Forever an optimist? Investigating the temporal consistency of optimism within and across life phases in rats

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Abstract

It is long known from human psychology that people differ in their perception of the world, with some judging ambiguous information more positively (i.e., “optimists”) and some more negatively (i.e., “pessimists”). About 20 years ago, this knowledge was transferred to animal welfare science to assess emotional states in animals by quantifying optimistic or pessimistic judgement biases. More recently, with increasing interest in animal personalities, researchers have also begun to explore whether differences in optimism and pessimism remain consistent across time. While first evidence suggests that optimism and pessimism represent a stable trait, less research has focused on their consistency within and across specific life phases. Using laboratory rats, we therefore aimed at systematically investigating the consistency of optimism and pessimism within and across two life phases during adulthood. Specifically, a cognitive judgement bias test relying on tactile cues was conducted twice during early and full adulthood, respectively. Temporal consistency within and across life phases was assessed by analysing the repeatability of individual optimism levels. While we did not detect consistent individual differences in optimism levels during early adulthood, they stabilized across phases and remained consistent during full adulthood. These findings align with human psychological research, suggesting that personality traits consolidate over lifetime. Despite this consolidation process, however, we also found considerable within-individual variation in overall optimism levels, indicating a high degree of behavioural plasticity. We therefore encourage future research on the ecological relevance of consistent versus flexible decision-making and highlight the significance of considering life phase when assessing behavioural traits.

Introduction

Originating from human psychology, the concept of “optimism” and “pessimism” (terms used without quotation marks hereafter) has been transferred to animal welfare science to assess emotional states in animals. Using so-called “cognitive judgement bias” (CJB) tests, the basic idea is thereby to assess the animals’ responses towards ambiguous cues, assuming that an animal in a positive affective state judges ambiguous cues more optimistically than an animal in a more negative affective state (Mendl et al., 2009). Accordingly, optimism and pessimism are conceptualized as opposite ends of an optimism/pessimism continuum (Hecht, 2013) and defined as the propensity of an individual to anticipate more rewarding or more aversive outcomes in ambiguous situations. For the ease of reading, we will refer to any individual score on this optimism/pessimism continuum as “optimism level” in the following.

CJB tests are now applied in various species, ranging from mammals and birds to fish, and even insects (reviewed by Lagisz et al., 2020; Neville et al., 2020), mostly interpreting optimistic choices as reflecting better and pessimistic choices as reflecting poorer welfare. More recently, however, researchers have also started to explore the concept from the perspective of behavioural ecology. Along these lines, it has been argued that being more or less optimistic can have an immense impact on survival and fitness: Under natural conditions, animals frequently make crucial decisions when encountering ambiguous situations. For example, rustling noises could indicate the presence of a predator or merely a windy day. In high predator density areas, a more pessimistic approach (i.e., fleeing) could be advantageous, while in low predator density areas, a more optimistic approach (i.e., staying) could save energy. Thus, optimistic and pessimistic decision-making can both represent adaptive strategies that contribute to improved fitness depending on the ecological context (Bračić et al., 2022; Espigares et al., 2022; Fawcett et al., 2014; Garnham et al., 2019; McNamara et al., 2011).

Indirectly, such a reasoning builds on the idea that individuals differ consistently in their optimism levels, assuming that optimism and pessimism might not only serve as an indicator of emotional states but also cover a trait dimension. In line with these considerations, it is increasingly acknowledged that individuals of the same population show great, yet consistent variation in behaviour: some are, for example, bolder, some are shyer, some are more and some are less aggressive than others (Dingemanse & Réale, 2005; Réale et al., 2007; Sih et al., 2020). Formally, such inter-individual differences in behaviour that are consistent over time and/or across contexts are referred to as animal personality (Kaiser & Müller, 2021; Réale et al., 2007; Sih et al., 2004; Stamps & Groothuis, 2010; Wolf & Weissing, 2012).

Applying this personality definition to the study of optimism and pessimism, time consistent inter-individual differences have already been described, for example, in wild house mice (Verjat et al., 2021) and bottlenose dolphins (Clegg et al., 2017) over a time span of three days. Likewise, consistency of optimism levels was shown in dairy calves across a time period of 25 days (Lecorps et al., 2018) and in laboratory mice across a time span of seven weeks (Bračić et al., 2022). Thus, while there is already some evidence for optimism and pessimism to represent a stable trait over varying time spans (Lecorps et al., 2021), less research has been done on the consistency of optimism levels within and across different life phases.

From human psychology, it is known that "dispositional optimism", the generalized tendency to expect positive outcomes in the future, is relatively stable over time but subject to changes due to lifetime events and aging (Carver & Scheier, 2014; Chopik et al., 2015; Chopik et al., 2020; Segerstrom, 2007). Similarly, Bell and Stamps (2004) have argued that also in animals, certain behavioural traits can be relatively consistent over time, but might nonetheless change throughout life. A study on common voles, for example, revealed that some behaviours like exploration and activity were highly consistent over short time scales, but varied depending on life phase and environmental context over longer time scales (Herde & Eccard, 2013). Likewise,

in a study on wild mouse lemurs, Dammhahn (2012) showed that males exhibit high behavioural consistency within a mating season, but not across different years. Finally, a recent meta-analysis showed that personality traits like boldness and activity tend to be consistent within but not across developmental life phases (Cabrera et al., 2021). But there are also some contrary findings: For instance, Schuster and colleagues (2017) analysed the consistency of exploration, activity and boldness in Eurasian harvest mice in both juveniles and adults, showing that the animals behaved consistently within as well as across life phases. However, systematic research on this topic is still scarce, with most studies focusing on the consistency of behavioural traits across shorter time scales and/or within single life phases (Stamps & Groothuis, 2010), and less studies addressing consistencies across longer time scales and/or across different life phases.

To contribute to this emerging field of research, we here aimed to examine the consistency of optimism levels in laboratory rats within and across different life phases. To this end, two life phases were defined during adulthood (i.e., early adulthood and full adulthood), and CJB testing was carried out twice within each phase using a cognitive judgement bias test relying on tactile cues. More specifically, the first phase started closely after adolescence (Schneider, 2013), with sexual and cognitive maturation being in the final process of completion (de Boer & Koolhaas, 2024; Mengler et al., 2014), while in the second phase, the rats could be considered sexually as well as socially mature (Sengupta, 2013). Moreover, it is known from previous studies that 2 and 5 months old rats differ in exploration and anxiety-like behaviour (Sudakov et al., 2021), indicating that behavioural changes occur between early and full adulthood that might also affect optimistic and pessimistic decision-making. As the majority of studies found personality traits to be consistent within but not across life phases (Cabrera et al., 2021) and repeatability estimates to become lower with increasing time intervals (Bell et al., 2009), we hypothesized optimism levels to be consistent within early and full adulthood, respectively, but to show less consistency across the two life phases.

Methods

Animals and housing conditions

24 female Lister Hooded rats (LIS:Crl) were purchased from a professional breeder (Charles River Laboratories, Research Models and Services, Germany GmbH, Sulzfeld, Germany) at an age of four weeks. They were housed in groups of four animals per cage and identified by their fur pattern. The cages (model "Furat", ferplast S.p.A., Castelgamberto, Italy; 78 x 48 x 70 cm^3) contained wood shavings as bedding material (TierWohl Super, J. Rettenmaier and Söhne GmbH & Co KG, Rosenberg, Germany) and had two additional levels to enable climbing and jumping. A transparent red plastic tunnel (ZOONLAB GmbH Animal Husbandry Experts, Germany; 15 cm x 9 cm and 9.5 cm high) and house (ZOONLAB GmbH Animal Husbandry Experts, Germany; 20.5 cm x 15.7 cm and 11.5 cm high), a cardboard tunnel (ZOONLAB GmbH Animal Husbandry Experts, Germany; length: 12.5 cm, Ø 9 cm) and two plastic hammocks (Sputnik, SAVIC, Belgium; 29 cm x 26 cm and 19 cm high) hanging from the cage top were used to enrich the cages with shelter options. Additionally, the cages were equipped with paper towels and four wooden cubes. In the housing rooms, the temperature was kept at approximately 22 °C and a relative humidity of about 50%. The light/dark cycle (12:12 h) was reversed with lights off at 9:00 am. Food (Altromin 1324, Altromin GmbH, Germany) and water were available *ad libitum* until the beginning of the experimental phase. During CJB training and testing phases, the animals were kept under a mild food restriction by providing food once per day after CJB training or testing. Before the age of 10 weeks, the amount of food provided allowed a weight increase according to the average weight increase of female Lister Hooded rats ("Growth Chart" for Lister Hooded rats, Charles River Laboratories, 2024). From 10 weeks of age on, the animals were maintained at 90-95 % of their current bodyweights. While the primary aim was to increase the animals' motivation to participate in the CJB training and testing by working for food rewards, such moderate food

