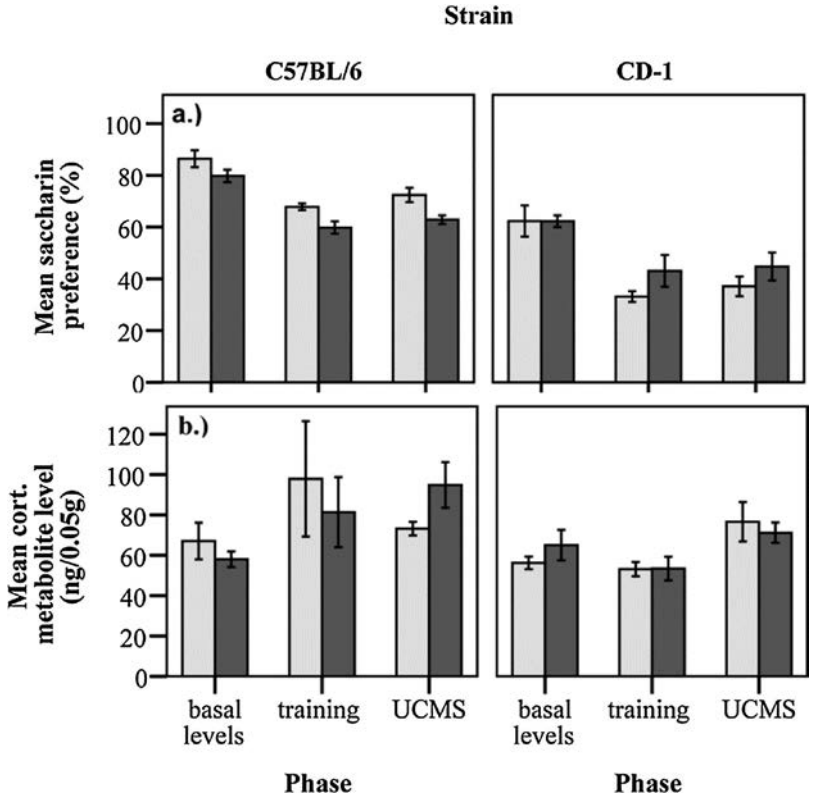

Fig. 5. Saccharin levels (a) and corticosterone metabolite levels (b) in C57BL/6 and CD-1 mice. Data for CON (light grey) and UCMS mice (dark grey) are presented as mean ± SEM.

