Once an Optimist, Always an Optimist? Studying Cognitive Judgment Bias in Mice

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Abstract

Individuals differ in the way they perceive the world. From human psychological research, it is known that these differences become particularly evident in ambiguous situations: while some individuals interpret ambiguous information pessimistically, others bias their interpretations in a more optimistic way, referred to as cognitive judgement bias (CJB). CJBs have also been studied in non-human animals as tools for the assessment of affective states. However, the ecological and evolutionary relevance of CJB has so far been overlooked. We here aimed to transfer the concept of CJB to behavioural ecology. More specifically, we investigated the causes of differences in CJB in mice, focusing on both genetic and environmental factors. Furthermore, we assessed whether individual differences in CJB are repeatable over time, addressing the question whether "optimistic" and "pessimistic" decision styles, respectively, may represent stable traits. Thus, two strains of mice (C57BL/6J and B6D2F1N) were housed in two different environmental conditions: "scarce" or "complex". While mice living in the "scarce environment" experienced standard housing conditions, those living in the "complex environment" had regular access to a super-enriched "playground". To calculate the repeatability of "optimistic" and "pessimistic" decision styles, we assessed CJB four times across the course of seven weeks. Moreover, we assessed anxiety-like behaviour to detect potential differences in the effects of genetic or environmental factors on CJB and anxiety. While the selected genotypes and environments influenced some aspects of anxiety-like behaviour, no influence on CJB could be detected, indicating that CJB and anxiety might represent distinct systems. Remarkably, CJB was moderately repeatable, suggesting that decision-making under ambiguity constitutes a relatively stable trait and might even be considered an aspect of animal personality.

Keywords

Genotype-environment interaction, behavioural repeatability, animal personality, decision-making under ambiguity, cognitive judgement bias, anxiety, spatial learning

Introduction

Individuals differ in the way they perceive the world. From human psychological research, it is known that these differences become particularly evident in ambiguous situations, in which

individuals have to decide between different options. Symbolic for such situations is the often-quoted question: “Is the glass half-full or half-empty?” Whereas some individuals would say the glass is half-full, others would describe it as half-empty. Thus, some individuals (i.e. ‘pessimists’) interpret ambiguous information negatively, while others (i.e. ‘optimists’) bias their interpretations in a more positive way, referred to as cognitive judgement bias (CJB) in the scientific literature (e.g. Mathews and MacLeod, 2005). This framework has been transferred from psychology to animal welfare science in 2004 with the aim of using CJB as an indicator of emotional background state in non-human animals (henceforth: animals; Paul et al., 2005). In a seminal study, Harding and colleagues developed a paradigm to detect CJB in rats (Harding et al., 2004). More precisely, the authors assessed whether rats behaved as expecting either a positive or a negative outcome in an ambiguous situation. In a first step, rats learned to press a lever for a food reward when a high tone was played (“go” response), and to refrain from pressing the lever to avoid a punishment when a low tone was played (“no-go” response). Next, to create an ambiguous situation, an intermediate tone was played, and the rats had to decide whether to go and press the lever (“optimistic” decision) or to refrain from pressing it (“pessimistic” decision). Since its introduction, the paradigm has revolutionised animal welfare science: It enables scientists to distinguish between “optimistic” and “pessimistic” individuals in a variety of different animal species (e.g. Matheson et al., 2008; Brydges et al., 2011; Douglas et al., 2012; Richter et al., 2012; Destrez et al., 2014; Bethell and Koyama, 2015; Löckener et al., 2016; Lalot et al., 2017).

While CJB assessment has become a key technique in animal welfare research, the ecological and evolutionary relevance of CJBs has largely been overlooked so far (but see Bateson, 2016). Under natural conditions, however, animals are confronted daily with plenty of different decisions: they need to choose when to retreat during contests with conspecifics, and whether to continue foraging under predation risk. When in such contexts decisions are made in the face of ambiguity, their outcomes are crucially related to survival and fitness. From an ecological perspective, “optimistic” and “pessimistic” decision styles may therefore represent adaptive strategies, conferring fitness advantages depending on the ecological context. Thus, it would be of major interest to transfer the concept of decision-making under ambiguity from animal welfare science to behavioural ecology.

To achieve a comprehensive understanding of the ecological relevance of CJB, it is important to shed light on the causes underlying optimistic and pessimistic decision-making. So far, studies using the CJB paradigm point towards the effects of both environmental and genetic factors (reviewed in Lagisz et al., 2020; Neville et al., 2020). In particular, several environmental manipulations, such as the provision of enrichment (e.g. Matheson et al., 2008; Brydges et al., 2011; Bethell and Koyama, 2015) or different social experiences (e.g. Bučková et al; Papciak et al., 2013; Daros et al., 2014), have been shown to induce shifts in CJB. Likewise, there are indications from studies on mouse and rat strains that genetic effects influence CJB, although existing evidence is not yet fully convincing (e.g. Enkel et al., 2010; Kloke et al., 2014; Novak et al., 2016; Hintze et al., 2018; Sorato et al., 2018). Nearly all of these studies, however, concentrate on single modulating factors, thereby not considering more complex interactions between genotype and the environment. A comprehensive understanding of the mechanisms driving “optimistic” and “pessimistic” decision-making is thus still missing.

Besides the thorough understanding of the proximate mechanisms underlying differences in behaviour, another central topic has gained increasing attention in behavioural ecology over the recent years: the stability of individual differences in behaviour over time and/or across different contexts, widely referred to as “animal personality” (Dall et al., 2004; Réale et al., 2007). Intriguingly, it has been recognized that in many animal species, individuals exhibit repeatable behavioural differences independent of features such as sex, age, or size (e.g. Dall et al., 2004; Sih et al., 2004; Réale et al., 2007; Stamps and Groothuis, 2010; Dall et al., 2012; Kaiser and Müller, 2021). For example, individuals may differ considerably in their exploratory tendencies, with some individuals being bolder as well as more risk-seeking, and others being less bold and more risk-averse (Groothuis and Carere, 2005; Dammhahn and Almeling, 2012). In light of such findings, the question arises whether decision-making under ambiguity may likewise represent a stable trait. To date, only a few studies addressed this question (Clegg et al., 2017; Lecorps et al., 2018a; Verjat et al., 2021), with just one study systematically investigating longer-term temporal stability of between-individual differences in CJB (Lecorps et al., 2018a, but for a different approach see Rygula et al., 2013).

The aims of the present study were twofold: First, we systematically investigated the influence of the environment and the genetic background on CJB in laboratory mice. Therefore, mice of

two different strains (C57BL6/J and B6D2F1N) were housed in two different environmental conditions ("scarce" and "complex"). We assessed the animals' CJB using a touchscreen-based paradigm, a method featured by a set of automation-related advantages (Krakenberg et al., 2019b). Second, we investigated whether CJB can be considered a stable trait. Therefore, we measured CJB four times across the course of seven weeks and calculated the repeatability as a measure of temporal stability. Based on the literature summarized above, we hypothesized differences in CJB to be driven by both genotype as well as the environment, and CJB to be repeatable across the course of several weeks. Additionally, anxiety-like behaviour and spatial learning were assessed in a battery of standardized tests to detect potential differences between genetic and/or environmental effects on CJB, anxiety and spatial learning.

Animals, Materials, and Methods

Animals and Housing Conditions

We used 36 female C57BL/6J and 35 female B6D2F1N mice purchased from a professional breeder (Charles River Laboratories, Research Models and Services, Germany GmbH, Sulzfeld, Germany) at the age of four weeks. Mice were housed in same-strain groups of three individuals per cage (Makrolon cages type III, 38 × 23 × 15 cm^3). Cages were equipped with wood shavings as bedding material (Allspan, Höveler GmbH & Co. KG, Langenfeld, Germany), a paper towel, a wooden stick, a semi-transparent red plastic shelter (11.1 × 11.1 × 5.5 cm^3, Tecniplast Deutschland GmbH, Hohenpeißenberg, Germany) and a semi-transparent red handling tunnel (length: 98.55 mm, diameter: 50.8 mm, ZOONLAB GmbH, Castrop-Rauxel, Germany). Housing rooms were kept at a reversed light/dark cycle of 12:12 h with lights off at 8.00 a.m., a temperature of approximately 23°C and relative humidity of about 50%. Water and food (Altromin 1314; Altromin Spezialfutter GmbH & Co. KG, Lage, Germany) were provided *ad libitum* until the beginning of the experimental phase. During the experimental phase, a restrictive feeding regime was provided, i.e. animals received food once per day to maintain 90-95% of their *ad libitum* feeding weights. Body weights of the mice were monitored daily using a digital scale (resolution: 0.1 g; KERN CM 150-1N pocket balance, KERN&Sohn GmbH, Balingen, Germany). This food restriction schedule aimed to enhance their motivation to work for food rewards, without inducing any known negative impact on welfare (Feige-Diller et al., 2020). We used tunnel handling (i.e. gently guiding the mice into the

handling tunnel and transferring them to the target location within the tunnel), a method suggested to reduce stress compared to tail handling (Gouveia and Hurst, 2017).