restrictions are also known to improve the health of laboratory rats by preventing the animals from overfeeding under laboratory conditions (Feige-Diller et al., 2020; Keenan et al., 1996; Tucci et al., 2006). Their weight was monitored daily with a break of max. 2 days using a digital scale (PCE-BT 2000, PCE Deutschland GmbH, Meschede, Germany; weighing capacity: 2100 g, resolution: 0.01 g). The amount of food per cage was calculated based on the lightest rat in the respective cage.

Experimental design

To investigate the consistency of optimism levels within and across different life phases, CJB testing was conducted four times across two life phases. More precisely, two life phases were defined during adulthood, and CJB testing was carried out twice within each phase. While within each phase, the time interval between two repetitions was set to 1.5 weeks, the time interval between the two life phases spanned 8 to 9 weeks, thereby guaranteeing a longer interval between than within phases (see Fig. 1). This allowed for an analysis of within-phase consistency compared to across-phase consistency of optimism levels. Following this experimental design, the first phase ("early adulthood", (EA))) began at postnatal day (PND) 73 ± 4 and lasted until PND 96 ± 4, while the second phase ("full adulthood", (FA)) started at PND 160 and ended at PND 191. Thus, the first phase started closely after adolescence (ending at PND 60; Schneider, 2013) and during the final process of sexual and cognitive maturation (de Boer & Koolhaas, 2024; Mengler et al., 2014), while in the second phase, the rats were several weeks older and could be considered sexually as well as socially mature (at 5-6 months; Sengupta, 2013). Initial training started already at PND 48 since the training duration for the first CJB test was longer compared to the short re-training (3-4 days) for subsequent CJB tests. The rats were trained on a daily basis until they met the learning criterion of 80% correct responses (see "Training" for more details) to proceed to the CJB test. Since not all animals reached the criterion at the same time and training had to be paused for several days in batch 1 and 3 (see "Cognitive Judgement Bias Test"

for further details), the initial CJB training lasted 3-4 weeks, followed by 3 days of CJB testing in the subsequent week. Thus, the animals finished the first test on PND 75 ± 4. After a break of 1.5 weeks, rats were shortly re-trained before being tested again for their CJB. Until the start of the second phase on PND 160, no further tests were conducted, and the food restriction was paused. On PND 160, the CJB training and the food restriction were resumed. The first CJB test of the second phase ended on PND 170-171. After a break of 1.5 weeks, the second test was conducted (see Fig. 1).

All testing was conducted by the same experimenter. Due to the nature and design of the experiment, it was not feasible to blind the experimenter for optimistic and pessimistic choices made by the rats. However, all data was only analysed after the last batch finished the tests and no treatment was applied, reducing the risk of bias.

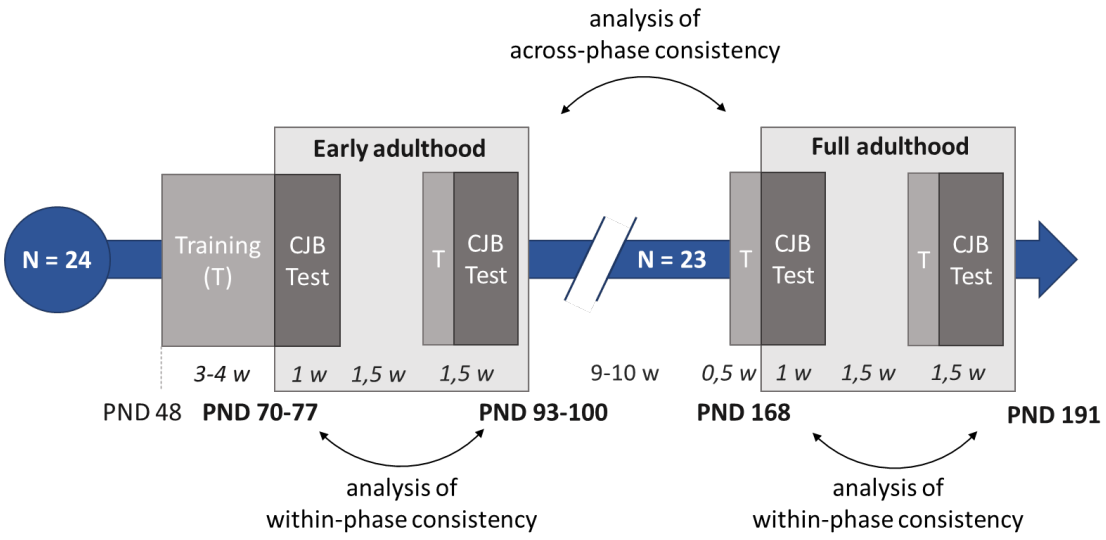

Figure 1: Experimental Design. Female rats were tested for their cognitive judgement bias (CJB) during two different life phases: early adulthood and full adulthood. The CJB test was conducted twice per phase. Durations of training, test and intervals are shown in weeks (w).

Cognitive Judgement Bias Test

For the determination of optimism levels, we used a CJB test originally developed by Brydges and Hall (2017) and modified the procedure with respect to several aspects (see "Paradigm" for details). The basic idea of the test is to utilize differently grained sandpaper as tactile cues to

study the reactions towards ambiguous cues. As widely done for CJB tests, we included three cues of different ambiguities (see below), all contributing to the characterization of the individuals' optimism levels.

Apparatus

The test apparatus was made of a type IV cage (floor space: 33 x 14 cm) without cage lid, divided into different sections (see Fig. 2). On one end, it had a start chamber containing a lid made of Plexiglas (floor space: 33 cm x 13 cm, height: 20 cm), cues were presented in the middle compartment (floor space: 33 cm x 22 cm) and on the other end, it split up in two reward chambers (floor space: 16.5 cm x 19 cm). All chambers had openings (8.2 cm x 8.2 cm) to the middle compartment which could be closed by sliding doors. To prevent the rats from jumping out of the apparatus, the walls of the type IV cage were extended by a 15 cm high Plexiglas attachment. In the middle part, sandpaper sheets (klebemeister.eu, Adbeere Com Marketing Unternehmergesellschaft & Co. KG, Gerbrunn, Germany; 230 mm x 280 mm; waterproof) of different grits (60, 120, 180, 400 and 1200) could be secured to the ground by magnets. Additionally, a large slider (33 cm x 38 cm) containing another sheet of sandpaper was used in the middle part. In each of the reward chambers, a food bowl (10 cm in diameter) was placed which contained a Petri dish with holes in the lid. Half a Honey Loop (Honey Loops, Kellogg Europe Trading Limited, Dublin, Ireland) was placed in each of these and the Petri dishes were sealed with transparent tape. This way, the rats could not choose a reward chamber by olfactory cues. Before the first and after each session, the apparatus was wiped with 70 % ethanol to

exclude any olfactory cues from conspecifics as well. After training or testing, the sandpaper sheets were washed under running water.