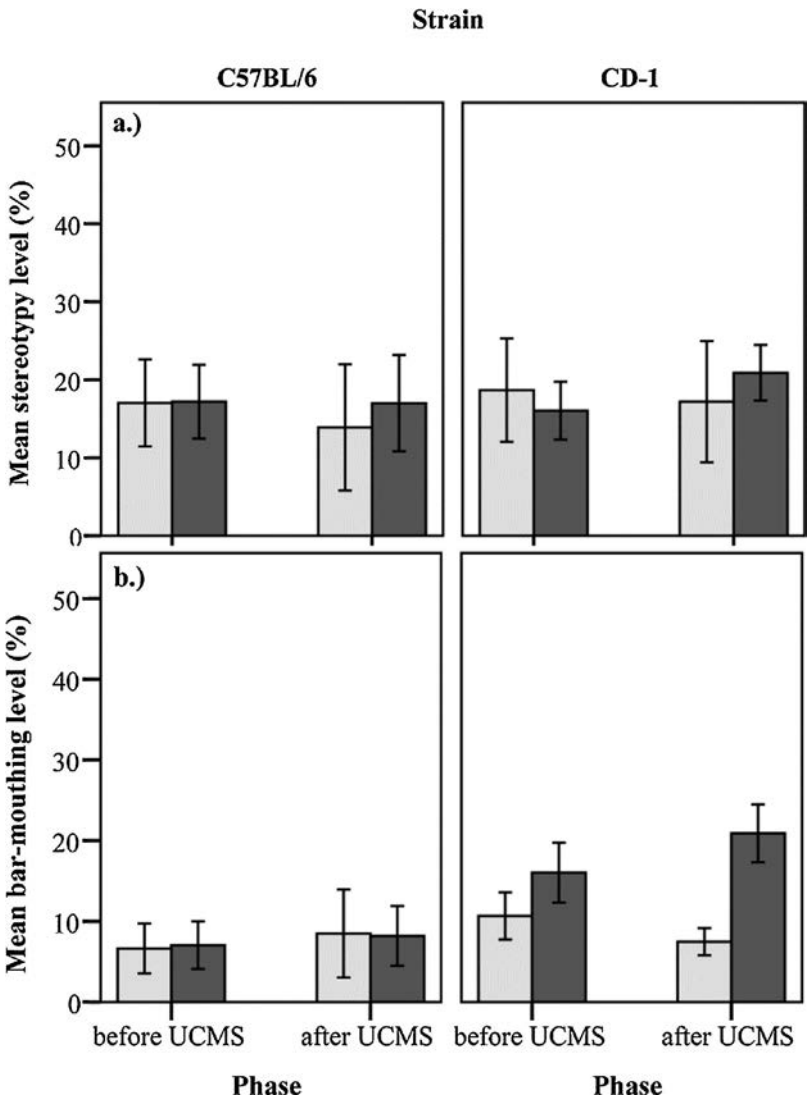

Fig. 6. Total stereotypy levels (a) and bar-mouthing levels (b) in C57BL/6 and CD-1 mice. Data for CON (light grey) and UCMS mice (dark grey) are presented as mean ± SEM.

Levels of activity, total stereotypy and bar-mouthing before and after the treatment phase were positively correlated ($r = 0.58$, $P = 0.01$, $df = 23$; $r = 0.64$, $P = 0.001$, $df = 23$; $r = 0.65$, $P = 0.001$, $df = 23$, respectively; partial correlations controlled for cage).

4.4. Relationship between stereotypy and optimistic choices in judgement bias test

Neither total stereotypy nor bar-mouthing levels predicted the number of sessions needed to reach the learning criterion during training of the judgement bias test ($r = -0.31$, $P = 0.19$, $df = 23$ and $r = -0.04$, $P = 0.78$, $df = 23$, respectively). For the analysis of the relationship between stereotypy performance and judgement bias, we combined responses (choices and latencies) to the three ambiguous cues. Mice with higher levels of total stereotypy made more optimistic choices to ambiguous cues ($r = 0.43$, $P = 0.049$, $df = 23$, Fig. 7), however the level of stereotypic behaviour was not correlated to latency to make a choice to ambiguous cues ($r = -0.37$, $P = 0.16$, $df = 23$). A similar correlation, although not significant, between stereotypy level and optimistic choices was found when looking at each treatment group separately (UCMS: $r = 0.43$, $P = 0.19$, $df = 9$ and CON: $r = 0.43$, $P = 0.11$, $df = 11$).

When looking at bar-mouthing only, we found no correlation between the level of bar-mouthing and optimistic choices

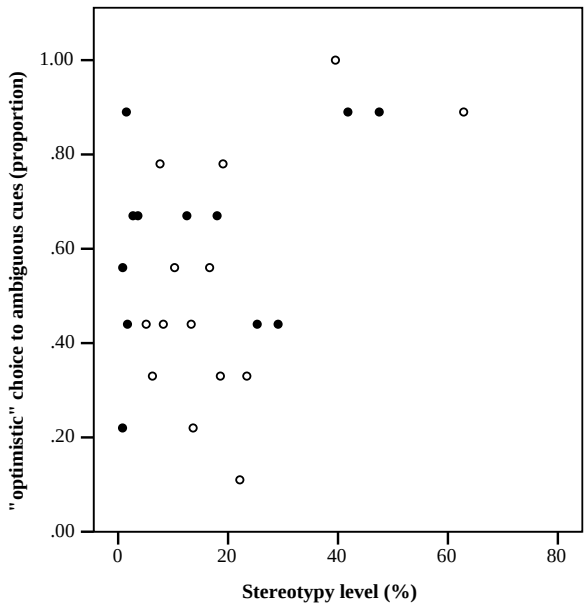

Fig. 7. Correlation between stereotypy level and optimistic choices made to ambiguous cues in C57BL/6 (black) and CD-1 mice (white).

to ambiguous cues ($r = 0.02$, $P = 0.97$, $df = 23$). Since bar-mouthing was affected by UCMS in CD-1, but not C57BL/6, mice, we further analysed if the difference in bar-mouthing before and after the treatment phase was associated with the proportion of optimistic choices to ambiguous cues, but did not find a significant correlation ($r = 0.20$, $P = 0.52$, $df = 11$). Similarly, there was no significant correlation between the change in bar-mouthing and the latency to make a choice to ambiguous cues ($r = -0.40$, $P = 0.20$, $df = 11$).