Ethical Statement

All procedures complied with the regulations covering animal experimentation within Germany (Animal Welfare Act) and the EU (European Communities Council DIRECTIVE 2010/63/EU) and were approved by the local (Amt für Gesundheit, Veterinär- und Lebensmittelangelegenheiten, Münster, Nordrhein-Westfalen, reference number: 39.32.7.1) and federal authorities (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen "LANUV NRW").

Experimental Design

We investigated the influence of genotype and environment on cognitive judgment bias, anxiety-like behaviour, and spatial learning by housing mice of two strains in two environmental conditions: a "scarce environment" and a "complex environment". Half of the mice per strain were pseudo-randomly assigned to the "scarce environment". These mice were housed as described above during the whole experimental phase. The other half of the mice were assigned to the "complex environment". These animals were also housed as described above but had once per day access to a super-enriched environment, the "playgrounds", consisting of varying social and structural elements (for details see section "Complex Environmental Condition"). Thus, four different treatment groups were created (Figure 1): "scarce environment" C57BL/6J (scarce-C57), "scarce environment" B6D2F1N (scarce-F1), "complex environment" C57BL/6J (complex-C57), and "complex environment" B6D2F1N mice (complex-F1).

The experiment consisted of four different phases: a touchscreen training phase, first cognitive judgement bias (CJB) test phase, repeated CJB testing phase, and behavioural test phase. Three mice housed in the same cage belonged to the same treatment group, but participated in different phases of the experiment, creating a split plot design with different sample sizes for each phase (for design details and visualisation see Figure 1). The touchscreen training phase started at the age of ten weeks. Mice participating in this phase underwent daily training sessions to learn the discrimination task required for CJB testing. Once trained mice succeeded in learning the discrimination task, they entered the CJB testing phase, at the age of 26±7 weeks, to determine the influence of genotype and environment on CJB. After the

first CJB test, one group of the mice underwent repeated CJB testing to estimate the repeatability of individual differences in CJB. Subsequently, the repeatedly tested mice, together with the non-trained mice, were tested in a behavioural test battery to investigate the influence of genotype and environment on anxiety-like behaviour and spatial learning. The behavioural test battery included an elevated plus maze (EPM), an open field test (OFT), a free exploration test (FET), and a labyrinth maze (LM).

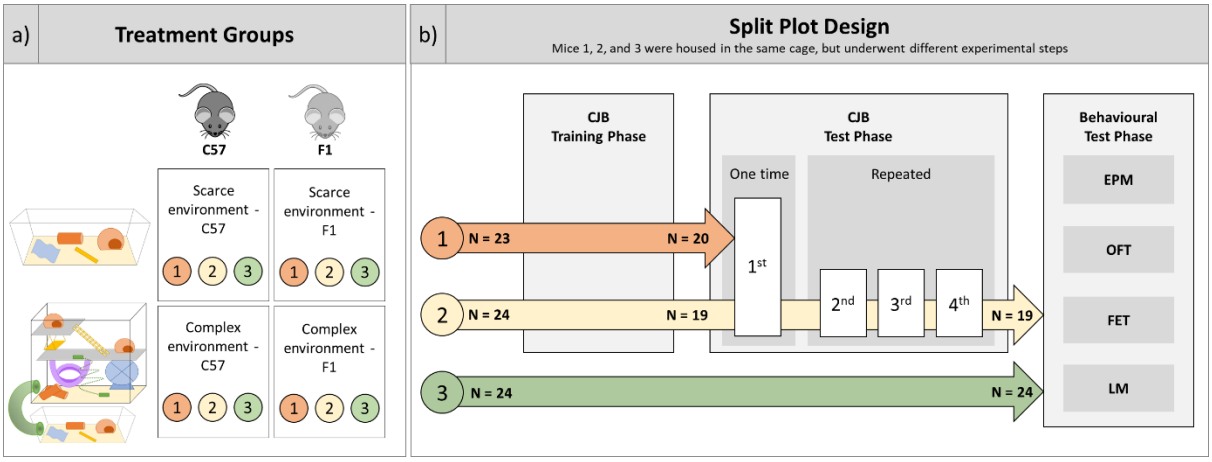

Figure 1: Experimental Design. a): Treatment groups. Mice of two different strains (C57BL/6J and B6D2F1N) were housed under one of two environmental conditions ("scarce" or "complex"). Mice from the "complex environment" had 1 h per day access to the "playgrounds". The three mice housed in the same cage belonged to the same treatment group but participated in different phases of the experiment. To represent this split plot design, we refer to a subset of mice that had the same experimental procedure with mice "1", "2", or "3" mice. **b): Split Plot Design.** Mice 1 and 2 participated in touchscreen training and the first CJB test. Mice that did not complete touchscreen training were not tested, indicated by the reduced sample sizes (N) after the CJB training phase (for details see section "Exclusion Criteria"). Mice 1 were relocated and used in another study after the first CJB test. Mice 2 continued with repeated CJB testing and subsequently entered the behavioural test phase together with mice 3. Mice 3 were not exposed to training-related procedures, but were otherwise treated as mice 1 and 2. CJB: cognitive judgement bias, EPM: Elevated plus maze, OFT: Open field test, FET: Free exploration test, LM: Labyrinth maze.

Randomisation was performed wherever possible: cages with same-strain mice were pseudo-randomly assigned to the "scarce" and "complex environment" and positioned in the rack in a balanced way. To avoid researcher bias, experimenters who handled mice did not know to which treatment group the mice belonged to (blinded study).

Complex Environmental Condition

In contrast to the "scarce environment", the "complex environment" offered mice a highly versatile environment, providing composite structural as well as social enrichment. The

system for providing the “complex environment” consisted of six adjacent “playgrounds” (50 x 32 x 52 cm^3), with a variety of items that allowed mice to express an array of natural behaviours, like climbing, gnawing, hiding, and digging (Figure 1). Grid walls between “playgrounds” allowed for tactile, visual, and olfactory contact with individuals other than their cage mates.

Each working day after touchscreen sessions, home cages were connected to one of the “playgrounds” for the duration of 1 h. This was done by taking the respective cages out of the rack and placing them underneath their assigned playground. Cages had a connector to which a transparent tunnel was attached, connecting the mice’s home cage with the playground. Mice could travel freely between their home cage and their playground. To control for handling effects, cages of the “scarce environment” group were placed on the table next to the "playgrounds" during the same period. After 1 h, all mice received their daily amount of food in the home cage food hopper. When mice left the playground to feed (if not, they were gently guided back), the connection tunnel was detached, and cages were returned to the rack. The tunnel connector was closed by a cap (diameter: 6 cm, FPI 4820, Ferplast S.p.A., Castelgomberto, Italy) when not in use.

To sustain the novelty of the structural enrichment, each “playground” was furnished differently and mice accessed different “playgrounds” on different days. All mice experienced all “playgrounds” and did not encounter the same “playground” more than two days in a row (order pseudo-randomised). Additionally, all “playgrounds” were cleaned and furnished with a new set of structural enrichment after six weeks of use.

To sustain the novelty of social enrichment, “playgrounds” were either separated by aluminium grid walls which allowed mice to see and sniff mice from other cages (social condition) or opaque red PVC walls that prevented such contact (non-social condition). Mice did not encounter the same condition for more than three days in a row (order pseudo-randomised).

Cognitive Judgment Bias (CJB) Test

Apparatus

For the CJB tests and the preceding touchscreen training, we used a commercially available touchscreen system (Bussey-Saksida Mouse Touch Screen Chambers, Model 80614, Campden

Instruments Ltd., Loughborough, United Kingdom). The system consisted of four independent chambers. Each chamber was equipped with a tone generator, an overhead illumination, an infrared-sensitive touchscreen at the front, and a reward magazine with a well for reward collection at the rear end. As a reward, we used servings of diluted sweet condensed milk (Nestlé "Milchmädchen gezuckerte Kondensmilch"; diluted 1:4 in tap water). The touchscreen itself was separated into three adjoining windows by a Perspex mask. The central window was used to display cues in form of white bars (6 × 1 cm^2) and the two side windows served as the response windows: mice needed to nose-poke a grey cross (width: 6 cm, height: 6 cm) displayed inside these windows in response to a cue presented in the central window. Data from the touchscreen training and cognitive judgement bias tests were automatically recorded by the ABET II software (version 2.20., Campden Instruments Ltd., Loughborough, Leics., UK).

Procedure

During touchscreen training and CJB test phase, mice had one session approximately every 24 h with 1-2 days of a break after five sessions. They were transported to the touchscreen system from the housing room using a semi-transparent red transport box. Sessions ended after a certain time limit or when the scheduled number of trials was reached, depending on the respective training step (for details see Krakenberg et al., 2019b). When the session was finished, the mice were carried back to their home cage. After all mice received their touchscreen training for the day, their weight was measured, and the respective enrichment regime applied. All touchscreen sessions were conducted after 8.15 a.m. during the dark phase of the day.

