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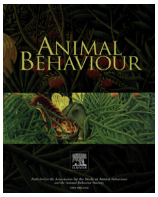

What it is like to be an optimist: temporal stability of cognitive bias in rats and its link to other individual traits

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Originating from human psychology, the concept of optimism/pessimism has become increasingly popular in animal welfare science. Typically, so-called judgement bias tests are used to study optimism levels in response to different affect manipulations. Recently, however, evidence is growing that judgement biases not only reflect affective states but might also cover a trait dimension meaning that the optimism level of an individual is stable over time. Contributing to this emerging field of research, we here aimed to comprehensively characterize optimistic and pessimistic rats by repeatedly testing a cohort of Lister Hooded rats in a tactile judgement bias test. To investigate potential links to other behavioural traits, we also assessed the rats' anxiety-like and exploratory behaviour, their laterality and their vocal response to human-induced play behaviour. Furthermore, we studied concentrations of basal faecal corticosterone metabolites to record hypothalamic–pituitary–adrenocortical activity. Therefore, we took all measures twice to assess the stability over time. In contrast to previous studies, we did not find optimism levels to be temporally stable in the present study, encouraging further research on potential factors that might affect or modulate the stability of optimism levels within and across different life phases. Furthermore, we did not detect suites of correlated behaviours, calling for more research on the impact of context and/or species on the emergence of such links. Nevertheless, our results contribute to the accumulating evidence that certain interindividual differences in behaviour, such as exploratory locomotion, vocalizations, anxiety-like behaviour and laterality as well as hypothalamic–pituitary–adrenocortical activity, are indeed stable across time and hence can be considered aspects of an animal's personality.

Originating from human psychology (Mathews & MacLeod, 1994), the concept of optimism/pessimism has become increasingly popular in animal welfare science. In general, optimism/pessimism is defined as a tendency to judge an uncertain and ambiguous situation in a more positive or more negative way, thereby creating a continuum with ‘optimism’ and ‘pessimism’ on opposing ends (Hecht, 2013; Houston et al., 2012). Importantly, in contrast to research in humans, the use of this concept in animal-based research does not imply a conscious component of these states and may involve a variety of cognitive processes (Mendl et al., 2009). For ease of reading, we will refer to any individual score on this optimism/pessimism continuum as ‘optimism level’ hereafter.

In animal welfare research, such optimistic or pessimistic judgements are used to investigate affective states in animals (Harding et al., 2004; Paul et al., 2005). It was shown that animals in a positive affective state make more optimistic judgements compared with animals in a more negative affective state that tend to make more pessimistic decisions (Lagisz et al., 2020; Neville et al., 2020). Specifically, so-called cognitive judgement bias tests are widely utilized to study the effects of varying factors on decision-making under ambiguity (Bethell, 2015). Therefore, the animals are trained to respond in different ways to a positive and a negative condition (e.g. pressing a lever on the left to get a food reward, when tone A is played and pressing a lever on the right to avoid punishment, when tone C is played). Then, an uncertain situation is modelled by presenting an ambiguous cue (tone B), and the animals' response reveals their anticipation of a positive or negative outcome based on the two

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learned reference conditions (in this example a left lever press would resemble an optimistic choice and a right lever press would constitute a pessimistic choice). Hence, differences in optimism levels are usually interpreted to reflect a higher expectation of reward or a lower expectation of punishment (Bateson, 2016). Please note that, however, individual differences might also be affected by the individual's sensitivity to the used reward and/or punishment. Using these tests, it was for instance shown that the provision of enrichment caused rats, *Rattus norvegicus* f. *domestica*, to judge an ambiguous cue more optimistically (Brydges et al., 2011; Richter et al., 2012), whereas the removal of enrichment triggered the opposite (more pessimistic) response (Bateson & Matheson, 2007; Burman et al., 2009). Recently, however, evidence is growing that judgement biases not only can inform about the animals' affective states but might also have components of enduring traits, as individual optimism levels have been found to be stable over time (Lecorps et al., 2018; Rafa et al., 2016; Rygula et al., 2013, 2015).

In the literature, stable interindividual differences across time and/or context are often referred to as animal personality (Kaiser & Müller, 2021; Stamps & Groothuis, 2010). Interestingly, animal personalities have already been described in many different taxa, ranging from primates to insects (Gosling, 2001), and concerning many different measurements. For example, stable interindividual differences were found with respect to not only behavioural measures, such as exploration and vocalization (Mällo et al., 2007; Réale et al., 2007), but also concerning endocrinological measures, for example glucocorticoid levels (Fanson & Biro, 2019; Taff et al., 2018). According to Kaiser and Müller (2021), an animal's personality is composed of different personality traits, like for instance 'boldness', that can be reflected by multiple behaviours. From the perspective of behavioural ecology, the emergence of such personality traits is particularly interesting, as they indicate reduced behavioural plasticity. This challenges the idea that behavioural variation is centred around an optimum that is shaped by natural selection, calling for research on how animal personalities could have evolved and how they are maintained within a population (Sih et al., 2004).

Among various hypotheses that are proposed in this regard (e.g. reviewed in Bergmüller & Taborsky, 2010; Wolf & Weissing, 2010, 2012), it is, for example, suggested that constant adaptation to changeable conditions might be too costly. Instead, stable behavioural phenotypes are favoured that allow appropriate responses on an average level, leading to similar fitness effects for each of the different phenotypes (Wolf & Weissing, 2010). In this regard, the existence of stable individual optimism levels is especially interesting, as low optimism levels are commonly associated with negative consequences, like a higher vulnerability to stressful situations (Lecorps et al., 2021) and a higher risk for psychopathology (Mathews & MacLeod, 2002, 2005). High optimism levels, in contrast, have been linked to positive consequences, such as better immune responsiveness and a longer life span (Carver et al., 2010; Lee et al., 2019). However, from the perspective of behavioural ecology, an adaptive value is suggested for both high and low optimism levels, depending on the context (Bateson, 2016; Siewert et al., 2024). For instance, during foraging in a safe environment, a more optimistic approach could be advantageous, as this behavioural response (i.e. staying instead of fleeing in an uncertain situation) is likely to increase foraging success and reduce energy costs. In an environment with a high predator density, in contrast, a pessimistic approach might be crucial to ensure survival (Bračić et al., 2022). Therefore, the existence of temporally stable differences in optimism levels covering the whole continuum (for a review see Lecorps et al., 2021) constitutes a fascinating topic for the study of animal personality.

Besides investigating single personality traits, the focus of research is also on identifying potential links between different personality traits. In this respect, a very early study showed, for instance that aggressiveness is positively correlated to boldness in sticklebacks (Huntingford, 1976). Likewise, it has been shown that boldness and aggression are linked to a certain coping style, with proactive animals being more aggressive and bold than reactive ones (Koolhaas et al., 1999). With respect to optimism, however, far less is known about potential links to other traits. Theoretically, one could argue that optimism might be associated with traits, such as fearfulness, exploration, lateralized behaviour and/or vocalization. More specifically, although the link to fearfulness might root in a tendency of optimistic individuals to expect positive outcomes (Bateson, 2016) and, hence, perceive uncertain and ambiguous situations as less threatening, an association with exploration could be explained on the basis of an overall increased motivation to gain rewards in these individuals (Rygula et al., 2015). Moreover, the so-called 'Valence-Hypothesis' from laterality research provides a theoretical framework for linking lateralized behaviour and optimism. Specifically, this hypothesis states that the left hemisphere is specialized for processing positive and the right hemisphere for processing negative emotions (Davidson et al., 1987). Keeping in mind that there is a contralateral connection between the brain and the body, stronger activation of the left hemisphere could favour a right-side preference in more optimistic individuals. Finally, optimism might also correlate with other measures of affective states, such as, for example, different vocalization types, as higher optimism levels are widely interpreted to reflect positive affect, which is also the case for certain call types (e.g. 50 kHz vocalizations in rats, Knutson et al., 2002).