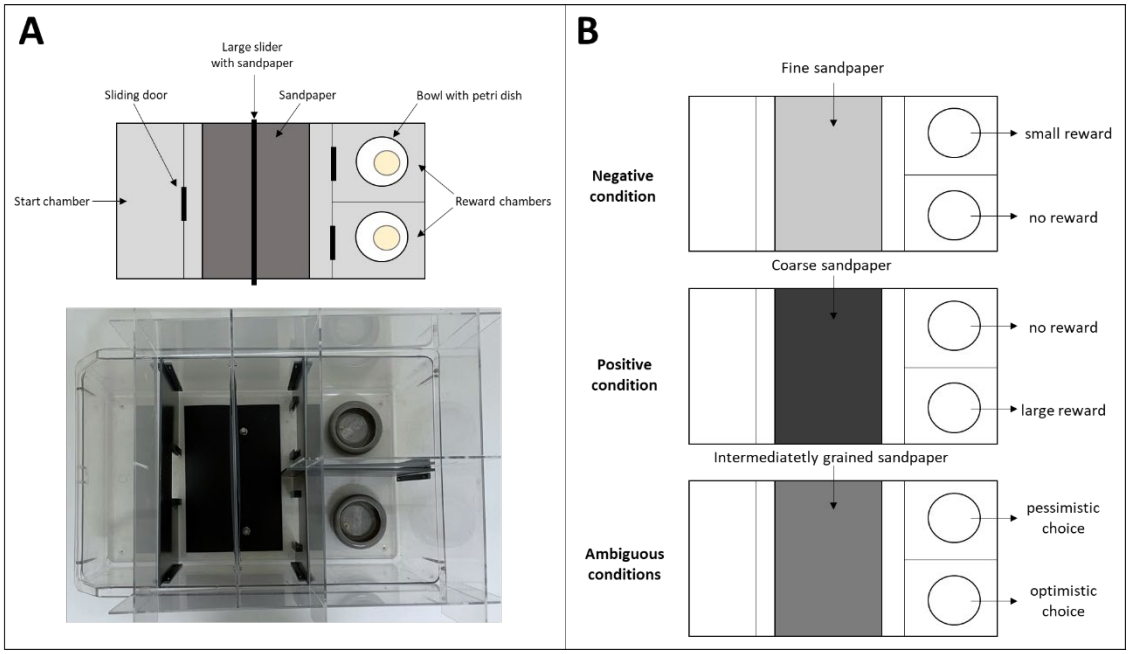

Figure 2: Cognitive judgement bias test apparatus and paradigm. A) A type IV cage with different intersections was used, containing a start chamber, a middle part and two reward chambers. Sandpaper was presented in the middle part and honey loops were offered as rewards in either of the two reward chambers. **B)** In the cognitive judgement bias test, rats learned to associate coarse or fine sandpaper with a left or right reward chamber containing either a large or small reward as a positive or negative reference condition. Then, in ambiguous conditions (near positive, middle and near negative), sandpapers of intermediate coarseness were presented and optimistic and pessimistic responses were recorded based on the rat's choice.

Procedure

During CJB training and tests, the rats participated in one session per day with a maximum of 9 days break in between. The rats were trained in an adjacent room to the housing room. All training and testing sessions were conducted during the dark phase after 9.15 a.m. under red light. The order of rats trained or tested was randomized. The training or test sessions ended when the rats completed all trials or when a cut-off point of 40 min was reached. Sessions which were not completed within this time were repeated the next day for the respective rat. Then, the individual was carried back to its home cage. After all rats finished their training or test session, they were fed the estimated amount of food pellets based on their weight.

Paradigm

In line with the CJB paradigm originally developed by Brydges and Hall (2017), the rats were first trained to associate coarse and fine sandpaper (60 and 1200 grit) with either the left or the right reward chamber. In the positive condition, they were presented with half a Honey Loop while they received only one-sixth of a Honey Loop in the negative condition. Which chamber and grit were combined in the positive and negative condition was balanced throughout the sample of animals. As soon as the rats met a certain learning criterion (see "Training II"), they proceeded to the test. In the test, they were presented with three ambiguous cues, sandpaper of intermediate grits to those presented during training (120, 180 and 400). These were close to the negative ("near negative", NN) or the positive condition ("near positive", NP), or were intermediate ("middle", M). If the rat chose the chamber associated with the positive condition when presented with an ambiguous cue, that response was considered to be optimistic while choosing the reward chamber associated with the negative condition was considered to be pessimistic.

In contrast to the test design of Brydges and Hall, however, we modified and/or added the following aspects: Firstly, the test apparatus was equipped with wall extensions to prevent the rats from jumping out. Secondly, a large slider with an additional sheet of sandpaper was added to slow the rats down and ensure sufficient tactile contact with the sandpaper cues. Thirdly, sand and scents were omitted from the reward chambers to simplify the procedure. Fourthly, smaller reward sizes were used to avoid overfeeding of the rats during multiple sessions per day. Fifthly, small modifications were applied to the training schedule and a slightly stricter learning criterion was used before animals could proceed to the test. Lastly, two more ambiguous cues were added, one closer to the positive and one closer to the negative cue. The use of more than one ambiguous cue is favoured to receive robust CJB test results (Lagisz et al., 2020). Please note that so far, it is not entirely clear how the perceived ambiguity of cues close to the reference

cues influences the judgement of the rats. Thus, we present and discuss data from all three ambiguous cues to characterize the rats with respect to their optimism levels. However, since there should be the greatest uncertainty about the outcome for the middle cue (Neville et al., 2020), we regard the reactions towards this cue as most relevant for interpreting the findings concerning the consistency of optimism/pessimism.

Training

Before the rats could be tested for their optimism levels in the CJB test, several training steps were necessary to prepare the basic discrimination of reference cues. More specifically, rats needed to associate the two reference sandpapers with the left or right reward chamber and the large or small rewards. Briefly, in the first step, the rats were habituated to the apparatus and the rewards (Habituation). In the second step, the two reference sandpapers and the two different reward sizes were presented for the first time with only the correct reward chamber open (Pre-Training). In the third step, both chambers were open and the rats learned to choose the correct chamber to receive the reward (Training I). In the last training step, randomly unrewarded trials were interspersed to accustom the rats to unrewarded ambiguous trials in the following test (Training II).

Habituation:

During the one-day habituation phase, sandpaper was omitted from the apparatus. Both chambers were opened, each containing a ¼ honey loop on a Petri dish. The designated rat was gently removed from its home cage and placed in the closed start chamber. After a 1-minute acclimatization, the rat was allowed to move freely when the slider opened. The choice of the left or right reward chamber was noted down and after consuming the reward, the rat was guided or put back into the start chamber to prepare the next trial. The process was repeated until each rat chose both chambers at least 5 times, with the reward chambers being restocked

with food rewards between trials. This habituation aimed to familiarize the individuals with the environment. Thus, this step was omitted upon re-training for CJB test repetition.

Pre-Training:

In the one-day pre-training phase, rats were introduced to sandpaper and different reward sizes. A sandpaper sheet (60 or 1200 grit) was placed in the large slider, with an additional sheet of the same grit magnetically fixed to the floor. Rats were individually placed in the starting chamber with the slider closed for a 1-min acclimatization time. In the positive condition, half a Honey Loop and in the negative condition, one-sixth of a Honey Loop was placed in the correct chamber, leaving the other one empty. Pre-training exclusively allowed access to the correct reward chamber by leaving the other one closed. The rat, initially in the start chamber with the slider closed, moved onto the sandpaper when the slider opened. The large slider was then removed upon tactile interaction (i.e., the rat touching the sandpaper with its nose or paw). After the rat consumed the reward, it was guided back to the start chamber, the slider closed, and preparations were made for the next trial. The pre-training comprised 30 trials (15x 60 and 15x 1200 sandpaper grit). The same sandpaper could be presented three times consecutively. Successful completion of all 30 trials within 40 minutes allowed rats to progress to the next training step the following day.

