Research Topics

Captive animal behavior: Individual differences in learning and cognition, and implications on animal welfare

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Captive animal behavior: Individual differences in learning and cognition, and implications on animal welfare

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Editorial: Captive animal behavior: Individual differences in learning and cognition, and implications on animal welfare

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Editorial on the Research Topic
Captive animal behavior: Individual differences in learning and cognition, and implications on animal welfare

To provide adequate welfare for animals in captivity, it is important to consider not only the needs of the species, but also those of the individual. In this context, knowledge about individual differences in learning and cognitive functioning are of particular importance, as it can help to assess the extent to which captive animals are able to adapt and respond to changing housing conditions.

In the last decades, individual differences in learning and cognition have been studied systematically across a wide range of taxa (1). However, the underlying factors that cause this variation, as well as its potential welfare consequences, are still under debate (2). While ultimate factors tend to play a minor role in explaining behavioral variation in captive animals, a variety of proximate factors could be responsible for the individual variation we see in animals' performance in learning and cognitive tasks (Finkemeier et al.). These factors include a variety of genetic and environmental components, ranging from breed or feeding type, to housing conditions (single vs. group housed), to idiosyncrasies of different research sites (3–5). The observation of robust intra-species variation in behavior under identical environmental conditions has led to a significant increase in research on inter-individual behavioral variation, often coined as personality, in many animal taxa, especially in the field of behavioral ecology (6). This behavioral variation can be observed in levels of activity, as well as exploratory and social behaviors, beside others. While the influence of genetic, physiological and behavioral factors on individual response patterns in farm, laboratory, and zoo animals has received considerable attention in recent years, few studies have addressed the role of these traits in predicting inter-individual differences in learning and cognition.

The objective of this Research Topic was to promote interdisciplinary research approaches on the link between individual variation in genetics, physiology and behavior, and learning and cognition—ranging from fields such as developmental psychology to applied ethology and addressing this variation in animals under human care, with particular emphasis on farm, companion and zoo animals. The manuscripts included in this Research Topic have examined the impact of genetics, neurotransmitters, hormones, critical life stages, and certain personality traits on learning and cognitive phenomena such as cooperation and self-control and range from farm animals (goat, pig, and chicken) to companion (dog, horse) and laboratory animals (rat) as studied species.

Studies in our Research Topic often focused on the association between different behavioral parameters and inter-individual differences in learning and other cognitive phenomena. In a study in goats, Finkemeier et al. investigated the relationship between distinctive personality traits and discrimination learning. Stability in the personality trait boldness was found to have an impact on learning performance in a visual reversal-learning task, with less bold goats performing better than bolder ones. These results support the general hypothesis that proactive animals tend to stick to once-learned routines and to react less flexibly to changing stimulus combinations (7). To study cooperative behavior in pigs, Rault et al. developed an ecologically relevant feeding paradigm, the so-called "joint log-lift task". To complete the task, two pigs must cooperate in lifting a log to receive a reward. While kinship had no influence on the cooperation behavior of individual dyads, inter-individual differences in sociability influenced the willingness to cooperate in pigs. The relationship between social competence and the impact of intranasal oxytocin on social behavior of dogs was studied by Turcsán et al. While oxytocin has been reported to have a general positive effect on social behavior, intranasal administration of oxytocin in this study increased social behavior in dogs toward humans only in animals that showed already a low baseline performance of interacting with humans. This indicates that the effect of oxytocin on social behavior is dependent on personality traits and the specific context. A study by Brucks et al. investigated self-control in horses. They found that horses wait until a maximum delay of 60 s to receive a highly valued reward rather than to get an immediately available reward of lower quality. While horses fed hay *ad libitum* instead of receiving a restricted diet achieved higher delay times, the trainability or patience of the horses had no influence on the maximum delay level.

Two of the submitted studies aimed to establish a relationship between different genetic predispositions and learning performance or flexibility in learning behavior. To test whether animals bred for high productivity have lower learning performance, Nawroth et al. compared the performance of dwarf goats and dairy goats in a visual discrimination and reversal-learning task. The results suggest that selection for high performance may have negatively affected the goats' behavioral flexibility with dwarf goats outperforming dairy goats in reversal learning. Dudde et al. investigated the role of the serotonin transporter (5-HTT) on anxiety and learning performance in chicken. Chicken from selection lines with different 5-HTT polymorphisms were tested with regard to their fearfulness and performance in a simple discrimination task. Chicken with reduced 5-HTT expression showed increased anxiety-like behavior, as has also been demonstrated in humans. However, and in contrast to human research, animals with reduced 5-HTT expression were also the slowest learners compared to hens with moderate or high expression.

Finally, three of the included studies addressed the effects of ontogeny or specific critical life stages on the cognitive abilities in chicken and pig. Garnham et al. investigated the relationship between affective states and inhibitory control in the red jungle fowl. Inhibitory control was measured using a detour task, while measures for affective states derived from the tonic immobility test and a cognitive judgement bias test. While inhibitory control was associated with affective states in younger chicks, no such association was found in older hens. The study shows that the link between affective states and inhibitory control can change during ontogeny. In another study in pigs by Bushby et al., a spatial judgement bias task was used to investigate the extent to which gestation affects the mood of pregnant sows. The reaction of gilts to ambiguous probe locations were tested at different stages of gestation. The results suggest that the mood of pigs can change during pregnancy, which could have an impact on the assessment of the welfare of captive multiparous animals. A study by Nagano examined that modified training to handle a rake-shaped tool in relation to an unreachable reward did not improve the rats' tool manipulation ability.

The studies, summarized in this Research Topic will improve our understanding of the internal and external factors that influence the expression of cognitive abilities in companion, laboratory, and farm animals, and how this in turn can have implications for their welfare.

Author contributions

Both authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Joint Log-Lift Task: A Social Foraging Paradigm

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Behavioural cooperation is under intense research. Yet, popular experimental paradigms often employ artificial tasks, require training, or do not permit partner choice, possibly limiting their biological relevance. We developed the joint log-lift task, a social foraging paradigm in which animals have to jointly lift a log to each obtain a food reward. The task relies on an obligate strategy, meaning that the only way to benefit is to work jointly. We hypothesised that (1) animals learn to spontaneously solve the task, and that (2) kin and (3) more sociable individuals would engage more often together in the task and achieve greater success than non-kin and less sociable individuals, respectively. We presented the task to 8 groups of juvenile domestic pigs (*Sus scrofa domesticus*) in their home pen for 30 min daily. Over the course of 9 days, the pigs showed evidence of learning by progressively switching from individual to joint behaviours, leading to 68% (62 out of 91 pigs) spontaneously solving the task. Success was influenced by sociability, but not kinship. There were large differences in success among dyads, hinting at the possible role of social dynamics and inter-individual differences in the ability and/or motivation to solve the task. The joint log-lift task allows researchers to investigate spontaneous cooperative tendencies of individuals, dyads and groups in the home environment through *ad libitum* engagement with the apparatus. This ecologically relevant paradigm opens the way to investigate social foraging experimentally at large scale, by giving animals free choice about when and with whom to work jointly.

Keywords: affiliation, coordination, cooperation, joint action, prosocial, spontaneous, sociability, social learning

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INTRODUCTION

Cooperation, a behavioural strategy in which agents achieve a common goal through coordinated action (1), has been investigated in a variety of animal species ranging from mammals (2–5) and birds (6) to social arthropods (7–9). Some experimental paradigms have been particularly popular to investigate cooperation [reviewed in (10)], such as the "string pulling task" (or "loose-string paradigm") employed in more than 160 bird and mammal species (11). Such approaches have provided insightful knowledge on the socio-cognitive abilities of various species. Nevertheless, the biological relevance of some tests has been questioned because they use artificial paradigms (12) or cognitively complex tasks (13). Furthermore, most experiments did not provide the opportunity for partner choice, neglecting social factors as an important facet of cooperation. Consequently, support is growing to investigate cooperation across animal species using ecologically-relevant contexts and species-specific paradigms (10).

We aimed to develop a task that would fulfil the requirements of being biologically relevant, intuitive enough to be solved spontaneously, and applicable to a group setting so that animals can choose their partner and work voluntarily. We developed the joint log-lift task (JLLT), in which two animals have to jointly lift a log to each obtain a food reward. Hence, the approach simulates a social foraging task. The task relies on an obligate strategy, meaning that the individuals only benefit if they work jointly and do not get a (lower) reward for performing the task individually, mimicking many conditions of cooperation found in nature when the only way to access a resource is to cooperate (14). Furthermore, the apparatus is designed so that it can be placed in the home environment and presented to a social group, thus allowing free partner choice and voluntary engagement with the apparatus.

There are conflicting findings on the importance of social factors on the propensity to cooperate. The kinship selection theory (15) was initially the predominant explanation, by cooperating with genetically related individuals to enhance one's inclusive fitness. However, cooperation between non-kin occurs based on alternative mechanisms such as reciprocity (16). In addition, factors at group level related to social dynamics or social relationships have been found to be important for cooperation [reviewed in (10)]. Unfortunately, relatively few studies used set-ups that allow partner choice in order to better understand the social factors involved in cooperation (10, 12, 17–20).

We tested domestic pigs (*Sus scrofa domesticus*) on the JLLT, as they are highly social animals that engage in cooperative behaviours such as coordinated nursing solicitation by piglets (21), nursing synchronisation (22), and occasional communal rearing (23). Pigs also consider social cues while foraging (24, 25). Their wild boar ancestors are capable of efficient temporal and spatial coordination while foraging, when cooperation prevails over competition (26). Foraging in pigs is typically done with the snout, rooting in the soil or under organic materials laying on the ground to find food items, and pigs possess a particular strong force in their snout to dig or lift (27). Hence, the JLLT solicits a biologically-relevant behaviour for pigs by asking them to lift a log.

We hypothesised that (1) pigs learn to spontaneously solve the task by working jointly, (2) kin would engage more often together in the task and achieve higher success than non-kin, and (3) more sociable individuals would engage more often in the task and achieve greater success than less sociable individuals.

METHODS

Animals and Housing

Eight groups of mixed-sex young pigs ($N = 91$ pigs) of a commercial farm breed were studied from 7 to 9 weeks of age. Pigs were housed in 3.1 × 4.7 m partly-slatted floor pens, with a 3 × 1 m covered heated sleeping area, *ad libitum* access to feed through a multi-space feeder containing a commercial pig meal diet, and *ad libitum* access to water through four drinkers. They were provided with environmental enrichment in the form of straw, wood shavings in the sleeping area, and a small wood log hanging on a chain in the pen. Room temperature was recorded at noon on each testing day, with an average temperature of 17.3°C (range: 15.1–18.8°C).

Joint Log-Lift Task Apparatus

The testing apparatus requires that two animals lift up a wooden log simultaneously to each receive a food reward (**Figure 1**; see **Supplementary Video**). If the log is lifted up on both sides simultaneously a magnet holds the log in the upper position and a food reward is released on each side (**Figure 1C**). If only one individual lifts up the log, or if individuals lift the log on both sides but each to a different height, the log cannot fix to the magnet due to the inclination and the food rewards remain inaccessible (**Figure 1B**).

The test apparatus was 75 cm wide and 63 cm high and had two openings at the front, each measuring 15 × 25 cm (width × height) separated by 25.5 cm from each other. The sides were made of wood whereas the front was a 4 mm transparent Plexiglas to allow the animals and the camera to see the log moving. Two 33 cm round plastic food bowls were placed below each opening to receive the food rewards. The 70 cm long and 2 kg wooden log laid out horizontally in the apparatus, resting on the food bowls. A metal strip was attached in the middle of the top surface of the log to lock in with the magnet. The magnet was placed at a height of 30 cm in the middle of the apparatus above the middle of the log and held the log in place when the log was lifted high enough on both sides to come in contact with at least 50% of the magnet's surface area. The direction of movement of the log was guided by a metal rail in the centre that guided the log to move vertically. The magnet locked only when the log was lifted from both sides simultaneously to the required height.

Habituation

A food preference test was conducted in each group prior to testing by offering to the group simultaneous choices between a total of eight heaps of 60 g of either chocolate-coated raisins, apples cut into 0.5 × 0.5 cm cubes, Solettis (salted sticks), and dried mealworms, with two heaps per feed type. Preferences were ranked based on which heap of feed items was depleted first, on group level. Across the eight groups of pigs, 89% of the first depleted feed items were the apple pieces, whereas 75% of the second depleted feed items were the chocolate raisins, with the rest divided between the salted sticks and dried mealworms.

Thereafter, on 2 consecutive days prior to the test, the apparatus was placed in each pen for 10 min per day while the log was locked up on the magnet. This way, pigs could explore the apparatus and discover that it contains food rewards (apple pieces mixed with chocolate raisins) in the food bowls, which were rebaited with small portions when pigs had finished consuming the food rewards.

Testing Days

Tests took place over 9 consecutive days with 30 min daily testing sessions, starting from when pigs were 7 weeks of age. Tests were carried out between 09:30 and 15:00 h. Pigs were marked on their back with an animal marker spray for individual recognition and remarked, when necessary, after the test session of that day was completed. The test order of the groups were randomised across

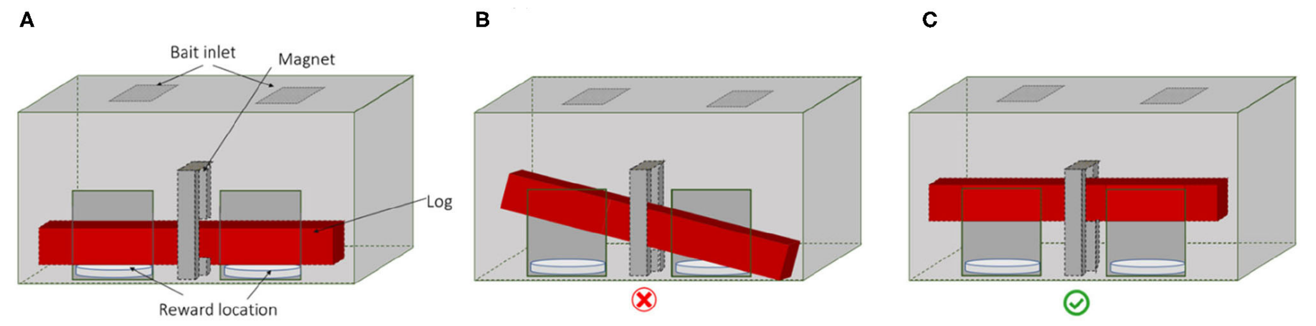

FIGURE 1 | The apparatus in three positions: **(A)** log is down; **(B)** if only one pig lifts the log or if two pigs lift the log but unevenly or not high enough then the magnet does not hold the log and no reward is provided; **(C)** two pigs lift the log to the top magnet and food rewards are provided.

TABLE 1 | Ethogram to record the behavioural interactions with the joint log-lift task apparatus.

Behaviour	Definition
Touch	A pig makes physical contact with the log with its snout
Attempt alone	One pig raises the log from one side alone, i.e., without another pig lifting on the other side
Attempt together	Two pigs jointly raise the log but do not manage to get it locked to the magnet at the top, because the movement is too uncoordinated or quick
Successful lift	Two pigs lift the log and successfully lock it to the magnet so that the log remains in the upper position

the testing days. Before the test, the experimenter stepped into the pen and set up a camcorder to video record the test from the front view of the apparatus. Entering the pen always woke up the pigs in the few occasions that they were asleep prior to testing, ensuring that they were aware of the start of the testing session. The test was started by placing the testing apparatus, with the log down, into the pen resting on the floor along the pen's door side. The pigs could then freely interact with the apparatus for 30 min per group. A single observer noted the timing, frequency and type of interactions with the apparatus according to an ethogram (**Table 1**) and the identities of the pigs displaying the behaviour. The observer stood outside the pen and behind the apparatus. When two pigs successfully lifted the log so that it remained fixed to the magnet, emitting a soft click sound, they received a food reward within 2 s of success with the experimenter manually placing a small amount (5–10 g) of small cut apple pieces (0.5 × 0.5 × 0.5 cm, Jonagold apples) mixed with chocolate raisins in their food bowl from two openings on the top of the apparatus. The apparatus was reset immediately after the pigs had consumed the reward. Food was not present in the bowl until after the pigs had succeeded.

On the first 3 testing days, the apparatus was pre-baited (i.e., food placed in the reward location) to increase motivation so that the pigs could smell the food reward below the log but not reach it unless the log was lifted successfully by both pigs. Hence, one pig was unable to reach the food rewards alone. There were occasionally pigs who displaced others and therefore, to avoid this, we stopped pre-baiting after day 3 and rewarded the pair of pigs immediately after success to avoid free-riding. Therefore, on the remaining days, the food bowls were not pre-baited unless the group had not been successful in the previous day in which case we pre-baited the apparatus to maintain the pigs' interest in the task. This was the case for group 1 on test days 6, 7, and 8; group 3 on day 8; and group 7 on days 7 and 9.

Group Composition and Kinship

After weaning at 4 weeks of age, pigs were either kept with their original littermates ("littermate" groups; $n = 4$ groups), or a new group was composed by mixing four different litters ("mixed" groups; $n = 4$ groups) to generate variation in group composition. In the littermate groups, the number of pigs per group varied between 10 and 14, based on initial litter size and as the initial group composition (i.e., litter) was kept intact. The mixed groups were matched to have a similar variation in group size as the littermate groups, with an average group size of 12 (Std. dev.: 1.825; range: 10–14) pigs in the littermate groups and 11 (Std. dev.: 0.816; range: 10–12) pigs for the mixed groups. Due to routine practices at the farm, piglets were fostered-off only when the number of piglets exceeded the number of functional teats of the sow and within 72 h after birth. There were 15 fostered pigs of which four were in the littermates groups and 11 in the mixed groups. The only four fostered pigs in the "littermate" groups were considered as kins of the other pigs in their litter of adoption, because they spent most of their life up to weaning with them, although they were not genetically related, but in pigs familiarity prevails over genetic relatedness (28). None of the pigs were lost-to-follow-up (i.e., removed from the study, e.g., due to health reasons), so group composition remained unchanged throughout the experiment.

The variable "kinship" indicated for each possible dyad combination whether the individuals within a dyad interacting with the apparatus were littermates or not. The effect of kinship was nested within group composition, as pigs in littermate groups were all kin whereas pigs in mixed groups could interact with kin and non-kin. Depending on the size of the mixed groups (between 10 and 12 pigs originating from four litters), an animal could have between 77.8 and 88.9% of unrelated animals in the mixed groups, i.e., non-kin.

Sociability

Sociability was recorded for 4 h daily for 5 days (3 days in the week after weaning and once weekly for the following 2 weeks). The following behaviours were recorded: nose-to-nose contact, nosing in proximity, nosing head, nosing body, allo-grooming, ano-genital nosing, exploring together, social play, lying together, mounting, agonistic behaviour, oral manipulation, belly nosing, other behaviour, or being out of sight, with the ethogram detailed in (29). Three observers, using the app Animal Behaviour Pro (version 1.4.4., Animal Behaviour Pro) installed on iPads, recorded the behaviour using 5-min scan sampling for each individual, resulting in 48 scans per animal per day (240 scans per animal in total). Intra- and inter-observer reliability was tested as intraclass correlation coefficient in R (version 3.6.3; R Core Team). The intra-observer reliability reached values of over 96.3% and the inter-observer reliability reached an agreement of 83.5%. The frequencies from all observation days were summed by behaviour by animal, and thereafter expressed as a proportion of the total number of scans. We created the variable "sociability" as the sum of the proportion of scans when an individual was observed initiating nose-to-nose contact, nosing in proximity, nosing head, nosing body, allo-grooming, exploring together and social play. As discussed in Camerlink et al. (29) ambiguous or potentially negative social behaviours such as ano-genital nosing, belly nosing, mounting, agonistic behaviour, and oral manipulation, or inactive behaviour like lying together were discarded from the sociability score. This resulted in one score per individual based on the frequency of the socio-affiliative behaviours initiated toward group members. We then calculated sociability scores for all possible dyads in the group by averaging the sociability of both individuals associating as a dyad in the task, i.e., for the joint behaviours Attempt together and Successful lift.