Touchscreen Paradigm

The paradigm applied here was the same as described previously in Krakenberg et al. (2020) with minor modifications in the discrimination training (Table S1 in Supplementary Material). Briefly, mice were trained to discriminate between two reference cues: positive and negative. The positive reference cue was a bar on the lower part of the central window (5 cm below the upper edge) and the negative reference cue was a bar on the upper part of the central window (1 cm below the upper edge). In trials with the positive cue, mice received a big reward (12 µl diluted condensed milk) for touching the correct side of the screen or a small reward (4 µl) for touching the wrong side. In trials with the negative cue, a bar displayed at the top of the

central window, mice received a small reward for touching the correct side of the screen or a mild punishment (5-sec timeout with lights on) for touching the wrong side. The location of the correct side for the cues was counterbalanced between mice: e.g., one mouse per cage had to touch the right-hand side in response to the positive cue to get a big reward, while the other mouse had to touch the left-hand side in response to the positive cue. A detailed description of our touchscreen paradigm can be found in Krakenberg et al. (2019b).

Once mice had learned to discriminate between the positive and negative cue, they proceeded to the CJB test. In the test, mice were presented with ambiguous cues, interspersed between reference cues. As ambiguous cues, we used three bars displayed at three intermediate positions: “near positive” (4 cm below upper edge), “middle” (3 cm below upper edge), and “near negative” (2 cm below upper edge). Using multiple ambiguous cues is recommended to achieve a robust CJB test (Lagisz et al., 2020). In total, the CJB test had 240 reference and 30 ambiguous cues, equally divided into five sessions spread over five days. In each session, each type of ambiguous cue was presented twice and pseudo-randomly interspersed between 48 reference cues. Response to ambiguous cues was unrewarded and unpunished.

Mice could either react toward the ambiguous cues as if predicting the positive cue outcome (“optimistic” choice) or as if predicting the negative cue outcome (“pessimistic” choice), from which we calculated their choice score:

$$Choice\ Score = \frac{N\ choices\ ("optimistic") - N\ choices\ ("pessimistic")}{N\ choices\ ("optimistic" + "pessimistic")}$$

The choice score can take values between -1 and +1, higher values indicating more “optimistic” choices and lower values indicating more “pessimistic” choices. Thus, the choice score serves as a relative measure of CJB.

Repeated CJB Test

After the first CJB test, one of two tested mice in each cage was randomly chosen to continue with repeated CJB testing (n = 19), to estimate the repeatability of individual differences in CJB. The test was repeated three times, resulting in four CJB tests per mouse over seven weeks. Between repeated tests, mice had a one-week time gap (following Mitchell et al., 2019

and Dingemanse and Wright, 2020) with two training sessions (one day apart) as reminders to maintain learning accuracy (discrimination training step 6 was used: see Table S1).

Battery of Behavioural Tests

Two weeks after repeated CJB testing, animals (including trained and non-trained mice) were tested in a battery of behavioural tests: the elevated plus maze (EPM), open field test (OFT), free exploration test (FET), and labyrinth maze (LM). The EPM and the OFT were used to assess the mice's state anxiety, in the FET we assessed their trait anxiety. Finally, in the LM we tested the mice's spatial learning abilities.

All tests were performed in a room that met the same conditions as described above for the housing room. Tests were video recorded (Logitech Webcam Pro 9000) and automatically tracked by software (ANY-maze, version 5.33, Stoelting Co., Wood Dale, IL, USA). All setups were cleaned with 70% ethanol between consecutive tests.

Mice were transported into the testing room either in a semi-transparent red transport box (EPM, OFT) or in their home cage covered with a black cloth (FET, LM). When the home cage was used, the test mice's cage mate(s) were transferred into waiting cages, furnished the same way as their home cage. In the testing room, tested mice had 1 min of waiting time in the transport box to accommodate before being tested. After placing the mice into the start position, the experimenter started the tracking software before leaving the room (except for the LM, where the experimenter was in the room during the test). All tests were performed during the dark phase between 8.15 a.m. and noon.

Elevated Plus Maze Test (EPM)

The apparatus was elevated by 50 cm from the ground and had four arms (30 × 5 cm^2 each) and a central area (5 × 5 cm^2) where the four arms met (Pellow et al., 1985; Lister, 1987, 1990). Two opposing arms were enclosed by 20 cm high walls and the other two opposing arms were open. All surfaces of the maze were made of grey PVC. The apparatus was illuminated by an LED lamp producing 25 lux in the central area. For testing, mice were placed in the central area of the apparatus facing the same closed arm. They had 5 min to freely explore the apparatus. The two cage mates were tested on the same day. We quantified relative number of open arm entries, relative time spent in the open arms, and distance travelled.

Open Field Test (OFT)

The apparatus was a plywood box with a square area (80 x 80 x 42 cm^3) painted with white varnish (Archer, 1973; Treit and Fundytus, 1988). The area 20 cm away from the walls was considered the centre zone. The apparatus was illuminated by an LED lamp producing 35 lux in the centre. Mice were placed in the same corner of the apparatus, facing the corner. Mice had 5 min to freely explore the apparatus. The two cage mates were tested on the same day. We quantified entries into the centre zone, time spent in the centre zone, and distance travelled.

Free Exploration Test (FET)

The apparatus was a modified version of the open field test which allowed mice to enter the apparatus by choice (Griebel et al., 1993). Light intensity in the centre of the arena was set to 35 lux. The apparatus measured 60 × 60 cm^2 and was framed by 35 cm high walls with an opening in one of them. The mice's home cage was attached to the opening (during the accommodation time in a transport box). Mice were placed in the home cage and had 15 min to freely explore the apparatus. The two cage mates were tested on consecutive days. We quantified latency to enter the apparatus, number of entries, time spent in the apparatus, and distance travelled.

Labyrinth Maze (LM)

The apparatus (40 cm × 24 cm) was divided by transparent walls 15 cm in height, forming a labyrinth that offered the mice’s home cage as the goal (Bodden et al., 2019). Light intensity in the centre of the arena was set to 12 lux. Mice were placed into the labyrinth and were given a maximum of 5 min to explore the labyrinth and find the exit to their home cage. Once the mice reached their home cage, the home cage was detached from the labyrinth. This test consisted of two trials with a 5-minute break in between, in which the mice remained in their home cage and the apparatus was cleaned with 70% ethanol. We quantified latency to reach the home cage, number of mistakes, and distance travelled. A mistake was scored when the mouse either took a wrong passageway or when it took a correct passageway but went back afterwards. To evaluate an individual´s learning performance, we calculated the relative difference between the first and second trial for each test parameter.

Data Analysis

Data were analysed using linear mixed-effect models (LMM). We assumed a Gaussian distribution and visually checked the distribution of model residuals to confirm reasonable goodness of fit (Schielzeth et al., 2020). When in doubt, we compared model residual histograms of raw and transformed data: if the histograms for models without transformations showed a strong deviation from a normal distribution and the Shapiro–Wilk test (Shapiro and Wilk, 1965) was significant, we chose the transformations which produced residual histograms that fit normality assumption the best (Table S2). Between-subject factors were centred for better interpretability of main effect estimates (Schielzeth, 2010), with C57BL/6J strain, "scarce environment", and "non-trained" as models reference levels. To calculate F-statistic and p-values for fixed factors, ANOVA type III tables were produced with the Satterthwaite method for denominator degrees of freedom. Differences were considered significant at $p \leq 0.05$.

Influence of Genotype and Environment on CJB

We analysed influences of genotype, environment, and their interaction on choice scores by fitting a model with the following factors: *cue* as fixed within-subject factor (three levels of ambiguous cues: *near positive*, *middle* and *near negative*), *genotype* and *environment* as fixed between-subject factors, including a genotype-by-environment interaction, and *individual* and *cage* as random between-subject factors. Before selecting this model, we explored the influences of design effects on a data set with randomised factors of interest (environment and genotype) to prevent bias (MacCoun and Perlmutter, 2015). Neither design effects nor training duration (which approximates the mouse age at test) significantly influenced the choice score.

Repeatability of CJB

The repeatability of CJB was estimated by calculating adjusted repeatabilities (R) of the choice score. Adjusted repeatability removes fixed effect variance from the estimate and is a useful tool to quantify the stability of an individual's trait over time (Nakagawa and Schielzeth, 2010). We calculated repeatability for each ambiguous cue by fitting a separate model for each: four repeated *CJB tests* were modelled as a fixed within-subject continuous variable and *individual* as random between-subject factor. Additionally, as each individual can respond differently to each cue, we fitted a model that allows different slopes for each *individual* across ambiguous

cues. This random slope model resulted in similar estimates as the above-described models so we report the results of those simpler models. The statistical significance of repeatabilities was tested by likelihood-ratio tests and uncertainty intervals were estimated by parametric bootstrapping (n = 1000, confidence level = 95%).