5. Discussion

The main aim of this study was to examine the relationship between the level of stereotypic behaviour and affective state in mice. To this end, we developed a novel active choice judgement bias test based on naturalistic foraging behaviour and discrimination of tactile stimuli, and used unpredictable chronic mild stress (UCMS) to assess the validity of this test.

5.1. Behavioural and physiological effects of UCMS

UCMS is commonly used to model depression in animals (Willner, 1997), and in rodents it triggers a number of neuroendocrine and behavioural changes (Cryan and Mombereau, 2004; Willner et al., 1987). These include enhanced hypothalamic–pituitary–adrenal axis (HPA) activity and increased anxiety, as well as anhedonia as measured by a reduced preference for saccharin solution (Pothion et al., 2004; Schmidt et al., 2010; Willner et al., 1987). Both strains showed an initial saccharin preference, and consistent with previous studies, preference was higher in C57BL/6 mice (Pothion et al., 2004). However, saccharin preference decreased gradually across training and test sessions in both strains and both treatment groups. It is possible that sampling of saccharin consumption during four days every week resulted in gradual habituation to the solution. Indeed, we found some evidence in the literature that saccharin can lose its hedonic value, and the difference between treatment groups may disappear after several weeks of UCMS (Pothion et al., 2004). Basal corticosterone metabolite levels were similar in both strains and consistent with other studies (Touma et al., 2004, 2003). However, we also found no significant effect of UCMS on this measure of HPA-activity. Effectiveness of, and sensitivity to UCMS is known to vary among strains, facilities and studies, with differences in the magnitude and persistence of the effect (Willner, 1997). However, the lack of significant effects on anhedonia and HPA-activity does not necessarily mean that UCMS had no effect of the animals' affective state and several other studies also failed to find evidence for anhedonia despite UCMS inducing a negative judgement bias (Harding et al., 2004; Parker et al., 2014). A possible explanation for the lack of effect is that the method used may not have been appropriate, or sensitive enough, to detect the specific changes. In rodents, the preference for sweet substances (such as sucrose or saccharine) is the standard method to characterise anhedonia (Pothion et al., 2004; Willner et al., 1987). However, due to its variability between different strains and the unreliability of the procedure among laboratories (D'Aquila et al., 1994; Forbes et al., 1996), its interpretation as a reliable measure of anhedonia has been questioned (Reid et al., 1997). It is also possible that UCMS animals did not develop a decrease in reward responsiveness. Anhedonia is one of the many symptoms of depressive disorders (Buckner et al., 2008) and its absence does not necessarily indicate that the animals were not experiencing negative affective states.

5.2. Judgement bias test

Both CD-1 and C57BL/6 mice learned to discriminate the two reference grades of sandpaper and exhibited a similar preference for almond over oat flake, judging from consistent correct responses in positive trials. The preference for almond was further confirmed by their shorter latencies to make a choice in positive trials, where correct responses were rewarded by an almond compared to negative trials where correct responses were rewarded by an oat flake. There was a strain difference in the acquisition of the test, as C57BL/6 mice needed more training sessions to reach the learning criterion than CD-1 mice. This difference was mainly due to a higher number of sessions needed by C57BL/6 mice to inhibit digging in the unbaited goal pot and reach criterion in negative trials. Strain differences in learning are common in mice, and C57BL/6 mice are known to be relatively slow in discrimination learning compared to other strains (Colacicco et al., 2002; Izquierdo et al., 2006). However, they were similarly quick as CD-1 mice in learning the positive trials, indicating that their learning ability was not generally impaired. C57BL/6 mice generally show poorer attentional set shifting compared to other strains (Colacicco et al., 2002; Onori et al., 2014), and it is possible that they made a strong initial association between the presence of sandpaper and the higher value reward while paying less attention to the grade of sandpaper, which could explain the delayed learning of negative trials in the present study. Alternatively, the difference in discrimination learning may be explained by differences in food restriction (Forestell et al., 2001), with a more severe restriction resulting in faster discrimination learning (Makowiecki et al., 2012). In the present study, the mice were mildly food deprived during training, and both strains showed a similar decrease in body weight (down to 95% of body weight compared to *ad libitum* feeding). Despite a similar weight loss, however, the mice of the two strains may have differed in their motivation for food, which is supported by the shorter choice latencies in CD-1 mice.