Evidence of Understanding of the Social Nature of the Task

To determine whether the pigs showed evidence of understanding the need for a partner to solve the task, video footage of the six most successful groups on the last testing day (Day 9), when most pigs had learned how to solve the task, was analysed. We recorded lifting alone in presence or absence of a partner facing the second opening of the apparatus. We also recorded whether the lift was synchronised, defined as two pigs performing a Successful lift by lifting the log starting out from a horizontal position, or non-synchronised, defined as a Successful lift after one pig lifted the log up completely on its side (such that the log is in a diagonal position) followed by the second pig lifting the log on the other side.

Statistical Analysis

Hypothesis 1 regarding learning of the task was tested based on the changes in behaviour over time (test days). Hypothesis 2 regarding kinship was tested based on the predictor variables "group composition" (littermates vs. mixed) for all behaviours, and "kinship" for the joint behaviours (Attempt together and Successful lift) as the variable kinship depended on dyadic associations. Hypothesis 3 regarding sociability was tested based on the predictor variable "sociability" for the joint behaviours (Attempt together and Successful lift) as this variable also depended on dyadic associations.

The frequencies of the four apparatus-directed behaviours "Touch," "Attempt alone," "Attempt together," and "Successful lift" were analysed in separate models, models 1–4 as described below.

Statistical Analyses of Touch and Attempt Alone

The frequencies at which each individual touched the apparatus ("Touch"; model 1) and attempted to lift the log alone ("Attempts alone"; model 2) were analysed with Generalised Linear Mixed Models [GLMM; (30), with Poisson (model 1) or Negative binomial (model 2) error structures, and log link function (31)]. The key predictor variables were test day (1 to 9), group composition (mixed vs. littermate), sex (male vs. female), and the interaction between test day and group composition or sex if significant, as fixed effects. We also included fostering (yes vs. no) and the individuals' birth weight to account for possible variation related to early life experience and dominance. To avoid pseudo-replication we included random intercept effects for the individual, the litter from which it originated, the group, and the day of test. In order to avoid an "overconfident" model, to keep type I error rate at the nominal level of 0.05, and to model the effect of fixed effects terms potentially varying between, for instance, groups or dyads, we included all theoretically identifiable random slopes (32, 33); see **Supplementary Table 1** for an overview of all models fitted and the random slopes included). As an overall test of the effects of the key predictors, and to avoid cryptic multiple testing (34), we compared this full model with a null model lacking these fixed effects but being otherwise identical. The full and respective null models were compared by means of a likelihood ratio test (35) and the significance of individual fixed effects determined by dropping them one at a time R-function drop1; (33). The full-null model comparisons were significant (model 1: $\chi^2 = 23.073$, df = 4, $P < 0.001$; model 2: $\chi^2 = 20.566$, df = 4, $P < 0.001$), indicating that the test predictors as a collective had a clear impact on those response variables.

Statistical Analyses of Attempt Together and Successful Lift

The frequencies of dyadic attempts to lift the log together ("Attempt together"; model 3) and to successfully lift together ("Successful lift"; model 4) were analysed with GLMMs with Poisson error structure and log link function. The predictor variables were test day (1 to 9), group composition (mixed vs. littermate), kinship (whether the two members of a dyad originated from the same litter or not), the interactions between

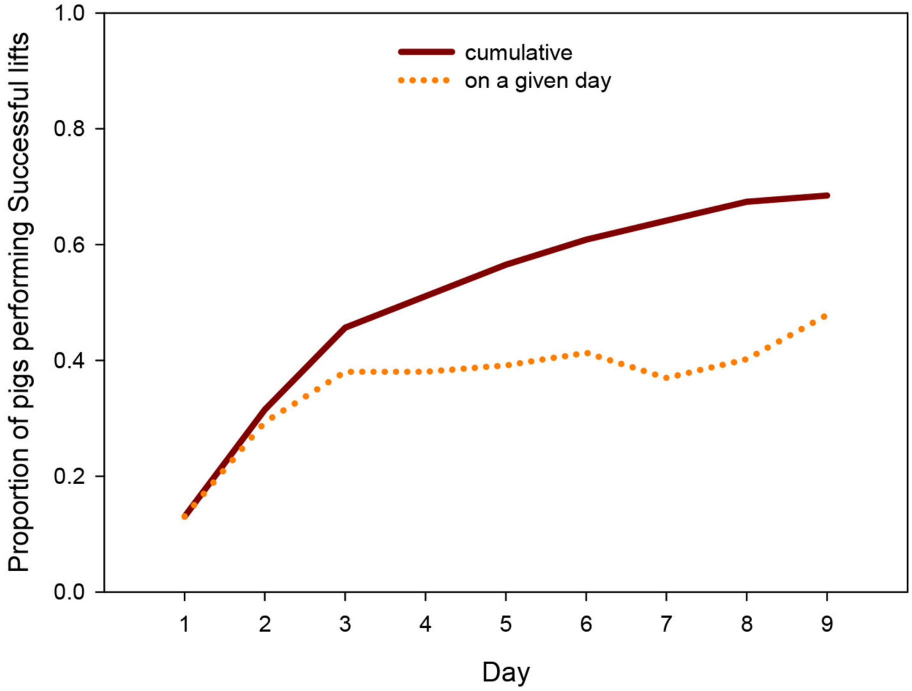

FIGURE 2 | Proportion of pigs per day and cumulative proportion that successfully lifted the log across days.

test day and group composition or kinship, the average sociability score per dyad, and the sex combination of the dyad (female-female, female-male, or male-male). We included random intercept effects for the pen, the dyad, the two members of the dyad, the two litters from which they originated, and test day nested within pen. Again, we included all theoretically identifiable random slopes (**Supplementary Table 1**). As animals could associate with various partners, we included all possible dyads and allocated "0" for dyads that never interacted. We compared this full model with a null model lacking the fixed effects but being otherwise identical. The full-null model comparisons were not significant for the test predictors as a whole (model 3: $\chi^2 = 3.945$, df = 5, $P = 0.557$; model 4: $\chi^2 = 7.403$, df = 5, $P = 0.192$), but significant main effects appeared in a reduced model after removal of the non-significant interactions (see results).

General Aspects of the Statistical Analysis

Data were fitted with models in R (version 3.6.3; R Core Team) using the function glmer of the package lme4 [version: 1.1-21; (36)]. We confirmed model stability by dropping levels of random effects one at a time and comparing the estimates derived for models fitted to those subsets with those obtained for the model for the full data set. We determined 95% confidence intervals of model estimates and fitted values by means of parametric bootstraps (N = 1,000 bootstraps; function bootMer of the packages lme4). None of the models was overdispersed (dispersion parameters model 1: 0.988; model 2: 0.715; model 3: 0.605; model 4: 0.352), and collinearity was no issue. Samples sizes are reported in **Supplementary Table 1**.

RESULTS

Behavioural Interactions With the Apparatus

Across the days, 68% ($N = 62$) of the animals performed at least once a Successful lift, i.e., solved the task through a joint action with another group member. The proportion of successful individuals on a given day rose quickly until day 3 and more slowly thereafter (**Figure 2**), with almost half of the pigs being successful on the last day ("on a given day" count) and two thirds of the pigs having succeeded at least once over the course of the 9 days ("cumulative count").

In total, there were 2261 Successful lifts over the 9 test days (**Figure 3**). Nevertheless, there was a large variation among the 91 individual animals in the number of Successful lifts. The top nine individuals from four different groups accounted for 50.5% of all Successful lifts, and there were large differences in the quantiles of Successful lifts across the 91 animals (minimum = 0, 1st decile = 0, 1st quartile = 0, median = 3, 3rd quartile = 64, 9th decile = 179 and maximum = 319 Successful lifts).

The frequency of behavioural interactions with the apparatus averaged 27.0 (min: 21.7, max: 34.4) interactions per pig and 30-min test across days, when all behaviours were summed up. Individual behaviours (Touch and Attempt alone) were progressively replaced by joint behaviours (Attempt together and Successful lift) (**Figure 3**).

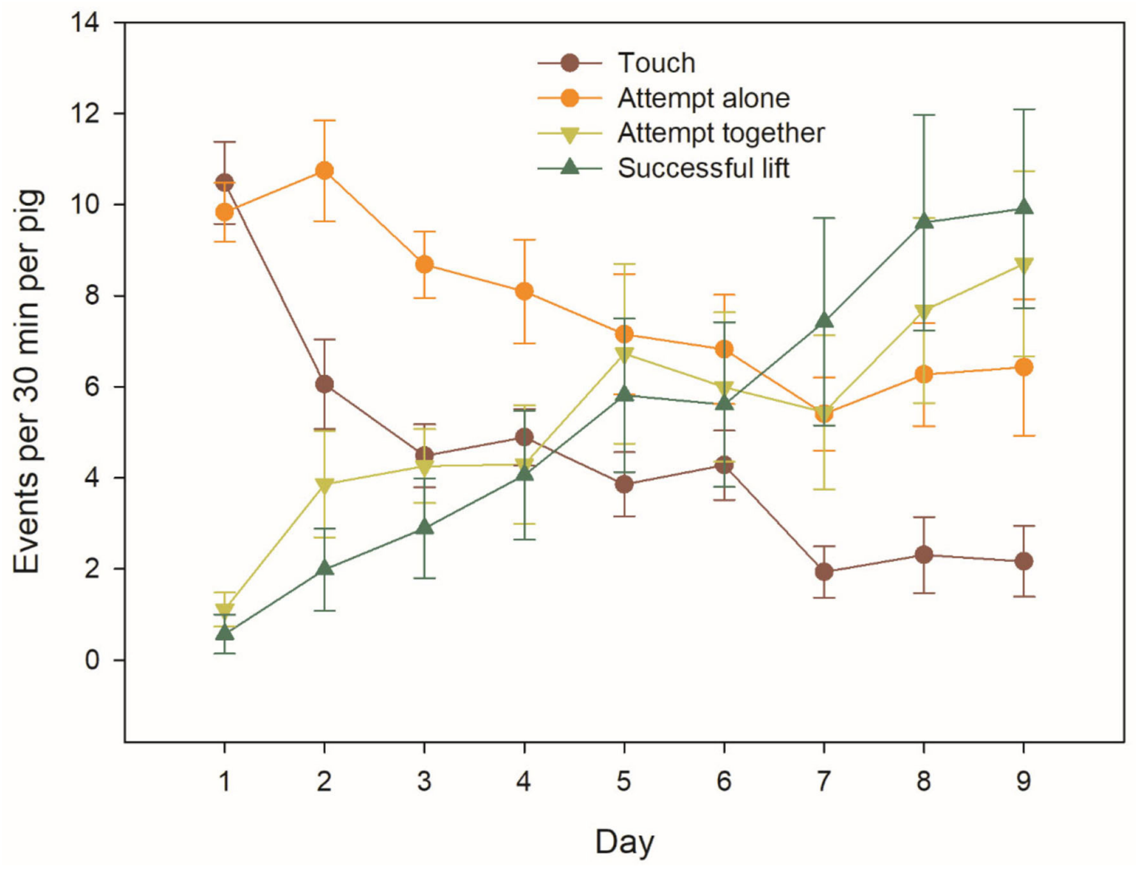

FIGURE 3 | Behavioural interactions with the apparatus over the testing days, based on group averages ($n = 8$) of individual pig behaviour. The bars represent the standard errors.

Evidence of Learning

The frequency of Successful lifts increased over the course of the test days ($P = 0.004$; **Figures 3**, **4** and **Supplementary Table 2**). The frequency of Attempts together also tended to increase over the course of the test days ($P = 0.08$; **Figure 3** and **Supplementary Table 3**). The frequency of Attempts alone and Touches differed or tended to differ, respectively, according to the interaction of test day and group composition (see below).

Group Composition and Kinship

The frequency of Successful lifts and Attempts together did not significantly differ according to group composition or kinship (**Supplementary Tables 2, 3**, respectively).

The frequency of Attempts alone decreased according to the interaction of group composition and test day ($P = 0.03$; **Figure 5** and **Supplementary Table 4**), whereby it decreased over the course of the test days in both group compositions but was steeper in the mixed groups as compared to the littermate groups (MIX: $-0.65+0.11$, $z = -5.71$, $P < 0.001$; LIT: $-0.33+0.1$, $z = -3.46$, $P = 0.001$). The frequency of Touches also tended to decrease according to the interaction of group composition and test day ($P = 0.06$; **Supplementary Table 5**) whereby the decrease over the course of the testing days also tended to be steeper in mixed groups as compared to the littermate groups.

Sociability

The frequency of Successful lifts increased with greater sociability score ($P = 0.03$; **Supplementary Table 2**). The frequency of Attempts together did not significantly differ according to sociability (**Supplementary Table 3**).

Dyads

Over the days, successful pigs gradually paired up with an increasing number of different partners (**Figure 6**), and over the last 3 days several pigs associated with 6 or more different partners. There was a large variation in the total number of success between dyads, indicating that the dyadic associations were strongly non-random. The average number of Successful lifts was 0.5 per 30 min daily session and 4.7 per dyad over the course of the 9 test days, but the distribution was highly skewed between dyads. On the one hand, only 26% (124 out of 479 possible) dyadic combinations of pigs successfully lifted together, in spite of the fact that pigs had the choice of partners within their group over the duration of the experiment. On the other hand,

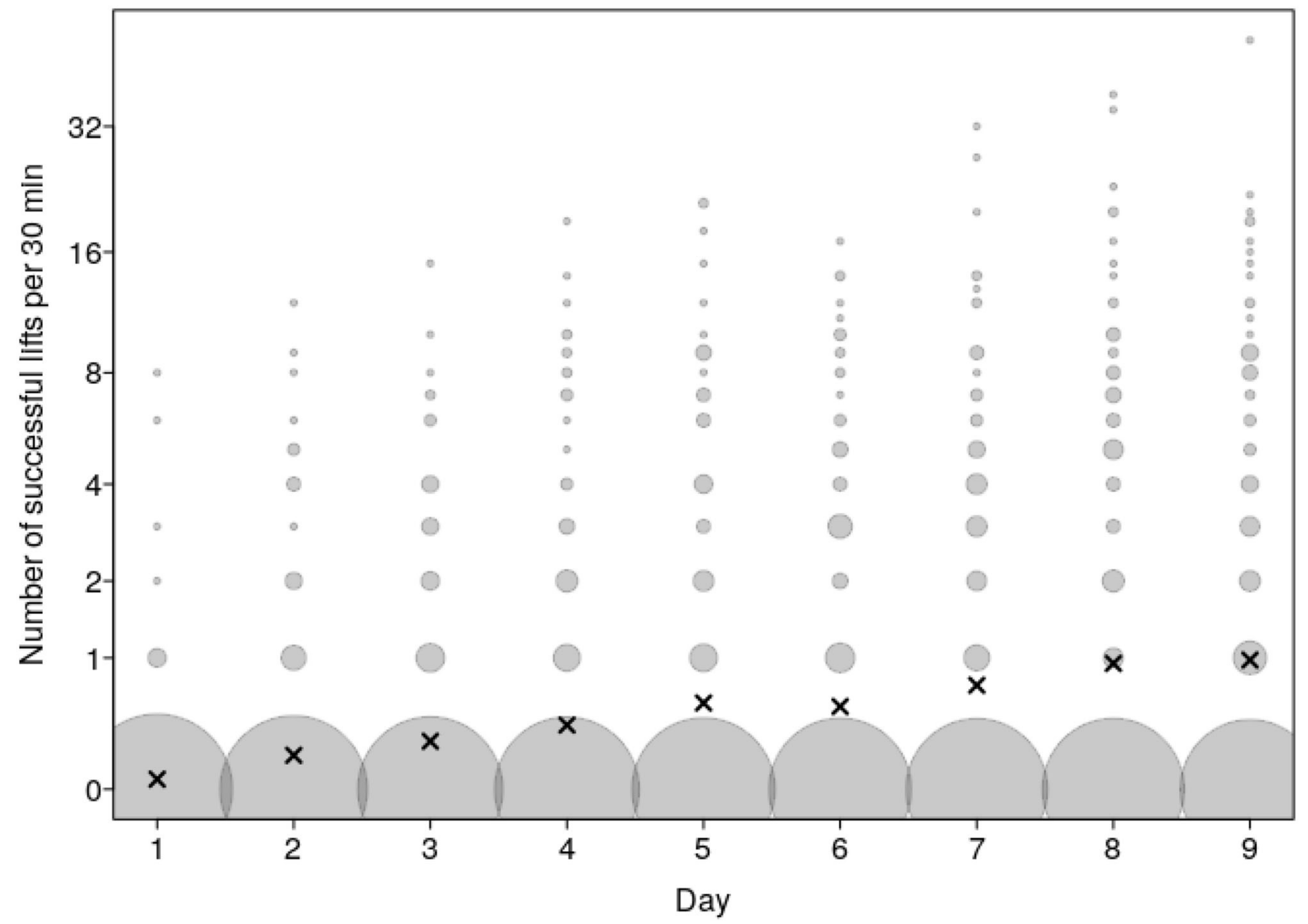

FIGURE 4 | Number of Successful lifts as a function of test day. Dots show the number of Successful lifts per test day and dyad whereby the area of the dots depicts the number of cases per combination of test day and number of Successful lifts (range: 1 to 468). The laying crosses depict the mean per day. A value of 0 was allocated to any of the potential dyadic combination of pigs in a group that did not perform a Successful lift on that day; hence the large number of 0 values.

some of these dyads were highly successful, with 39 dyads with 20 or more Successful lifts over the nine sessions, 12 dyads with 50 or more Successful lifts, and even three dyads with over 100 Successful lifts.

Evidence of Understanding of the Social Nature of the Task

From the subset of data analysed for the six most successful groups on the last testing day, out of 462 occurrences of lift alone, 434 (93.9%) were lifts in the presence of a partner facing the second opening of the apparatus, and the rest in absence of a partner. Of 448 lift successes, 112 (25%) were synchronised, and the rest non-synchronised.

DISCUSSION

Pigs solved the JLLT spontaneously, progressively switching from individual to joint behaviours, with almost half of the pigs being successful on the last day and two thirds of the pigs having succeeded at least once over the course of the 9 days. The JLLT relies on an obligate strategy, meaning that the individuals only benefit if they engage in the task jointly and do not get a (smaller) reward for performing the task individually. Thus, the task is more demanding than other situations where succeeding alone is possible (12, 14). This task can easily be adapted to socially foraging species in an ecologically-relevant manner, for instance for primates that may attempt to lift the log with their hands, elephants with their trunks, or other suids with their nose. The principle of the task can be also used for construction of a device, with a modified mechanical design, for species that use other specific motor skills during foraging, such as pulling a branch or scratching the ground. We posit that such a device could be designed for various species in which individuals often forage synchronously in close vicinity of each other and frequently use a specific motor pattern to access a food source.