Influence of Genotype and Environment on Anxiety-like Behaviour and Spatial Learning

To investigate if genotype and environment (interactively) influence the mice's behaviour, for each behavioural parameter we fitted a model with *touchscreen training* (two levels: *trained* and *non-trained*), *genotype* (two levels: *B6D2F1N* and *C57BL/6J*), and *environment* (two levels: *complex* and *scarce*) as fixed between-subject factors, including a genotype-by-environment interaction, and with *cage* as a random factor.

Software

Data analysis and plotting were done in R 4.0.0 (R Core Team, 2020) with lme4 package for fitting mixed-effect models (Bates et al., 2015), lmerTest package for quantifying p values (Kuznetsova et al., 2017), and the rptR package for estimating repeatability (Stoffel et al., 2017). Figures were created using the ggplot2 package (Wickham, 2016).

Exclusion Criteria

Animals were excluded from the experiment if they did not reach the CJB test in 90 touchscreen training sessions. From 47 mice trained, 39 successfully finished the discrimination training and were then tested in the CJB test. All 19 mice that entered the repeated CJB testing phase were successfully tested. Due to a setup error in the LM, three mice had to be excluded from the LM analysis.

Results

Influence of Genotype and Environment on CJB

We analysed the influence of genotype and environment on mice´s reaction towards the three ambiguous cues (calculated as a choice score) in the touchscreen paradigm. The reaction to the three ambiguous cues was significantly different ($F_{(2,76.00)} = 243.883$, $p < 0.0001$; see Table S2 for a detailed overview of results in the following sections). We did not, however, find a significant influence of genotype ($b = -0.021 \pm 0.084$, $F_{(1,19.00)} = 0.059$, $p = 0.810$), environment ($b = -0.142 \pm 0.084$, $F_{(1,19.00)} = 2.795$, $p = 0.111$) or their interaction ($b = -0.061 \pm 0.170$, $F_{(1,19.00)} = 0.128$, $p = 0.724$) on choice scores (Figure 2).

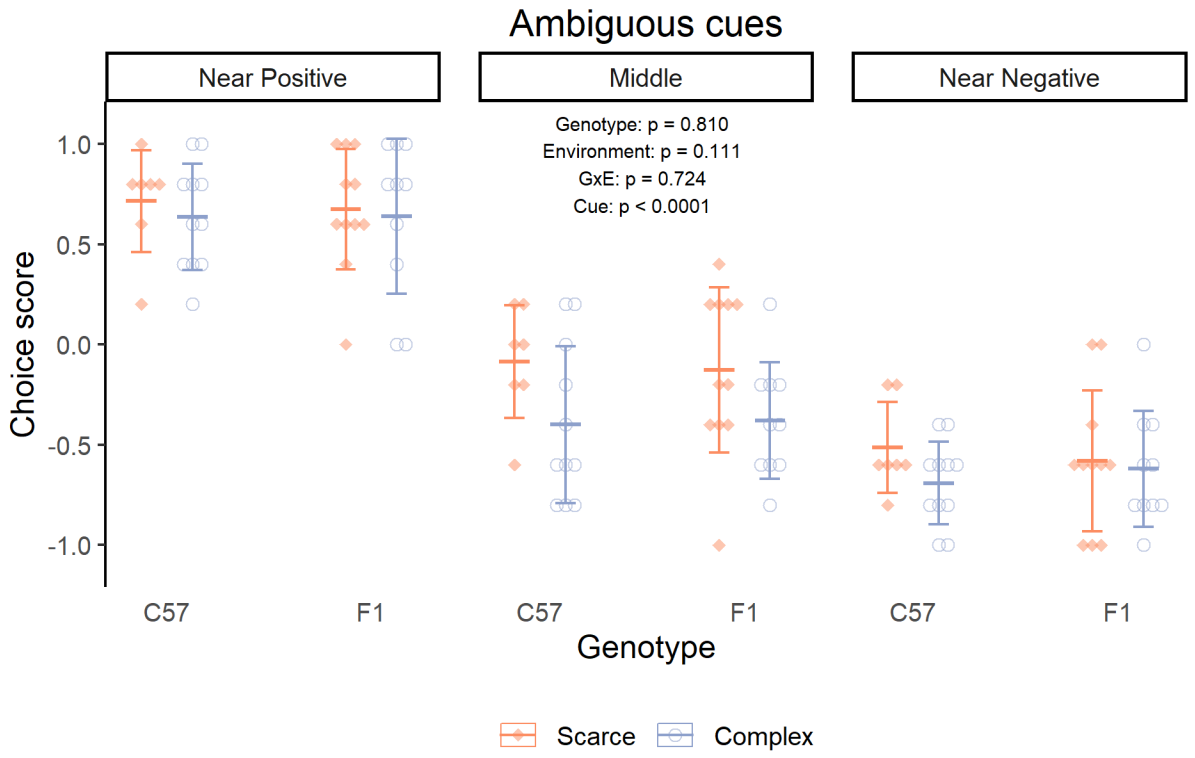

Figure 2: Cognitive judgment bias. Two mouse strains (C57BL/6J and B6D2F1N) were housed in two environmental conditions: the "scarce environment" (red) and the "complex environment" (blue). Data for each ambiguous cue are presented as means (horizontal mark) ± SD for each treatment group and points for the individual choice score. Statistical analysis based on the linear mixed-effects model. Number of individuals per treatment: $n_{scarce\text{-}C57} = 7$, $n_{complex\text{-}C57} = 11$, $n_{scarce\text{-}F1} = 10$, $n_{complex\text{-}F1} = 11$.

Repeatability of CJB

The stability of between-individual differences in CJB was assessed by estimating the repeatability of the choice score for each of the three ambiguous cues. Repeatability was

significantly different from zero for the "near positive" (R = 0.30, 95% CI [0.05, 0.54], p = 0.003) and the "middle" cue (R = 0.23, 95% CI [0.02, 0.46], p = 0.016), but not for the "near negative" cue (R = 0, 95% CI [0.00, 0.20], p > 0.999, Figure 3).

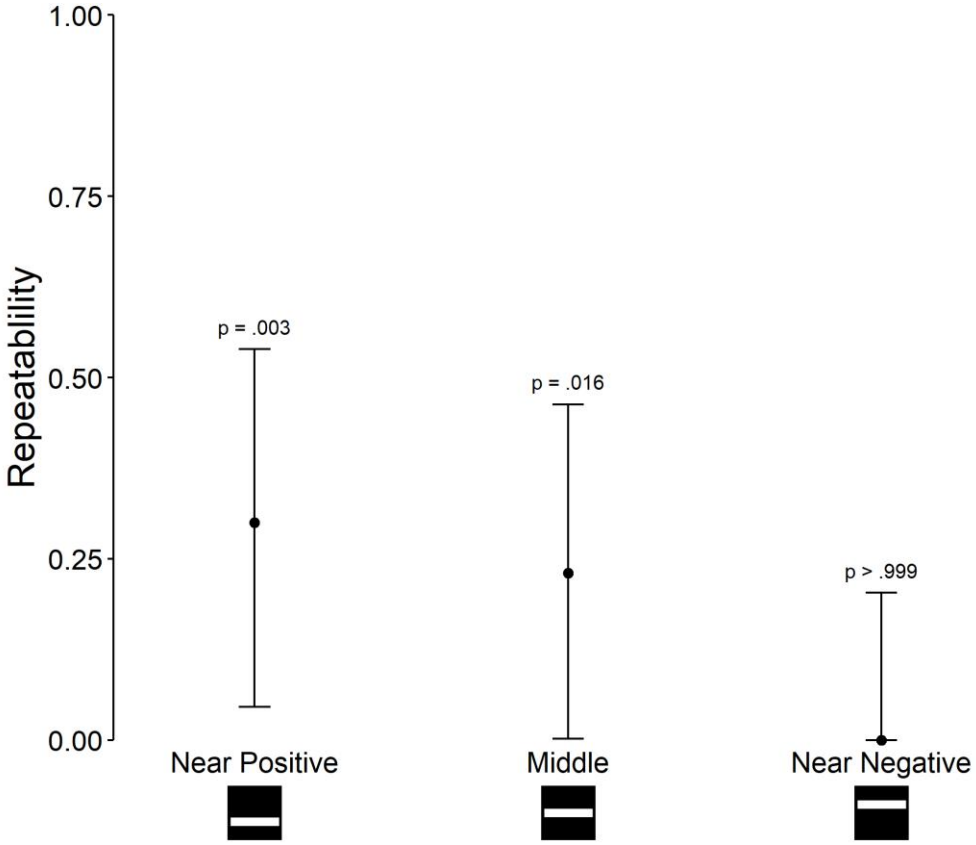

Figure 3: Repeatability of cognitive judgment bias. Repeatability estimates for choice scores from three ambiguous cues are represented by dots and corresponding 95% confidence intervals. Statistical analysis of adjusted repeatability based on the linear mixed-effects model. Number of individuals: n = 19.