Indeed, some first studies on various species support the existence of such links. For instance, a study conducted in dairy calves found more optimistic individuals to be less fearful (Lecorps et al., 2018), and another study conducted in mice reported more optimistic individuals to be less explorative (Verjat et al., 2021). Although the former is in line with the arguments presented above, the latter stands in contrast to the theoretical considerations. To explain this opposition, the authors suggest that lower optimism levels might be accompanied by higher levels of perceived environmental uncertainty, resulting in increased exploratory behaviour. In terms of lateralized behaviour, the individual optimism level was found to be linked to handedness [a characteristic specified as a personality trait in rodents (Groneberg et al., 2025; Stieger et al., 2021)] in common marmosets, with left-handed individuals being more pessimistic than right-handed ones (Gordon & Rogers, 2015). Also, evidence for a link between individual optimism levels and certain vocalization types is already existent: Hinchcliffe et al. (2020) observed increased optimism levels in rats that were vocally more responsive to human-induced play behaviour, hinting towards an association between judgement bias and vocalization during play behaviour. In addition, Mällo et al. (2007) detected a correlation between higher rates of 50 kHz calls and less activity in an exploration task, a trait also linked to higher optimism levels in mice (Verjat et al., 2021). Moreover, since such behavioural responses often interact with supporting physiological reactions that increase the individuals' fitness (Cote et al., 2006), certain physiological markers might also be associated with optimism. In this regard, a study in chickens already found an association between optimism and corticosterone levels, the latter being a physiological marker for hypothalamic–pituitary–adrenocortical activity, with higher corticosterone concentrations being linked to more pessimistic decision-making (Iyasere et al., 2017). Likewise, a connection between corticosterone levels and coping style was found (Cockrem, 2007), which is also linked to individual optimism levels in pigs (Asher et al., 2016). Thus, while there is first evidence that such links do exist, it is less clear if they (1) evolved for adaptive reasons, (2) are caused by optimism levels being a cognitive component of already established personality traits or (3) are based on optimism levels constituting a higher-level trait under which different personality traits can be grouped (Siewert et al., 2024). To solve this evolutionary riddle, however, more comprehensive data on the temporal stability of optimism, its link to other traits as well as their underlying mechanisms are needed.

Contributing to this emerging field of research, we here aimed to investigate what it is like to be an optimist by systematically studying potential links between optimism and other personality traits. Taking into account what is already known from the literature, we repeatedly tested Lister Hooded rats regarding their behaviour and faecal corticosterone metabolites (FCMs) by conducting a battery of standardized tests. More specifically, we assessed the animals' optimism levels in a tactile judgement bias test, which is assumed to be of high ecological relevance for a nocturnal rodent species like rats and should therefore increase the construct validity of test results (Olsson et al., 2003). Furthermore, we included standardized tests commonly used to assess anxiety-like and exploratory behaviour in rodents. Although originally validated for measuring affective states (Pellow et al., 1985; Treit & Fundytus, 1988), these tests also find increasing application in animal personality research, where they are conducted repeatedly to assess temporal stability of behavioural measurements (for a recent discussion, see Améndola et al., 2022). In addition, we included a test for laterality, analysed the vocal response to human-induced play behaviour and determined FCMs, which reflect hypothalamic–pituitary–adrenocortical activity (Palme, 2019). In line with the literature, we hypothesized rats display repeatable individual differences in behavioural parameters as well as FCMs. Furthermore, we hypothesize optimism levels to correlate negatively with measures for anxiety-like and exploratory behaviour, the rate of 22 kHz calls and corticosterone concentrations. Moreover, we hypothesize optimism levels to correlate positively with the degree of being right-side lateralized and the rate of 50 kHz calls.

METHODS

Animals and Housing Conditions

The study included 12 intact female and 12 intact male Lister Black Hooded rats, ordered from Charles River Laboratories (Research Models Services, Sulzfeld, Germany) at postnatal day (PND) 28. Lister Black Hooded rats are an ideal model species for phenotypic characterization, as they have widely been used in behavioural studies (e.g. Finlayson et al., 2016). All animals could be identified by their individual fur pattern. Hence, no further marking was necessary. The rats were delivered successively in three batches, with four females and four males per batch. Animals of each batch were housed in same-sex groups of four individuals with olfactory, acoustic and visual contact to the other sex. The cages (Furat, Ferplast, Italy; 48 cm × 78 cm and 70 cm high) contained wood shavings as bedding material (TierWohl Super, J. Rettenmaier and Söhne, Rosenberg, Germany), two additional floors, paper tissues as nesting material, four wooden cubes, a cardboard tunnel, two hanging houses (Sputnik, SAVIC, Belgium; 29 cm × 26 cm and 19 cm high), a semitransparent red plastic house (ZOONLAB Animal Husbandry Experts, Germany; 20.5 cm × 15.7 cm and 11.5 cm high) and a semitransparent red plastic tunnel (ZOONLAB Animal Husbandry Experts, Germany; 15 cm × 9 cm and 9.5 cm high). Thereby, the housing conditions exceeded the standard housing conditions for laboratory rats regarding floor space (approximately two times a standard Makrolon Typ IV cage) and a number of enrichment items (Supplementary Material). Water and food (Altromin 1324, Altromin Spezialfutter KG, Lage, Germany) were offered ad libitum, except during specific phases of the experiment that required a restricted feeding regime (for details see below). The housing room was maintained at a reversed light:dark cycle with lights off at 0900 hours, a temperature of ~22 °C and a relative humidity of about 50%.

Experimental Design

The experiment consisted of two characterization phases with a break of 25 days in between. Tests within a characterization phase were closer together in time than tests across the two characterization phases, thereby creating two distinct phases, with a break in between that covers a time span recommended for assessing stability of behaviour and personality (Stamps & Groothuis, 2010). In each of the two characterization phases, the same behavioural and FCM measurements were taken (Fig. 1). Before the start of the first characterization phase, a 5 week-long acclimatization phase to the laboratory conditions and 1 week of habituating the animals to the daily handling and weighing by the experimenter were conducted. Rats were exclusively cup-handled at all times from their arrival until the end of the experiment. Subsequently, the training phase for the Cognitive judgement bias test (CJBT) began (PND: 69). In order to investigate the link of optimism to other personality traits, all animals were tested in the CJBT (PND 91–93) first. Afterwards, the Elevated plus maze (EPM; PND 97), the Open field combined with a Novel object test (OF + NOT; PND 98), the collection of faeces for a FCM analysis (PND 99), a Food reaching test (FRT; PND 101) and a Play test (PT; PND 107) were conducted (for details regarding the different test procedures, see below). In order to determine stability over time, a second characterization phase was performed after a break of 25 days, starting on PND 133. Included was the repetition of the abovementioned tests (CJBT: PND 133–135, EPM: PND 139, OF + NOT: PND 140, FCM: PND 141, FRT: PND 142, PT: PND 143). The reminder training sessions for the CJBT were already performed from PND 126 onwards. The slight difference in duration between characterization phase 1 (17 days) and characterization phase 2 (11 days) was caused by 1 day of habituation for the FRT and 3 days of habituation required for the PT before the very first performance of these tests.

Food Restriction

With the start of the CJBT training until the end of the CJBT, the rats were mildly food-restricted in a way that they maintained 90%–95% of their highest weight measured during the week of handling or the first day of CJBT training. Such a restricted diet is often applied during CJBT training to increase the animals' motivation to gain food rewards (e.g. Krakenberg et al., 2019). To avoid large bodyweight fluctuations, each cage received an individually adjusted amount of food, based on the individuals' current percentage bodyweights that were calculated by daily weighing in the morning (PCE-BT 2000, PCE Deutschland, Meschede, Germany; weighing capacity: 2100 g, resolution: 0.01 g). If individuals from one cage differed a lot in their percentage bodyweights, the amount of food was adjusted in a way that the individual with the lowest percentage bodyweight did not weigh less than 90%–95% of its starting weight. The feeding was carried out after all individuals finished their daily CJBT training so that the period without food was never more than 24 h.

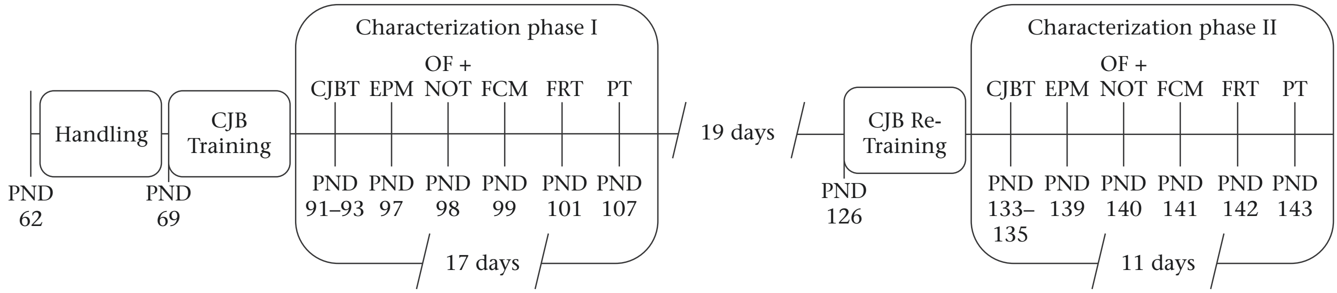

Figure 1. Experimental design. After the rats were habituated to handling (PND 62-68), they were trained for the cognitive judgement bias test (CJBT; start: PND 69). Then, the first characterization phase was performed (PND 91-107), including the CJBT, the Elevated plus maze (EPM), the Open field and Novel object test combination (OF + NOT), the faecal corticosterone metabolite (FCM) collection, the Food reaching test (FRT) and the Play test (PT). After a break of 19 days CJBT retraining began (start: PND 126), and subsequently, the second characterization phase was performed (PND 133-143). Due to habituation required for the FRT and PT, the first characterization phase is 6 days longer than the second characterization phase.