Our findings do not provide evidence about the specific cognitive mechanisms behind the successful joint action of the pigs at the apparatus. We presume that the task may reflect cooperation, based on the definition by Noë (1) of "a behavioural strategy in which agents achieve a common goal

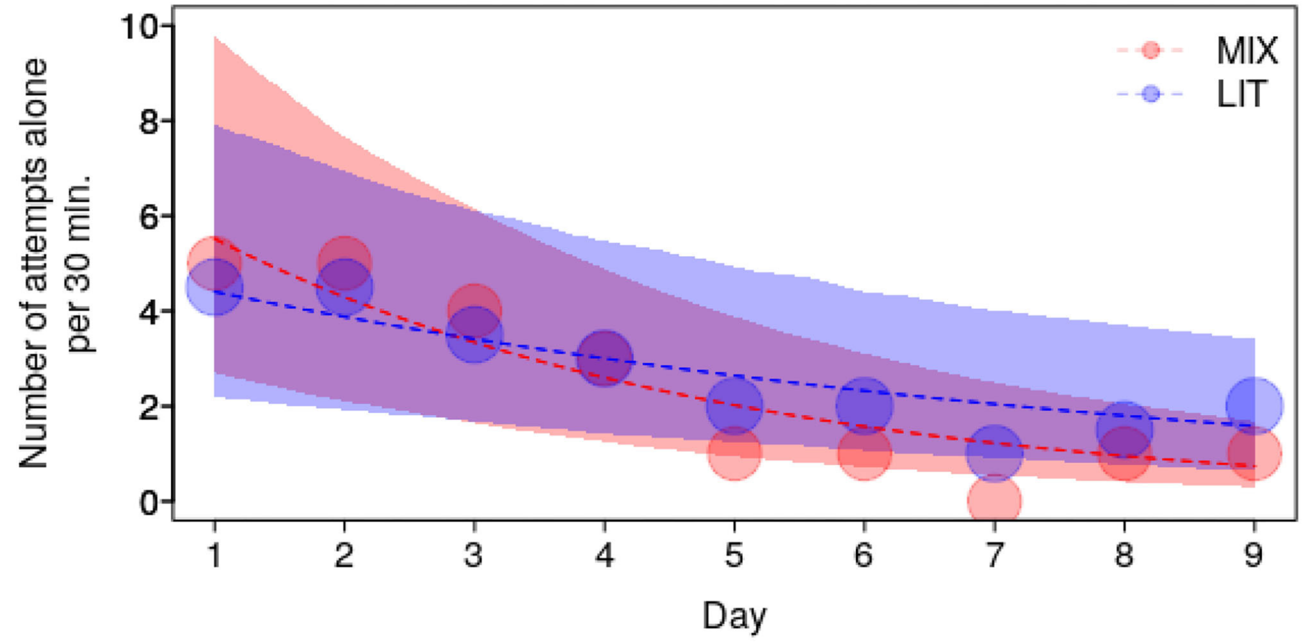

FIGURE 5 | Frequency of Attempts alone as a function of test day, for the two group compositions with mixed groups in light red (MIX) and littermate groups (LIT) in dark blue. Dots show the median number of Attempts Alone per test day whereby the area of the dots depicts the number of counts (range: 43 to 48). The dashed lines and the shaded polygons depict the fitted model and its confidence limit.

through coordinated action." However, the term "cooperation" remains debated (37), from simple definitions such as two or more animals acting simultaneously or sequentially to solve a problem (12, 38), to much stricter definitions requiring the capacity to understand the role of the partner and to share intentions (39). Consequently, terms such as similarity or independent cooperation (38), coordination (40), collaboration (41), and intentional cooperation (38) have been proposed to distinguish between different types of joint actions (i.e., "cooperation" in the wide sense of the term). Even within the same act of cooperation, the participating individuals may differ in what cognitive mechanisms they employ (42). The mechanisms involved in solving the JLLT remain to be elucidated.

The animals were able to solve the JLLT spontaneously. Spontaneous cooperation [e.g., (5, 18, 20, 43, 44)] has been much less studied than instrumental cooperation when animals are trained at the task (12, 13). Spontaneous learning was likely facilitated by the design of the apparatus, enticing an elementary natural foraging behaviour. It also allowed researchers to circumvent the need for prior separate individual training, which can be problematic when testing cooperation that requires coordination rather than the mere combination of two individuals having learnt to succeed independently (12, 45). Kune Kune free-ranging pigs failed to spontaneously solve the JLLT when presented with it for 18 trials over 3 sessions in selected pairs, but the Kune Kune pigs succeeded at it after training (46). Pigs in the present study managed to successfully solve the JLTT when presented with the task in the home environment and in their group with *ad libitum* trials over 30-min for 9 days, allowing for more trial-and-error opportunities than this other study. The success then depended solely on coordinating the action in time with a suitable partner within the group. The progressive change in behaviour from single to joint actions supports that the pigs learned how to solve the task, as experience is often necessary for cooperation (47). The time from the first exposure to the apparatus to solving the task was among the shortest ever recorded in studies with other tasks including primates (48), social canids (49), and parrots (50).

The highly skewed distribution of success across dyads indicates that mutual free choice of partners may be a key aspect for success. More sociable pigs were found to be more likely to perform Successful lifts, although their engagement in Attempts together did not differ, suggesting greater proficiency at the task in more sociable pigs. This possibly reflected social tolerance as a factor conducive to cooperation in other species (18, 51). Offering the task at group level also allowed us to test animals in their home environment, hence without disturbance (e.g., handling, novel environment), and with free-choice about when and with whom to work jointly. Similarly chimpanzees free to interact with an apparatus in their home enclosure selected their preferred cooperation partner to solve the task (5). Testing in the home environment allows accounting for social and other contextual features that may have core influences on the propensity of animals to interact with others (26, 45, 52, 53).

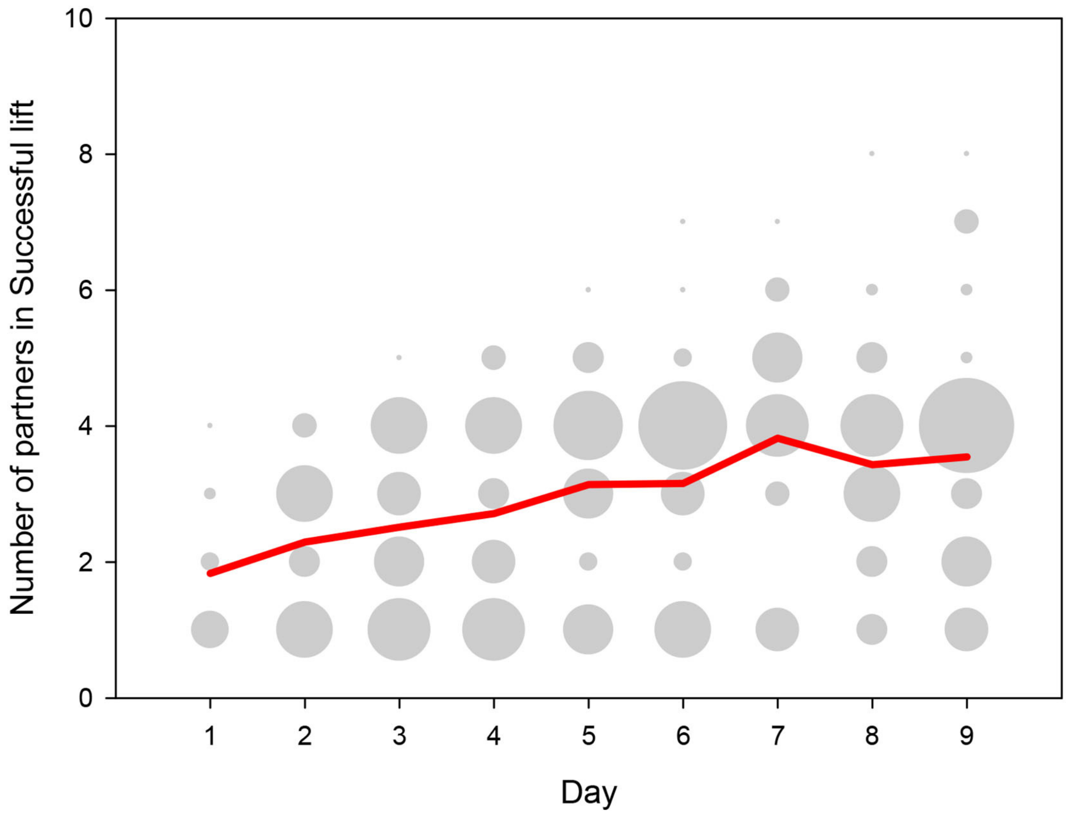

FIGURE 6 | Number of partners with whom a pig successfully lifted the log, depicted for those pigs that lifted the log at least once on a given day. The y-axis shows the number of partners per test day for a given pig whereby the dot size depicts the number of pigs that lifted for that specific number of partners. The red line depicts the mean number of partners across days.

Kin were however not more likely to engage or succeed in the task than non-kin. The only difference was that pigs in mixed groups reduced their attempts alone quicker than pigs in littermate groups, but with no difference in attempting together or successful lifts. Pigs are able to recognise familiar individuals from a young age (28) and can form affiliation with kin (54) and non-kin (55). Nevertheless, pigs in the wild live in groups of genetically related females with little fission-fusion dynamics (56), possibly explaining their lack of kin discrimination. Kinship through inclusive fitness is a common explanation for cooperation, but cooperation can also operate based on reciprocity (16). The highly skewed distribution of lifts across the dyads indicates that successful pigs appeared to form preferential dyadic associations to solve the task. Thus, pigs may learn to associate with more proficient partners over the course of their trials, or based on their affiliative preferences (55).

The majority of pigs were highly motivated to work to access the high-value food reward, despite having *ad libitum* access to feed. Pigs show a high motivation for foraging when provided the opportunity (57). The number of successful individuals on a given day rose until day 3, the last day in which the apparatus was pre-baited, but pigs maintained interest thereafter, and whether pre-baiting the apparatus heightens interest requires further research. Given that pigs became increasingly successful over the days but the total number of interactions with the apparatus remained similar, it appears that a steady motivation was driving the learning process that was faster in some individuals, slower in others, and failed (within the given timeframe) in yet other individuals. Although the task theoretically allows each pig to engage with the apparatus when they want and with whom they want, a third of the pigs did not succeed, which suggests that either they were unable to solve the task, would have needed longer, or possibly were unable to access the apparatus during the relatively short testing sessions due to the monopolisation of the apparatus by other pigs. These limitations could be solved by providing access to the apparatus for longer test sessions and over a longer time period.

The underlying cognitive mechanism of the JLLT remains to be elucidated. One possibility is that pigs are using their capability to synchronise their behaviour (58) to achieve the time-coordinated joint action at the apparatus. It has been shown both through modelling and experimental research that synchronisation in pairs could be achieved without explicit communication and that it can promote joint performance (59, 60). However, the pigs in this study showed synchronised lifting in only one quarter of the coded test sessions. Nevertheless, other mechanisms can facilitate joint action or coordination (12), including social facilitation (61). Success in the JLLT task could also result from chance associations of individuals acting simultaneously, as seen in other social foraging tasks [e.g., (20)].

At least, pigs lifted in a large majority of cases (94%) when another partner was present at the other hole, rather than when no partner were present. Further research should use appropriate controls to test whether pigs take account of the partner's presence and behaviour [e.g., (46, 62)].

CONCLUSIONS

The feasibility of the JLLT makes it a versatile tool for experimental investigations of social foraging at large scale, by giving animals free choice about when and with whom to work jointly. This approach offers the possibility to study the role of partner choice, group social dynamics, personality, and proficiency in the task (ratio of success to failure) on the performance of different species of animals. At the same time, the variability of success in the JLLT between individuals and dyads within groups in the current study indicates that the task may be a valuable paradigm to assess various factors that affect social associations at different levels of animal social organisation.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: http://dx.doi.org/10.17632/4vb5xnm393.1.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Ethics Committee of the University of Veterinary Medicine, Vienna, Austria (Research Protocol No. ETK-95/06/2019).

AUTHOR CONTRIBUTIONS

J-LR, IC, MŠ, and SG designed the apparatus, wrote the manuscript, and participated in data analysis and interpretation. J-LR and IC conceived and designed the study. IC carried out the experimental work. RM carried out the statistical analyses. All authors gave final approval for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2021.745627/full#supplementary-material

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BRIEF RESEARCH REPORT
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Goats (*Capra hircus*) From Different Selection Lines Differ in Their Behavioural Flexibility

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Given that domestication provided animals with more stable environmental conditions, artificial selection by humans has likely affected animals' ability to learn novel contingencies and their ability to adapt to changing environments. In addition, the selection for specific traits in domestic animals might have an additional impact on subjects' behavioural flexibility, but also their general learning performance, due to a re-allocation of resources towards parameters of productivity. To test whether animals bred for high productivity would experience a shift towards lower learning performance, we compared the performance of dwarf goats (not selected for production, 15 subjects) and dairy goats (selected for high milk yield, 18 subjects) in a visual discrimination learning and reversal learning task. Goats were tested individually in a test compartment and were rewarded by choosing either a white or a black cup presented by the experimenter on a sliding board behind a crate. Once they reached a designated learning criterion in the initial learning task, they were transferred to the reversal learning task. To increase the heterogeneity of our test sample, data was collected by two experimenters at two research stations following a similar protocol. Goats of both selection lines did not differ in the initial discrimination learning task in contrast to the subsequent reversal learning task. Dairy goats reached the learning criterion slower compared to dwarf goats (dairy goats = 9.18 sessions; dwarf goats = 7.74 sessions; $P = 0.016$). Our results may indicate that the selection for milk production might have affected behavioural flexibility in goats. These differences in adapting to changing environmental stimuli might have an impact on animal welfare e.g., when subjects have to adapt to new environments or changes in housing and management routines.

Keywords: animal cognition, discrimination learning, farm animals, reversal learning, ungulates

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INTRODUCTION

To survive, animals need to flexibly adapt to their environment (Shettleworth, 2010). Their ability to learn, and associated levels of behavioural flexibility, have been linked to many socio-ecological parameters, such as the diversity of food sources and habitats (Rosati, 2017), and to complex social group structures (Amici et al., 2008, 2009). Behavioural flexibility refers to the adaptive change in the behaviour of an animal, e.g., an animal's ability to learn a now reversed learning contingency. Another factor with the potential to impact behavioural flexibility, or learning ability in general, is artificial selection by humans, either by means of domestication (Price, 1999; Lindqvist and Jensen, 2009) or subsequent selection for specific production traits (Dudde et al., 2018). These differences can be of relevance in the context of various welfare-related issues in farm animals, such as adaptation to new environments or changes in housing and management routines, but remain relatively unexplored.

To assess behavioural flexibility, researchers often rely on the assessment of an individual's reversal learning ability (Berg, 1948). Although the test design is identical, reversal learning necessitates different and more complex cognitive mechanisms compared to simple discrimination learning (Diekamp et al., 1999). After meeting a certain learning criterion in an initial discrimination task, subjects will have to inhibit responses to the originally rewarded stimulus and to respond to a previously unrewarded stimulus in a reversal task. By using learning and reversal learning tasks, one can thus not only measure the general ability of an individual to learn, but also how flexible it can adapt its learned response.

During the course of domestication, with more stable food security and environmental conditions, selection pressure for improved learning performance and flexible adaptation to novel contexts might have been altered in domestic animals (Price, 1999). Research on learning performance comparing domestic species and their wild ancestors has come up with inconclusive results. While Lindqvist and Jensen found impaired spatial learning in domestic fowl compared to their non-domesticated counterparts (Lindqvist and Jensen, 2009), Gunther et al. showed that domestic guinea pigs (*Cavia porcellus*) learned an association faster than non-domesticated cavies (*Cavia aperea*), while both groups did not differ in their reversal learning performance (Brust and Guenther, 2015). Wolves (*Canis lupus*), in turn, differ in their behavioural flexibility compared to domestic dogs (*Canis lupus familiaris*) (Marshall-Pescini et al., 2015). However, the directionality of this difference was dependent on the task that was used (Marshall-Pescini et al., 2015).

Not only domestication, but also further selection and breeding of farm animals for high productivity can have an indirect impact on behavioural traits due to an assumed re-location of resources, according to the so-called Resource Allocation Theory (Beilharz et al., 1993). As animals bred for high performance may invest more resources into production traits and less in other biological processes, these changes might also potentially affect their ability to learn and their flexibility to adapt to new or variable environments. Indeed, selection for high productivity has already been found to have altered foraging and exploration behaviour in farm animals (Schütz and Jensen, 2001; Colpoys et al., 2014). In terms of potential impacts on mental processes, recent work on different production lines of laying hens found no, or contradictory, associations between selection for production and learning performance: hens that have been bred for higher egg yield were faster to reach a learning criterion in a visual discrimination task, compared to lines that have not been selected for high egg yield. However, both lines did not differ in their performance in the subsequent reversal learning task (i.e., do not show differences in behavioural flexibility; Dudde et al., 2018). Further investigations in the context of different production traits are necessary to assess potential associations between the selection for production traits, learning performance and behavioural flexibility.

In this study, we assess learning and behavioural flexibility in goats. Goats, as grassland foragers and prey animals, rely heavily on vision when navigating and have already shown to be able to master visual and spatial reversal tasks (Langbein et al., 2008; Meyer et al., 2012). Thus, a visual discrimination task and a subsequent reversal learning task were used to investigate whether goats not selected for production traits (dwarf goats) and goats selected for high milk yield (dairy goats) differ in their (reversal) learning ability. Based on the Resource Allocation Theory (Beilharz et al., 1993), we hypothesised dwarf goats outperforming dairy goats in the learning task, as well as in the reversal learning task.

METHODS

Subjects, Housing, and General Procedure

To increase the heterogeneity of our sample, data was collected by two researchers at two research sites (Agroscope Tänikon in Ettenhausen, Switzerland, and the Research Institute for Farm Animal Biology in Dummerstorf, Germany) (Voelkl et al., 2018, 2020). 18 non-lactating female Nigerian dwarf goats (mean age ± SD; Ettenhausen: 364.4 ± 3.2 d, Dummerstorf: 361.7 ± 19.2 d at start of habituation) and 18 non-lactating female dairy goats (Ettenhausen: 339 ± 12.4 d, Dummerstorf: ∼396 d at start of the initial visual discrimination task) participated in the experiment, that consisted of a visual discrimination task and a subsequent reversal learning task. The number of subjects for the current study was logistically limited due to their assignment for a specific treatment for a subsequent study (Nawroth et al., 2021). I.e., goats were a randomly chosen sub-sample of a larger group and were group-housed in 6 pens with 10 subjects each (of which three subjects per pen participated in this experiment and were thus assigned as one treatment group for a study that investigated the impact of test experience on individuals' performance in subsequent conceptually different tests) at both locations (Ettenhausen: $n = 9$ for dwarf and dairy goats each; Dummerstorf: $n = 9$ for dwarf and dairy goats each).

Dwarf goats for both locations were bred in Germany, Dummerstorf. Dairy goats were bred at different Swiss farms (Saanen and Chamois coloured goats) and one large German farm (Deutsche Edelziege). The Nigerian Dwarf goat is commonly kept as pet and zoo animal in Europe and not selected for productivity traits. The only selection aim in the Dummerstorf population was to avoid inbreeding. The potential milk yield of dwarf goats does likely not exceed 0.3 kg per day (Akinsoyinu et al., 1977). As it was common practise in Dummerstorf, dwarf goat kids stayed with their dams for 6 weeks before they were weaned. We used three of the most common high-producing dairy breeds in Switzerland and Germany (Saanen and Chamois coloured goats, Deutsche Edelziege). These animals had a potential milk yield of up to 3 kg per day (Vacca et al., 2018). In accordance with common practise in the dairy goat industry, the dairy goat kids had been separated from their dam shortly after birth and were artificially raised.