Training I

Unlike pre-training, both chambers were accessible in this training step, requiring active decision making from the rat. However, after the 1-min acclimatization period in the start chamber, each session started with four only-correct trials to guide the rat towards the correct side. Then, the actual training phase commenced, consisting of a total of 30 trials, again evenly distributed between positive and negative conditions. If a rat chose the wrong reward chamber, it was prevented from entering the correct chamber and was guided back into the start chamber. The

respective trial was repeated until the rat chose the correct chamber but only up to three times. If the rat still chose the wrong reward chamber upon the second “correction trial”, an only-correct trial followed and the session continued with the next trial afterwards. The Training I step was repeated until the rat achieved at least 80 % correct choices in both the negative and the positive condition (Bračić et al., 2022), corresponding to a minimum of 12 out of 15 correct trials per condition with less than 10 correction trials. Meeting this criterion allowed the rat to progress to Training II. However, as the test (see “Test”) followed directly after Training II and was conducted on three consecutive days, the rats remained in Training I until the time schedule allowed training and testing on four consecutive days. If the rat took more than 19 correction trials, it returned to pre-training (please note that this threshold was based on experiences made during piloting).

Training II

Similar to the previous training step, Training II started with a 1-min acclimatization period and four only-correct trials, followed by 30 trials with both reward chambers open. However, in this step, wrong choices were not followed by correction trials. Moreover, within the 30 training trials, six (three of each condition) were unrewarded to accustom the rats to unrewarded ambiguous trials in the following test. These unrewarded trials were evenly distributed within the 30 training trials. The session could not start or end with an unrewarded trial and consecutive unrewarded trials were prohibited. After an unrewarded trial, at least one training trial of each condition needed to follow before another unrewarded trial could be presented. Again, a learning criterion of 80 % correct trials in each condition was necessary to proceed to the next step, which was the CJB test. If the rat did not meet the criterion, it was set back to Training I.

Test

The test protocol, similar to Training II, included 4 initial only-correct trials followed by 30 open trials. Among 24 reference cues (N and P), six ambiguous cues (NP, M and NN) were interspersed: sandpaper of 120, 180 and 400 grit, presented two times each. Criteria for introducing ambiguous cues included prior presentations of both reference cues before the first trial with an ambiguous cue, and occurrences of both reference cues between two ambiguous cues. Additionally, the test could not end with an ambiguous cue, and each ambiguous cue on a test day followed the sandpaper of 60 and 1200 grit once to mitigate potential influences. The test did not include correction trials and all ambiguous trials were unrewarded to avoid reinforcing optimistic or pessimistic responses. The animals were accustomed for unrewarded trials in the previous training step which is a common practice in CJB studies (Bethell, 2015; Bračić et al., 2022; Brydges & Hall, 2017). The CJB test was conducted on three consecutive days, equalling six presentations of each of the three ambiguous sandpaper cues. Thus, the two reference cues N and P were each shown 36 times while each of the ambiguous cues NN, M and NP were shown six times per test repetition.

Statistical analysis

The experiment was conducted using 24 rats after performing an *a priori* power analysis with an average effect size (f = 0.3) and a power of 80% (G*power, version 3.1.9.7). During full adulthood, the sample size was reduced to N = 23 because one animal had to be euthanized due to unrelated medical reasons.

Data were analysed in R 4.4.0 (R Core Team 2020) using the “lme4” (Bates et al., 2015; version 1.1-35.3) and “lmerTest” (Kuznetsova et al., 2017; version 3.1-3) packages for mixed model fitting. All plots were created using the “ggplot2” package (Wickham, 2016; version 3.5.1).

Differences in choices towards the five cues in the CJB test

In a first step, we used a general linear mixed model (GLMM) to estimate general differences in choices between the three ambiguous and the two reference cues to validate that the animals could distinguish between the sandpapers with different grain sizes. The response ("choice") was fitted as a binomial data distribution (optimistic choice = 1; pessimistic choice = 0). GLMMs also always included the type of "cue" (P, NP, M, NN, N) as well as "test repetition" (from 1 - 4) as fixed effects as the main points of interest. Note that the two reference cues N and P were shown 36 times while the ambiguous cues NN, M and NP were shown six times per test repetition (please see above "Test"). To find the optimal random-effects structure, we next built models including "ID" (describing the individual) and "age". Comparing the Akaike information criterion (AIC) between these models (Zuur et al., 2009), we retained "ID" as a relevant random effect (see Supplementary Table S1). Comparing AICs, we then similarly tested if the fixed effect "batch" added improvement to the model fit, but did not find support for this (Supplementary Table S1). Thus, our final and most parsimonious model included the type of cue and the test repetition number as fixed effects as well as ID as a random effect (choice ~ cue + test repetition + (1|ID), family = binomial). Model residuals were checked using the "DHARMa"-package (Hartig, 2024; version 0.4.6). Pairwise comparison of different choices between the cues was conducted using the "emmeans" package (Lenth, 2024; version 1.10.3; adjust = "sidak").

For the visualization of optimism levels, choice scores were calculated from the responses of the rats towards these ambiguous cues based on the following equation:

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

353 Choice scores could range from values between -1 and +1, with lower values indicating more
354 pessimistic choices and higher values indicating more optimistic choices (Bračić et al., 2022;
355 Krakenberg, von Kortzfleisch et al., 2019; Krakenberg, Woigk, et al., 2019; Papciak et al., 2013;
356 Rygula et al., 2013).

357 **Differences in optimism levels within and across life phases**

358 In the next step, we assessed general differences in optimism levels within and across life
359 phases.
360 For the analysis, we divided our data into three subsets according to the three ambiguous cues
361 (NP, M and NN). We then built a GLMM for each subset using the model structure "choice ~ test
362 repetition + (1|ID), family = binomial". Subsequently we pairwise compared the different levels
363 of the fixed effect "test repetition" using the "emmeans" package to assess differences in
364 optimism within and across life phases for each cue.

365 **Consistency of optimism within and across life phases**

366 In a second step, we analysed the consistency of optimism levels within and across the two life
367 phases. Such consistency can be estimated by calculating the repeatability (R) of behaviours.
368 The repeatability describes the proportion of total phenotypic variance explained by between-
369 individual differences for repeated measures of a behaviour in a population (Nakagawa &
370 Schielzeth, 2010). Using the package "rptr" (Stoffel et al., 2017; version 0.9.22) we calculated
371 adjusted repeatabilities (R_{adj}) that are estimated after controlling for confounding fixed effects
372 (Nakagawa & Schielzeth, 2010). For the **within-phase consistency**, we estimated the
373 repeatability of optimism levels separately for early and full adulthood, respectively. For each of
374 those two phases, we always used the respective first and second CJB test, separated by a short
375 time interval of 1.5 weeks. For the analysis of **across-phase consistency,** we assessed the
376 repeatability based on the second test of the early adulthood phase and the first test of the full

adulthood phase. This way, we compared the two tests that directly followed upon each other, but came from different life phases with an interval of 10 week in between.

Again, subsets of the data were used in the same way as before. The model response was therefore also the “choice” (optimistic choice = 1; pessimistic choice = 0) and the datatype set as "binary". “Test repetition” was added as a fixed effect to control for the test session and the ID included as a random effect. To calculate the confidence intervals (CIs), and thus the uncertainty of the repeatability estimates, we used parametric bootstrapping (n = 1000, confidence level = 95%). The statistical significance of repeatability estimates was tested by likelihood ratio tests. We also assessed whether the repeatability of optimism levels differed significantly between life phases as well as between within- and across-phase repeatability using the bootstrapped samples of the different repeatability estimates. The asymptotic two-tailed p-value was calculated as twice the proportion of samples where the difference (e.g., $R_{EA} – R_{FA}$) was smaller (or greater) than zero, based on the direction of the average difference. However, there were overall no significant differences (see Supplementary Table S2).

Comparison of within- and between-individual variation

We were also interested in understanding how the between-individual differences in optimism levels compare to within-individual variation. Therefore, we further quantified and compared the variance explained by these sources of variation. From the repeatability models we extracted not only the adjusted repeatability (R_{adj}) for the “ID” (as the between-individual difference) but also the repeatability for the residuals (as the within-individual difference). Besides comparing variances as proportions of phenotypic variance (R_{adj}), individual variance components (i.e., the absolute estimates of variance of between-individual and within-individual differences) were also compared as described for the repeatability estimates (see above), however, without any significant differences detected (see Supplementary Table S2).

Ethical note:

All experimental procedures complied with the regulations covering animal experimentation within Germany (Animal Welfare Act) and the EU (European Communities Council DIRECTIVE 2010/63/ EU). The study was approved by the corresponding local (Gesundheits- und Veterinäramt Bielefeld, Nordrhein-Westfalen) and federal authorities (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen „LANUV NRW", reference number 81-02.04.2022.A101).