Cognitive Judgement Bias Test

To assess optimism levels, a CJBT was performed, which was adapted from the CJBT developed for rats by Brydges and Hall (2017). The test is based on the animals' learned response to two different reference cues and uses the response to an ambiguous cue that represents exactly the middle between the two learned reference cues as an outcome measurement.

Apparatus

The test apparatus was a Makrolon Type IV cage, which was divided into a start box (floor space: 33 cm × 13 cm), a middle compartment (floor space: 33 cm × 22 cm) and two (left and right) reward chambers (floor space: 16.5 cm × 19 cm; Fig. 2). Passing between compartments could be regulated with sliders (opening: 8.2 cm × 8.2 cm). The start box was closed with a lid, and all walls were extended by 15 cm to prevent the animals from jumping out of the apparatus. In each of the reward chambers, there was a food bowl (MultiFit Tiernahrungs, Krefeld, Germany; diameter: 10 cm), with a tape-shut Petri dish inside that contained a piece of the used reward (Honey Llama Loops, Kellogg Europe Trading, Dublin, Ireland), to outbalance the smell.

Training

The CJBT was performed on PND 91—93 and PND 133—135, with the initial training starting at PND 69 and the retraining starting at PND 126. The retraining was performed in order to check if the required criteria for testing were still fulfilled, which was the case for all animals. The animals were trained five times a week, starting around 1000 hours, after the daily weighing. The rats were tested under red light in a randomized order in the room next to the housing room. Each training and test session started with a 1 min habituation in the start box and had a maximum duration of 40 min but was finished earlier, as soon as the rat completed all trials required. Before the first rat as well as between rats, the apparatus was cleaned with 70% ethanol and paper tissues.

In the first step of CJBT training, the 'Habituation', the rats had 1 day to familiarize themselves with the test apparatus and the reward. The rats could freely move between all chambers of the apparatus, and in both reward chambers, one-fourth of a honey loop was presented on top of the Petri dish. Whenever the rat chose one of the reward chambers and ate the reward, it was gently guided back to the start box, so that the reward chamber could be refilled, before the rat was released again. The training was finished, when the rat consumed the reward five times in each of the reward chambers.

In the second step, the 'pretraining', the rats were supposed to associate two reference cues (coarse grit and fine grit sandpaper) with a big or a small food reward (half a honey loop versus one-sixth a honey loop) in either the left or the right reward chamber. Therefore, one sheet of sandpaper (coarse grit: klebemeister.eu, Adbeere Com Marketing Unternehmergesellschaft KG, Gerbrunn, Germany; 230 mm × 280 mm, grit: 60, waterproof; fine grit: klebemeister.eu, Adbeere Com Marketing Unternehmergesellschaft KG, Gerbrunn, Germany; 230 mm × 280 mm, grit: 1200, waterproof) was presented and fixed with magnets on the floor of the middle compartment. Another sheet was attached to a big slider (33 cm × 38 cm) that was held in the centre of the middle compartment to encourage interaction of the rat with the sandpaper. As soon as the rat touched the sandpaper on the slider with its nose or paws, the slider was removed, and the rat could access the correct reward chamber. The incorrect reward chamber was closed during this step of training, so that the rat could only make correct choices. After retrieving the reward, the rat was guided back to the start box, and the next trial was prepared before releasing the animal again. It was counterbalanced across individuals and sexes, if the coarse or the fine sandpaper was used as a cue for the positive (big reward) or negative (small reward) condition. Similar to the findings from Brydges and Hall (2017), rats tended to learn the response to the positive condition faster (i.e. in fewer trials) compared with the negative condition, indicating that they perceive a difference in reward size. To proceed to the next step, the rats had to accomplish 30 trials in total, whereas each condition was presented 15 times but never more than three times in a row. All sandpaper sheets were cleaned under running water after each training session.

The third step, called 'training I', started with four trials of 'pretraining'. In the following 30 trials, the set-up was the same as during 'pretraining'; however, this time both reward chambers were open, so that the rat could also make incorrect choices, where no food reward could be obtained. After each incorrect trial, correction trials were presented, in which the incorrectly completed trial was repeated until the rat did it correctly. To finish this step, the rats had to complete all trials with correctness of 80% in both conditions and less than 10 correction trials in total. If a rat needed 20 or more correction trials, it was returned to 'pretraining'.

For 'training II', the fourth and last step of training, the setting was as in the preceding step. After four trials of 'pretraining', 30 trials of 'training I' followed. However, this time no correction trials were conducted. Moreover, six random trials (three of each condition) were unrewarded to prepare the animals for the CJBT, which included unrewarded trials as well (see below; Brydges & Hall, 2017). This habituation to unrewarded trials was supposed to lower reward expectations of the animals in order to prevent the

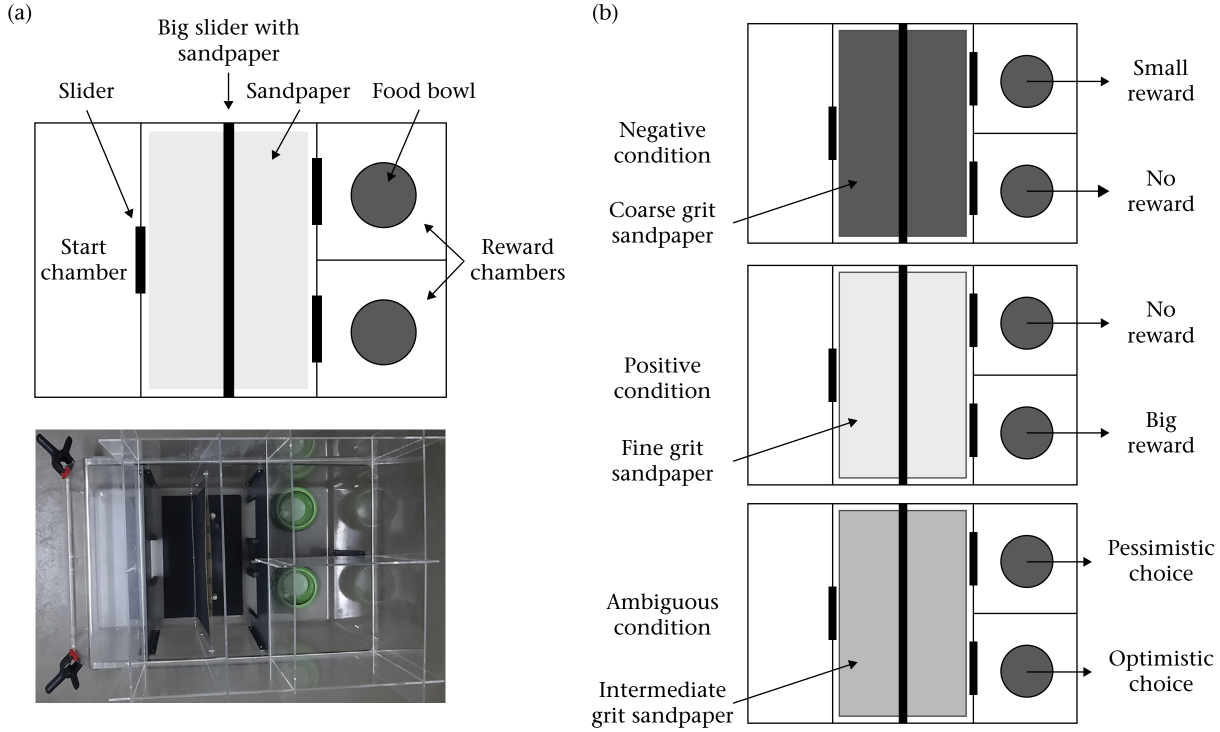

Figure 2. Cognitive judgement bias test (CJBT). (a) Schematic view (top) and a photo (bottom) of the CJBT apparatus, composed of a start box, a compartment with sandpaper on the ground and on a big slider and two reward chambers containing food bowls. All compartments are connected by small sliders. (b) Schematic view of the CJBT paradigm with a small reward in the left reward chamber for coarse grit sandpaper (negative condition) and a big reward in the right reward chamber for fine grit sandpaper (positive condition). For the intermediate grit sandpaper (ambiguous condition), a decision for the left chamber would therefore be a pessimistic choice (expectation of small reward) and a decision for the right chamber would be an optimistic choice (expectation of big reward).

loss of ambiguity across test days (for a discussion see Roelofs et al., 2016). The training never started or finished with an unrewarded trial and two unrewarded trials never followed each other directly. To be admitted to the CJBT, the rats had to complete all trials with a correctness of 80% in both conditions. If this criterion was not met, the rat was returned to ‘training I’.