Initially, dwarf and dairy goats were housed in one large group pen (per selection line) at each location. At the age of 7–8 months, all goats were then moved to pens of 10 goats each. The total area of each dwarf goat pen was 14 m^2 (∼3.6 × 3.9 m), consisting of a deep-bedded straw area of 11 m^2 (∼2.8 × 3.9 m) and a 0.5 m elevated feeding place (1.4 m^2). The total area of each dairy goat pen was 17.7 m^2 (∼3.9 × 4.55 m) consisting of a deep-bedded straw area of 13.4 m^2 (∼4.55 × 2.95 m) and a 0.65 m elevated feeding place (1.82 m^2). Hay was provided behind a feeding fence at the feeding place twice a day at around 8 am and 4 pm in Ettenhausen and at around 7 am and 1 pm in Dummerstorf. Each pen had one watering place and a mineral supply. Additional structures in the straw-bedded area included a wooden bench (for dwarf: 2.3 m long, 0.5 m high, 0.5 m wide; for dairy: 2.4 m long, 0.6 m high, 0.62 m wide) along the wall of the pen and a round wooden table (0.8 m high, Ø 1.1 m) in the centre of the pen. Pens and handling regimes were kept as similar as possible at both locations.

For individual habituation, shaping, training, and testing, goats were physically and visually separated from their pen-mates in a test area (450 × 200 cm), but kept acoustic contact to their pen-mates that were located in an adjacent waiting area (600 × 200 cm). The experimenter sat in another adjacent compartment (150 × 200 cm) separated from the tested animal by a grate, allowing subjects to insert their snouts through the bars. A sliding board (60 × 20 cm) was placed on the experimenter side of the grate on a small table (105 × 40 cm) at a height of ∼35 or 40 cm (for dwarf goats and dairy goats, respectively) in front of the grate (**Figure 1**). Subjects were not food restricted before testing. Goats were tested once a day (∼between 9:00 and 12:00, with time of testing counterbalanced between subjects). To decrease potential experimenter biases, two experimenters (CN and KR) were alternating between each test session at both research sites.

Habituation

Subjects were first introduced as a group (all subjects of each pen) for 2 days to the test arena and an adjacent waiting area for ∼20 min per day. Subsequently, goats were introduced as pairs to the test arena. Each pair was provided ∼10 pieces of reward (uncooked piece of pasta; positive reinforcement) over a period of 5 min *via* the sliding board (dwarf goats: 6 days; dairy goats: 4 days). Finally, subjects were habituated alone for 2 min, using the same procedure as for the paired habituation (dwarf goats: 13 days; dairy goats: 7 days). Habituation sessions were repeated until the individual showed no signs of arousal or stress during food delivery. All subjects reached the criterion to proceed with the shaping procedure.

Shaping

Shaping was introduced to familiarise subjects to the test procedure and to train them how to indicate a choice. In shaping trials, one flat plastic bowl (brown, diameter 14 cm, height 2 cm) was located in the middle of the sliding board. In the first four trials of a shaping session, the experimenter put a food reward into the bowl and then pushed the platform towards the grate. If the animal put its nose through one of the middle gaps in the grate, it received a reward (positive reinforcement). This was repeated for six additional trials, but for these, the experimenter covered the bowl with a cup (light brown, diameter 12 cm, height 10.5 cm) before letting the subject make its choice. Shaping sessions were repeated until the individual showed no signs of arousal or stress during participation and instantly chose the baited position. If a subject did not make a choice within 60 s, a trial was repeated. If a trial had to be repeated twice, the session was terminated. Dwarf goats received a total of five shaping session, while dairy goats received a total of two sessions. Afterwards, all goats proceeded to the training. Dwarf and dairy goats differed in their emotional reactivity, so providing all goats with the same time of habituation and shaping might have led to different absolute levels of habituation/shaping for the two groups (Rosenberger et al., submitted)[1]. In addition, we chose a criterion on the group, rather than the individual level, as these goats were part of the treatment group (the group that received cognitive stimulation) for a subsequent study (Nawroth et al., 2021).

FIGURE 1 | Example illustration of the test setup during the visual discrimination and reversal learning task. © Nordlicht/FBN.

Training

Training sessions were conducted for both selection lines and consisted of 10 trials each. All subjects received a maximum of two sessions per day. Two bowls were placed on the left and right side of the sliding board at a distance of 30 cm. The experimenter baited only one bowl in full view of the subject, then covered both bowls with identical cups (same size and colour as before) and pushed the board towards the grate. The subject made its choice by putting its snout through one of the outer left or right gaps in the grate (opposite the respective bowl) and the experimenter

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[1] Rosenberger, K., Simmler, M., Nawroth, C., Langbein, J., and Keil, N. (2021). *Reactivity of Domesticated Goats Towards Various Stressors Following Long-Term Cognitive Test Exposure* (submitted).

only delivered the reward if the subject was choosing the baited bowl. Each side was baited pseudorandomly five times per session and a maximum of five sessions were administered. Subjects were considered to have completed training when they achieved at least 8 out of 10 correct choices in two consecutive sessions (binomial test; $P = 0.012$).

Visual Discrimination Task

All subjects participated first in the visual discrimination learning task. In this task, the experimenter (E) baited one of two different coloured cups (black or white, diameter 14.5 cm, height 12.0 cm) surreptitiously. The two different coloured cups were placed with the opening upwards on the left and right side of the sliding board at a distance of ∼30 cm (**Figure 1**). Half of the subjects of each selection line were rewarded for choosing one particular coloured cup, whereas the other half was rewarded by choosing the other colour. After a presentation of ∼2 s, E pushed the board towards the grate. The subject was able to make a choice by putting its snout through one of the outer left or right gaps in the grate (opposite the respective bowl) and, if the correct choice (e.g., the baited cup) was made, the goat obtained the reward. To avoid olfactory cueing, a piece of uncooked pasta was attached inside each cup. Each test session consisted of two initial motivation trials (one piece of pasta placed on either the left or right uncovered bowl on the board) at the beginning and 12 subsequent test trials as described above. The location of the baited cup was presented in a pseudo-randomised order across trials, but the baited cup was never positioned on the same side more than two trials in a row. After the first and second incorrect choice in each session, the goat had the opportunity to correct its choice: the non-rewarded cup was withdrawn, and the rewarded cup was kept on the board. After the goats chose the rewarded cup, it received the reward and a new trial started. This correction trial was still scored as incorrect. The inclusion of correction trials was done to prevent frustration and the development of side biases. Any subsequent error resulted in an immediate withdrawal of both cups, leaving the goat unrewarded. Subjects received as many sessions as needed to reach the specific learning criterion (10 out of 12 correct in two consecutive sessions).

Reversal Learning Task

In the reversal learning task, the procedure was the same as in the initial discrimination task (including correction trials), except that in this task the previously unrewarded cup was rewarded, i.e., the reward contingencies were reversed. Again, we scored the number of sessions that goats needed to reach the learning criterion (10 out of 12 correct in two consecutive sessions).

Note that due to logistical reasons, each subject received a combined maximum number of 20 sessions for the visual discrimination and the reversal learning task. If a subject did not indicate a choice after 60 s, the trial was repeated. If the subject did not make a choice in the repeated trial, the session was terminated. After three consecutive terminated sessions, a subject was excluded from further testing. Consequently, two dwarf goats (Dummerstorf) were excluded from subsequent testing as they did not indicate a choice (visual discrimination learning session 1 and 8, respectively).

Ethical Note

Animal care and all experimental procedures were in accordance with the ASAB/ABS Guidelines for the Use of Animals in Research (Association for the Study of Animal Behaviour). All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Ministry of Agriculture, Environment and Consumer Protection of the federal state of Mecklenburg-Vorpommern, Germany (Ref. Nr. 7221.3-1.1-062/17) and by the Swiss Cantonal Veterinary Office, Thurgau (Approval No. TG04/17 – 29343). Housing facilities met the Swiss welfare requirements for farm animals. All measurements were non-invasive, and a session lasted no more than 10 min for each individual goat. If the goats had become stressed, e.g., were frequently vocalising and not paying attention to the test procedure during a test session, the test would have been stopped.

Data Scoring and Analysis

A digital video camera (Ettenhausen: Sony HDR-CX240E; Dummerstorf: Panasonic HDC-SD60) was used to record all trials. We scored which cup (correct or incorrect) the test subject chose for each trial. A "correct" choice was scored if the subject chose the baited cup (i.e., by putting its snout through the respective gap in the grate). Fifteen dwarf goats and 18 dairy goats reached the learning criterion in the initial visual discrimination task. As one dairy goat took 10 sessions to finish in the visual discrimination task, it was subsequently not able to reach the criterion within the remaining 10 reversal learning sessions while all other goats did reach the criterion here, too. We assigned it to 12 reversal learning sessions, as this would have been the minimum number of total sessions for this task for this individual to reach the criterion.

To assess inter-observer reliability, 10% of the videos were coded by a second coder who was unfamiliar to the initial hypothesis. Inter-observer reliability for choice analysis showed a high level of agreement (Cohen's $\kappa = 0.996$, $P < 0.001$).

Statistical analyses were carried out in R v.3.6 (R Core Team, 2017). We scored the number of sessions a subject needed to reach the learning criterion [i.e., choosing correctly in 10 out of 12 trials (binomial test, $P = 0.019$) in two consecutive sessions] for both tasks. The number of sessions needed to reach this criterion was used as outcome variable and was analysed with a linear mixed-effects model (LMM) fit with gaussian family distribution (LMM; lmer function, lme4 library) (Pinheiro and Bates, 2000). Performances in the discrimination and reversal learning task were analysed separately in two models, because the underlying mechanisms to solve both tasks cannot be assumed to be identical (Diekamp et al., 1999). Both models included "Selection line" (factor with two levels: dwarf, dairy) and "Colour" of the rewarded container cup (factor with two levels: white, black) as well as their interaction as fixed factors. "Identity" of the goats nested in "Pen" (1–12) nested in "Location" (Ettenhausen, Dummerstorf) was included as a random factor to control for repeated measurements. For both models, we

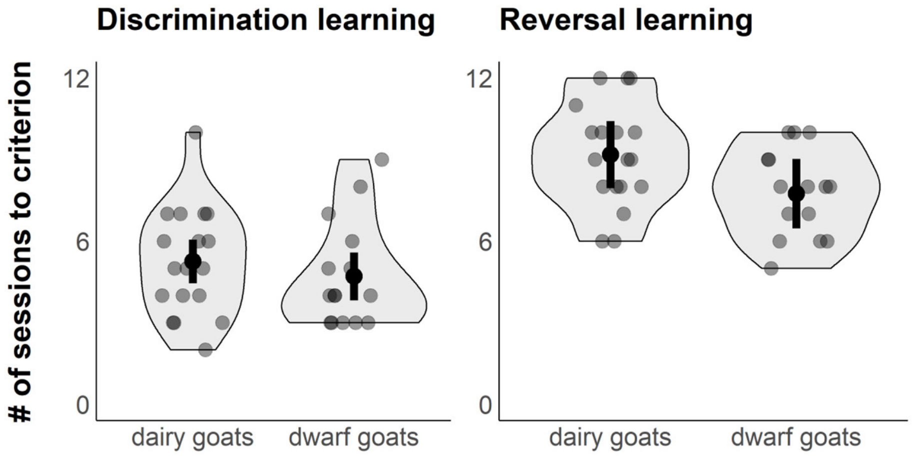

FIGURE 2 | Violin plots with jittered raw data points (grey dots) showing the performance of dwarf and dairy goats in the initial visual discrimination learning task (left) and the reversal learning task (right). Figure shows also model estimates (black dots) and 95% CIs (error bar).

checked the residuals of the models graphically for normal distribution and homoscedasticity (simulateResiduals function, DHARMa library). *P*-values were calculated using parametric bootstrap methods (1,000 bootstrap samples, PBmodcomp function, pbkrtest library). *P*-values calculated with parametric bootstrap tests give the fraction of simulated likelihood ratio test (LRT) statistic values that are larger or equal to the observed LRT value. This test is more adequate than the raw LRT because it does not rely on large-sample asymptotic analysis and correctly takes the random-effects structure into account (Halekoh and Højsgaard, 2014).

Code and raw data are available at the Electronic **Supplementary Material** (ESM) and here: https://osf.io/tfmwc/.

RESULTS

Training

Dwarf goats needed 2.94 ± 1.06 (mean ± SD) sessions to reach the criterion (Ettenhausen 2.50 ± 0.71 sessions; Dummerstorf: 3.33 ± 1.15 sessions), while dairy goats needed 2.78 ± 0.85 sessions to reach the criterion (Ettenhausen 2.78 ± 0.92 sessions; Dummerstorf: 2.78 ± 0.79 sessions). One subject (dwarf goat, Ettenhausen) did not reach the criterion and was excluded from further testing.

Visual Discrimination Task

"Selection line" did not affect the number of sessions to reach the learning criterion in the initial visual discrimination task (est. ± CI: dwarf goats: 4.70 ± 0.43; dairy goats: 5.25 ± 0.39; $P = 0.38$, **Figure 2**). The colour of the rewarded cup ("Colour") had an impact on the learning performance in the discrimination learning task, with the black stimulus being more easily learned than the white (est. ± CI: black stimulus: 4.05 ± 0.42; white stimulus: 5.89 ± 0.41; $P = 0.005$). There was no interaction for "Colour" and "Selection line" ($P = 0.39$). The variation (SD) explained by the random effects "Location" and "Pen" was < 0.001 for both.

Reversal Learning Task

Dwarf goats needed fewer sessions compared to dairy goats to learn the reversal task ("Selection line," est. ± CI: dwarf goats 7.74 ± 0.62, dairy goats 9.18 ± 0.60; $P = 0.016$, **Figure 2**). "Colour" did not affect performance in the reversal learning task (est. ± CI: black stimulus: 8.10 ± 0.62; white stimulus: 8.93 ± 0.61; $P = 0.12$). There was no interaction for "Colour" and "Selection line" ($P = 0.77$). Variation (SD) explained by the random effect "Pen" was < 0.001, while the variation for "Location" was 0.7.

DISCUSSION

To determine how selection for high productivity impacts general learning capacity and behavioural flexibility, we investigated the ability of dwarf (not selected for production traits) and dairy goats (selected for milk production) to solve a visual discrimination and a subsequent reversal learning task. While both groups performed similarly in the initial learning task, we found that dwarf goats performed better in the reversal task compared to dairy goat. Although other confounding factors need to be addressed in future research to rule out alternative explanation, our results provide first support for the hypothesis that selection for high productivity may be associated with decreased behavioural flexibility, but not initial learning performance, in goats.

The performance of goats in the initial learning task in this study (ranging from 2 to 10 session to reach the learning criterion, totalling in 24–120 trials) did not differ between both selection lines. At least for goats, and contrary to the Resource Allocation theory, the selection for high production does appear to not notably affect learning performance in a visual discrimination task. The goats' general performance in the task appears to be comparable to the performance of sanctuary goats tested with a similar design, but a slightly different protocol (8–96 trials to reach a learning criterion Nawroth and Prentice, 2017). When dwarf goats were presented with a four-choice visual discrimination task using an automated learning device with a screen (i.e., presented 2D stimuli), they needed 180–620 trials until they reached a designated learning criteria (Langbein et al., 2007), indicating that providing live stimuli appears to enhance learning by making the set up less arbitrary (O'Hara et al., 2015). Many other factors, ranging from different thresholds regarding a learning criterion to differences in the design of these visual discrimination tasks have likely also contributed to this variation and make rigorous comparisons not feasible. Future research should aim to better standardise test protocols in order to increase comparability of results.

In the reversal task dwarf goats outperformed dairy goats which lends support to the hypothesis that the Resource Allocation theory can also be applied to some cognitive functions in farm animals (Beilharz et al., 1993). However, Dudde et al. did not find such a relationship in their reversal learning task (Dudde et al., 2018) between two lines of hens that have been bred for higher egg yield compared to two lines that have not been selected for high egg yield. One reason for a lack of this pattern could be a relatively high drop-out rate in the lines not selected for high egg yield (40 and 60% of individuals, respectively, at the start of the initial learning task), which could have led to a biassed overall comparison of both groups. In our study, the only drop-outs were as well from the line that was not selected for high performance (dwarf goats) but with a moderate rate only (∼17%). However, the difference in performance between dwarf and dairy goats in the reversal learning was relatively small, making it difficult to infer the biological meaningfulness of the observed effect when applied to other contexts in a farm setting in which learning might occur (e.g., locating and remembering new drinker and feeder positions after transfer to new environments).

Although both selection lines were handled in a similar manner at the research facilities, early ontogenetic factors could have also played a role and might explain the observed differences. As it is common for the dairy industry, the dairy goats used in this study had been separated from their dam right after birth. In contrast, dwarf goats had been allowed to stay with their mothers for 6 weeks. Research has shown that early separation from the dam and rearing by humans increased tameness scores in goats (Lyons et al., 1988). If this would have been the case with our subjects, we would have expected a better performance in dairy compared to dwarf goats, as tamer individuals exhibit less stress during handling, and stress, in turn, can affect memory and learning (Mendl, 1999; Valenchon et al., 2013; Brajon et al., 2016). In addition, differences in sociality, rather than behavioural flexibility, might account for the detected differences. Subjects of both selection lines might differ in how they cope with isolation during the test situation, which in turn could have impacted reversal learning performance. Another factor limiting the general applicability of our results is that we could only test one breed of the non-production line, heightening the risk that the found differences in the reversal learning task might be due to specifics of this breed (or even population), rather than a result of non-selection for production.

Interestingly, goats were faster to learn a colour association when the stimulus was a black cup rather than a white cup in the learning, but not reversal learning task. Goats might have been biassed due to the setup of the training sessions. Although light brown cups have been used during training, we cannot exclude the possibility that goats transferred this association to the darker cup in the test trials. So other settings, such as the colour and shape of the stimulus, can impact learning performance and future designs should aim to strongly adhere to species-specific limitations regarding visual acuity and colour discrimination ability. Although in our study we found no interaction with learning performance, this finding also highlights the need for a balanced presentation of the stimuli and/or stimulus preference tests prior to the test situation.

CONCLUSIONS

Differences in behavioural flexibility could affect the ability of specific selection lines to adapt to new environments or changes in housing and management routines and thus might be relevant for the welfare of domestic animals. Our results provide first support to the hypothesis that selection for high milk yield in goats might be associated with decreased behavioural flexibility.

DATA AVAILABILITY STATEMENT

Code and raw data are available in the **Supplementary Material** and in a public repository here: https://osf.io/tfmwc/.

ETHICS STATEMENT

All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Ministry of Agriculture, Environment and Consumer Protection of the federal state of Mecklenburg-Vorpommern, Germany (Ref. Nr. 7221.3-1.1-062/17) and by the Swiss Cantonal Veterinary Office, Thurgau (Approval No. TG04/17 /17rova). Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

CN, NK, and JL conceptualised the study. CN and KR collected the data. CN analysed the data and wrote the main part of the manuscript. All authors provided critical feedback on the manuscript and gave approval for submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2021.796464/full#supplementary-material

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Intranasal Oxytocin Improves Social Behavior in Laboratory Beagle Dogs (*Canis familiaris*) Using a Custom-Made Social Test Battery

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For a long time, oxytocin has been thought to have a generally positive effect on social cognition and prosocial behavior; however, recent results suggested that oxytocin has beneficial effects only under certain conditions. The aim of the present study was to explore potential associations between social competence and the effect of intranasal oxytocin on the social behavior of laboratory beagle dogs. We expected oxytocin treatment to have a more pronounced positive effect on dogs with lower baseline performance in a social test battery. Thirty-six adult dogs of both sexes received 32 IU intranasal oxytocin and physiological saline (placebo) treatment in a double-blind, cross-over design, with 17–20 days between the two sessions. Forty minutes after the treatment, dogs participated in a social test battery consisting of eight situations. The situations were carried out within one session and took 20–30 min to complete. Principal component analysis on the coded behaviors identified four components (Willingness to interact, Preference for social contact, Non-aversive response to nonsocial threat, and Non-aversive response to social threat). The subjects' behavior during the placebo condition was used to assess their baseline performance. We found that oxytocin treatment had a differential effect on the behavior depending on the baseline performance of the individuals in all components, but only two treatment × baseline performance interactions remained significant in a less sensitive analysis. In accordance with our hypothesis, oxytocin administration increased dogs' contact seeking and affiliative behaviors toward humans but only for those with low baseline performance. Dogs with low baseline performance also showed significantly more positive (friendly) reactions to social threat after oxytocin administration than after placebo, while for dogs with high baseline performance, oxytocin administration led to a more negative (fearful) reaction. These results indicate that similar to those on humans, the effects of oxytocin on dogs' social behavior are not universally positive but are constrained by individual characteristics and the context. Nevertheless, oxytocin administration has the potential to improve the social behavior of laboratory beagle dogs that are socially less proficient when interacting with humans, which could have both applied and animal welfare implications.