Throughout the whole experiment and beyond, the welfare of the animals was carefully monitored. Moreover, exclusively non-invasive methods were used and all testing was conducted during the active phase of the animals. The housing conditions of the rats consisted of spacious cages, structured with multiple levels and various enrichment items. After the experiment, the rats were either kept in our housing facility, rehomed, or transferred to a collaborating partner.

Results

Differences in choices towards the five cues in the CJB test

As a first step, we fitted a GLMM to assess how choices differed between the five cues (N, NN, M, NP, P). The analysis revealed a significant effect of "cue" on optimism levels (χ^2 = 2719.664, df = 4, p < 0.001; see Supplementary Table S3 for all effects). Subsequent pairwise comparisons showed significant differences between all cues (see Supplementary Table S4), indicating that the rats perceived all cues differently. Specifically, the positive training cue was interpreted the most optimistically with a gradual decrease in the optimism score from this cue over the NP, M and NN cue and the most pessimistic interpretation of the negative training cue (Fig. 3). Moreover, the comparably low variance in the responses towards the positive and negative reference cues indicated the overall success of the training (Fig. 3).

Differences in optimism levels within and across life phases

In a next step, we fitted GLMMs for each ambiguous cue (NN, M, NP) separately to assess differences in optimism levels between the CJB test repetitions within and across life phases. Optimism levels did not significantly change within and across life phases for the NP cue (χ^2 = 6.417, df = 3, p = 0.093) (see Supplementary Table S5 for all effects) (Fig. 3). In contrast, we found a significant effect of "test repetition" in the models of the NN (χ^2 = 9.248, df = 3, p = 0.026) and the M cues (χ^{2*} = 14.208, df = 3, p = 0.002), indicating a change of optimism levels over time. Subsequent pairwise comparisons showed significantly higher optimism levels in the first test repetition compared to the second test repetition of the EA phase for the NN (estimate = 0.727 ± 0.297, z = -2.453, p = 0.042) and the M cues (estimate = -0.942 ± 0.265, z = -0.53, p = 0.001) (Fig. 3). With respect to the across-phase comparison as well as to the within-phase comparison

during full adulthood, optimism levels did not differ significantly (see Supplementary Table S6) (Fig. 3).

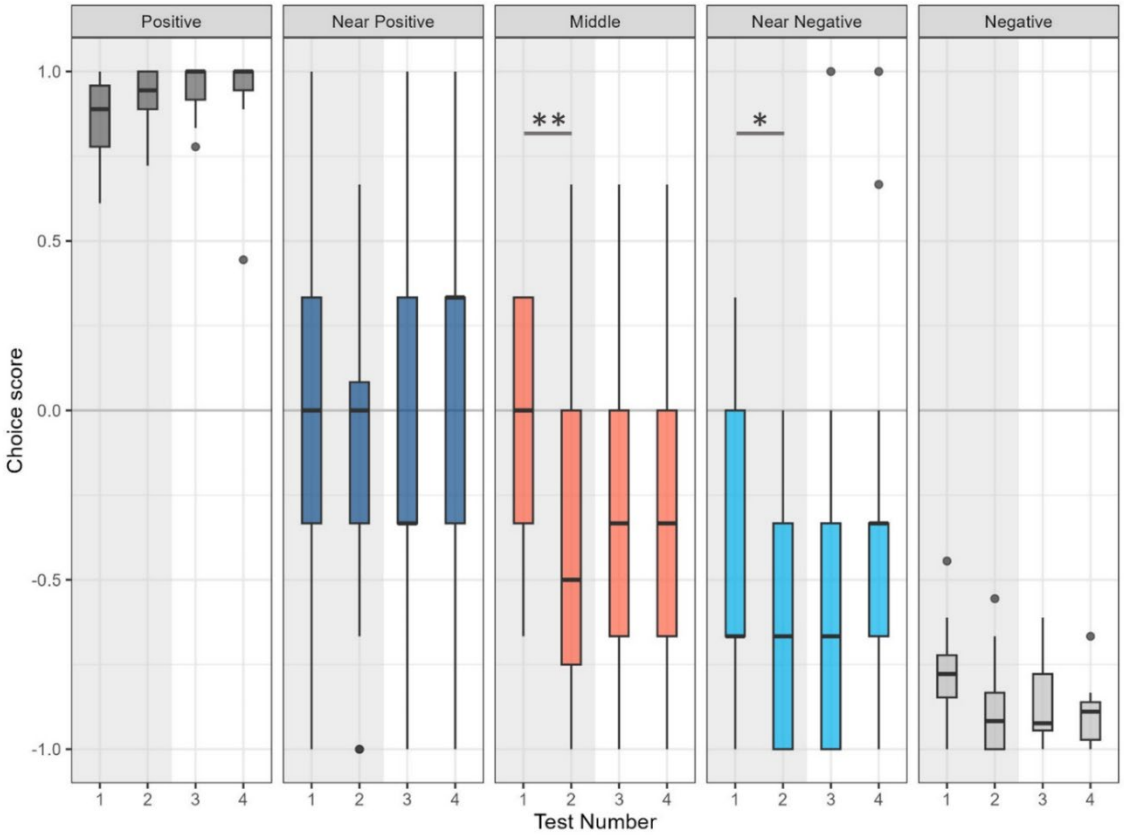

Figure 3: Choice scores over the two phases and test repetitions. Rats were tested in the CJB test for their optimism levels two times during early (indicated by the grey background) and full adulthood (indicated by the white background), respectively. The choice score ranges from +1 (most optimistic) to -1 (least optimistic). Shown are the inter-quartile ranges (shapes) with medians (horizontal lines), 1.5*inter-quartile range (whiskers) and outliers (dots). The sample size was N = 24 during early and N = 23 during full adulthood with different numbers of trials per test repetition for each of the cues ("reference cues" P and N: each 36 trials; ambiguous cues NP, M and NN: each 6 trials). GLMMs with subsequent pairwise comparisons showed significant differences between the five cues as well as significantly higher optimism levels in the first compared to the second test repetition for the M and NN cue. Significance levels as follows: * = $p \leq 0.05$, ** = $p \leq 0.01$, ***= $p \leq 0.001$.

Consistency of optimism within and across life phases

Next, repeatabilities were estimated within and across the two life phases to analyse the consistencies of choices towards the ambiguous cues. Generally, repeatability estimates were highest for the NP cue and lowest for the NN cue, regardless of the phase (Fig. 4). Interestingly, within early adulthood, optimism levels were only significantly repeatable for the NP cue (NP: $R_{adj} = 0.103$, CI = [0.003, 0.208], $p = 0.001$; M: $R_{adj} = 0.041$, CI = [0, 0.118], $p = 0.085$; NN: $R_{adj} = 0.022$, CI = [0, 0.095], $p = 0.214$; Fig. 4). Across phases, optimism levels were also

significantly repeatable for the M cue (NP: R_{adj} = 0.111, CI = [0.012, 0.209], p = 0.001; M: R_{adj} = 0.063, CI = [0, 0.149], p = 0.025; NN: R_{adj} = 0.046, CI = [0, 0.123], p = 0.066; Fig. 4). Finally, within full adulthood, optimism levels were significantly repeatable for all three cues (NP: R_{adj} = 0.097, CI = [0.005, 0.19], p = 0.002; M: M: R_{adj} = 0.089, CI = [0, 0.195], p = 0.003; NN: R_{adj} = 0.085, CI = [0, 0.183], p =0.004), indicating consistent interpretations of all these cues (Fig. 4).