The CJBT was conducted on 3 consecutive days and the setting was like during ‘training II’. However, instead of the randomly unrewarded trials, three unknown sandpapers were presented, twice each, which were also unrewarded. These unknown cues comprised one, close to the fine sandpaper (near fine; klebemeister.eu, Adbeere Com Marketing Unternehmergesellschaft KG, Gerbrunn, Germany; 230 mm × 280 mm, grit: 400, waterproof), one, close to the coarse sandpaper (near coarse; klebemeister.eu, Adbeere Com Marketing Unternehmergesellschaft KG, Gerbrunn, Germany; 230 mm × 280 mm, grit: 120, waterproof) and one intermediate (ambiguous; klebemeister.eu, Adbeere Com Marketing Unternehmergesellschaft KG, Gerbrunn, Germany; 230 mm × 280 mm, grit: 180, waterproof). Both reference cues were displayed at least once before as well as between the presentation of an unknown cue. Each unknown sandpaper followed each of the two reference cues once to control for an effect of the previously shown cue. The test was never started or finished with an unknown cue. The near-positive and near-negative cues were included to get evidence that unknown cues were rated in reference to the learned cues, which can be seen in a graded response curve (Lagisz et al., 2020). The ambiguous cue, which was shown twice per test day, was used to assess the cognitive judgement bias of the animal. If, as a response to that cue, the animal chose the side of the learned positive condition (expectation of a big reward), this choice was considered to be optimistic. If the animal chose the side of the learned negative condition (expectation of a small reward), this choice was considered to be pessimistic.

Elevated Plus Maze

The EPM is a common behaviour test for rodents that is based on their natural avoidance of open and bright spaces (Lister, 1987; Pellow et al., 1985). It is used to assess anxiety-like behaviour and exploratory locomotion (Rodgers & Johnson, 1995; Treit et al., 1993). Nowadays, the EPM also finds increasing application in animal personality studies, where the temporal stability of different behaviours measured in the EPM is assessed by repeated testing (e.g. Rödel et al., 2012). The apparatus of the EPM was plus-shaped and made out of grey plastic, with two opposing closed arms (52 cm × 10 cm), two opposing open arms (52 cm × 10 cm) and a square centre zone (10 cm × 10 cm). The closed arms were surrounded by 30 cm high walls with transparent flexible plastic protectors on top to prevent the rats from jumping and walking on the walls. The open arms were surrounded by a 0.4 cm high border to secure the rats when leaning over the edge. The whole apparatus was elevated 60 cm above the ground and placed in a fixed orientation inside the test room, which remained stable across test repetitions. The test apparatus was illuminated from above with a light intensity of ~25 lx.

In this study, the EPM was performed on PND 97 and PND 139 between 1000 hours and 1200 hours, respectively. The rats were tested in a randomized order in a separate room. For the transport of the animals to the test room, a semitransparent red plastic box (22 cm × 22 cm and 15 cm high) was used, which was cleaned between individuals as mentioned before. Before the start of each test, the rat spent 1 min inside the transport box for acclimatization to make sure that all animals were in the same state of arousal when being tested (Izídio et al., 2005). Then, the rat was put in the centre zone of the test apparatus, facing the open arm of the maze. The behaviour of the rat was recorded and automatically tracked by using a camera (Logitech HD Pro C920 Full HD-Webcam 1920 × 1080 Pixel, Logitech, Apples, Switzerland) and tracking software (ANY-maze Video Tracking Software, version 6.32, Stoelting, Wood Dale, United States). The test duration was 5 min and during testing, the experimenter left the room to prevent bias. As described above, the apparatus was cleaned before the first rat as well as between rats. Measured parameters were the relative time spent on the open arms, the number of entries into the open and closed arms and the total distance travelled (Rodgers & Johnson, 1995; Treit et al., 1993).

Open Field and Novel Object Test

As the EPM, the OF is used to assess anxiety-like behaviour and exploratory locomotion in rodents by relying on their natural aversion to open and bright spaces (Treit et al., 1993; Treit & Fundytus, 1988). In animal personality research, the OF has become one of the most widely used behaviour tests, where it is repeatedly performed in order to assess ‘boldness’ (e.g. Yuen et al., 2017). The apparatus of the OF was square-shaped and made out of grey plastic, with a floor space of 104 cm × 104 cm and a wall height of 40 cm, which were moreover topped with transparent flexible plastic protectors to prevent the rats from jumping and walking on the walls. The space 26 cm apart from the walls was defined as the peripheral zone and the space in the middle of the arena (52 cm × 52 cm) was defined as the centre zone. The test arena was illuminated from above with a light intensity of ~35 lx.

The OF + NOT were performed on PND 98 and PND 140 between 1000 hours and 1400 hours, respectively. The rats were tested in a randomized order in a separate room. For the transport of the animals to the test room, a semitransparent red plastic box (22 cm × 22 cm and 15 cm high) was used, which was cleaned between individuals. Before the start of each test, the rat spent 1 min inside the transport box for acclimatization, to make sure that all animals were in the same state of arousal when being tested (Izídio et al., 2005). In the following, the rat was placed inside the front left corner of the arena, facing the wall. The behaviour of the rat was recorded and automatically tracked by using a camera (Logitech HD Pro C920 Full HD-Webcam 1920 × 1080 Pixel, Logitech, Apples, Switzerland) and tracking software (ANY-maze Video Tracking Software, version 6.32, Stoelting, Wood Dale, U.S.A.). The test duration was 5 min and during testing, the experimenter left the room to prevent bias. The apparatus was cleaned before the first rat as well as between rats. Measured parameters were the time spent in and the number of entries made to the centre of the apparatus as well as the total distance travelled (Archer, 1973; Treit & Fundytus, 1988).

Following the OF, the NOT was performed to moreover assess the animals' exploration of a novel object (Verjat et al., 2021). Therefore, the rat was captured from the OF arena and put back into the transport box. If necessary, the arena was cleaned of faeces and urine. Then, the novel object was placed in the middle of the arena and again, the rat was put inside the front left corner of the arena, facing the wall. The zone within a radius of 31 cm around the object was designated as an exploration zone. The test duration was 5 min and the experimenter left the room to prevent bias. For the NOT, two different objects (inverted transparent plastic bottle: 15.5 cm high and 5 cm in diameter; tin can: 11 cm high and 7.5 cm in diameter) were used in characterization phases 1 and 2 of the experiment to exclude habituation effects. The order of object presentation was pseudorandomized, as it was balanced for sex and phase. Measured parameters were the latency to enter, the time spent in and the number of entries made to the exploration zone of the apparatus as well as the mean distance to the novel object and the total distance travelled (Richter et al., 2010; Verjat et al., 2021).

Faecal Corticosterone Metabolites

Faecal corticosterone metabolites, which reflect pituitary–adrenocortical activity (Palme, 2019), were used to investigate the animals baseline corticosterone values in characterization phase 1 (PND 99) and characterization phase 2 (PND 141) of the experiment. Sampling took place between 1000 hours and 1200 hours in the rats' housing room on both sampling dates. For exactly 2 h, the animals were housed singly in Makrolon Typ IV cages that were prepared in advance with a small amount of bedding, a paper tissue, a semitransparent red plastic house and food and water ad libitum. After all rats were transferred back to their home cages, faecal boluses were collected with gloves, whereby all faeces from one sampling cage were stored in a distinct, labelled 25 ml Eppendorf tube (Eppendorf, Hamburg, Germany) at −27.5 °C.

For the analysis of the FCMs, the wet weight of the faecal samples was determined (scale: 510-23, Kern, Ballingen, Germany; weighing capacity: 300 g, resolution: 0.001 g). Subsequently, the samples were dried for 3 h at 80 °C in an oven (Modell 500, D-06061, Memmert, Schwabach, Germany). The dried faeces were weighed again and stored in 25 mL Eppendorf tubes. In the following, the faeces were pulverized with a mixer mill (Mixer Mill MM 400, Retsch, Haan, Germany) by using a stainless-steel ball (diameter: 12–15 mm, Retsch, Haan, Germany). Seventy milligrams of the faeces powder were then filled into a new 2 ml Eppendorf tube and mixed with 1.4 ml methanol (80%). The mixture was vortexed for 30 min (Multi-vortex, V-32, Kisker, Steinfurt, Germany) and centrifuged for 10 min with a speed of 5200 rpm (Centrifuge 5415 R, Eppendorf, Hamburg, Germany). Subsequently, 500 μL of the supernatant that contained FCMs was transferred to a 2.0 ml safe-lock Eppendorf tube and stored at −20 °C. In the following, FCM concentrations were analysed by using a 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassay (see Lepschy et al., 2007; Touma et al., 2003).