Influence of Genotype and Environment on Anxiety-like Behaviour and Spatial Learning

State Anxiety (Elevated Plus Maze and Open Field Test)

Mice from the C57BL/6J strain entered the open arms of the EPM more often (b = -0.270 ± 0.170, $F_{(1, 20.128)}$ = 107.839, p < 0.0001, Figure 4) and spent more time on the open arms of the EPM than B6D2F1N mice (b = -0.254 ± 0.144, $F_{(1, 16.11)}$ = 48.682, p < 0.0001, Figure 4b). In addition, C57BL/6J travelled significantly more in the OFT than B6D2F1N mice (b = -6.510 ± 13.592, $F_{(1, 38)}$ = 5.351, p = 0.026). We did not find a significant influence of genotype on the three other behaviours measured in the EPM and OFT (Table S2).

Mice from the "complex environment" entered the open arms more often (b = 0.068 ± 0.170, $F_{(1, 20.128)}$ = 5.406, p = 0.031, Figure 4a) and spent more time there than mice from the "scarce environment" (b = 0.100 ± 0.144, $F_{(1, 16.11)}$ = 10.074, p = 0.006, Figure 4b). There was no significant influence of the environment on distance travelled in the EPM, nor on any of the three behaviours measured in the OFT.

We found two significant genotype-by-environment interactions in the EPM: C57BL/6J from the "complex" – but not the "scarce" – environment entered the open arms more often (b = -0.164 ± 0.340, $F_{(1, 20.261)}$ = 6.873, p = 0.016, Figure 4a) and spent more time on the open arms than B6D2F1N from the same environment (b = -0.197 ± 0.289, $F_{(1, 16.278)}$ = 7.152, p = 0.016), Figure 4b). There was no significant genotype-by-environment effect on distance travelled in the EPM, nor on any of the behaviours measured in the OFT.

We also included touchscreen training as a factor in the statistical model to control for differences between touchscreen-trained and non-trained mice. Training had a significant effect on three of the six behaviours measured in the EPM and the OFT. Trained mice entered the open arms of the EPM less often (b = -0.107 ± 0.027, $F_{(1, 21.827)}$ = 14.012, p = 0.001), spent less time on the open arms (b = -0.117 ± 0.031, $F_{(1, 17.481)}$ = 12.753, p = 0.002), and spent less time in the centre zone of the OFT than non-trained mice (b = -5.957 ± 3.127, $F_{(1, 38)}$ = 4.749, p = 0.036). There was no significant effect of training on distance travelled in the EPM, nor on entries to the centre or total distance travelled in the OFT.

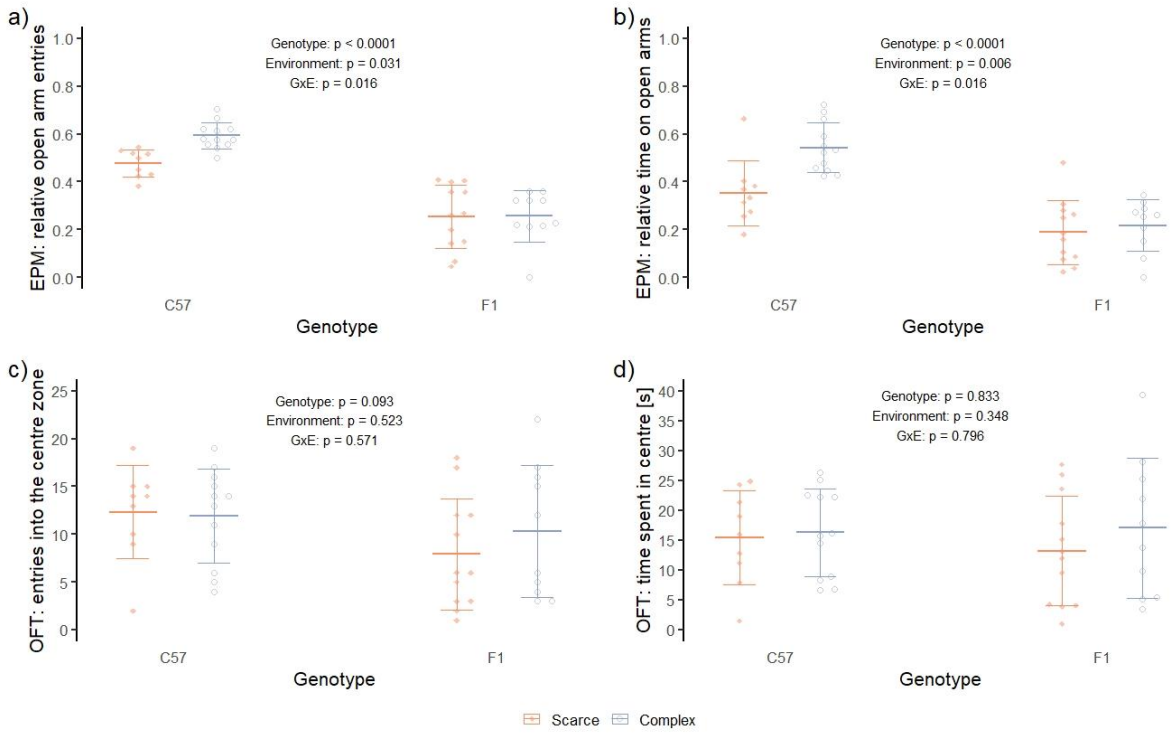

Figure 4: State anxiety. Two mouse strains (C57BL/6J and B6D2F1N) were housed in two environmental conditions: the "scarce environment" (red) and the "complex environment" (blue), and tested in an EPM and in an OFT to assess their state anxiety levels. a) number of entries to the open arms relative to the number of closed arm entries on the EPM; b) time mice spent in the open arm relative to the time the mice spent in the closed arm on the EPM; c) number of entries into the centre of the OFT; d) time mice spent in the centre zone of the OFT. Data are presented as means (horizontal mark) ± SD for each treatment group and points for the individual scores. Statistical analysis of adjusted repeatability based on the linear mixed-effects model. Number of individuals: $n_{scarce\text{-}C57}$ = 9, $n_{complex\text{-}C57}$ = 12, $n_{scarce\text{-}F1}$ = 12, $n_{complex\text{-}F1}$ = 10. EPM: Elevated plus maze test, OFT: Open field test.

Trait Anxiety (Free Exploration Test)

Mice of the C57BL/6J strain waited longer to enter the FET arena than B6D2F1N (b = -0.681 ± 2.284, $F_{(1, 20.476)}$ = 4.808, p = 0.04, Figure 5a). Apart from latency to enter the arena, we did not find any strain differences in the other three behaviours measured in the FET (Figure 5b).

Mice from the "complex environment" entered the FET faster (b = -0.998 ± 2.284, $F_{(1, 20.476)}$ = 10.621, p = 0.004, Figure 5a) and more often (b = 10.059 ± 5.846, $F_{(1, 38)}$ = 16.709, p < 0.0001, Figure 5b). In addition, mice from the "complex environment" travelled a greater distances (b = 7.011 ± 6.104, $F_{(1, 18.984)}$ = 6.933, p = 0.016) than mice from the "scarce environment". Only the time spent in the FET arena was not significantly influenced by the environment.

Neither genotype-by-environment interaction, nor touchscreen training had a significant effect on any of the three behaviours measured in the FET.

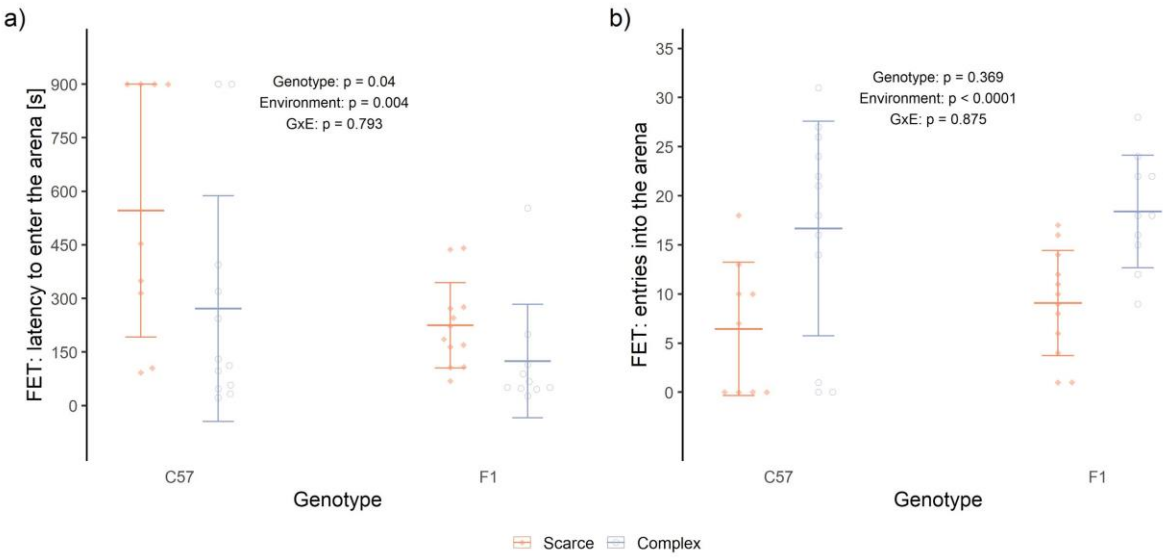

Figure 5: Trait anxiety. Two mouse strains (C57BL/6J and B6D2F1N) were housed in two environmental conditions: the "scarce environment" (red) and the "complex environment" (blue) and tested in a FET: a) Latency of the mice to first enter the FET arena; b) number of entries into the FET arena from the home cage. Data are presented as means (horizontal mark) ± SD for each treatment group and points for the individual scores. Statistical analysis of adjusted repeatability based on the linear mixed-effects model. Number of individuals: $n_{scarce\text{-}C57} = 9$, $n_{complex\text{-}C57} = 12$, $n_{scarce\text{-}F1} = 12$, $n_{complex\text{-}F1} = 10$. FET: Free exploration test.