Keywords: oxytocin, dog, social behavior, individual differences, laboratory beagle

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INTRODUCTION

Although the majority of research supports a generally positive effect of oxytocin on social behaviors, this neuropeptide also seems to play critical (and complex) roles also in agonistic or antisocial interactions (1, 2), such as maternal aggression (3). Moreover, a growing number of studies have demonstrated that the effect of oxytocin is not as uniformly beneficial as was previously thought but depends on the characteristics of the individual, on the (perceived) characteristics of the social partner, and on the situation and context itself [see review in Bartz et al. (4)]. In humans, there are numerous studies demonstrating such individual effects, with the best-documented example being the effect of oxytocin in social dilemma games. Oxytocin treatment could promote or actively hinder trust and cooperation in these tasks, depending on the participant's individual traits [e.g., attachment anxiety and avoidance (5, 6)], and on how the participants perceive/categorize their social partner [in-group vs. out-group: (7, 8); trustworthy vs. untrustworthy: (9); known vs. unknown: (10)], or the interaction of these two factors (11). These studies altogether suggest that oxytocin primarily affects anxiously attached, rejection-sensitive participants and promotes higher trust and cooperation toward familiar, trustworthy, or in-group partners, while in less anxious participants and when interacting with unknown, untrustworthy, or out-group partners, it has no or even negative effects (i.e., promotes distrust and less cooperation). These results indicate that oxytocin is not a universal pro-social enhancer but seems to selectively improve social behaviors in individuals who are in need of such enhancement, while others without such need do not benefit from oxytocin. In direct support of this, Bartz et al. (12) showed that oxytocin administration improved performance in an empathic-accuracy task but only in individuals who were less socially proficient during the baseline measurement, whereas it had no effect on more socially proficient individuals.

Contrary to humans, there are only a handful of studies that found similar interactional effects in other species, including dogs. Even though dogs are famous for their unique, human-like social competence (13), their social behavior toward humans is not at all uniform [e.g., Persson et al. (14)]. While plenty of research has shown that oxytocin administration improves the dogs' social approach and human-directed affiliative behaviors [reviewed in Kis et al. (15), Buttner (16)], the question remains whether the effect of oxytocin treatment would be more pronounced in individuals with lower baseline social competence. Two studies, however, lend support to the existence of the differential effect of oxytocin treatment on dogs, depending on their basal levels of oxytocin and the genetic variants they carry on their oxytocin receptor gene (OXTR). More specifically, Romero et al. (17) reported that, after oxytocin nasal intake, social proximity seeking (the tendency to approach their partners) was significantly higher in dogs with low levels of endogenous oxytocin than in dogs with high levels of endogenous oxytocin (measured before intranasal administration). Persson et al. (18) showed that oxytocin treatment increased physical contact seeking with the owners in golden retrievers carrying the AA genotype of the 19131AG OXTR-SNP, but it decreased this behavior in individuals with GG genotype.

Social competence is a multifaceted construct consisting of a range of social–emotional and cognitive skills needed for successful social adaptation. This is one of the central phenomena of social-developmental psychology [see, e.g., Junge et al. (19)], which, however, can also be used to conceptualize the general social ability of an animal during different types and contexts of social interactions (20). Social competence is also a central concept of studies of social cognition in dogs (13) and is often defined as the ability of the dog to exploit available social information to adaptively optimize their social behavioral responses. In dogs, social competence can be measured in the degree to which dogs are effective in their social interactions with other dogs and humans (i.e., their ability to adjust their behavior to meet the demands of different social contexts) when demonstrating cooperative and communicative skills and when to make and sustain social interactions, etc. It is also worth mentioning that different aspects of social competence can be improved through socialization with humans. One reason for the lack of direct empirical evidence regarding the potential moderating effect of the individual's baseline social competence on the effect of oxytocin treatment could be that the majority of studies in this field have been carried out on well-socialized family dogs.

The current study is thus aimed to fill this gap and investigate the effect of oxytocin on dogs with low social competence. It is reasonable to assume that beagles raised with limited human contact under controlled laboratory conditions represent a group with markedly lower social competence than family dogs. However, this group still retains a measure of variability in this regard (21), which makes them ideal subjects to study how baseline social competence alters the effect of oxytocin administration. In this study, we used a double-blind within-subject design; that is, the dogs' responses were investigated in a test battery twice, once after receiving intranasal oxytocin and once after receiving a placebo treatment (in a randomized order). The baseline social competence of each dog was assessed based on their behavioral performance during a placebo treatment. Based on the results of Bartz et al. (12), we hypothesized that the dogs' baseline performance and their treatment would interact, and that oxytocin treatment would have a more pronounced positive effect for dogs with lower baseline performance.

MATERIALS AND METHODS

Subjects

Thirty-seven laboratory beagle dogs participated in the experiment: 15 females and 22 males (none had been neutered). The age of the dogs varied from 2 to 8 years (mean ± SD: 4.75 ± 2.39 years). All dogs were bred by the same commercial breeder who specialized in breeding dogs for laboratory use (WOBE Kft., Budapest, Hungary). The dogs were brought to the research facility where the behavior test took place 1 month before the onset of testing for acclimatization. All dogs were kept indoors, mostly in same-sex pairs in kennels (2×2 m), except one male and one female kennel with three dogs in double-sized kennels. The dogs had visual contact with each other. All dogs participated in the same daily care routines including feeding, cleaning, and handling. The dogs were fed once a day at the same time and had an *ad libitum* supply of water, and their interaction with humans was limited to the daily feeding and cleaning of their kennels.

Procedure

The subjects participated in the test battery twice, with 17–20 days between the two sessions. Two female experimenters (BT and EP) conducted the tests. Half of the dogs were randomly assigned to one experimenter, who played the role of the caretaker for that dog in both test sessions, while the other female experimenter participated in the tests as the "unfamiliar" experimenter. The roles of the experimenters (i.e., caretaker or unfamiliar experimenter) were reversed for the other half of the dogs.

Pre-assessment and Familiarization

Two weeks before the first test session took place, the experimenters had visited each kennel to assess whether the dogs were capable of tolerating the presence of an unfamiliar human. One experimenter (the prospective "caretaker") entered the kennel, crouched down, called both dogs in a friendly manner, petted them, and offered them dry food (the same as their normal daily diet) from her hand. Dogs passed the assessment if they approached the experimenter within 1 min, tolerated petting (did not flee), and accepted the food ($N = 30$ dogs did so).

Dogs that did not meet these criteria ($N = 7$ dogs) participated in a familiarization session (ca. 10 min of social interaction with their assigned caretaker) the next day, with the aim of getting the dogs used to the presence of and physical contact from their caretaker. Familiarization took place in the same room as the behavior test, and the dogs were carried to the testing room by the caretaker. The procedure consisted of four phases.

Phase 1 (Passive Interaction, 3 Min)

The caretaker crouched next to the wall and remained passive. Whenever the dog approached her on its own, she gently petted the dog's head and back but did not force the contact and did not initiate interaction.

Phase 2 (Active Interaction, 2 Min)

The caretaker stood up, walked to the opposite wall, crouched down, and called the dog.

- If the dog approached her within 10 s, she praised and petted the dog for 20–30 s.
- If the dog did not approach her within 10 s but did not avoid her either, she stepped closer to the dog and gently petted it for 10 s and then stepped back to the wall and waited for 10 s.
- If the dog actively avoided her, she continued to talk to the dog for another 20 s. Whatever the dog's reaction was, after 30 s, the caretaker stood up, walked 3–4 m away, and repeated the above procedure three more times (altogether 4 × 30 s).

At the end of Phase 2, all seven of these dogs were willing to approach the caretaker within 1 min and tolerated physical contact from her.

Phase 3 (Food, ∼2 Min)

The caretaker showed a piece of dry food (the same as their normal daily diet) to the dog and then put it on the floor and stepped away. If the dog picked up the food within 10 s, she repeated it four more times. If the dog did not eat the food within 10 s, she put down another piece next to it and stepped farther away, repeating this procedure until the dog ate the food (or until 2 min had elapsed). Once the dog ate the food off the floor, the caretaker repeated the same procedure, this time offering food from her hand. At the end of Phase 3, all seven of these dogs were willing to accept food from the caretaker.

Phase 4 (Collar and Leash, ∼3 Min)

The caretaker put a collar and leash on the floor and a few pieces of dry food next to them. Once the dog sniffed the collar and leash, the caretaker put the collar on the dog, continuously praised and petted the dog for 10 s, and then removed the collar. After ∼30 s, she put the collar back on the dog, attached the leash (but left it lying on the floor), continuously praised and petted the dog for 10 s, and then removed the leash. After ∼30 s she put the leash back on and walked around the room with the dog on the leash, continuously calling and praising the dog.

Oxytocin/Placebo Treatment

The same two female experimenters (BT and EP) who carried out the behavior tests performed the drug treatments, and both were trained in these tasks. The caretaker held the dog while the experimenter administered the dose. Both experimenters were blind as to which treatment the dogs received. Dogs received a single intranasal dose of 32 IU (8 puffs in total, with a half dose administered to each nostril) of oxytocin (Syntocinon, Novartis, Basel, Switzerland) and placebo (physiological saline) treatments in a repeated-measures design. Intranasal administration procedure was unfamiliar to the dogs; thus, some dogs resisted the administration. If a puff was clearly missed (i.e., the dog moved its head right before the experimenter administered the puff), it was repeated. One dog (a 2.1-year-old female) actively and strongly resisted the intranasal administration and thus was excluded from the study. The order of the treatments was balanced between the remaining dogs: $N = 18$ dogs (10 males, 8 females, mean age 5.22 years) received oxytocin before the first test session, and placebo before the second test session; $N = 18$ dogs (12 males, 6 females, mean age 4.43 years) received the treatments in the reversed order. Intranasal administration of oxytocin or placebo was followed by an incubation period (22) spent in the kennels of 35–45 min. After the waiting period, the dog was removed from the kennel and transported to the testing room by the caretaker.

Social Test Battery

The test battery consisted of a warm-up phase and 8 tests, and it took ∼20 to 30 min to complete. The tests were carried out on the same day and in the same order for all subjects. In some of the tests, the dogs remained leashed by default. However, since the dogs were not familiar with wearing a leash, if the dog showed strong resistance to the leash at any point of the test (i.e., constantly struggled, tried to escape, or displayed freezing behavior) and the caretaker could not calm the dog, she removed the leash and the test continued without it.

Warm-Up

The purpose of this phase was to familiarize the subject with the testing environment. The caretaker (hereafter C) carried the dog to the testing room (3 × 5 m) and put it down, and then she sat down on the floor next to the wall. There were objects (including a cardboard box, bags, newspapers, plastic bottles, and a tennis ball) placed around the room. The dog could move freely, while C remained passive in her position. If the dog approached C and initiated the interaction, C briefly petted the dog but otherwise ignored it. The warm-up was terminated after 3 min if the dog explored at least two objects (i.e., spent at least 5 s sniffing or manipulating them) and also approached C at least once during this period. If the dog did not meet these criteria, the warm-up continued until the dog did so or for a maximum of 10 min.

Test 1. Interaction With the Caretaker

The aim of this test was to assess how much the dog tolerates/seeks positive interaction with C. The dog was unleashed during the test. The test consisted of 4 trials; each trial was 30 s long.

In the first trial, C stood up and approached the dog in a normal upright (walking) posture and at a normal speed of walk while talking to it in a friendly manner. If the dog was friendly or passive (i.e., showed no avoidance or aggression), C petted the dog and talked to it until the end of the trial (30 s). If the dog moved away, C waited for 15 s and then tried to approach the dog again. If the dog did not move away this time, C petted the dog, and if the dog moved away again, C waited passively until the end of the trial. At the end of the trial, C stepped away from the dog (3–4 steps). In the second trial, C crouched down and called the dog in a friendly manner. If the dog approached her, she petted the dog and talked to it until the end of the trial (30 s). If the dog did not approach her, C waited for 15 s and then approached the dog and petted it. If the dog moved away, C waited passively until the end of the trial. The third and fourth trials were identical to the second one.

Test 2. Food Motivation

The aim of this test was to assess the dogs' preference for different food rewards (dry food or sausage) in a social context. The dry food was familiar to the dogs, but the sausage was not (at least during the first food motivation test session). C put a piece of dry food and a piece of sausage on the floor and verbally encouraged the dog to eat them (for 20 s). Then C offered the same food types to the dog from her hand and encouraged the dog to eat them. If the dog ate only the sausage or both food types, the sausage was used in further tests, and if the dog chose only the dry food or none of the foods, dry food was used.

Test 3. Greeting by an Unfamiliar Experimenter

The aim of this test was to assess how much the dog tolerates/seeks positive interaction with the unfamiliar experimenter. It was based on the procedure of Turcsán et al. (21). The test consisted of 3 trials. The dog was held on a loose leash (if it tolerated being leashed) by C, who was standing next to it. In the first trial, the unfamiliar experimenter (hereafter E) approached the dog at ∼1.5 m and called the dog for 15 s. If the dog stepped toward E, she petted the dog for 7–8 s. If the dog did not approach E, E stepped closer and tried to pet the dog. If the dog showed active avoidance, E talked to the dog for 7–8 s. At the end of the trial, E stepped away from the dog (2–3 steps). The second and third trials were similar to the first, except that E did not approach the dog at the beginning of the trial; she just called it from her location.

Test 4. Training for Eye Contact

The aim of this test was to assess how much the dog tolerates/seeks eye contact with C. It was based on the procedure of Wallis et al. (23). The dog was unleashed during the test. The test consisted of a pretraining phase and a test phase.

Pretraining Phase. C sat down on the floor next to the wall, called the dog, and offered a piece of food. Then she tried to make eye contact with the dog (by talking in a high-pitched voice, clapping, whistling, etc.). When the dog established eye contact, C praised the dog verbally and gave the food to the dog (if the dog refused to eat, C used only verbal praise). This procedure was repeated once more. C had 3 min to establish eye contact with the dog twice. If C succeeded, the test continued with the test phase; otherwise, the test was terminated.

Test Phase. C sat passively by the wall, avoiding any noise or sudden movement that would attract the dog's attention, and watched the dog for 3 min. Every time the dog established eye contact with her on its own, C rewarded the dog (with food and/or verbal praise), and then she continued to passively watch the dog.

Test 5. Potentially Threatening Moving Object

The aim of this test was to assess how the dog reacts to the approach of an apparently self-moving object. The object was a remote-controlled toy car (30 cm long, 15 cm wide, and 8 cm high) with a 50 × 30 × 15 cm cardboard box placed over it. The test consisted of two trials. In the first trial, the dog and C were standing at one end of the room. The dog was held on a loose leash. If the dog did not tolerate the leash (i.e., struggled, tried to escape, or froze and did not move), C removed the leash and gently held the dog's body until the test started. E placed the object at the opposite end of the room (∼5 m from the dog) and used a remote control to direct the object toward the dog, moving it slowly and haltingly (moving ∼1.5 m, then stopping for a few seconds, and then moving again). The approach was terminated if (1) the dog showed active avoidance (retreated to the wall or moved behind C); (2) the dog approached the object; or (3) the object moved within 1 m of the dog. After the approach was terminated, C unleashed the dog (if it was on a leash), carried the object back to its starting point, and then took the dog back to its starting point. The second trial was identical to the first one, except that when the object started moving toward the dog, C stepped ∼2 m away from the dog. When the trial ended—either because the dog showed avoidance/approach or because the object moved within 1 m to the dog—C unleashed the dog (if it was on a leash), went to the object, called the dog in a friendly manner (for a maximum of 30 s), and encouraged it to approach/interact with the object.

Test 6. Directional Gesturing

The aim of this test was to assess the dog's ability to rely on human directional signals (including pointing and gazing) when choosing between two objects. It was based on the procedure of Hernádi et al. (24). The test consisted of a pretraining phase and a test phase with three trials.

Pretraining Phase. C gently held the dog's body. E kneeled on the floor ∼1.5 m from the dog and put a plastic plate with a piece of food 20–40 cm in front of the dog. The dog was allowed to eat the food. If the dog refused to eat it, both C and E encouraged the dog until it approached and (at least) sniffed the food/plate. Then they returned to their starting positions.

Test Phase. In the first trial, E placed two identical plastic plates on the floor ∼1.8 m apart, both containing a piece of food. E attracted the dog's attention (by talking in a high-pitched voice, clapping, whistling, etc.), and once the dog looked at her, E pointed (sustained pointing) and gazed at one of the plates and then looked back at the dog. Then C released the dog. If the dog approached one of the plates at <10 cm, E picked up the other plate. If the dog did not approach any of the plates, E waited for 15 s and then picked up both plates. The second and third trials were similar to the first one, except for the cues given by E. In the second trial, E used momentary pointing, E pointed and gazed at one of the plates (the opposite one to the first trial) for 2–3 s, and then lowered her arm and looked back at the dog before it was released. In the third trial, E did not use pointing but only gazed at one of the plates (the same as in the first trial) for 2–3 s and then looked back at the dog before it was released. The side of the cued plate in the first trial was balanced between the dogs.

Test 7. Emotion Recognition

The aim of this test was to assess the dog's ability to rely on the human emotional expressions of joy and fear when choosing between two objects. It was based on the procedure of Turcsán et al. (25). The test consisted of two trials. In the first trial, C held the dog's collar or the dog's body, and E crouched down ∼1.5 m from the dog and placed two objects of similar size (a yellow wooden toy and a red plastic cup) on the floor ∼1.8 m apart. E stepped to one of the objects, picked it up, and showed the assigned emotional expression (joy or fear) for ∼5 s. She expressed the emotion with facial expression, verbal exclamations, and body language and looked at the dog at least once during this time. Then E put down the object, went to the other object, and performed the other emotional expression. When E took up her original standing position (halfway between the two objects), C released the dog. If the dog made a choice (approached one of the objects within 10 cm), E picked up the other object. If the dog did not make a choice, E waited for 15 s and then picked up both objects. The second trial was similar to the first one, except that E demonstrated the two emotions in the reverse order. The objects' location (right or left side), their assigned emotions, and the emotional expression displayed first by E were balanced between the dogs.

Test 8. Threatening Approach

The aim of this test was to assess how the dogs respond to a threatening approach by the experimenter. It was based on the procedure of Vas et al. (26). The dog and C were standing at one end of the room, and the dog was held on a loose leash. E stood ∼5 m from the dog and called the dog's attention. When the dog looked at her, E started to slowly approach the dog with a slightly bent upper body, staring steadily into the eyes of the dog and without any verbal communication. If the dog interrupted the eye contact with E, she tried to attract the dog's attention by making some noise (coughing or tapping the ground with her foot) and then continued the approach. The approach was terminated if (1) the dog showed active avoidance (retreated to the wall or moved behind C), (2) the dog approached E within arm's reach, or (3) E reached the dog. After the approach was terminated, C unleashed the dog, and E went back to her starting point, crouched down, called the dog in a friendly manner, and petted the dog to resolve the situation.