Food Reaching Test

The FRT is a behaviour test that is used to investigate paw preference as a measure of lateralization (Collins, 1975). The test apparatus was a semitransparent red plastic box (29 cm × 29 cm and 29.5 cm high), with a small opening (1.5 cm × 2 cm) in the centre of the lower edge of the front side. In the present study, the FRT was performed on PND 101 and 142 between 1000 hours and 1400 hours, respectively. Before the test was conducted for the first time, a habituation to the test apparatus was done (PND 100), to teach the animals where to reach for the food reward (1/6 a honey loop; Honey Llama Loops, Kellogg Europe Trading, Dublin, Ireland). Therefore, the rat was put inside the apparatus and was given approximately 5 min to freely explore the box. Then, the same reward also used on the test day was presented with tweezers inside the box, close to the opening, to make sure that the animals associate it with a food reward. The habituation lasted for a maximum of 15 min but was finished earlier if 10 rewards were consumed (McDowell et al., 2018). The habituation as well as the test were conducted under red light and the rats were tested in a randomized order in the room next to the housing room. On the test day, the rat spent 1 min in the box for habituation, before the reward was presented with tweezers in front of the opening, which the animal needed to reach for. The test duration was set to a maximum of 15 min, but was finished earlier if 35 rewards were successfully pulled inside the box. Before the first rat as well as between rats, the apparatus was cleaned. For subsequent video analysis, the procedure was filmed at an angle from above (Sony Handycam FDR-AX33, Sony Europe, Weybridge, U.K.). Left and right paw reaches, respectively, were counted as a measure of direction, whereas the absolute discrepancy between the two measurements was used to assess the strength of lateralization (Wells, 2003).

$$\text{strength of lateralization} = |\text{left paw reaches} - \text{right paw reaches}|$$

Play Test

The PT was used to record the rats' vocal response to human-induced play behaviour ('tickling'), which imitates the rats' natural rough-and-tumble play (LaFollette et al., 2017, 2018; Panksepp & Burgdorf, 2000). The test apparatus ('tickling arena') was a Makrolon Type IV cage, with bedding material (TierWohl Super, J. Rettenmaier and Söhne KG, Rosenberg, Germany) and a wall extension (height: 15 cm) to prevent the rats from jumping out. In the present study, the PT was performed on PND 107 and 143 between 1000 hours and 1400 hours, respectively. The rats were tested under red light in a randomized order but separated by sex to enable the reuse of bedding material for individuals from the same cage, in the room next to the housing room. Before the PT was conducted for the first time, a habituation to the tickling arena, and the tickling method itself was done (PND 104–106). For this purpose, the animals were placed in the arena for 3–5 min on three consecutive days before the actual test. On the first day, the rat could freely explore the arena for 3 min. In the following minute, the rat also had the opportunity to explore the hand of the experimenter, which was placed flat against one side of the arena. The last minute of the first habituation session was used to familiarize the rat with the tickling method. The second and third days of the habituation as well as the test day started directly with the habituation to the experimenter. On the test day, according to the protocol developed by LaFollette et al. (2018), the rat was then tickled for 15 s, which was followed by a 15 s pause interval. In total this procedure was repeated four times, so that the test duration was 2 min. The rats' vocalizations were recorded with a microphone attached to the corner of the tickling arena (Avisoft-Bioacoustics CM16/CMPA with Avisoft-UltraSoundGate 416Hb, Avisoft Bioacoustics e.K., Glienicke/Nordbahn, Germany; Burgdorf & Panksepp, 2001), and the number of 50 kHz and 22 kHz calls, respectively, was counted subsequently in the pause and tickling interval, respectively. Before the first rat as well as between rats, the apparatus was cleaned.

Statistics and Sample Sizes

The study was planned with a sample size of 24 individuals (12 females and 12 males), based on an a priori power analysis performed in G*Power (version 3.1.9.7) that aimed to detect an average effect size ($f = 0.3$) with a power of 80%. Two females were excluded from the study before testing started, and one male was excluded during the experiment, before the test repetition, due to health issues that were not related to the experiment. This results in a sample size of 22 individuals (10 females and 12 males) for the first characterization phase and a sample size of 21 individuals (10 females and 11 males) for the second characterization phase.

All analyses were done in R version 4.4.0 (R Core Team). For the statistical analysis, we first calculated repeatability estimates (R) for all measured parameters. In the context of animal behaviour, repeatability estimates are often used to quantify the stability of individual differences (e.g. Bell et al., 2009; Nakagawa & Schielzeth, 2010). The repeatability was estimated in a univariate, linear mixed effects model framework using the rptR package in R (Stoffel et al., 2017). In these models, choices in the CJBT (optimistic versus pessimistic), paw reaches in the FRT (left versus right) and arm entries in the EPM (open versus closed) were modelled as proportions. Vocalization data, the strength of lateralization in the FRT, as well as the entries made to the exploration zone in the NOT and the centre in the OF were modelled as Poisson distributions. All remaining parameters were modelled as Gaussian distributions and square root transformed if necessary to improve the distribution of model residuals (Supplementary Material). All models were fitted with the characterization phase as a fixed effect and individual ID as a random effect. Using the repeatability estimates calculated from these models, we continued the subsequent analysis to detect links between different personality traits. Here, only parameters that reached $R \geq 0.3$ were included, as this is the average repeatability reported for behavioural parameters (Bell et al., 2009).

Before continuing the analysis, we dropped parameters measured within the same test, which were highly correlated (Supplementary Material), as they did not provide any meaningful additional information. This resulted in simpler models that are less prone to overfitting (Zuur et al., 2010). Hence, we kept the number of 50 kHz calls during tickling in the PT but not during the pause, as well as keeping the time spent in the exploration zone of the NOT but not the mean distance to the novel object in the NOT. Moreover, we kept the number of entries made to the centre of the OF and dropped the time spent there. For the PT, we chose the tickling interval over the pause interval, since this was the most direct response to the tickling measured. For the NOT and OF, we chose the parameter that was measured with a higher accuracy by the automated tracking software. To further reduce the number of dependent variables, we conducted a principal component analysis on parameters collected across different tests, that were (1) correlated (Supplementary Material) and could (2) be grouped thematically as one behavioural aspect. Thus, the distance travelled in the EPM, OF and NOT was transformed into one locomotion parameter (Supplementary Material). Hence, in the next step, the multivariate mixed model was calculated for the following 10 behaviour outcomes: (1) relative time spent on the open arms of the EPM, (2) latency to enter the exploration zone in the NOT, (3) time spent in the exploration zone of the NOT, (4) number of entries made into the centre of the OF, (5) locomotion in the EPM, OF and NOT, (6) direction of lateralization in the FRT, (7) strength of lateralization in the FRT, (8) number of 50 kHz calls emitted during tickling in the PT, (9) number of 22 kHz calls emitted during tickling in the PT and (10) FCM.

In order to reveal the links between different personality traits, we applied Markov chain Monte Carlo techniques in a Bayesian framework using the MCMCglmm package (Hadfield, 2010) to fit a multivariate generalized linear mixed model. Vocalization data and the strength of lateralization in the FRT were modelled as a Poisson distribution, whereas the direction of lateralization in the FRT was analysed with a multinomial distribution. All other parameters were analysed with Gaussian distributions and scaled to support model fit.

Similar to Houslay and Wilson (2017), we included sex and characterization phase as fixed effects and individual ID as a random effect. The covariance structure was defined by rcov = us(trait):ID, as the different measurements were paired in time and repeated at the individual level. We used an expanded prior defined to deal with potential autocorrelation, with $V = \text{diag}(10)$, $\text{nu} = 10$, $\text{alpha.mu} = \text{rep}(0,\ 10)$ and $\text{alpha.V} = \text{diag}(1000, 10, 10)$. A burn-in period set to 20 000 and thinning intervals of 100 were used to yield effective sample sizes of > 2000 and satisfy convergence criteria. Following de Villemereuil (2023), models were checked for convergence and autocorrelation.

Using the posterior distributions, we estimated the among-individual correlations, the mean covariance, as well as the lower and upper bound of the 95% credible interval (CI) for each possible trait combination (Houslay & Wilson, 2017). Significance levels of $P \leq 0.1$ were considered a trend, whereas $P \leq 0.05$ was considered significant. For the correlations between personality traits, statistical significance was determined by a 95% credible interval that does not span zero.