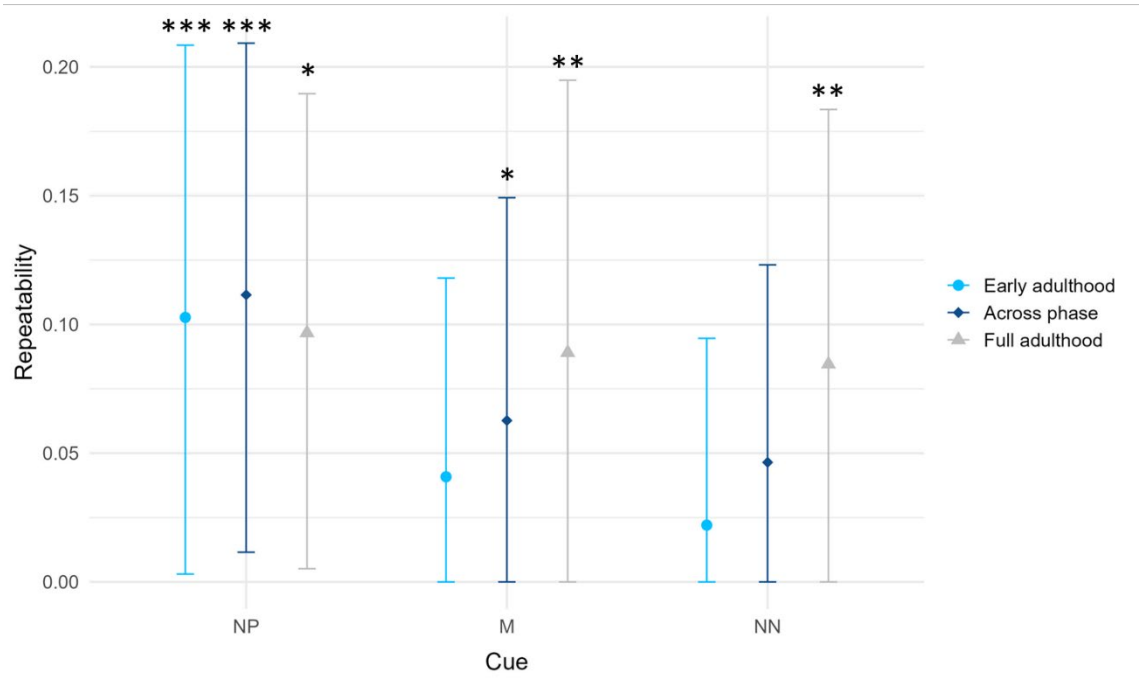

Figure 4: Adjusted repeatability estimates of optimism levels within and across the two life phases. Rats were tested for their optimism levels twice in early and full adulthood, respectively. Shown are adjusted repeatabilites after accounting for fixed effects. The shapes show the repeatability estimates based on the choices the rats made towards the three ambiguous cues during two tests in each phase, lines represent the 95 % confidence intervals. Significance levels as follows: * = p ≤ 0.05, ** = p ≤ 0.01, ***= p ≤ 0.001.

Comparison of within- and between-individual variation

For a more detailed analysis of variance, we also compared the individual variance components for between-individual and within-individual differences. This way, we could compare the amount of variation attributed to consistent individual differences with the variation attributed to changes in individual optimism levels over the repeated measurements. Strikingly, with 89-98% over all cues and phases, the relative amount of variation described by differences within

individuals was substantially higher than the amount of variation attributed to differences between individuals with only 2-11 %, indicating highly flexible decision-making (Fig. 5). Comparing the cues with each other, the rats' responses towards the NP cue always showed the highest amount of variation regarding differences between individuals (Fig. 5).

Additionally, we also compared the absolute values of the individual variance components (i.e., within- and between-individual differences), referring to the raw variance without relating it to the total amount of variation. Descriptively, the absolute values for within-individual differences did not change across cues and phases, except for the NN cue during early adulthood and across phases. Here, within-individual variation was considerably higher than for other cues and phases. Interestingly, at the same time variation attributed to between-individual differences was lowest for the NN and M cue during early adulthood compared to other cues and phases (Tab. 1), thereby also influencing the repeatability estimates.

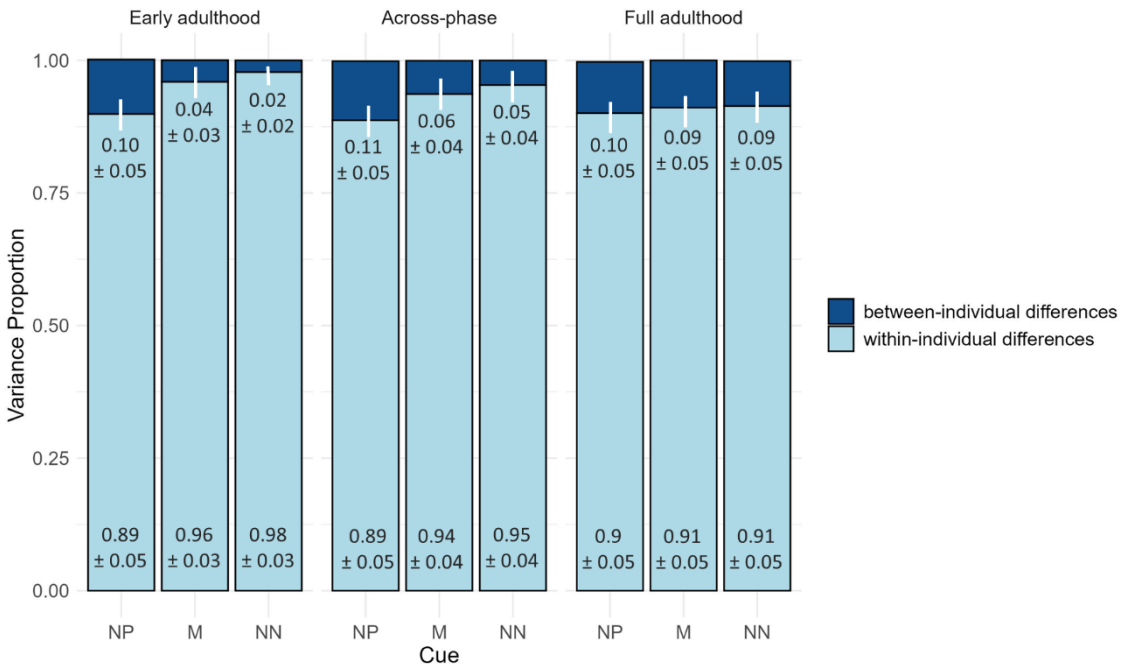

Figure 5: Variance proportions of between- and within-individual differences in optimism levels. Variance decomposition showed that the majority of variance in optimism levels within- and across phases could be attributed to within-individual differences.

Table 1: Repeatability and individual variance components of optimism levels within and across two different life phases. Overview of adjusted repeatabilities (R_{adj}) including confidence intervals (CI) and individual variance components (between-individual variance, within-individual variance) for the three ambiguous cues in early and full adulthood. The sample size for early adulthood was N = 24 and for full adulthood N = 23. Significant repeatabilities are highlighted in bold.

		R_{adj} [CI]	Between-individual variance	Within-individual variance
Early adulthood	NP	**0.103** [0.003, 0.208]	0.452	4.023
	M	0.041 [0, 0.118]	0.184	4.372
	NN	0.022 [0, 0.095]	0.129	5.722
Full adulthood	NP	**0.097** [0.005, 0.19]	0.442	4.003
	M	**0.089** [0, 0.195]	0.445	4.551
	NN	**0.085** [0, 0.183]	0.522	4.554
Across-phase	NP	**0.111** [0.012, 0.209]	0.517	4.066
	M	**0.063** [0, 0.149]	0.323	4.781
	NN	0.046 [0, 0.123]	0.335	6.856

Discussion

Using laboratory rats, we aimed at assessing the consistency of optimism levels within and across early and full adulthood. We therefore conducted a cognitive judgement bias test twice during each of these life phases and analysed within- and across-phase repeatabilities. In early adulthood, responses towards the majority of ambiguous cues, including the most ambiguous middle cue, were not consistent. Across phases, however, optimism levels consolidated to a certain degree until full adulthood, where significantly repeatable optimism levels were found for all three ambiguous cues. Still, between-individual variation was rather low, indicating that the judgements of the rats remained highly flexible with a large amount of within-individual variation. Moreover, the rats showed a "pessimism shift" in early adulthood as they were significantly more optimistic during the first test than in the second one. Overall, our results hint at a consolidation process of optimism levels during adulthood rather than optimism levels being consistent within specific life phases.