Behavior Coding

All tests were videotaped and analyzed using Solomon Coder (beta 190802 by András Péter, *http://solomoncoder.com/).* Altogether, 28 variables (20 scores, 1 frequency, 7 latencies) were coded in the 8 tests of the battery (**Table 1**). Neither the coders nor the experimenters had any information about the treatment that the dog received. Note that the number of coded variables was high relative to the number of dogs investigated, and the range and variance of the continuous variables (frequency and latency) were markedly different compared to the score-type variables. We therefore reduced the number of variables and homogenized the range of the different variable types using the following steps.

First, the continuous variables (frequency and latencies) were recoded into 4 to 6 categories to match the range of the score variables and also to control for extreme values. The threshold values of the categories were decided based on the variables' histograms. Second, for the tests that included repeated trials (Interaction with caretaker, Greeting, and Potentially threatening moving object), we calculated the mean of the trials for each variable coded in that test. Third, in the cases of the Directional gesturing and Emotion recognition tests, the number of dogs that did not make a choice in any given trial was too high (ranging from 35.6 to 58.9%) to assess the dogs' ability to follow directional gestures or emotional cue. Thus, for these two tests, we analyzed the total number of valid choices the dog made. Fourth, we also created a composite score from the two variables assessing the dogs' willingness to eat food (measured in the Food motivation and Directional gesturing tests) by taking the sum of these scores. The final set contained 13 variables; their descriptions can be found in **Table 1**. Inter-observer agreements for all variables were assessed by double coding $N = 15$ dogs (41.6% of the whole sample) by two

TABLE 1 | Definition of the variables coded in the tests and their inter-observer reliability [Cohen's kappa or intraclass correlation (ICC)].

Subtest, variable	Definition	Data processing
1. Interaction with the caretaker		
Reaction to petting	When C tried to pet the dog, the dog: 0, avoided contact (turned or moved away); 1, passively tolerated contact (no sign of contact seeking or avoidance); 2, showed a little contact seeking (shortly sniffed C, kept eye contact); 3, actively sought contact with C (cuddle up, lick, and climb in lap). If the dog behaved differently at the beginning vs. the end of the trial, the mean of the scores assigned to the two behaviors was given.	Coded separately for the four trials. For the analysis, the mean of the four trials was calculated. ICC = 0.927, $F_{14,14} = 13.792$, $p < 0.001$
Latency of approach	From the moment C stepped outside the dog's reach until the dog got within arms' reach of C. If the dog never stepped out of reach, the latency was 0; if the dog did not approach C, the maximum (15 s) was given.	Coded separately for the three approaches. For the analysis, the raw latency was recoded into categories based on its histogram: 0, 0–0.9 s; 1, 1–2.3 s; 2, 2.4–4.5 s; 3, 4.5–9 s; 4, 9–13 s; 5, 13–15 s. Then the mean score of the three approaches was calculated. ICC = 0.816, $F_{14,14} = 9.863$, $p < 0.001$
2. Food motivation and 6. Directional gesturing		
Accept food	If the dog ate food (1) or not (0).	Coded separately for the two tests. For the analysis, the two variables were summed. Cohen's kappa = 0.694, $N = 15$, $p < 0.001$
3. Greeting by an unfamiliar experimenter		
Reaction to petting	When E tried to pet the dog, the dog: 0, avoided contact (turned or moved away); 1, passively tolerated contact (no sign of contact seeking or avoidance); 2, showed a little contact seeking (shortly sniffed E, kept eye contact); 3, actively sought contact with E (cuddle up, lick, and climb in lap). If the dog behaved differently at the beginning vs. the end of the trial, the mean of the scores assigned to the two behaviors was given.	Coded separately for the three trials. For the analysis, the mean of the three trials was calculated. ICC = 0.683, $F_{14,14} = 3.020$, $p = 0.024$
Latency of approach	From the moment E called the dog/stepped outside the dog's reach until the dog got within arms' reach of E. If the dog approached E before her call or never stepped out of reach, the latency was 0; if the dog did not approach E, the maximum (15 s) was given.	Coded separately for the three trials. For the analysis, the raw latency data were recoded into categories based on its histogram: 0, 0–0.9 s; 1, 1–2.3 s; 2, 2.4–4.5 s; 3, 4.5–9 s; 4, 9–13 s; 5, 13–15 s. Then the mean score of the three trials was calculated. ICC = 0.973, $F_{14,14} = 47.054$, $p < 0.001$
4. Training for eye contact		
Frequency of eye contacts	The number of eye contacts the dog established during the test phase (3 min). If the dog did not pass the pretraining phase, 0 (the minimum) was given.	For the analysis, the raw frequency data were recoded into categories based on its histogram: 0, 0–1; 1, 2–10; 2, 11–21; 3, >21. Cohen's kappa = 1.000, $N = 15$, $p < 0.001$
5. Potentially threatening moving object		
Type of reaction	The object stopped because the dog: 0, moved away, in the opposite direction as C; 1, moved behind C; 2, was passive; 3, approached the object.	Coded separately for the two trials. For the analysis, the mean of the two trials was calculated. ICC = 0.997, $F_{14,14} = 341.714$, $p < 0.001$
Distance from object	How far the object was from the dog when it stopped: Score 0, ≥4 m; Score 1, ≥2 and <4 m; Score 2, ≥1 and <2 m; Score 3, <1 m.	Coded separately for the two trials. For the analysis, the mean of the two trials was calculated. ICC = 0.995, $F_{14,14} = 200.714$, $p < 0.001$
Latency of sniffing	From the moment C called the dog to the object until the dog approached it at <10 cm. If the dog approached the object on its own while it was still moving, the latency was 0; if the dog did not approach the object at all, the maximum (30 s) was given.	For the analysis, the raw latency data were recoded into categories based on its histogram: 0, 0 s; 1, 1–5 s; 2, 5–20 s; 3, 20–30 s. Cohen's kappa = 1.000, $N = 15$, $p < 0.001$
6. Directional gesturing		
Choice	The plate the dog approached at <10 cm: 0, none; 1, any plate.	Coded separately for the three trials. For the analysis, the number of valid choices out of three was calculated. Cohen's kappa = 1.000, $N = 15$, $p < 0.001$
7. Emotion recognition		
Choice	The object the dog approached at <10 cm: 0, none; 1, any object.	Coded separately for the two trials. For the analysis, the number of valid choices out of two was calculated. Cohen's kappa = 1.000, $N = 15$, $p < 0.001$

(Continued)

TABLE 1 | Continued

Subtest, variable	Definition	Data processing
8. Threatening approach		
Type of reaction	The dog's final reaction (when the test was terminated): 0, active avoidance (moved away or behind C); 1, passive (no movement toward or away from E); 2, ambivalent (few hesitant steps toward/away from E, may show tail wagging); 3, friendly/appeasing (approached E).	Remained the same for the analysis. Cohen's kappa = 1.000, $N = 15$, $p < 0.001$
Distance from E	How far E was from the dog when she terminated the approach: Score 0, >2 m; Score 1, 1–2 m; Score 2, <1 m; Score 3, the dog approached E.	Remained the same for the analysis. Cohen's kappa = 1.000, $N = 15$, $p < 0.001$

E, experimenter; C, caretaker.

independent coders. The inter-observer reliability, assessed by Cohen's kappa or intraclass correlation coefficient (ICC; two-way mixed model, absolute agreement) of all variables, was good or excellent (**Table 1**).

Statistical Analysis

The 13 variables coded in the first test session were subjected to principal component analysis (PCA) with Varimax rotation. We used the eigenvalue > 1 rule (27) to determine the number of components retained, and variables that failed to load > 0.5 on any component were excluded in a stepwise manner (28). Cronbach's α was calculated to assess the internal consistency of the resulting components. To calculate the component scores for each dog, we standardized the variables using z-transformation and then calculated the mean of the variables loading with at least 0.5 on a given component (variables that loaded negatively on a component were first multiplied by −1).

We used the component scores of the dog during the placebo treatment condition as a measure of their baseline social competence.

The component scores were used as dependent variables in generalized estimating equation models using restricted maximum likelihood estimation. Dog ID was set as a random factor. For fixed effects, we included the *Treatment* (oxytocin and placebo), the *Test session* (1st/2nd), and the *Sex* (male/female) of the dogs. The *Baseline performance* of that component (the corresponding component score of the dog during the placebo treatment) was entered as a covariate. Two-way interactions between treatment and the other variables were included in the models. Non-significant effects were removed from the model sequentially, in the order of their decreasing significance, starting with the interactions; non-significant main effects were removed only if they had no interaction left in the model (29). Regarding *Baseline performance*, we expected a significant interaction with treatment, which would indicate that oxytocin has a differential effect on the behavior of dogs with high and low social competence. If it was significant, to interpret the interaction, we split the *Baseline performance* at its median to create high and low groups and re-ran the model with this categorical variable. We used sequential Bonferroni correction to adjust the *p*-values of the *post-hoc* tests for multiple comparisons. If this interaction was not significant and was removed from the model, the main effect of *Baseline performance* was also removed, because—being part of the component score itself—this variable on its own was redundant with the dependent variable. All statistical tests were carried out using SPSS v.22.0.

RESULTS

Principal Component Analysis

The 13 variables were grouped into four components (**Table 2**), which together explained a relatively high proportion (76.7%) of the total variance. The first principal component included variables from the Directional gesturing, Emotion recognition, Food motivation, and Training for eye contact tests. Higher scores on this component indicate the dogs' willingness to accept food from a human and to make a choice in response to human directional gestures and emotional displays, as well as their increased tendency to make eye contact with the experimenter. Consequently, this component is referred to as "Willingness to interact."

The second component contained variables from the Interaction with the caretaker and Greeting by an unfamiliar experimenter tests. Higher scores on this component correspond to a more positive reaction to both familiar and unfamiliar humans (i.e., increased contact seeking toward both C and E, and more positive responses upon being petted by both C and E). Based on these, the second principal component is referred to as "Preference for social contact."

The third component, labeled as "Non-aversive response to non-social threat," contained variables only from the Potentially threatening moving object situation. A high score on this component indicates a more positive (less fearful) reaction to the approaching unfamiliar object.

The fourth principal component, labeled as "Non-aversive response to social threat," included variables from the Threatening approach test. A high score on this component is associated with a more positive (less fearful) response to the threatening approach of the experimenter. Cronbach's alpha values were high (>0.8) for all principal components (**Table 2**), indicating a good degree of internal consistency.

TABLE 2 | Results of the principal component analysis.

Variable (*test*)	**Principal components** **Willingness to interact**	 **Preference for social contact**	 **Non-aversive response to non-social threat**	 **Non-aversive response to social threat**
Number of valid choices *(Directional gesturing—test 6)*	**0.913**	0.051	0.200	0.104
Accept food *(Directional gesturing + Food motivation—tests 2 and 6)*	**0.851**	0.164	0.166	−0.036
Number of valid choices *(Emotion recognition—test 7)*	**0.846**	0.014	0.049	0.225
Number of eye contacts *(Training for eye contact—test 4)*	**0.695**	−0.016	0.227	0.031
Reaction to petting *(Interaction with C—test 1)*	−0.137	**0.905**	0.010	0.064
Reaction to petting *(Greeting by E—test 3)*	0.051	**0.890**	0.079	0.068
Latency of approach *(Interaction with C—test 1)*	−0.068	**−0.815**	0.041	0.046
Latency of approach *(Greeting by E—test 3)*	−0.260	**−0.719**	0.061	−0.232
Distance from object *(Potentially threatening moving object—test 5)*	0.066	−0.087	**0.911**	0.040
Type of reaction *(Potentially threatening moving object—test 5)*	0.285	−0.049	**0.856**	0.105
Latency of sniffing *(Potentially threatening moving object—test 5)*	−0.329	−0.182	**−0.697**	−0.245
Type of reaction *(Threatening approach—test 8)*	0.087	0.170	0.129	**0.893**
Distance from E *(Threatening approach—test 8)*	0.118	0.027	0.133	**0.891**
Eigenvalue	4.282	2.788	1.593	1.308
Explained variance	32.940	21.443	12.252	10.060
Cronbach's α	0.861	0.806	0.816	0.819

Loadings > 0.5 are in boldface.
E, experimenter; C, caretaker.

The Effect of Familiarization, Age, and Sex on the Baseline Social Competence of the Dogs

Seven dogs that initially did not tolerate the presence of physical contact from their prospective caretaker participated in an additional familiarization procedure, with the aim to lower their (social) fear to a level where they could be tested in the battery without causing severe stress for the individuals. Given that the familiarization took place in the same room and utilized a procedure that was similar to later tests, we cannot exclude the possibility that the familiarization may have been more successful than intended, increasing the baseline social competence of these individuals from the lower end to the higher end of the spectrum. Before integrating these dogs together with the rest for later analyses, we first investigated if their baseline social competence was higher than that of the rest of the dogs.

We found that these dogs ($N = 7$) had lower scores in the placebo condition (baseline social competence, **Figure 1**) as compared to the rest of the dogs ($N = 29$) in all but one component (Mann–Whitney U test, Willingness to interact: $z = 1.963$, $p = 0.049$; Preference for social contact: $z = 2.378$, $p = 0.016$; Non-aversive response to non-social threat: $z = 2.546$, $p = 0.009$). In the case of Non-aversive response to social threat, there was no significant difference between these 7 and the rest of the dogs ($z = 0.609$, $p = 0.557$). Thus, the results indicated that the baseline social competence of these dogs was still lower or at the same level as the rest of the dogs, which justified pooling the data together for further analyses.

The dogs' component scores during the placebo condition did not correlate with the dogs' age (Spearman correlation, $N = 36$, highest $|\rho| = 0.095$, $p > 0.449$ for all) and did not significantly differ between males and females (Mann–Whitney U-test, $N = 36$, $p > 0.133$ for all).

Main and Interaction Effects of Treatment, Test Session, Sex, and Baseline Performance

Regarding the Willingness to interact component, we found a significant interaction between *Treatment* and *Test session* ($\chi^2 = 17.292$, $p < 0.001$), and also between *Treatment* and *Baseline performance* ($\chi^2 = 5.279$, $p = 0.022$). However, when we divided the dogs into low and high groups by the median of the baseline performance and entered this categorical variable in the model to interpret this latter interaction, it was no longer significant ($\chi^2 = 2.214$, $p = 0.137$). *Post-hoc* pairwise comparisons also did not show a significant difference in the oxytocin treatment between the low and high groups. When this interaction (and consequently, the categorical baseline performance variable) was removed from the model, the *Treatment* × *Test session* interaction also became non-significant ($\chi^2 = 0.005$, $p = 0.942$), and the final model contained only the main effect of *Test session* (1st/2nd; $\chi^2 = 12.562$, $p < 0.001$). The dogs showed a higher motivation to engage in the two-way object-choice tasks, were more willing to accept food from the experimenter, and engaged in more eye contact with her during the second (repeated) test session than during the first occasion (**Figure 2**).

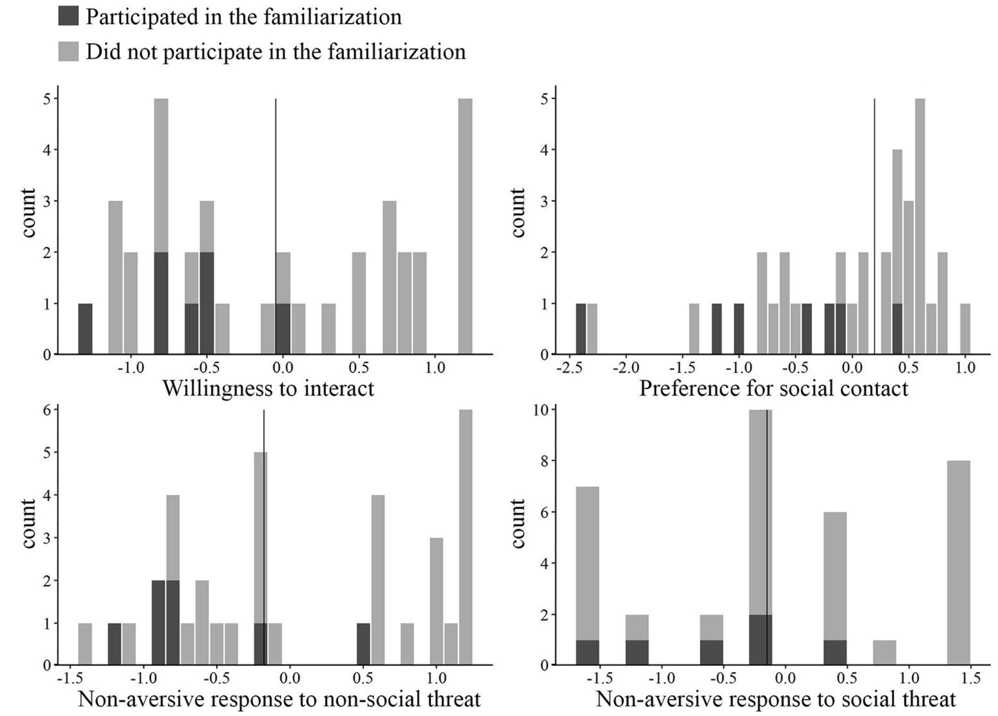

FIGURE 1 | Histograms of the four components during the placebo treatment condition. Vertical lines represent the median that was used as the threshold for dividing the dogs into high and low groups ($N = 18$ in all groups). Dogs that participated in the familiarization are marked with dark gray.

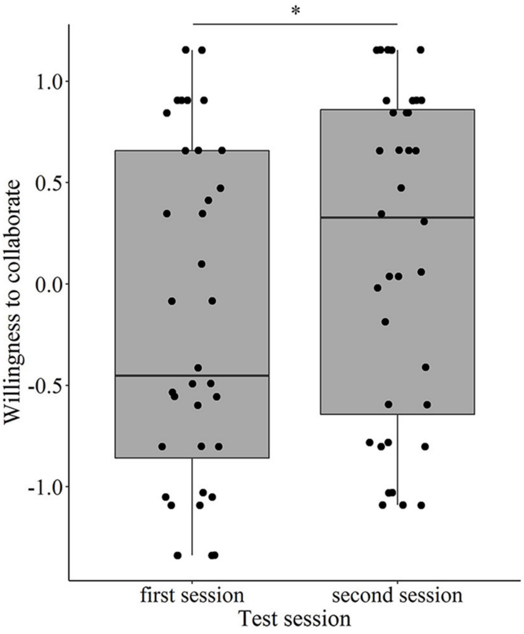

FIGURE 2 | The effect of repeated testing on the dogs' Willingness to interact with human partners (*$p < 0.001$).

Regarding the Preference for social contact component, *Sex* (male/female) and *Test session* (1st/2nd) had no main and interaction effects ($p > 0.308$ at removal); however, we found a significant interaction between *Treatment* and *Baseline performance* of the dogs ($\chi^2 = 5.509$, $p = 0.019$). When the *Baseline performance* was entered in the model as a categorical variable, its interaction with *Treatment* was still significant ($\chi^2 = 10.389$, $p = 0.001$). *Post-hoc* pairwise comparisons revealed that the effect of oxytocin administration was significant only in dogs with low baseline performance (Bonferroni-adjusted $p = 0.004$): these dogs showed a higher preference for social interaction with both familiar and unfamiliar humans in terms of contact seeking and response to human-initiated physical contact after intranasal administration of oxytocin than in the placebo condition. We found no effect of treatment in the case of dogs with high baseline performance ($p = 0.357$) (**Figure 3**).

Similar to the previous component, we found no main or interaction effect of *Sex* (male/female) or *Test session* (1st/2nd) on dogs' Non-aversive response to non-social threat ($p > 0.156$ at removal), but the *Treatment* × *Baseline performance* interaction was significant ($\chi^2 = 7.647$, $p = 0.006$). However, when we divided the dogs into low and high groups by the median, its interaction with *Treatment* was no longer significant ($\chi^2 = 2.287$, $p = 0.130$), and *post-hoc* comparisons also did not show a significant difference between the low and high groups in the effect of oxytocin vs. placebo treatment. When this interaction (and consequently, the categorical baseline performance variable) was removed from the model, the main effect of *Treatment* was not significant ($\chi^2 = 0.013$, $p = 0.909$).