Ethical Note

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 (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).

The welfare of the animals was carefully monitored during the whole experiment and beyond. Testing was conducted during the active phase of the animals only and was based solely on noninvasive methods. The housing conditions included spacious cages with multiple levels and a variety of enrichment items. After the experiment, rats remained in our housing facility and were rehomed or handed over to a cooperation partner.

RESULTS

Optimisms Levels Varied Across the Two Characterization Phases

Comparing the two characterization phases regarding optimisms levels in the CJBT, seven rats increased their individual optimism level (four females, three males), two rats maintained their optimism levels (one female, one male) and 12 rats decreased their optimism levels (five females, seven males) in the CJBT repetition (Fig. 3). Besides that, relatively low optimism levels were reported in both sexes, with the most optimistic female showing three of six optimistic choices and the most optimistic male showing four of six optimistic choices.

Not Optimism But Other Behaviours Show Temporal Stability

Regarding the repeatability analysis of our main outcome measure, that is, optimistic choices versus pessimistic choices in the CJBT, optimism levels were not found to be repeatable ($R = 0.000$, Confidence interval (ConI): [0, 0.053], $P = 1.000$; Fig. 4). In contrast, 15 of the remaining 18 parameters were found to have considerably high repeatability scores (for an overview, see Supplementary Material). More specifically, all measures reflecting anxiety-like behaviour had repeatability between 30% and 50%. Namely, these were the relative time spent on the open arms of the EPM ($R = 0.425$, ConI: [0.012, 0.716], $P = 0.022$), the time spent in the centre of the OF ($R = 0.460$, ConI: [0.070, 0.749], $P = 0.021$) and the entries made to the centre of the OF ($R = 0.319$, ConI: [0, 0.682], $P = 0.089$), the time spent in the exploration zone of the NOT ($R = 0.441$, ConI: [0.022, 0.724], $P = 0.016$), the latency to enter the exploration zone of the NOT ($R = 0.336$, ConI: [0, 0.660], $P = 0.054$) and the mean distance to the object in the NOT ($R = 0.554$, ConI: [0.159, 0.797], $P = 0.002$). Furthermore, parameters reflecting exploratory locomotion, that is, the distance travelled in the EPM ($R = 0.549$, ConI: [0.173, 0.781], $P = 0.003$), the OF ($R = 0.803$, ConI: [0.586, 0.918], $P < 0.001$) and the NOT ($R = 0.741$, ConI: [0.490, 0.883], $P < 0.001$), were found to be stable across time. Likewise, all but one vocal response of the rats to human-induced play behaviour showed temporal stability, ranging between 40% and 70% repeatability. These were 50 kHz calls emitted during the tickling ($R = 0.689$, ConI: [0.438, 0.871], $P < 0.001$) and pause interval of the PT ($R = 0.698$, ConI: [0.493, 0.864], $P < 0.001$) as well as 22 kHz calls displayed during the tickling interval of the PT ($R = 0.437$, ConI: [0, 0.765], $P = 0.037$). Additionally, both laterality parameters, the direction ($R = 0.482$, ConI: [0.247, 0.761], $P < 0.001$) and strength ($R = 0.559$, ConI: [0.150, 0.823], $P = 0.002$) of lateralization in the FRT, were found to be repeatable. Moreover, FCM was repeatable ($R = 0.407$, ConI: [0, 0.695], $P = 0.025$), indicating temporal stability of hypothalamic–pituitary–adrenocortical activity. Only a low repeatability score below 5% was found for two parameters reflecting exploratory locomotion, that is, the number of open

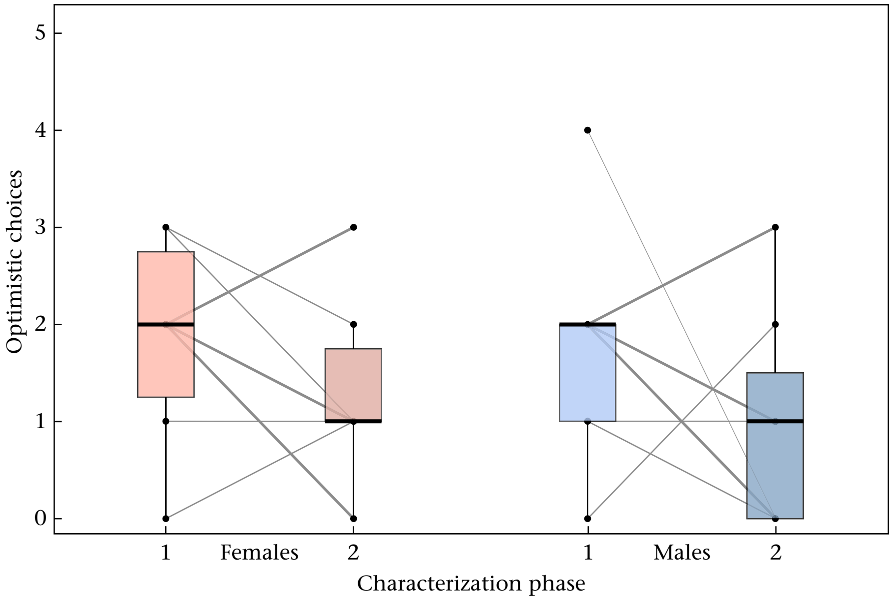

Figure 3. Individual optimism levels in the CJBT. Shown is the number of optimistic choices (max. 6) in the CJBT divided by characterization phase and sex. Box plots show the median (lines in boxes), the 25% and 75% quartiles (boxes), the minima and maxima (whiskers) and individual data points (points). Lines connect individual data points across the two characterization phases and thickness of lines indicate multiple individuals with the same coordinates.

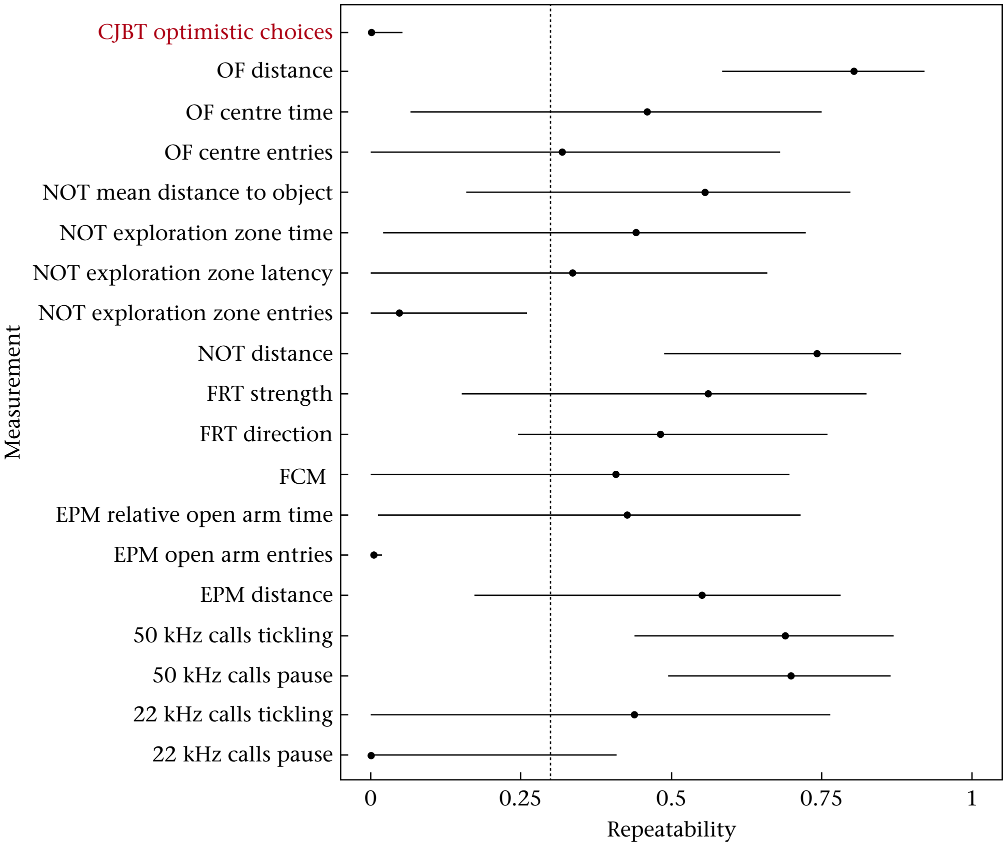

Figure 4. Repeatability of test measurements. Repeatability estimates (points) and confidence intervals (lines) calculated for measurements from the cognitive judgement bias test (CJBT), elevated plus maze (EPM), open field (OF), novel object test (NOT), faecal corticosterone metabolite (FCM) collection, food reaching test (FRT) and play test (PT). The dashed line indicates the average repeatability of behavioural parameters (Bell et al., 2009).

arm entries in comparison to closed arm entries in the EPM ($R = 0.005$, ConI: [0, 0.019], $P = 0.241$) and the number of entries made into the exploration zone of the NOT ($R = 0.048$, ConI: [0, 0.260], $P = 0.379$). Finally, the number of 22 kHz calls emitted during the pause interval of the PT was not repeatable ($R = 0.000$, ConI: [0, 0.410], $P = 1.000$).