Optimism levels consolidate over adulthood

Within early adulthood, optimism levels, as derived from all three ambiguous cues, were significantly repeatable only for the near positive cue. Across phases, optimism levels were already significantly repeatable for both the near positive and the middle cue. Finally, within full adulthood, responses towards all three ambiguous cues were significantly repeatable, covering also the near negative cue. Especially when focusing on the most ambiguous middle cue, these results indicate that optimism levels become more consistent over adulthood and remain consistent for a time interval of up to 10 weeks. This not only contradicts our hypothesis, but also indicates that – in contrast to what has been reported before for the majority of studies (Cabrera et al., 2021) – personality traits not necessarily tend to be more consistent within than between life phases. Please note, however, that in contrast to previously studied developmental

life phases there is no clear definition of “early” and “full adulthood”, making it difficult to simply contrast within and between phase consistency without considering further study details. However, when interpreting the findings on the basis of the much longer time interval between than within the two phases, our results also stand in contrast to previous studies showing that repeatability estimates tend to decrease with increasing intervals between measurements (Bell et al., 2009). Findings on behavioural consistencies may thus depend on both the specific life phase as well as the time intervals between the repeated measurements with no universally valid pattern explaining high or low repeatability estimates, respectively.

Interestingly, however, our findings align with research on human personality, where behavioural traits are known to gradually consolidate with increasing age (Roberts & DelVecchio, 2000). Specifically, our results indicate that optimism levels in rats, similar to personality traits in humans, may become more consistent with age, as theoretical considerations suggest for animals as well (Stamps & Krishnan, 2014). Likewise, a meta-analysis on the consistency of personality traits in domestic dogs indicated a high variability in puppies but stabilization of personality traits in adulthood (Fratkin et al., 2013).

Compared to other studies on repeatabilities of optimism levels, the overall repeatability estimates seemed to be rather low, with the highest estimates being $R_{adj} = 0.11$. By contrast, a previous study in mice reported repeatability estimates of $R_{adj} = 0.3$ and $R_{adj} = 0.23$ for the NP and M cue, respectively (Bračić et al., 2022). However, we used a binomial dataset for the data analysis, as the rats had only two possible choices, an optimistic or a pessimistic response. With behavioural tests like this, it may be more unlikely to yield high repeatability estimates than with continuous data. Moreover, confidence intervals tend to be rather large in binomial datasets due to the limited amount of information (Nakagawa & Schielzeth, 2010) which sometimes led to the inclusion of zero in our results although the repeatability estimate was significant. Therefore, the results, although significant, have to be interpreted with caution.

Despite being consistent, optimism levels retain a high degree of flexibility

While we found overall repeatable optimism levels with increasing age, a closer examination of the variance composition showed that optimism levels only consolidated to a certain degree: With only up to 11% of the observed variation being attributed to between-individual differences, it is striking that most of the variance in optimism levels was explained by within-individual differences. Although residual within-individual variation often represents the largest proportion of the total variation for labile traits, it typically accounts for only up to 60% (reviewed by Westneat et al., 2015). Thus, with approximately 90% of within-individual variation, our results indicate that individuals – despite their overall tendencies to choose consistently more optimistically or pessimistically – remained highly flexible in their judgements. When discussing these results from an evolutionary perspective, it has originally been suggested that selection should favour behavioural plasticity over consistency in certain changing environments (Dall et al., 2004; Sih et al., 2004). From this point of view, individuals remaining flexible in their judgements would be better adapted to react to environmental heterogeneity, such as changing predator density or varying resource availability. However, with the emergence of animal personality research, this viewpoint has been challenged (Dall et al., 2004; Sih et al., 2004). For example, it has been argued that individuals showing behavioural consistency may be favoured by conspecifics during mate choice because of a higher genetic quality. More precisely, it has been argued that if expressing a particular personality trait comes with certain costs, it is more difficult for individuals of poor genetic quality to bear these costs, leading to less consistent behaviour in these individuals. Consequently, individuals showing more consistent behaviour are supposed to have a higher genetic quality (Schuett et al., 2011; Kight et al., 2013). Moreover, a higher predictability derived from behavioural consistencies may be beneficial for social interaction partners whenever behaviours have to be coordinated, for example during cooperative foraging or predator avoidance (Kight et al., 2013; Dall et al., 2004;

Wolf et al., 2010). Since different behaviours are thought to be shaped by distinct selection pressures favouring either consistency or flexibility (Kight et al., 2013), the implications for optimistic and pessimistic decision-making within this framework remain to be clarified (but for more theoretical considerations see Siewert et al., accepted manuscript).

Rats were more optimistic at the onset of early adulthood

Besides investigating the individual consistency of optimism levels, we also analysed the data on the group level. First of all, we observed gradually decreasing optimism levels from the positive training cue (P), over the three ambiguous cues (i.e., near positive (NP), middle (M) and near negative (NN) cues) to the negative training cue (N). Such a gradual response curve has been considered important to confirm that the rats perceived the ambiguous cues in reference to the learned training cues and not merely experienced them as novel (Gygax, 2014; Hintze et al., 2018).

Next, we analysed differences in optimism levels over time, comparing the four test repetitions. The rats were significantly more optimistic when confronted with the M and NN cues during the very first test repetition in early adulthood compared to the following test repetition. Consequently, optimism levels differed neither across phases nor within full adulthood, with the rats continuing to choose more pessimistically. Interestingly, Hodges and colleagues (2022) found similar patterns: They compared three differently aged rat groups in their cognitive bias by training the animals to associate a negative condition with foot shocks and measuring freezing responses to an ambiguous condition. Following this procedure, they found “young” (PND 100) and “middle-aged” adults (PND 210) to display a more pessimistic cognitive bias than “adolescent” rats (PND 40), a finding that was mostly explained by immature risk assessment during adolescence (Hodges et al., 2022; Rodham et al., 2006). In our study, the age range of the rats during early adulthood was just in between the “adolescents” and “young adults” of the

588 Hodges study. The observed “pessimism shift” from test repetition 1 to 2 might therefore reflect
589 a similar maturation process that takes place sometime during early adulthood.
590 However, as we did not use a negative reinforcer that would have posed a real threat to our
591 animals, the rats might indeed have been more optimistic at the beginning, rather than
592 displaying any kind of immature risk assessment during adolescence or early adulthood.
593 An alternative explanation could be that the novelty of the test leads to different reactions
594 towards ambiguous cues during the first compared to the following test repetitions. In fact, loss
595 of ambiguity with repeated testing has been extensively discussed before (Roelofs et al., 2016).
596 The idea here is that, as animals are repeatedly presented with ambiguous cues, they might
597 learn about the outcome of these trials (i.e. receiving no reward), influencing choices in
598 subsequent trials and confounding the observed effects (Doyle et al., 2010). However, to
599 counteract, we already included a training step in which some of the training trials were
600 randomly not rewarded to accustom the rats to this outcome during ambiguous trials.

601 **The role of cue ambiguity for the interpretation of optimism levels**

602 Comparing the responses towards the three ambiguous cues revealed clear differences in the
603 degree of consistency depending on the cue. More specifically, the rats responded most
604 consistently towards the NP cue both within and across life phases, while responses towards the
605 NN cue were observed to be least consistent. Interestingly, a similar pattern was reported for
606 mice (Bračić et al. 2022), with highest repeatability estimates for the NP cue and lowest for the
607 NN cue. In the mouse study, the lower response accuracy could be explained by differences in
608 the animals’ perception of the visual cues that were employed. Here, we ruled out such
609 perceptual inconsistencies by counterbalancing rough and fine sandpaper for negative and
610 positive trials.
611 Alternatively, the underlying cause of this pattern might be found in the variance composition
612 of optimism levels, directly influencing the repeatability estimates. When analysing the absolute

variance components, it is striking that the variance attributed to within-individual differences was equally large overall, except for higher values for the NN cue in early adulthood and across phases. In addition, the variance explained by between-individual differences was smaller for the M and NN cue compared to the NP cue in early adulthood. Together, this indicates that the different ambiguous situations were evaluated differently with respect to outcome probability and payoff (Bateson, 2016; Mendl et al., 2009). More specifically, when confronted with the NN and M cue, the rats might have perceived the probability of receiving a large reward to be low and the cost of retrieving no reward to be high, therefore responding rather pessimistically throughout testing. In contrast, higher between-individual variation in responses towards the NP cue indicate greater differences in the rats' judgements. While some individuals interpreted the NP cue more pessimistically, others gave a higher value to obtaining a large reward, interpreting the cue in resemblance to the positive reference cue.