Taken together, our data indicate that training on this active choice test based on differential food rewards produced graded responses across the three ambiguous cues. The closer the ambiguous cue was to the positive cue, the more optimistic responses the mice made. There are only a few studies in rodents that found such a clear and almost linear relationship across variation of ambiguity (Enkel et al., 2010; Papciak et al., 2013; Rygula et al., 2014, 2012). However, these studies included aversive outcomes, which may be easier for the animals to learn, but may also result in a higher risk of negative baseline bias (Hales et al., 2014). Tests based on differential positive reinforcers may therefore provide more neutral responses to ambiguity, thereby facilitating the detection of subtle differences in affective state (reviewed by Hales et al. (2014)).

5.3. Effect of UCMS on judgement bias

Similar to findings in rats by Harding et al. (2004), UCMS mice tended to show decreased responses for the higher value reward in positive trials. This is an interesting result, as responses to the unambiguously reinforced cues should remain unaffected by UCMS. One possible explanation is that the UCMS treatment may have resulted in reduced accuracy of decision making or decreased feeding motivation. Stress is known to increase the speed of responding in clinical studies (Keinan, 1987) and to influence decision making in depressed individuals (Cella et al., 2010). Furthermore, UCMS has been shown to impair decision making in mice, by decreasing choice evaluation and increasing response speed (Pardon et al., 2000), which may result in reduced accuracy in responding (Mendl, 1999). However, we found no evidence of reduced accuracy in choices made to the previously learned negative reference cues. Thus, UCMS mice did not make more errors than CON mice in negative trials, indicating that the reduced proportion of optimistic responses in positive trials may not just be a result of impaired decision making. We also found no evidence for decrease in overall food intake in UCMS mice, as incorrect choices in positive

trials resulted in food (oat flake) consumption as well. An alternative explanation for decreased responding of UCMS mice may be a reduced rewarding value of the more palatable food used in positive trials, which could be indicative of anhedonia. Although we failed to find a treatment effect in the saccharin preference test, the standard measure of anhedonia (see Section 5.1), reduced preference for a more palatable food reward has been shown to effectively discriminate anhedonic behaviour in stressed rats (Mateus-Pinheiro et al., 2014). Therefore, decreased responding in positive trials by the UCMS mice may indicate a possible sign of anhedonia.

Furthermore, we found a difference in responses to the ambiguous cues between the two treatments, but interpretation of this effect is not straightforward. While CON mice showed the predicted graded response across the three ambiguous cues, UCMS mice of both strains did not seem to discriminate between them. Thus, UCMS did affect responding to ambiguous cues but it is unclear whether this was due to judgement bias or some other effect on decision making.

The difference in choices was associated with shorter response latencies in UCMS mice compared to CON mice and this is consistent with results from other studies using reward based judgement bias tests. For example, rats stressed as juveniles (Brydges et al., 2012) and rats undergoing UCMS (Parker et al., 2014) displayed shorter choice latencies compared to control animals. By contrast, Harding et al. (2004) found longer latencies to the positive reference cue and the near positive ambiguous cue in UCMS rats. However, this difference may be due to the different test paradigm. Harding et al. (2004) had used a go/no-go task with an aversive burst of white noise to condition the no-go response in the negative trials, which may have rendered the UCMS rats more hesitant when making a response to ambiguous cues.

Taken together, the fact that the same pattern of responses to ambiguous cues was observed across both mouse strains strongly suggests that both choices and latencies of responding were sensitive to variation in affective state induced by UCMS. However, it remains unclear whether these changes reflect judgement bias or some more specific stress-related change in decision making.