Spatial learning (Labyrinth Maze)

Neither genotype, nor environment or genotype-by-environment interactions had a significant effect on any of the three behaviours measured in the LM (Figure 6). However, touchscreen-trained mice had a higher relative difference in total distance travelled than non-trained mice ($b = 0.739 \pm 0.293$, $F_{(1,15.711)} = 4.808$, $p = 0.031$).

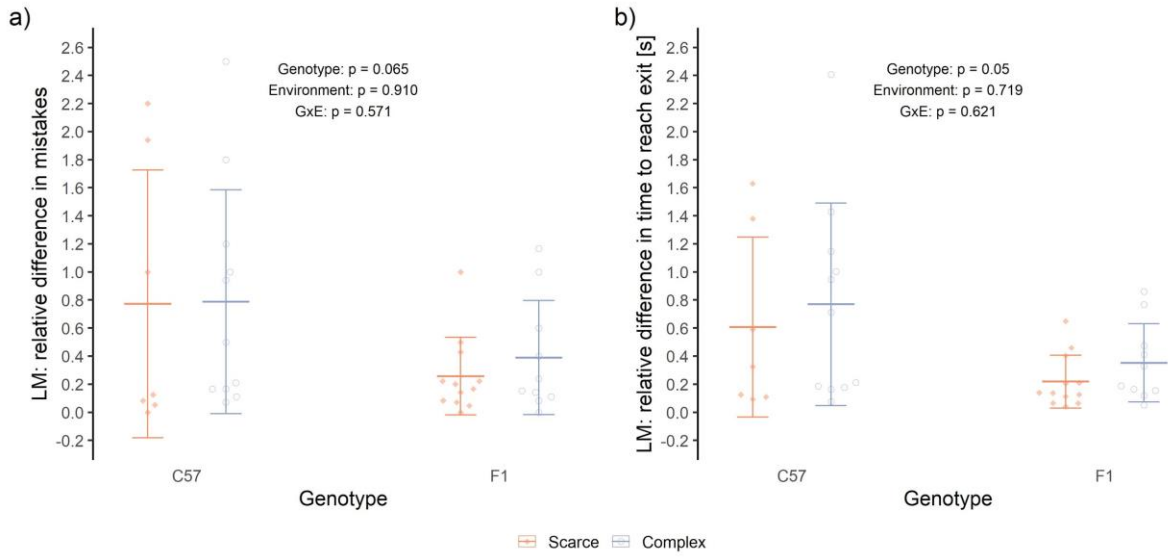

Figure 6: Spatial learning. Two mouse strains (C57BL/6J and B6D2F1N) were housed in two environmental conditions: the "scarce environment" (red) and the "complex environment" (blue) and tested in LM: a) relative difference in mistakes between second and first trial; b) relative difference in time to reach the exit between the second and first trial. Data are presented as means (horizontal mark) ± SD for each treatment group and points for the individual scores. Statistical analysis of adjusted repeatability based on the linear mixed-effects model. Number of individuals: $n_{scarce\text{-}C57} = 7$, $n_{complex\text{-}C57} = 11$, $n_{scarce\text{-}F1} = 12$, $n_{complex\text{-}F1} = 10$. LM: Labyrinth maze.

Discussion

This study had two major aims: First, we studied the role of genotype, environment and their interplay on cognitive judgement bias. Second, we investigated whether individual differences in CJB are stable over time. Moreover, we conducted a behavioural test battery to assess the influence of genotypes and environment on anxiety-like behaviours and spatial learning. Overall, genotype and environment did not significantly influence CJB or spatial learning in our laboratory mice, but they did influence some measures of anxiety-like behaviour. Importantly, individual differences in CJB were repeatable over the course of several weeks and thus partly reflect stable individual differences.

Influence of Genotype and Environment on CJB

Mice interpreted the three ambiguous cues differently in the CJB test. This is in line with the majority of judgement bias tests across species (e.g. Doyle et al., 2010; Lalot et al., 2017).

Contrary to our expectations, selected genotypes and environments did not significantly influence reactions towards ambiguous cues. In humans, a bias in interpreting ambiguous information seems to be influenced by both genetic variation and environmental influences (reviewed in Hirsch et al., 2016). In a twin study, the heritability of ambiguous word interpretations was estimated at 30% (Eley et al., 2008). Besides this, a candidate gene approach revealed that the serotonin transporter gene is associated with a bias in interpreting ambiguous information (Fox and Standage, 2012). Motivated by these findings in humans, an influence of genetic background on CJB was expected in animals, too. Only few studies addressed this and the evidence has been inconclusive so far. Rats selectively bred for helpless and non-helpless phenotype differed in their CJB (Enkel et al., 2010, but see Richter et al., 2012), as did different family groups of starlings (Bateson et al., 2015). Contrary to these findings, CJB was not influenced by a stress-susceptible genotype in pigs (Carreras et al., 2016) and was not heritable, based on the pedigree analysis of red junglefowl (Sorato et al., 2018). Similarly, CJB in laboratory mice was not significantly affected by a serotonin transporter genotype (Krakenberg et al., 2019a, but see Kloke et al., 2014), but there are some indications that mouse strains differ in their CJB (Novak et al., 2016; Hintze et al., 2018). In short, current

evidence is inconclusive regarding the heritability and genetic basis of CJB in animals (e.g. Sorato et al., 2018) and this could explain the lack of genotype effect in our study.

In contrast to the sparse publications about the impact of the genetic background, more studies investigated the influence of environmental factors on CJB in animals. Environmental enrichment was found to induce a positive CJB shift in multiple species of birds and mammals (e.g. Matheson et al., 2008; Brydges et al., 2011; Douglas et al., 2012; Richter et al., 2012; Destrez et al., 2014; Bethell and Koyama, 2015; Löckener et al., 2016; Lalot et al., 2017). By contrast, a smaller number of studies did not find a beneficial effect of enrichment (e.g. Brilot et al., 2010; Wichman et al., 2012; Keen et al., 2014; Bailoo et al., 2018; reviewed in Lagisz et al., 2020). In line with the latter studies, we also did not detect an influence of versatile structural and social enrichment. There are three possible reasons for this. First, in our study, mice had only limited access to the enriched environment (only 1 h per day), which might not have been enough to induce a positive shift in CJB. Second, even if enrichment would have had a positive effect on our mice, it might have been masked by the negative contrast between their permanent housing condition and restricted access to an enriched environment (Latham and Mason, 2010). Third, a recent meta-analysis provided conclusive support that the environment influences CJB (Lagisz et al., 2020); but the effect sizes of environmental manipulation are estimated to be small to moderate (and descriptively even lower for manipulation by enrichment). Consequently, stronger manipulations and large sample sizes are needed to determine effects of environmental enrichment.

Taken together, we suggest that individual differences in CJB are neither dominantly driven by an individual's genetic background, nor by environmental conditions typically manipulated in laboratory studies. Thus, the story of how one becomes an “optimistic” or “pessimistic” decision-maker might be more complex than assumed; it is the outcome of a lifelong interplay between (epi)genetic and numerous, partly stochastic, environmental influences, which cannot be easily disentangled (Traynor and Singleton, 2010; Groothuis and Trillmich, 2011; Lewejohann et al., 2011; Tikhodeyev and Shcherbakova, 2019).