Personality Traits Were Not Linked to Each Other

As optimism levels were not found to be repeatable in the first part of the analysis and therefore did not fulfil the definition to be considered a personality trait, this parameter could not be included in the further analysis, hence allowing no conclusion about potential links between optimism levels and other personality traits. A further investigation regarding the change rate in optimism levels and a potential correlation to the detected repeatable parameters did not yield significant results either (for details see Supplementary Material). Instead, to detect links between other described personality traits, an explorative analysis was conducted with parameters that were more than 30% repeatable, which is the average repeatability of behavioural parameters according to a meta-analysis from Bell et al. (2009). However, applying this threshold, no correlations among any of these parameters could be detected (all CIs spanning zero, for details see Supplementary Material). Nevertheless, on a descriptive level, two personality trait combinations stood out, as they showed a stronger correlation compared with the other personality trait combinations. More specifically, the findings hint towards a link between the number of 50 kHz calls during tickling in the PT and the direction of lateralization in the FRT, with rats frequently vocalising in the 50 kHz range being more likely to use the left paw in the FRT than rats rarely vocalizing in the 50 kHz range during tickling in the PT (mean correlation: 0.288, 95% CI: [−0.204, 0.753]; Fig. 5). Moreover, rats that spent more time in the exploration zone of the NOT arena, indicating increased levels of exploration and reduced anxiety-like behaviour, were more likely to emit 50 kHz calls during tickling in the PT (mean correlation: 0.315, 95% CI: [−0.437, 0.861]).

Apart from detecting potential links between different traits, the analysis revealed a significant effect of the characterization phase on the merged locomotion parameter from the EPM, OF and NOT, with the rats showing more locomotion in the first characterization phase (post-mean = −0.689, 95% CI: [−1.128, −0.205], $P = 0.007$). Moreover, a significant effect of sex was found on FCM (post-mean = 1.062, 95% CI: [0.412, 1.716], $P = 0.004$), the strength of lateralization in the FRT (post-mean = 0.748, 95% CI: [0.088, 1.383], $P = 0.029$), locomotion (post-mean = −1.467, 95% CI: [−1.970, −0.943], $P < 0.001$) and the number of entries made into the centre of the OF (post-mean = −4.203, 95% CI: [−1.28, −0.205], $P = 0.007$). Female rats had lower FCM values, were less strongly lateralized, had higher locomotor activity and entered the centre of the OF more often compared with male rats, the latter reflecting increased exploratory locomotion. Furthermore, females showed a trend to vocalize more in the 22 kHz range during tickling in the PT compared with males (post-mean = −0.895, 95% CI: [−1.805, 0.011], $P = 0.054$).

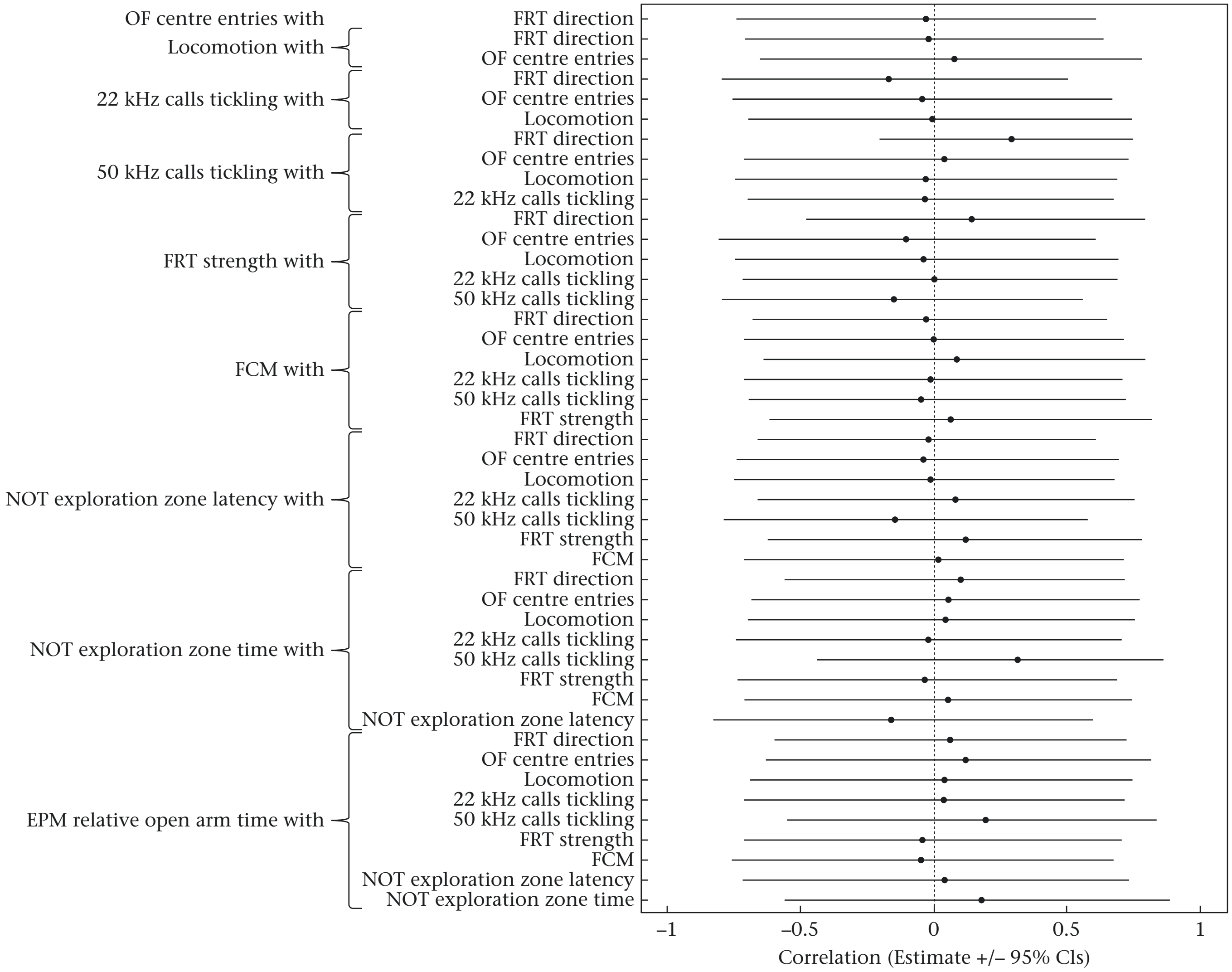

Figure 5. Correlations between traits. Correlation means (points) and credible intervals (CI; lines) calculated for all possible trait combinations from the cognitive judgement bias test (CJBT), elevated plus maze (EPM), open field (OF), novel object test (NOT), faecal corticosterone metabolite (FCM) collection, food reaching test (FRT) and play test (PT). The dashed line indicates the zero line. Statistical significance was determined by a 95% credible interval (CI) that does not span zero.

DISCUSSION

The aim of the present study was to comprehensively characterize individuals with different optimism levels. To achieve this goal, temporal stability of an individual's optimism level and its link to other personality traits were investigated. Optimism levels were not found to be temporally stable. Due to this finding, the links between optimism levels and other personality traits could not be further examined. However, several other behavioural parameters and FCMs were found to be stable across time, hence matching the definition for being considered a personality trait. An explorative analysis of links between these personality traits hinted towards a potential association of vocalizations and exploratory behaviour as well as lateralization.