Besides the differential consistency in responses towards the three cues, the observed "pessimism shift" was also only shown in responses towards the NN and M but not the NP cues. Interestingly, a meta-analysis on CJB in animals revealed that M and NN cues were most likely to show effects on CJB after non-pharmacological affect manipulations (Lagisz et al., 2020). Together, this might indicate that responses towards the NP cue are generally less susceptible for both acute affect manipulations and enduring developmental changes.

Conclusion

In the present study, we systematically investigated within- and across-phase consistency of rats' optimism levels during early and full adulthood. In line with previous studies, we observed significant repeatability of optimism levels, further supporting the assumption that optimism and pessimism might not only indicate emotional states but also cover a trait dimension. Furthermore, our results show that - similar to findings from human personality research - optimism levels consolidated over adulthood, supporting the theory that behavioural

638 phenotypes may become relatively “fixed” after being acquired at an early developmental stage
639 (Kight et al., 2013). Still, the rats showed remarkable flexibility in their decision-making, pointing
640 towards a certain degree of behavioural plasticity in optimism levels.
641 Considering possible implications of our results, we highlight the importance of taking the life
642 phase into account when testing for temporal consistency of behavioural traits as these might
643 consolidate only later in life. Finally, we encourage future research on the ecological relevance
644 of consistent versus flexible decision-making. In particular, we consider it important to identify
645 factors influencing the trait consolidation process of optimism and pessimism during adulthood,
646 including genetic, environmental, and social variables, as well as to test for the generality of
647 these patterns across traits and/or species.
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Author statement:

S.H.R. conceived the study. S.H.R., V.S. and L.B. designed the experiments. S.H.R. and S.K. supervised the project. L.B. carried out the experiments. L.B. and C.M. conducted the statistical analysis of the data. L.B. wrote the initial draft of the manuscript. All authors critically revised the manuscript and gave final approval for publication.

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Declaration of competing interest:

The authors declare to have no competing interests.

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Supplementary material

Table S1: Akaike information criterion (AIC) comparison for model simplification. Comparison of generalized linear mixed models (GLMMs) fitted with different (A) random and (B) fixed effect structures. In bold the best model according to the lowest AIC.

A) AIC comparison of random effect structures, in bold best model.

Model no.	Fixed effect structure	random effect	df	AIC
1	cue + test repetition + batch	**ID**	**11**	**1734.949**
2	cue + test repetition + batch	age	12	1743.317
3	cue + test repetition + batch	ID +age	12	1736.949

B) AIC comparison of fixed effect structures, in bold best model.

Model no.	Fixed effect structure	random effect	df	AIC
1	**cue + test repetition**	**ID**	**7**	**1732.618**
2	cue + test repetition + batch	ID	9	1734.949

Table S2: Comparison of repeatability and variance components within and across phases. Differences in repeatability as well as differences in absolute between- and within-individual variance between life phases were estimated using Monte Carlo simulations.

Comparison		R_{adj}	Between-individual variance	Within-individual variance
EA vs. FA	NP	p = 0.98	p = 0.994	p = 0.798
	M	p = 0.51	p = 0.394	p = 0.576
	NN	p = 0.294	p = 0.314	p = 0.784
EA vs. across-phase	NP	p = 0.894	p = 0.866	p = 0.724
	M	p = 0.724	p = 0.646	p = 0.244
	NN	p = 0.676	p = 0.574	p = 0.21
FA vs. across-phase	NP	p = 0.868	p = 0.892	p = 0.536
	M	p = 0.748	p = 0.696	p = 0.608
	NN	p = 0.524	p = 0.674	p = 0.212

Table S3: Model summary for the final GLMM. Shown are the results for the fitted GLMM, modelled as a binary response variable (choice ~ cue + test repetition + (1|ID), family = binomial).

	Estimate	SE	z	p
(Intercept)	3.438	0.120	28.542	<0.001
test2	-0.374	0.106	-3.527	<0.001
test3	-0.195	0.106	-1.836	0.066
test4	-0.157	0.106	-1.486	0.137
cueNP	-3.277	0.124	-26.408	<0.001
cueM	-3.910	0.128	-30.582	<0.001
cueNN	-4.447	0.135	-32.826	<0.001
cueN	-5.893	0.115	-51.421	0

Table S4: Pairwise comparisons of optimism levels for different cues. Optimism levels between the different cues were compared using the "emmeans" package.

Comparison	Estimate	SE	z	p
P vs. NP	3.278	0.124	26.408	<0.001
P vs. M	3.91	0.128	30.582	<0.001
P vs. NN	4.447	0.135	32.826	<0.001
P vs. N	5.893	0.115	51.421	<0.001
NP vs. M	0.632	0.124	5.119	<0.001
NP vs. NN	1.17	0.131	8.907	<0.001
NP vs. N	2.615	0.109	23.933	<0.001
M vs. NN	0.537	0.134	3.996	<0.001
M vs. NN	1.982	0.113	17.566	<0.001
NN vs. N	1.445	0.121	11.927	<0.001

Table S5: Model summary for the GLMMs of the ambiguous cues. To examine differences between test repetitions, data subsets for each of the three ambiguous cues were created (choice ~ test repetition + (1|ID), family = binomial).

NP				
	Estimate	**SE**	**z**	**p**
(Intercept)	0.023	0.216	0.108	0.914
test2	-0.273	0.246	-1.107	0.268
test3	-0.193	0.249	-0.773	0.439
test4	0.314	0.251	1.253	0.210
M				
	Estimate	**SE**	**z**	**p**
(Intercept)	-0.162	0.207	-0.781	0.435
test2	-0.941	0.265	-3.558	<0.001
test3	-0.562	0.256	-2.197	0.028
test4	-0.702	0.259	-2.711	0.007
NN				
	Estimate	**SE**	**z**	**p**
(Intercept)	-0.996	0.228	-4.361	<0.001
test2	-0.727	0.297	-2.453	0.014
test3	-0.497	0.288	-1.723	0.085
test4	0.016	0.273	0.058	0.954

Table S6: Pairwise comparisons of optimism levels in different tests. Optimism levels between the two tests in early and full adulthood as well as across phases were compared using the "emmeans" package.

Comparison		**Estimate**	**SE**	**z**	**p**
Early adulthood	NN	0.727	0.297	-2.453	0.042*
	M	-0.942	0.265	-0.53	0.001*
Full adulthood	NN	0.513	0.292	1.757	0.218
	M	-0.14	0.264	-0.53	0.934
Across-phase	NN	0.23	0.314	0.735	0.844
	M	0.379	0.27	1.403	0.408

Table S7: Comparison of variance proportions with confidence intervals [CI] explained by test repetition. Differences were estimated by Monte Carlo simulations. Sample sizes were N = 24 in the EA and N = 23 in the FA phase.

Cue		R^2	CI
Early adulthood	NP	0.009	[0, 0.045]
	M	0.044	[0.009, 0.103]
Full adulthood	NN	0.021	[0.001, 0.073]
	NP	0.014	[0, 0.053]
	M	0.001	[0, 0.023]
Across-phase	NN	0.013	[0, 0.05]
	NP	0.002	[0, 0.028]
	M	0.008	[0, 0.039]
	NN	0.001	[0, 0.021]
Comparison		**p**	
EA vs. FA	NP	0.784	
	M	0.034*	
	NN	0.734	
EA vs. across-phase	NP	0.64	
	M	0.138	
	NN	0.216	
FA vs. across-phase	NP	0.402	
	M	0.592	
	NN	0.386	