5.4. Home cage activity and stereotypic behaviour

Activity and stereotypy levels were similar to those normally reported for mice raised under conventional housing conditions (Engel et al., 2011 Gross et al., 2011a,b, 2012; Tilly et al., 2010). Overall levels of activity and stereotypy were not significantly affected by UMCS; however, levels before and after the treatment phase were positively correlated, indicating stable individual differences. The most prevalent stereotypy performed was bar-mouthing, which in CD-1 mice increased in UCMS mice and slightly decreased in CON mice after the treatment phase, but remained unchanged in the C57BL/6 strain. Bar-mouthing in CD-1 mice has been shown to originate from the attempt to escape the cage (Nevison et al., 1999; Würbel et al., 1996), while the origins of other mouse stereotypies (back-flipping, cage-top twirling, patterned climbing) are still unclear. Stressors used in the UCMS may have increased their motivation to escape the cage, resulting in higher levels of bar-mouthing. Similarly, removal of enrichment was found to increase stereotypy levels (mostly bar-mouthing) in CD-1 mice (Latham and Mason, 2010), indicating that performance of bar-mouthing may be highly context specific and different from other types of stereotypic behaviour. Furthermore, UCMS affected bar-mouthing levels only in CD-1 but not C57BL/6 mice. Bar-mouthing is the most common stereotypy in laboratory mice (Garner et al., 2011; Gross et al., 2011a,b; Tilly et al., 2010; Würbel et al., 1996). However, the frequency and bout length of bar-mouthing appears to differ between the two strains of mice, as it is performed at higher levels and for longer bouts in CD-1 mice (Gross et al., 2011a,b; Würbel et al., 1996) compared to C57BL/6 mice (Tilly et al., 2010). Our data therefore suggest that despite a similar behavioural pattern, bar-mouthing in different strains may actually be related to different motivational factors.

5.5. Relationship between stereotypic behaviour and judgement bias

It is generally assumed that higher levels of stereotypy reflect a more negative affective state. However, Mason and Latham (2004) showed that within the same environment, stereotypic behaviour sometimes correlates with signs of better welfare. In line with this, we found across both strains that mice performing higher levels of total stereotypy made more optimistic choices to ambiguous cues. While this may suggest that they were in a more positive affective state, the association is relatively weak and dependent on few data and should be interpreted with caution. Stereotyping animals also have impaired decision making and can be more impulsive (Garner and Mason, 2002; Garner et al., 2011); however, we found no evidence supporting this, as we found no effect of stereotypy level on discrimination learning or latencies to make a choice to ambiguous cues. Similar results were found in grizzly bears, where pacing correlated positively with optimistic choices, which was linked to anticipation of food (Keen et al., 2013). On the other hand, the level of head twirling, but not pacing, in monkeys (Pomerantz et al., 2012) and back-flipping in starlings (Brilot et al., 2010) were associated with negative judgement bias. Moreover, while we found a positive correlation between total stereotypy and optimistic choices, no such relationship was found for bar-mouthing, the most prevalent type of stereotypy among these mice. These conflicting findings suggest that the implications of stereotypic behaviour for animal welfare may vary greatly depending on the specific type of stereotypy. Further studies investigating specific types of stereotypy and how they relate to variation in affective state and careful validation of measures of affective state are therefore needed before we can draw any firm conclusions.

6. Conclusions

In conclusion, the active choice test of judgement bias used here produced consistent changes in responses to ambiguous cues in outbred CD-1 mice as well as inbred C57BL/6 mice. This test protocol may thus have great potential for measuring judgement bias in mice. Although UCMS did not significantly affect saccharin preference and faecal corticosterone metabolites, it affected choices made to both reference and ambiguous cues. Thus, UCMS mice of both strains exhibited a decreased preference for the higher value reward, which may indicate anhedonia. However, their lack of discrimination between the three ambiguous cues and the shorter choice latencies indicate that UCMS induced changes in decision making that cannot be fully explained by variation in judgement bias. Although this test clearly needs further validation, it represents the first test based on differential food rewards which may effectively measure judgement bias in mice. Our data also indicate that bar-mouthing increased during UCMS in CD-1, but not in C57BL/6 mice. Furthermore, mice with higher levels of stereotypic behaviour made more optimistic choices in the judgement bias test. However, no such relationship was found for bar-mouthing, underscoring the possibility that different types of stereotypy and stereotypies in different strains may have different underlying mechanisms.

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