Temporal Stability of Individual Differences in CJB

Reactions towards the ambiguous cues were repeatable for two out of three ambiguous cues, estimated at R = 0.30 for “near positive” and R = 0.23 for “middle” cue: this indicates moderately stable individual differences in CJB over seven weeks. To our knowledge, this is

549 the longest period for which the repeatability of CJB tests has so far been estimated. Rygula
550 et al. (2013; 2015; 2016) indicated that differences between rats categorised as “optimists” or
551 “pessimists” did not significantly change during ten weeks (no significant interaction between
552 repeated tests and assigned CJB category). However, it is difficult to interpret the lack of
553 statistical significances in terms of magnitude of individual difference and the repeatability
554 was not explicitly estimated. Only one study investigated repeatability across periods longer
555 than a few days: calves showed moderately repeatable individual differences in CJB across a
556 25-day interval ($R^2 = 0.41$, equivalent to unadjusted repeatability; Lecorps et al., 2018b). Three
557 other studies conducted over a short-term period found moderate to high repeatability
558 estimates (Clegg et al., 2017; Sorato et al., 2018; Verjat et al., 2021). It is expected that
559 repeatabilities over a longer period are smaller than over a short period (Bell et al., 2009).
560 Compared to other aspects of animal behaviour, our repeatability estimates seem to be in a
561 similar range (average R = 0.37; Bell et al., 2009). For example, the repeatability for activity
562 and mate preference was estimated at 0.20-0.25, and around 0.5 for aggressive and
563 explorative behaviour. Considering that CJB is a complex phenomenon emerging from the
564 interplay of cognition and affective states (e.g. Mendl and Paul, 2020), the moderately
565 repeatable individual differences observed in the present study are therefore notable.

566 When compared to the other two ambiguous cues, repeatability for the “near negative” cue
567 was surprisingly low. The reason for this is not clear but might be due to lower response
568 accuracy towards this cue. Mice had more difficulties in learning the correct response towards
569 the negative than to the positive reference cue, probably resulting in lower accuracy in our
570 paradigm (Krakenberg et al., 2019a; Krakenberg et al., 2019b; Krakenberg et al., 2020).
571 Because the “near negative” cue is visually the most similar to the negative cue, lower
572 accuracy in the negative cue could also lead to reduced accuracy in the “near negative” cue.
573 As reduced accuracy would inflate within-individual variation and hence reduce repeatability
574 (based on the equation for repeatability: Sokal and Rohlf, 1995), our paradigm, in fact, might
575 have underestimated the “true” repeatability for the “near negative” cue.

576 But what does the temporal stability of individual differences tell us about CJB? Our results
577 align with the notion that CJB does not merely reflect a short-lived emotional state directly
578 caused by recent experiences, but also a stable trait (e.g. Faustino et al., 2015; Asher et al.,
579 2016; Roelofs et al., 2016; Mendl and Paul, 2020). Indeed, since we detected stable individual

differences across seven weeks, we propose – in agreement with other recent publications – to consider CJB as an aspect of animal personality (Asher et al., 2016; Lecorps et al., 2018a) defined as individual differences in behaviour that are consistent across time and/or contexts (Dall et al., 2004; Réale et al., 2007). However, future studies need to deeper explore the stability of individual differences in CJB: How stable are these differences over even longer periods? Can they be modulated in different life phases? Do they hold across different contexts?

Influence of Genotype and Environment on Anxiety-like Behaviour and Spatial Learning

In our study, both genetic and environmental factors influenced some measures of anxiety-like behaviour. C57BL/6J strain mice entered the open arms of the EPM more often and spent more relative time on the open arms than B6D2F1N, which indicates lower levels of state anxiety in C57BL/6J compared to B6D2F1N mice. This is in accordance with a previous study comparing anxiety-like behaviours between these two strains (Kortzfleisch von et al., 2020). Furthermore, in comparisons between C57BL/6J and DBA/2 mice, the parental strains of B6D2F1, C57BL/6J mice expressed lower levels of anxiety than DBA/2 mice (e.g. Misra and Pandey, 2003; Võikar et al., 2005; DuBois et al., 2006; Mathiasen et al., 2008; Bodden et al., 2019, but see Trullas and Skolnick, 1993; Gard et al., 2001; Goldberg et al., 2012).

Surprisingly, the opposite picture emerged in the trait anxiety test: B6D2F1N mice entered the free exploration test arena significantly faster than C57BL/6J. However, differences between trait and state anxiety in rodents have already been reported before (Avgustinovich et al., 2000; Goes et al., 2009; Kloke et al., 2013; Bodden et al., 2019), further supporting the assumption that state and trait anxiety represent distinct systems (Lister, 1990; Belzung et al., 1994; Chapillon et al., 1999; Kopp et al., 1999; Kloke et al., 2013).

The complex environment reduced both state and trait anxiety. Again, this is in line with previous studies showing positive effects of environmental enrichment on state anxiety (Benaroya-Milshtein et al., 2004; Friske and Gammie, 2005; Meshi et al., 2006; Pokk et al., 2007; Coke-Murphy et al., 2014; Hendershott et al., 2016; Aujnarain et al., 2018; but see also Kloke et al., 2013; Goes et al., 2015). Interestingly, we found significant interactions between the genetic background and the environment. Specifically, the “complex environment”

610 reduced anxiety-like behaviour in EPM for C57BL/6J mice, but not for B6D2F1N: C57BL/6J mice
611 from the “complex environment” entered the open arms more often and spent more relative
612 time on the open arms than C57BL/6J from the “scarce environment”, but this was not the
613 case for B6D2F1N. This indicates that beneficial effects of enrichment can be strain-specific, a
614 phenomenon already known from other strain comparisons (e.g. Abramov et al., 2008; Õkva
615 et al., 2013; Åhlgren and Voikar, 2019). Consequently, even when positive effects of
616 enrichment regimes are detected for certain strains, a generalisation across other strains and
617 species remains difficult.

618 Albeit not in the focus of this study, some measures of anxiety-like behaviours were also
619 influenced by touchscreen training, with trained mice showing more anxiety-like behaviours
620 compared to non-trained mice. More specifically, trained mice entered the open arms of the
621 EPM less often and for shorter relative time, and spent less time in the centre of the OFT than
622 non-trained mice. Although surprising at first glance, Krakenberg et al. (2021) offer several
623 plausible explanations for this result. One explanation could be that touchscreen training in
624 itself represents an enrichment for mice. With the end of CJB testing, mice would lose this
625 enrichment, which could have induced a more negative state and consequently higher levels
626 of anxiety-like behaviour in the anxiety-like tests which followed CJB testing (for a detailed
627 discussion, see Krakenberg et al., 2021).

628 In our spatial learning task, neither genotype nor environment significantly influenced
629 performance in the labyrinth maze. Regarding genotype, to our knowledge, the only study
630 which compared the same two strains in a spatial learning task showed that B6D2F1 mice
631 outperformed both parental strains (Upchurch and Wehner, 1989). Studies comparing the
632 parental strains of B6D2F1 found better learning in C57BL/6 than in DBA/2 mice (Ammassari-
633 Teule and Caprioli, 1985; Nguyen et al., 2000; O'Leary et al., 2011; Bodden et al., 2019, but
634 see Brooks et al., 2005; Brown and Wong, 2007). Given all the literature providing evidence
635 for differences in spatial learning abilities between C57BL/6, DBA/2, and even B6D2F1, it
636 remains unclear why our study did not reproduce these findings. One possibility might be that
637 spatial learning in a labyrinth maze is somewhat different from spatial learning in the Morris
638 water maze, the most commonly used spatial learning task in the aforementioned studies.
639 Environmental enrichment is known to improve learning performance in mice (Meshi et al.,
640 2006; Loss et al., 2015; Hendershott et al., 2016, but see Prusky et al., 2000), so it was

surprising not to see a positive effect of environmental enrichment in our study. As discussed for the influence of environmental enrichment on CJB (see section Influence of Genotype and Environment on CJB), one possible reason for the lack of an effect might be the limited access to the enriched environment in our study. Bennett et al. (2006) compared aged and young mice from either a constantly enriched or temporarily limited enriched environment in the Morris water maze. Interestingly, they found that only old mice from the constantly enriched environment group, but not old mice from the 3h exposure/day group, behaved similar to young "controls". This points towards a differential effect of exposure time. Furthermore, some studies investigating the effect of environmental enrichment on spatial learning emphasise the importance of exercise: providing a running wheel was more effective than providing toys only (Praag von et al., 1999; Lambert et al., 2005; Mustroph et al., 2012). As in our study, enrichment items changed daily, mice were exposed to running wheels only irregularly, which in turn, might account for the different results. It might thus be rather subtle differences in the environment that have significant effects on behaviour.

Conclusion

We systematically investigated the influence of genotype and environment on cognitive judgement bias in laboratory mice. We found that albeit selected genotypes and environments influenced some aspects of anxiety-like behaviours, there was no influence of genotype and/or environment on CJB and spatial learning. Similar discrepancies between CJB and anxiety-like behaviours have already been reported in other studies (Brydges et al., 2012; Destrez et al., 2014; Bethell and Koyama, 2015; Verjat et al., 2021), indicating that CJB and state anxiety as well as trait anxiety represent distinct systems. Consequently, a "pessimistic" individual is not necessarily an anxious one. Furthermore, we identified CJB to be moderately repeatable, indicating that "optimistic" and "pessimistic" decision styles represent partly stable traits of individuals. Taken together, we suggest that individual differences in CJB are, themselves, an aspect of animal personality, which cannot easily be explained by other traits. Future research should aim to identify and quantify specific drivers of individual differences in CJB. Furthermore, studying the consequences of "optimistic" and "pessimistic" decision styles will provide valuable insights into ecological and evolutionary processes.