Regarding the dogs' Non-aversive response to social threat, while *Test session* had no main or interaction effect ($p > 0.374$ at removal), we found two significant interactions: *Treatment* × *Sex*

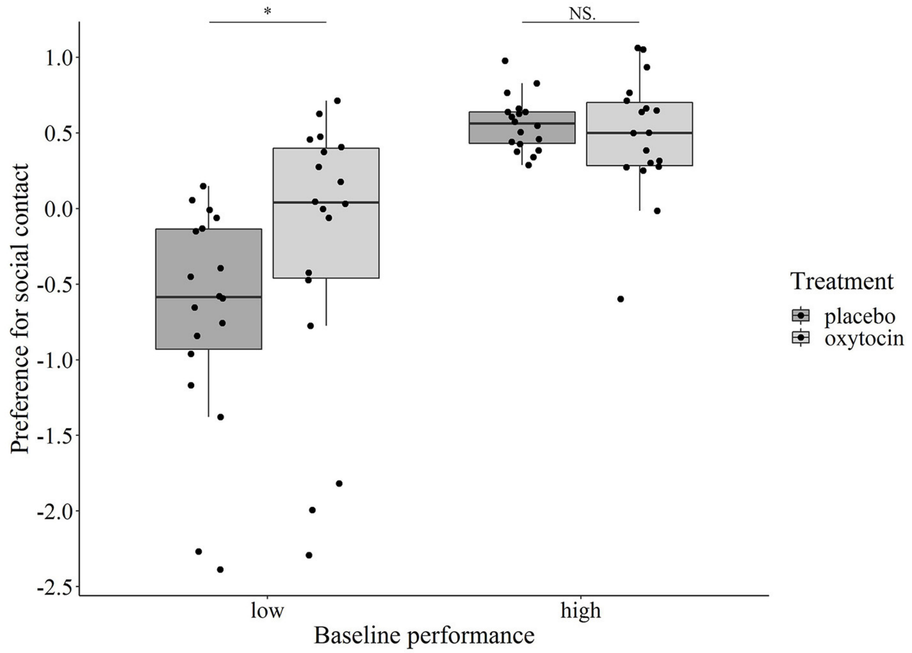

FIGURE 3 | Relationship between the dogs' baseline performance and oxytocin/placebo treatment on their Preference for social contact component score. Oxytocin treatment significantly increased the component score in dogs with low baseline performance (*$p = 0.004$), while the effect of treatment was not significant in dogs with high baseline performance ($p = 0.357$).

($\chi^2 = 3.901$, $p = 0.048$) and *Treatment* × *Baseline performance* ($\chi^2 = 34.559$, $p < 0.001$). Regarding the former, *post-hoc* group comparisons did not show a significant or trend-level difference of the oxytocin treatment between males and females. However, for the *Treatment* × *Baseline performance* interaction, when the *Baseline performance* was entered in the model as a categorical variable, its interaction with *Treatment* was still significant ($\chi^2 = 16.751$, $p < 0.001$). *Post-hoc* tests revealed that oxytocin had an opposite effect on dogs' reaction to the experimenter's threatening approach depending on their baseline performance: dogs with low baseline performance showed significantly more positive reaction (i.e., tolerated the approach longer before moving away and/or were more likely to react with approach instead of avoidance) after receiving oxytocin as compared to receiving placebo (Bonferroni-adjusted $p = 0.002$), while for dogs with high baseline performance, intranasal administration of oxytocin led to a significantly less positive reaction (Bonferroni-adjusted $p = 0.043$) (**Figure 4**).

DISCUSSION

Although there is abundant research pointing to the social effects of oxytocin, less is known about how individual factors may moderate the effect of oxytocin in species other than humans. In this study, we set out to investigate the potential moderating effect of laboratory beagle dogs' baseline social competence on the association between oxytocin treatment and different aspects of social behavior. In accordance with our hypothesis and similar to human studies [e.g., Bartz et al. (12)], we have found that oxytocin treatment has a differential effect on the behavior, depending on the baseline social competence of the dogs. Individuals with low social competence benefit more from oxytocin treatment than socially more proficient individuals.

We tested various aspects of social behavior in laboratory beagles; however, three of the tasks included in the test battery (Training for eye contact, Directional gesturing, and Emotion recognition tasks) proved unsuitable for testing laboratory dogs. Even though these three tests aimed to measure phenotypes that, according to previous studies on family dogs, are affected by oxytocin [following gestural cues (30); willingness to make eye contact with humans (31); emotion recognition (32)], these test situations proved unfit for these purposes in our sample of laboratory beagles. This was probably because food rewards seemed insufficient to motivate the laboratory dogs to participate in the task, and/or their willingness to participate could have been influenced by a variety of situational factors, such as the unfamiliarity of the situation and/or the proximity of the stranger. The finding that one of the principal components, Willingness to interact, was composed of the dogs' willingness to accept food as well as behavioral variables coded in the abovementioned three tasks, clearly indicates that the degree of individual willingness to participate is one of the key challenges in testing laboratory dogs [similar to some other animals, like cats; e.g., Smith et al. (33)].

It is perhaps not surprising that the repetition of the test battery (the 1st and 2nd test sessions) had a significant effect on the Willingness to interact component—but only on this component. Dogs showed more willingness to actively participate in the test—regardless of the treatment—during the second test occasion. This suggests that they gathered positive experiences

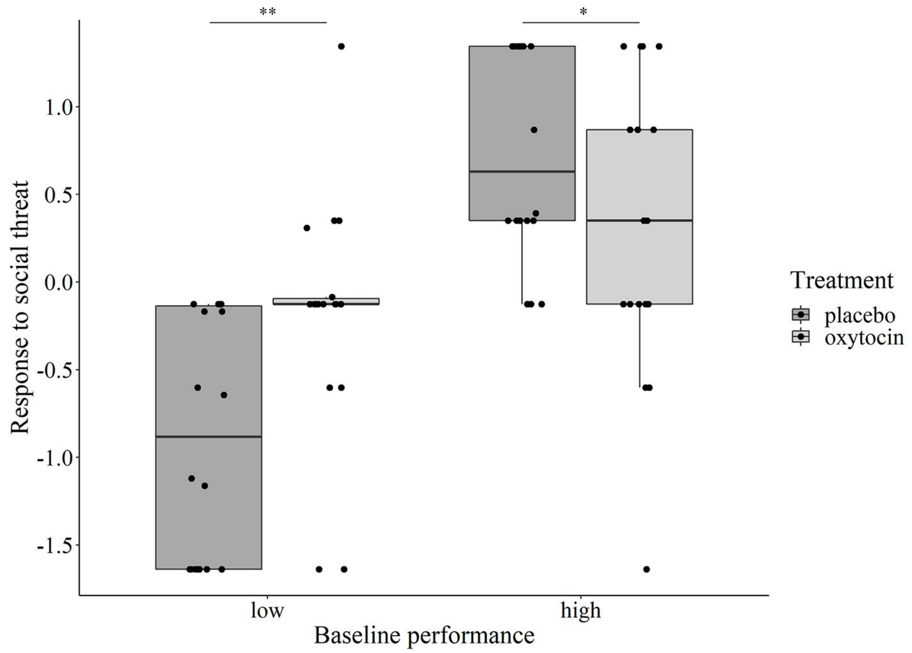

FIGURE 4 | Relationship between the dogs' baseline performance and oxytocin/placebo treatment on their Non-aversive response to social threat component score. Oxytocin treatment significantly increased the component score in dogs with low baseline performance (**$p = 0.002$), while the effect of treatment was the opposite in dogs with high baseline performance (*$p = 0.043$).

and/or habituated to the unfamiliarity of the situation enough to be able to focus on the task itself. Increasing food motivation probably also played an important role in this effect. Note that some of the dogs showed food neophobia during the first session (i.e., they were reluctant to consume the sausage probably because it was unfamiliar to them). During the repeated test session, however, all dogs that were willing to accept dry food readily accepted this new type of food reward.

The findings of the present study confirmed the differential effects of oxytocin treatment on the dogs depending on their baseline performance in the test battery (a measure of social competence). Although all four sets (components) of behavioral measures significantly interacted with treatment, these interaction effects were not uniformly strong in all principal components. Regarding the Willingness to interact and the Non-aversive reaction to non-social threat components, we found a significant treatment × baseline performance interaction only with a more sensitive assessment of the individual's baseline performance (i.e., when analyzed as a continuous score), whereas a comparison of the high- and low-performance groups (i.e., when grouped and analyzed as a categorical variable) did not show a significant difference between them. On the contrary, in the case of the other two components (Preference for social contact and Non-aversive reaction to social threat), significant differences remain between dogs even when categorized as high and low social competence groups in their reaction to oxytocin treatment. The results showed that oxytocin leads to a more positive reaction in both these components, but only in dogs with lower baseline performance. Regarding the Non-aversive reaction to social threat component, it is also interesting to note that oxytocin treatment led to a more negative (fearful) reaction in those dogs who showed a highly positive reaction to the threatening human in the placebo condition. This is in line with human studies suggesting that the effect of this neuropeptide depends on various contextual and inter-individual factors [for a review, see Bartz et al. (4)], and this phenomenon manifests itself also in the context-dependent influence of oxytocin on brain function (34). Results of another recent study indicate that oxytocin may serve as a warning system against potential threat cues in the environment and thus has the potential to facilitate active defensive behaviors (35). This finding contradicts the popular belief that oxytocin generally enhances social motivation and affiliative behaviors and further supports the results of human studies indicating that oxytocin can also induce antisocial effects, like aggression, envy, and distrust (8, 36), especially in negative situations like threat or competition (37).

Moreover, these results are also (partly) in agreement with the findings of Hernádi et al. (38). In their study, oxytocin treatment led to a less positive reaction to the threatening approach in dogs, compared to the placebo treatment, but only when the owner was performing the test, and no treatment effect was found when an unfamiliar experimenter approached the dog in a threatening manner. However, contrary to our results obtained on dogs with a low (fearful) baseline reaction, Hernádi et al. (38) did not find a positive effect of oxytocin, probably because their sample lacked dogs that showed severe fear in this situation (i.e., only 10%

of their subjects responded with avoidance in the test); thus, a low number of dogs could positively benefit from the effect of oxytocin.

The current study has some limitations; firstly, there were a limited number of dogs available for analysis; secondly, we had no *a priori* information about behavior measures indicating a high (or low) level of social competence in dogs. Thus, the dogs' baseline performance was obtained by direct measurement of the individual dog's behavior in the different tests of the battery during the placebo condition. Moreover, the categorization of the dogs into high- and low-performance groups was rather rough. These could be especially problematic if task repetition affects the dogs' performance, as half of our subjects received a placebo in the second test session. Although we found such an effect only in the case of the Willingness to interact component, the repetition effect may overshadow a possible interaction between the baseline performance and the oxytocin treatment in this component. We advocate future studies to develop an independent measure of social competence that allows for a more objective method of categorization.

All in all, in at least two of the four components, we found that baseline performance indeed moderates the effect of oxytocin on the dogs' social behavior: oxytocin promotes increased social behavior only in individuals with poor social competence (low baseline performance in the placebo treatment) while having no or even a negative effect on dogs with sufficient social competence (high baseline performance). These results are consistent with the social salience hypothesis (4, 36, 37, 39), which suggests that oxytocin generally increases the salience of social cues, and its subsequent effects (whether positive or negative) depend on how these cues are interpreted, based on contextual information and the individuals' inclinations. Accordingly, oxytocin enhances positive behaviors (social approach, prosocial behaviors, and reduced stress) only when the attributed salience of the social context is positive, while in negative (competitive, aggressive, or threatening) contexts, oxytocin enhances negative behaviors (anxiety, competitive, or aggressive behaviors). In our case, increasing the salience of the social cues in positive contexts (Interaction with the caretaker and Greeting by an unfamiliar experimenter) indeed led to more positive reaction for dogs with low baseline preference for social contact (those that were less attuned to positive social information), while in the negative situation (Threatening approach), increasing the salience of the negative social cues led to more negative reactions for dogs with a more positive baseline reaction (those that were less attuned to negative social information). However, in the case of the Non-aversive reaction to social threat component, oxytocin also led to a more positive reaction for dogs with more negative baseline reactions, which suggests that other factors (e.g., social stress) may also play a role aside from the salience of social cues.

Alternatively, our findings are also in accordance with a putative inverted U-shaped correlation between endogenous oxytocin availability and social performance found in rats and humans [e.g., (40–42)]. Although endogenous oxytocin levels were not measured in our study, one might speculate that baseline performance was associated with endogenous oxytocin levels. Accepting these preconditions, administration of oxytocin to low performers could have increased the otherwise low baseline oxytocin level and shifted them into a concentration range more optimal for social functioning [for a similar finding in dogs, see Romero et al. (17)]. On the other hand, in the case of high baseline performers, an already high endogenous oxytocin level was further increased by external oxytocin administration, which could have pushed them out of the optimum concentration range, resulting in a less positive social behavior. A similar phenomenon has been described in Syrian hamsters, where at the baseline level, females were more socially competent than males, and the same dose of externally administered oxytocin reduced the reward value of social interaction in females while increasing it in males (43). Note that oxytocin, especially when present in high concentrations, can also bind to vasopressin receptors, and vasopressin can have opposite effects on behavior compared to oxytocin (44).

SUMMARY AND CONCLUSIONS

In summary, the findings of the present study contribute to the growing body of evidence against the popular belief that oxytocin has a generally positive effect on social/prosocial behaviors and support the notion that oxytocin can help some individuals, but not others. This selective effect of oxytocin could explain at least some of the inconsistencies between previous dog oxytocin studies. Individuals with low social competence might be rare among family dogs, especially among pet dogs whose owners volunteer for research. Thus, different samples of family dogs could have different numbers of individuals or even no individuals on the negative end of the competence spectrum regarding the behavior in question, leading to a small or no association found in one study and significant associations in another. The social competence spectrum of laboratory dogs is supposedly much wider than that of family dogs, which made them a good choice for the purpose of our study. However, it is also reasonable to assume that for laboratory dogs on the negative end of the spectrum, even standard handling procedures can be stressful (21). In line with this notion, our findings show that oxytocin has a positive effect on the human-directed social behavior of these dogs, indicating that, in the absence of alternative opportunities (i.e., regular affiliative contact with humans), oxytocin administration has the potential to improve the wellbeing of these animals.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The research was carried out in accordance with the Hungarian regulations on animal experimentation and the guidelines for the use of animals in research described by the Association for the Study of Animal Behavior (ASAB). Ethical approval was obtained from the National Animal Experimentation Ethics Committee (Ref. No. PE/EA/3742-4/2016). This study was also approved by the Local Ethical Committee of Gedeon Richter Plc. and was carried out in strict compliance with the European Directive 2010/63/EU regarding the care and use of laboratory animals for experimental procedures.

AUTHOR CONTRIBUTIONS

JT, VR, BT, and BL contributed to the conception and design of the study. JT was responsible for the funding acquisition. BT and EP carried out the behavioral experiments. BT performed the statistical analyses. BT and JT wrote the first draft of the manuscript. All authors contributed to manuscript revision and read and approved the submitted version.

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How Inhibitory Control Relates to Positive and Negative Affective States in Red Junglefowl

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Individual differences in inhibitory control, an aspect of cognition, are found in many species. How this variation links to affective states is not much explored, and could be relevant for welfare. As less fearful, more optimistic, individuals may act more impulsively, inhibitory control could link to less negative, more positive, affective states. Alternatively, poorer inhibitory control could associate with more negative, less positive, affective states, as poorer inhibitory control can result in individuals being less able to adapt to changing environments and more likely to show stereotypies. We here explored in three cohorts ($N = 209$) of captive red junglefowl, the ancestor of domestic chickens, how inhibitory control associated with affective states. Specifically, we measured inhibitory control with a detour task, and negative and positive affective states with a tonic immobility test and a cognitive judgement bias test, respectively. Cognition and behaviour can differ between ages and sexes. Therefore, we investigated how inhibitory control related to affective states in younger chicks (≈2.5 weeks old), older chicks (≈5 weeks old) and sexually mature adults (≈28 weeks old) of both sexes. In younger chicks, poorer inhibitory control associated with less negative, more positive, affective states. We found no relationship between inhibitory control and affective states in older chicks or adults, nor sex differences regarding how inhibitory control related to affective states. Overall, our results suggest that inhibitory control can link to affective states and that the nature of these links can change over ontogeny.

Keywords: affective state, animal welfare, chicken, cognitive bias, fowl, impulsivity, tonic immobility

INTRODUCTION

Individual variation in cognition [i.e., how individuals acquire, process, store, and act on environmental information (1)] is repeatedly observed across taxa [e.g., (2–4)]. Nevertheless, the implications of this variation for individuals are not well-known. Determining how cognition links to affective states could help us ensure good welfare for animals. Affective states (i.e., emotional states) can be perceived as negative [e.g., stress (5), fear (6, 7)], or positive [e.g., pleasure

(8)]. In turn, more positive and less negative affective states could be considered an indicator of good welfare (9, 10). Thus, it is not surprising that how cognition and affective states relate has become a focus of recent research. Thus far, affective state has been found to influence various aspects of individual variation in cognition, including learning (11) and decision making (12). Nonetheless, there are still potential relationships between cognition and affective states that are not much explored.

One potential relationship between cognition and affective states that is scarcely investigated is how inhibitory control relates to affective states. Inhibitory control [an executive function which allows individuals to inhibit their prepotent responses (13)] is an aspect of cognition repeatedly shown to vary between individuals of the same species [e.g., (14–16)]. Inhibitory control can be measured using a detour task, which explores whether an individual can inhibit the prepotent response of trying to obtain a reward directly through a transparent barrier and instead obtain it by detouring around the barrier [e.g., (14, 17, 18)]. A potential functional cause for a relationship between inhibitory control and affective states in non-human animals could be neuropsychology, specifically, how risk-seeking vs. avoidant individuals are. Individuals with less negative, and more positive, affective states are likely to be less fearful [e.g., (6, 7)] and more optimistic [e.g., (19, 20)]. A lower fearfulness implies that these individuals will be less concerned about potential risks, while higher optimism implies that they are more likely to assume that taking risks will result in positive consequences. On the other hand, individuals with more negative and less positive affective states could be expected to be more fearful [e.g., (6, 7)] and less optimistic [e.g., (19, 20)], which could lead to these individuals being more avoidant. Taken together, we could expect individuals with more risk-seeking, impulsive behaviour (i.e., showing poorer inhibitory control) to show more positive, less negative, affective states. To our knowledge, this expectation has not yet been empirically explored. Inhibitory control could also connect to affective state via the consequences that poorer inhibitory control can have for individuals. First, individuals with poorer inhibitory control are less able to adapt to changing situations [e.g., (21)]. Second, individuals with poorer inhibitory control are generally more impulsive [e.g., (22–24)], which can make them more likely to display behaviours with seemingly negative consequences [e.g., stereotypies (25–27)]. Taken together, poorer inhibitory control could be predicted to have negative consequences for individuals, and thus link to more negative, less positive, affective states. That aspects of inhibitory control can be worsened by stress-inducing experiences [e.g., cortisol dosing (28), forced isolation (29)] supports such a link, though further studies are needed to confirm or reject this.