Optimism Levels Were Not Stable Across Time

The individuals' optimism levels did not show temporal stability across the two characterization phases of the experiment. This result contrasts previous findings from studies in which evidence for stability over time was found. In these studies, optimism levels were considered a personality trait after repeated testing with intervals of 3 or up to 25 days (Lecorps et al., 2018; Verjat et al., 2021). In the present study, the assessment of optimism levels was repeated after 42 days, which might indicate that optimism levels have a stable component but remain flexible across larger time spans. This is in line with the general finding that repeatability estimates decrease when measuring points are far apart (Bell et al., 2009). However, in mice, Bračić et al. (2022) found optimism levels to be moderately repeatable across 7 weeks, indicating species-specific differences in the temporal stability of optimism levels that are not simply caused by different lifespans. In their study, three test repetitions of the cognitive judgement bias test were conducted, which increases the robustness of repeatability results in contrast to a single repetition (Martin et al., 2011). Nevertheless, it is unlikely that these differences in the study design caused the discrepancy between our study and the one of Bračić et al. (2022), as a lower number of measurements per individual is assumed to lead to an over- and not underestimation of test outcomes (Wolak et al., 2012). Another explanation could be the age or life phase of the animals, respectively. In the present study, rats were tested during full adulthood (Schneider, 2013) in both characterization phases. From humans, it is known that behavioural parameters become more stable during ageing, indicating the gradual consolidation of personality traits across the lifespan (Roberts & Delvecchio, 2000). The same is also suggested for animals (Stamps & Krishnan, 2014); however, a meta-analysis found the opposite in ectotherms, with juveniles being more repeatable in their behaviour than adults (Bell et al., 2009). Therefore, the stability of personality traits across the life span or within certain life phases might vary between taxa and needs further investigation in rats.

As it is recently discussed that also individual differences in plasticity might be part of an animal's personality (O'Dea et al., 2022; Stamps, 2016), the collected data were also checked for links between the change in optimism levels, that is, plasticity in response to an ambiguous cue and repeatable behavioural outcomes. However, no significant associations were detected in this analysis (Supplementary Material). Still, the study reports interesting findings regarding individual optimism levels, as it provides further evidence for a population bias towards lower optimism levels in rats, which was also already reported elsewhere (Rygula et al., 2015). Interestingly, in contrast to Rygula et al. (2015), this was the case, although the test design was based on differences in reward values and not on punishment in the negative condition. In theory, this would be more likely to cause a shift towards higher optimism levels, as there is ‘not a lot to lose’ in case of a potentially wrong choice (Bateson, 2016). Nevertheless, under natural conditions, being too optimistic could lead to the death of an animal, whereas being too pessimistic would cause unnecessary energetic costs at worst. This trade-off could favour a population bias towards lower optimism levels in rats. Nevertheless, please note that this is a theoretical speculation, which needs empirical evidence for approvement.

Apart from optimism levels, our data showed temporal stability for several behavioural measurements and FCMs, which matches the definition for being considered a personality trait. Those can mainly be grouped into personality traits reflecting anxiety-like behaviour, exploratory locomotion, vocalizations, laterality or hypothalamic–pituitary–adrenocortical activity. Regarding these personality traits, our findings are in line with the literature. Standardized tests like the OF, the EPM and the NOT are widely used in research on personality traits and produce robust results with high repeatability estimates regarding anxiety-like and exploratory behaviour (reviewed in Améndola et al., 2022). Moreover, we successfully reproduced the finding that rats show stable individual responses to human-induced play behaviour (Mällo et al., 2007) and confirmed the result that the direction and strength of paw preference in food reaching task is temporally stable in rodents (Groneberg et al., 2025; Stieger et al., 2021). Furthermore, our findings support the suggestion that basal FCMs are repeatable (Taff et al., 2018), highlighting that both behavioural and endocrinological measures can be part of an animal's personality.

As argued before, animal personalities are discussed to result in limited plasticity (Sih et al., 2004). In the literature, differences in the stability of behaviour are explained by different selective processes (Kight et al., 2013). As unlimited behavioural flexibility is likely to be too costly (Wolf & Weissing, 2010), certain behaviours might underlie specific selection pressures that push towards higher stability. In social-living species, for example, it is reasonable to argue that behavioural stability might be advantageous to increase the predictability of the interaction partner. In this context, it has been suggested that in species with biparental care, females select behaviourally predictable males, as this might have benefits for the coordination of brood care (Cabrera et al., 2021). Apart from social contexts, a stable preference for one side, like for instance paw preference, might reflect lateralization of the brain, which is assumed to be advantageous (Rogers, 2000), particularly during complex tasks (Fagot & Vauclair, 1991; Stieger et al., 2021). Taken together, our results revealed repeatable individual differences in behavioural measurements as well as FCMs. However, contrasting the literature, optimism levels were not found to be stable across time, thereby encouraging further research on potential factors that might affect or modulate the stability of optimism levels within and across life phases.

Vocalization Might be Linked to Exploration and Lateralization

Since the focal parameter, that is, optimism levels, was not found to be repeatable and thus excluded from the analysis, we explored potential links between the detected personality traits. Only personality traits that reached a repeatability estimate above average, according to Bell et al. (2009), were included.

In the literature, such suites of correlated behaviours are also referred to as behavioural syndromes (Carter et al., 2013). In our analysis, no suites of correlated behaviours could be detected. This contrasts with previous studies, reporting, for instance, a positive correlation between vigilance in a novel object task and faecal glucocorticoid metabolites in squirrels (Clary et al., 2014). Moreover, for lateralization, links to other personality traits were already suggested (Irving & Brown, 2013), and indeed, handedness was shown to be connected to stress reactivity in mice (Neveu, 1996). Nevertheless, findings about potential links are overall still quite inconclusive, with several studies reporting negative results (Bell & Stamps, 2004; Rudolfová et al., 2017, 2022). Although these negative results might be caused by species-specific differences, Bell and Stamps (2004) go even further: After showing that two populations from closely related taxa differ in their suites of correlated personality traits, they argue that differences might also be caused by the different environmental conditions the populations are facing, presumably resulting in different selection pressures. Along these lines, it has therefore been suggested that links between personality traits could be context-specific and therefore only be detectable under certain circumstances (Sih et al., 2004).

Nevertheless, on a descriptive level, the present study revealed hints for a positive link between the frequency of 50 kHz vocalizations and time in the exploration zone of a novel object task as well as being left side lateralized in a food reaching task. Indeed, one study conducted with rats also found a positive correlation between 50 kHz vocalization rates and measures from an exploration task. Here, rats that vocalized a lot in the 50 kHz range were less active (Mällo et al., 2007). The second finding, hinting towards a positive correlation between the frequency of 50 kHz calls and a preference for using the left paw, is in line with the so-called ‘Right-Hemisphere-Hypothesis’. This hypothesis is often referred to in research regarding lateralization and implies that emotions are predominantly processed in the right hemisphere of the brain (Smith & Bulman-Fleming, 2005). The right hemisphere is also responsible for motor control of the left side of the body. Since 50 kHz calls are interpreted to reflect a positive emotional state in rats (Burgdorf & Panksepp, 2001), an increased activity of the right hemisphere can be assumed during the emission of these calls. In combination with the contralateral connection between the brain and the limbs, this could trigger a preference for the left paw. Considering all the abovementioned points in summary, the results of the present study suggest that some personality traits are more closely linked than others, thereby encouraging further research on potential correlations and their underlying selection pressures. Knowledge gained from such studies will finally help understand the costs, benefits and limitations of behavioural plasticity (Kight et al., 2013).

Conclusion

In contrast to previous studies, optimism levels were not found to be temporally stable in the present study, encouraging further research on potential factors that might affect or modulate the stability of optimism levels within and across different life phases. Furthermore, no suites of correlated behaviours were detected, highlighting the need for disentangling the impact of context and/or species on the emergence of such links. Nevertheless, our results contribute to the accumulating evidence that certain interindividual differences in behaviour, such as exploratory locomotion, vocalizations, anxiety-like behaviour and laterality as well as hypothalamic-pituitary-adrenocortical activity, are indeed stable across time, and hence can be considered aspects of an animal's personality.

Such findings are particularly interesting and important, as they might have implications for various research areas. For example, animals with different personalities are likely to react differently to stress and certain environmental conditions (Sih et al., 2004). This could result in different individual demands required to maintain good welfare, highlighting the relevance of animal welfare science (Carere & Locurto, 2011; Sroka et al., 2024). Moreover, in ecological research, the existence of animal personalities might lead to differences in niche realization and individual niche specialization (Krüger et al., 2021; Schirmer et al., 2020), thereby modulating the way in which individuals interact with their environment. This, in turn, might have consequences for, for example, the complexity of ecosystems as well as for practical conservation efforts (Carscadden et al., 2020). Finally, in the field of evolutionary biology, knowledge gained from research on animal personality helps understand how these differences in behaviour evolved and how they are maintained (Sih et al., 2004).

Author Contributions

Sophia Marie Quante: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Carolin Mundinger:** Formal analysis. **Rupert Palme:** Resources, Methodology. **Sylvia Kaiser:** Supervision. **S. Helene Richter:** Supervision, Funding acquisition, Conceptualization.

Data Availability

The data supporting this study are available as Supplementary Material with this article.

Declaration of Interest

The authors declare they have no competing interests.

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Supplementary Material

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