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Supplementary Material

Table S1: Discrimination training steps after Krakenberg et al. (2020) with modifications. Discrimination training consisted of six steps. All sessions ended after maximally 30 min unless the mouse reached the maximum number of trials before this time. During correction trials, animals were presented with the same cue until touching correctly. Pseudo-probe trials, i.e. balanced numbers of positive and negative trials that remained unpunished and/or unrewarded, were included to accustom the mice to the outcome of the probe trials during testing.

Step	Max. number of trials	Learning criterion	Return criterion	Correction trials	Number of pseudo-probe trials
1	50	Minimally 5 days in this step, 50 trials in 20 min on 2 consecutive days	50 trials in 20 min not reached in 25 days → pre-training	-	-
2	20	80% correct responses and ≤7 CTs on two consecutive days	>20 CTs or no CT reduction of 45% daily → Step 1	yes	-
3	50	80% correct responses and ≤13 CTs on two consecutive days	>30 CTs or no CT reduction of 45% daily → Step 1	yes	-
4	50	80% correct responses and ≤8 CTs on two consecutive days	Learning criterion not met on 1 out of 4 days → Step 3	yes (in trials 1-25)	2 (pseudo-randomly distributed across trials 26-50)
5	50	80% correct responses and ≤6 CTs on two consecutive days	Learning criterion not met on 1 out of 4 days → Step 3	yes (in trials 1-15)	4 (randomly distributed across trials 16-50)
6	50	80% correct responses and ≤5 CTs on two consecutive days	Learning criterion not met on 1 out of 4 days → Step 3	yes (in trials 1-5)	6 (randomly distributed across trails 6-50)

985 Table S2: Statistical analysis of CJB test, anxiety-like behaviour, and spatial learning.

Factors	Estimate (b) ± SE	Sum Sq	Mean Sq	NumDF	DenDF	F value	p value	transformation
CJB: choice score								
Cue (near positive)	0.668 ± 0.055							
Cue (middle)	-0.255 ± 0.055	33.685	16.842	2	76.000	243.883	**<0.0001**	
Cue (near negative)	-0.603 ± 0.055							
Genotype	-0.021 ± 0.084	0.004	0.004	1	19.003	0.059	0.810	none
Environment	-0.142 ± 0.084	0.193	0.193	1	19.003	2.795	0.111	
GxE interaction	-0.061 ± 0.170	0.009	0.009	1	19.003	0.128	0.724	
EPM: entries to open arms (%)								
Genotype	-0.270 ± 0.170	0.718	0.718	1	20.128	107.839	**<0.0001**	
Environment	0.068 ± 0.170	0.036	0.036	1	20.128	5.406	**0.031**	none
TS training	-0.107 ± 0.027	0.093	0.093	1	21.827	14.012	**0.001**	
GxE interaction	-0.164 ± 0.340	0.046	0.046	1	20.261	6.873	**0.016**	
EPM: time spent on open arms (%)								
Genotype	-0.254 ± 0.144	0.496	0.496	1	16.11	48.682	**<0.0001**	
Environment	0.100 ± 0.144	0.103	0.103	1	16.11	10.074	**0.006**	none
TS training	-0.117 ± 0.031	0.13	0.13	1	17.481	12.753	**0.002**	
GxE interaction	-0.197 ± 0.289	0.073	0.073	1	16.278	7.152	**0.016**	
EPM: total distance travelled (m)								
Genotype	-6.825 ± 20.759	682.691	682.691	1	20.836	2.43	0.134	
Environment	4.967 ± 20.759	97.945	97.945	1	20.836	0.349	0.561	BoxCox
TS training	-4.328 ± 5.593	31.666	31.666	1	22.496	0.113	0.74	
GxE interaction	-2.180 ± 41.573	20.303	20.303	1	20.972	0.072	0.791	

Factors	Estimate (b) ± SE	Sum Sq	Mean Sq	NumDF	DenDF	F value	p value	transformation
OFT: entries into the centre (#)								
Genotype	-2.810 ± 4.736	89.577	89.577	1	38	2.96	0.093	none
Environment	1.190 ± 4.736	Dez 56	12.56	1	38	0.415	0.523	
TS training	-3.612 ± 2.196	105.985	105.985	1	38	3.502	0.069	
GxE interaction	1.110 ± 9.500	9.874	9.874	1	38	0.326	0.571	
OFT: time spent in centre (sec)								
Genotype	-0.525 ± 7.124	3.427	3.427	1	38	0.045	0.833	none
Environment	2.591 ± 7.124	68.634	68.634	1	38	0.903	0.348	
TS training	-5.957 ± 3.127	360.766	360.766	1	38	4.749	**0.036**	
GxE interaction	1.268 ± 14.286	5.124	5.124	1	38	0.067	0.796	
OFT: total distance travelled (m)								
Genotype	-6.510 ± 13.592	455.925	455.925	1	38	5.351	**0.026**	none
Environment	0.780 ± 13.592	5.549	5.549	1	38	0.065	0.8	
TS training	-2.314 ± 3.754	25.401	25.401	1	38	0.298	0.588	
GxE interaction	2.025 ± 27.221	32.368	32.368	1	38	0.38	0.541	

986

Factors	Estimate (b) ± SE	Sum Sq	Mean Sq	NumDF	DenDF	F value	p value	transformation
FET: entries into the arena (#)								
Genotype	2.476 ± 5.846	49.723	49.723	1	38	0.826	0.369	none
Environment	10.059 ± 5.846	1.005.737	1.005.737	1	38	16.709	**<0.0001**	
TS training	-1.003 ± 2.620	2.98	2.98	1	38	0.05	0.825	
GxE interaction	-3.839 ± 11.724	1.513	1.513	1	38	0.025	0.875	
FET: latency to enter arena (sec)								
Genotype	-0.681 ± 2.284	3.979	3.979	1	20.476	4.808	**0.04**	log
Environment	-0.998 ± 2.284	8.79	8.79	1	20.476	10.621	**0.004**	
TS training	-0.031 ± 0.302	0.048	0.048	1	22.153	0.058	0.812	
GxE interaction	0.233 ± 4.570	0.058	0.058	1	20.611	0.07	0.793	
FET: time spent in the arena (s)								
Genotype	42.483 ± 85.011	16.486.366	16.486.366	1	38	1.199	0.28	none
Environment	50.599 ± 85.011	23.961.679	23.961.679	1	38	1.743	0.195	
TS training	8.259 ± 39.780	10.949.928	10.949.928	1	38	0.797	0.378	
GxE interaction	-123.116 ± 170.518	14522.68	14522.68	1	38	1.057	0.31	
FET: total distance travelled (m)								
Genotype	3.315 ± 6.104	111.653	111.653	1	18.984	1.561	0.227	none
Environment	7.011 ± 6.104	495.927	495.927	1	18.984	6.933	**0.016**	
TS training	-1.840 ± 2.662	0.025	0.025	1	20.878	0	0.985	
GxE interaction	-8.094 ± 12.240	50.191	50.191	1	19.097	0.702	0.413	

987

Factors	Estimate (b) ± SE	Sum Sq	Mean Sq	NumDF	DenDF	F value	p value	transformation
LM: number of mistakes, relative difference (Trial 1 / Trial 2)								
Genotype	-0.458 ± 0.364	0.552	0.552	1	17.904	3.865	0.065	none
Environment	0.011 ± 0.364	0.002	0.002	1	17.882	0.013	0.910	
TS training	0.135 ± 0.127	0.196	0.196	1	16.290	1.371	0.258	
GxE interaction	0.174 ± 0.728	0.048	0.048	1	18.043	0.333	0.571	
LM: total distance travelled, relative difference (Trial 1 / Trial 2)								
Genotype	-0.700 ± 0.742	0.975	0.975	1	16.505	1.221	0.285	log
Environment	0.025 ± 0.742	0.207	0.207	1	16.488	0.259	0.617	
TS training	0.739 ± 0.293	4.516	4.516	1	15.711	5.651	**0.031**	
GxE interaction	0.814 ± 1.484	0.113	0.113	1	16.663	0.141	0.712	
LM: time to reach exit, relative difference (Trial 1 / Trial 2)								
Genotype	-0.213 ± 0.306	0.161	0.161	1	18.230	4.388	0.050	sqrt
Environment	0.087 ± 0.306	0.005	0.005	1	18.208	0.134	0.719	
TS training	0.119 ± 0.065	0.126	0.126	1	16.697	3.445	0.081	
GxE interaction	0.022 ± 0.612	0.009	0.009	1	18.372	0.253	0.621	

988