To determine how variation in inhibitory control could influence affective states, we need to be able to measure affective states. Animals with more negative affective states [e.g., more fearful (6, 7), more stressed (30, 31)] remain longer in tonic immobility, that is they remain motionless for longer after having been restrained on their back and tonic immobility has been induced (32). Animals with more positive affective states show higher levels of positive judgement bias [i.e., optimism (e.g., 19, 20)]. More optimistic individuals are faster to approach a novel ambiguous cue that is intermediate between learnt positive and negative cues in a cognitive judgement bias test [e.g., (33, 34)]. If poorer inhibitory control links to more negative, less positive, affective states, it should correlate positively with negative affective states and negatively with positive affective states. Impulsivity (thus also inhibitory control) can link to affective states in humans. For example, in humans, higher impulsivity regarding alcohol consumption has been found to link to both more negative and more positive affective states (35), and increased impulsivity is associated with increases in daily stress (36). Overall, how impulsivity relates to affective state in humans appears to depend on the measures of impulsivity and affective states explored [reviewed in (37)]. Despite the interest in how inhibitory control connects to affective states in humans, this connection is yet to be investigated in other animals.

If inhibitory control links to affective states there is potential for these relationships to differ over ontogeny (i.e., different relationships may be found at different ages). For example, inhibitory control can be slow to develop {at least in some primates, [e.g., humans and rhesus monkeys, *Macaca mulatta*, (38, 39)]}. Further, individuals can learn to improve their inhibitory control over time (18, 40). Taken together, this suggests that individuals should improve in inhibitory control as they age. This has been observed in humans [e.g., (41, 42)], but lacks research in other animals. Less is known about changes in affective states over ontogeny, however that inhibitory control can change over ontogeny implies that the relationship between inhibitory control and affective states may also do so. Relationships between inhibitory control and affective states could also differ between sexes. Both inhibitory control and affective states show sex differences. Males typically have poorer inhibitory control (when it comes to avoiding impulsive actions), but are better at inhibiting impulsive choices (i.e., waiting for a larger, delayed reward rather than going for an instant, small reward), than females (43). The nature of sex differences in affective states differs between species (44). Overall, while sex differences in the relationship between inhibitory control and affective states could be expected, they are less clear to predict the nature of.

We here explored how inhibitory control relates to affective states in red junglefowl, *Gallus gallus*. If inhibitory control is linked to affective states via how risk seeking vs. risk avoidant individuals are, we hypothesised that individuals with poorer inhibitory control would have a less negative, more positive, affective states. If inhibitory control linked to affective states due to poorer inhibitory control having negative consequences, we hypothesised that individuals with poorer inhibitory control would have more negative, less positive, affective states would have. Red junglefowl, along with their descendant, the domestic chicken (45), are increasingly used for behavioural and cognitive studies [reviewed in (46)]. Chickens are one of the world's most intensively farmed animals [reviewed by (47, 48)], and face severe welfare issues such as feather pecking, vent pecking, and cannibalism (27, 49). The population of junglefowl we used for this study are known to show individual variation in inhibitory control [e.g., (40, 50)], which can show temporal consistency across time in both chicks (51) and adults (52). Fowl are known to show easily discernable tonic immobility reactions (53), thus tonic immobility can be used to measure negative affective states in this species. Cognitive judgement bias tests are validated for measuring positive affective states or optimism in non-human animals (54, 55) and the cognitive judgement bias test used here was specifically developed for use in junglefowl (34, 50, 56). As junglefowl and domestic chickens develop from chicks to adults, they may display changes in behaviour and cognition, as well how these relate to each other [e.g., (34, 57–59)]. Thus, we explored relationships between inhibitory control and affective states in three ages, young chicks (≈2.5 weeks), older chicks (≈5 weeks) and sexually mature adults (≈28 weeks). We also included both males and females in this study to explore whether relationships between inhibitory control and affective states differed between sexes.

MATERIALS AND METHODS

Animals and Housing

The red junglefowl used for this study came from a pedigree bred population belonging to Linköping University, Sweden [see (56) for further details]. Specifically, we used three cohorts (Cohort 1 was hatched in 2016, Cohort 2 in 2017, and Cohort 3 in 2019). Birds were tested between 1 and 6 weeks of age [i.e., as chicks, before the age at which they would typically become independent from their mothers, (60, 61)] and between 27 and 29 weeks of age [i.e., as sexually mature adults, sexual maturity occurs at around 20–25 weeks of age, (62, 63)]. We collected data from all cohorts when they were young chicks, ≈2.5 weeks old when their inhibitory control was measured (Cohort 1: $N_{\text{females}} = 36$, $N_{\text{males}} = 34$; Cohort 2: $N_{\text{females}} = 23$, $N_{\text{males}} = 29$; Cohort 3: $N_{\text{females}} = 35$, $N_{\text{males}} = 23$). We did not test all cohorts at all ages, rather we tested Cohort 1 also as adults, ≈28 weeks old when their inhibitory control was measured ($N_{\text{females}} = 51$, $N_{\text{males}} = 48$), and Cohort 3 also as older chicks, ≈5 weeks old when their inhibitory control was measured ($N_{\text{females}} = 35$, $N_{\text{males}} = 23$). Not all younger chicks tested in Cohort 1 were retested as adults (42 younger chicks from Cohort 1 were retested as adults), nor were all younger chicks tested in Cohort 3 retested as older chicks (54 younger chicks from Cohort 3 were retested as older chicks). Also, in Cohort 1, some of the birds that were tested as adults were not tested as younger chicks, which was due to that these birds had been control birds for other studies when they were chicks. Chicks were sexed at 6 weeks of age, when moulting into sex specific plumage. Thus, for both younger and older chicks, experimenters were blind to the chicks' sex. We used artificial incubators to hatch our birds, thus reducing potential maternal effects. We gave each bird a numbered wing tag soon after hatching to enable individual identification. As chicks, birds were housed in mixed-sex groups in cages (72 × 71 × 53 cm, L × W × H), which contained perches, heaters, and saw-dust for dustbathing. In 2016 and 2017, we distributed chicks evenly between the cages, whereas in 2019, we housed them either in small (consisting of seven individuals) or large (consisting of 16 individuals) groups as part of another study. In 2016 and 2019, we designated a home pen to each chick, so they lived in stable social groups, while in 2017, we regularly moved chicks between pens. Differences in how cohorts were kept, as chicks, were due to differences in other studies taking place in parallel. As adults, birds were kept in two single-sex enclosures (6 m^3) equipped with perches, shelters, saw-dust for dustbathing, and access to an outdoor area (400 × 260 × 250 cm, L × W × H). For both chicks and adults, we used artificial lighting that was set so that the lights were on between 7 a.m. and 7 p.m., and birds always had access to *ad libitum* commercial poultry feed and water. Testing took place between 8 a.m. and 6 p.m. and birds were tested singly. The experiments were consistent with Swedish ethical requirements (Linköping Ethical Committee, ethical permit numbers 50-13 and 288-2019).

Experimental Set-Up

Birds took part in tests in the following order: cognitive judgement bias test, detour task, tonic immobility test. Birds participated once in the tonic immobility and cognitive judgement bias tests (apart from in Cohort 1, where birds participated in these tests once as young chicks, and once as adults). Thus, we used the same measures of negative affective state and positive affective state for analyses of both younger chicks and older chicks. The detour task and cognitive judgement bias test took place in arenas which varied in their dimensions according to whether chicks or adults were being tested (dimensions of arena used for chicks: 48 × 39 × 15 cm; dimensions of arena used for adults: 90 × 50 × 60 cm, L × W × H). To minimise isolation stress during testing, we habituated all subjects, when they were chicks, to being alone in the testing arenas before they were tested [*sensu* (64)]. Rewards during testing and training always consisted of ≈1/3 of a fresh mealworm for chicks, and a whole fresh mealworm for adults. For tests that consisted of a training phase and a testing phase (i.e., detour task, cognitive judgement bias test), birds were returned to their cage for a minimum of 1 h after completing training before commencing testing, to maintain reward motivation and minimise duration of time spent in social isolation. Birds were sometimes initially helped during training to find rewards. This was done by either tapping near the reward with tweezers, leaving a trail of mealworms, or guiding with a hand. Birds were never helped to find rewards during testing. For tests that consisted of multiple trials (i.e., detour task, cognitive judgement bias test) a trial started after a bird was placed in the arena and ended either when the bird obtained the reward (both tests), approached a cue within 2 cm (cognitive judgement bias test, at this distance a bird can see if their chosen cue is rewarded or not), or left the arena (both tests). At the start of trials, in tests which involved interacting with testing equipment (i.e., detour task, cognitive judgement bias test), for both training and testing trials, birds were placed into the arena at one of the short ends of the arena opposite, and facing away, from the testing equipment. This latter prevented them from automatically approaching the testing equipment without intending to. Testing equipment was repositioned and rebaited between trials, without birds being able to watch this.

Measuring Inhibitory Control

To measure inhibitory control, we used a detour task [*sensu* (40, 50)]. For each age, each bird was only tested once. Inhibitory control shows moderate temporal consistency between younger and older chicks in this population [Cohort 3 used in this study, (51)], and in adults (52).

Training Phase

Before a bird could take part in the detour task, we needed to familiarise it with obtaining a reward by navigating around a barrier (18). Specifically, birds learnt, over a series of trials, to obtain a reward from the centre of an opaque tube (5Ø × 8L cm for chicks and 7Ø × 8L cm for adults) by walking to one of the ends of the tube and putting their head into one of the tube's openings. We considered a bird ready for testing once it had obtained the reward, without pecking at the tube or needing help to find the reward, in five consecutive trials.

Testing Phase

During the testing phase of the detour task, we presented each bird with a transparent tube with a reward at its centre. The dimensions of the tube, as well as the position of both the bird and tube, at the start of each trial, were identical to those in the training phase. We measured each bird's inhibitory control as the number of trials (out of five) in which it inhibited the impulsive response of trying to peck the reward directly through the transparent tube, and instead used the detour learnt in the training phase to obtain the reward. We termed this measure "Inhibitory control," where a higher measure indicated better inhibitory control (14). We used only five trials to reduce aspects of learning affecting this measure (18, 40). Variation in inhibitory control measures ranged from 0 to 5 for all ages.

Measuring Negative Affective State

To measure negative affective state, we used a tonic immobility test [e.g., (7, 65), *sensu* (50, 57)]. Tonic immobility has shown moderate temporal consistency in our population of junglefowl for both chicks (58) and adults (57). We induced tonic immobility by laying a bird on its back in a V-shaped wooden cradle (20 × 10 cm) and gently holding the bird down, for 15 s, with one hand over its chest, applying light pressure, and another over its eyes. After this, we slowly removed our hands and measured "Negative affective state" as the time taken (s) by the bird to return to standing; the longer this latency, the more negative [i.e., more fearful (6, 7), more stressed (30, 31)] the affective state of the bird [e.g., (65, 66)]. While testing, the experimenter avoided eye contact with the test bird. If, following restraint, the bird did not remain on its back for at least 3 s, we did not consider tonic immobility to be induced, so we repeated the restraint. In 2016 and 2017, we used a maximum of three attempts to induce tonic immobility, while, in 2019, this was increased to 5. If we were unable to induce tonic immobility in a bird, we gave it a "Negative affective state" measure of 0 s. If a bird remained immobile for 600 s, we gave it a "Negative affective state" measure of 600 s and then gently brought it out of tonic immobility by hand. Eight younger chicks, three older chicks and in 15 adults were given a measure of 600 s for "Negative affective state." Four younger chicks, and three adults, were not tested in the tonic immobility test. This was due to these birds being accidentally omitted from data collection due to experimenter error. Variation in our negative affective state measure ranged from 0 to 600 s for chicks and 4.37–600 s for adults.

Measuring Positive Affective State

To measure positive affective state, we used a cognitive judgement bias test [*sensu* (34)].

Training Phase

Before a bird could participate in our cognitive judgement bias test, it needed to learn to associate a white cue with a reward, and a black cue with the absence of a reward. A cue consisted of a bowl (5 × 3 cm, Ø × H), in front of a laminated card (9 cm^2) of matching colour. To teach the birds to associate the cues with their outcomes, we simultaneously presented them with both a rewarded white cue and an unrewarded black cue, separated by an opaque divider, several consecutive times. To prevent the development of side preferences, we varied which side the rewarded cue was presented on (left or right) according to a pre-determined, pseudorandom sequence. For each trial, there were three possible outcomes: "pass", in which the bird approached the rewarded cue without needing help, "fail", in which the bird approached the unrewarded cue or left the arena, or "helped", in which the bird was helped to find the rewarded cue (note that we only initially helped birds and not during testing). We deemed a bird ready to progress to the testing phase once it had scored six consecutive passes. This criterion was chosen as it is very unlikely to be reached by chance (56). The number of trials birds received in this training stage varied depending on how many they needed to reach our set learning criterion. In terms of sessions, birds typically needed 1 or 2 (max 4) to reach our set learning criterion.

Testing Phase

During cognitive judgement bias testing, we presented birds with single cues, either rewarded white, unrewarded black, or one of three novel, unrewarded grey cues (i.e., light grey: 25% white/75% black, mid grey: 50% white/50% black, or dark grey: 75% white/25% black) in a pseudorandom order over a series of trials. Three different grey cues were used due to other investigations which also used data from this test. For this study, we used response to the mid grey cue to measure positive affective state, as this cue is the most ambiguous between the learnt positive and negative cue. Birds saw the mid grey cue 2 times in 2016 and 3 times in 2017 and 2019. To measure "Positive affective state," we recorded average latency (s) to approach the mid grey cue. A shorter latency indicated higher optimism and, thus, a more positive affective state (9, 33, 34). Note the inverse nature of this measure. We also recorded each bird's average latency to approach the rewarded cue in this test. We gave chicks up to 30 s and adults up to 60 s to approach the cue (since adults were in larger arenas and can show lower food motivation than chicks). Ten younger chicks, eight older chicks and 13 adults were given max values in this test. Seventeen younger chicks, six older chicks, and 29 adults did not complete the cognitive judgement bias test, either because they failed to learn the cue reward association at the training stage or did not complete the test. The latter was due to low food motivation. Variation in our positive affective state measure ranged from 0.93 to 30 s for chicks and 1.70 to 60 s for adults.

Statistical Analyses

We used R studio version 4.1.2 (67) to analyse our data. As the data did not fit assumptions of normality, we used non-parametric tests. We considered *p*-values $\leq$ 0.05 to imply significant results.

We explored temporal consistency in our measures of inhibitory control and affective states between younger chicks and adults (in Cohort 1), and temporal consistency in our measure of inhibitory control between younger chicks and older chicks (in Cohort 3), using Spearman's rank correlation tests.

As fowl can display changes in behaviour and cognition, and relationships between these, over ontogeny [e.g., (34, 57–59)], we analysed data from younger chicks, older chicks, and adults separately. To explore how inhibitory control related to affective state in our birds, we created models using the package "lme4" (68). Before designing the models, we investigated how independent our two affective state measures were by exploring the relationship between them, within each age, using Spearman's rank correlation tests. We found that "Negative affective state" (i.e., latency to righten in a tonic immobility test) and "Positive affective state" (i.e., latency to approach a novel, ambiguous cue in cognitive judgement bias test) were not correlated in younger chicks ($R_s = 0.07$, $p = 0.38$, $N = 162$) or older chicks ($R_s = -0.22$, $p = 0.11$, $N = 50$) and were only moderately correlated in adults ($R_s = -0.29$, $p = 0.01$, $N = 69$). Therefore, we made separate models for "Negative affective state" and "Positive affective state" for all ages. Initially, we made a generalised linear mixed model for younger chicks, again using "lme4", with "Cohort" (1–3) as a random effect. However, as "Cohort" explained very little variation, we used generalised linear models instead, also made in "lme4". We did not include individual ID as a random effect, since only one measure per age was included in our data. In all our models, our measure of affective states (either "Negative affective state" or "Positive affective state") was our response variable. For adults and the older chicks, the predictor variables in our models were "Inhibitory control" and an interaction between "Sex" (male = 0, female = 1) and "Inhibitory control." These predictor variables were also included in the model for younger chicks, along with an interaction between "Cohort" and "Inhibitory control." We included interactions between "Sex" and "Inhibitory control," and "Cohort" and "Inhibitory control," in our models, but did not include "Sex" and "Cohort" as separate predictors. This was because we were specifically interested in whether the relationship between inhibitory control and affective states differed between sex or cohort, not whether affective states differed between sex or cohort. If, for any of our models, an interaction was not significant, we removed this interaction from the model. This resulted in the removal of the interaction between "Sex" and "Inhibitory control" from all our models. The interaction between "Cohort" and "Inhibitory control" was significant in the model for younger chicks, thus we did further analyses to investigate how the relationship between inhibitory control and affective state differed between cohorts. To do this, we first subsetted the data for younger chicks into the three separate cohorts and then ran simple models (affective state measure $\sim$ inhibitory control) for each subset separately. In all our models for "Positive affective state," we included average latency to approach the rewarded cue as a covariate, thus accounting for individual differences in response speed not due to differences in optimism (e.g., general speed, motivation). For all our models, our response variables were continuous and non-normal, thus we used a gamma distribution. As gamma requires only positive values in response variables, we replaced 0 s in the data with 0.01.

As results can be influenced by outliers or max values (the presence of max values could create ceiling effects that mask relationships in the data), we ran the analyses first with all data, and then (1) with outliers removed (first only extreme outliers and then also mild outliers, defined below), and (2) with max values removed. We defined extreme outliers as data points that were 3 × the interquartile range of the upper or lower quartiles, and mild outliers were as data points that were 1.5 × the interquartile range of the upper or lower quartiles (69, 70). Max values were measures of 600 for "Negative affective state," 30 for "Positive affective state" in younger and older chicks, 60 for "Positive affective state" in adults.

RESULTS

We found no qualitative effects of outliers or max values on any of our analyses, that is the patterns we detected, and what we found to be significant or non-significant, did not differ between analyses using all data, analyses using data with outliers removed, or analyses using data with max values removed. Therefore, we here only report results from analyses using all data. We found no sex differences in relationships between inhibitory control and affective states for any of our ages (in all models the interaction between sex and inhibitory control was $p > 0.1$).

Inhibitory control was not consistent between younger chicks and adults (in Cohort 1, $R_s = -0.07$, $p = 0.64$, $N = 42$), though it was consistent between younger chicks and older chicks (in Cohort 3, $R_s = 0.32$, $p = 0.02$, $N = 54$). Our measure of negative affective state did not show consistency between younger chicks and adults (in Cohort 1, $R_s = 0.006$, $p = 0.97$, $N = 42$), nor did our measure of positive affective state ($R_s = -0.13$, $p = 0.51$, $N = 41$).

How Inhibitory Control Linked to Affective States in Younger Chicks

In younger chicks (i.e., ≈2.5 weeks old), birds that had higher "Inhibitory control" had a significantly higher "Negative affective state" ($t = 2.49$, estimate $= 0.005$, SE $= 0.002$, $p = 0.01$, $N = 177$, **Figure 1A**). Further, the interaction between "Cohort" and "Inhibitory control" was a significant predictor of "Negative affective state" ($t = -2.89$, estimate $= -0.002$, SE < 0.001, $p = 0.004$). However, the relationships between "Inhibitory control" and "Negative affective state" between cohorts were all non-significant when cohorts were analysed separately (Cohort 1: $t = 0.88$, estimate < 0.001, SE $= 0.002$, $p = 0.88$, $N = 70$; Cohort 2: $t = 0.84$, estimate $= 0.02$, SE $= 0.002$, $p = 0.41$, $N = 49$; Cohort 3: $t = -0.37$, estimate < 0.001, SE < 0.001, $p = 0.69$, $N = 58$). Younger chicks with better inhibitory control also had a higher "Positive affect state" measure ($t = 2.75$, estimate $= 0.04$, SE $= 0.01$, $p = 0.006$, $N = 164$, **Figure 1B**; recall that a higher "Positive affective state" measure implies a less positive affective state, because the measure used was latency to approach the ambiguous cue). The interaction between "Cohort" and "Inhibitory control" was also a significant predictor of "Positive affective state" ($t = -3.44$; estimate $= -0.02$, SE $= 0.004$, $p < 0.001$). When the relationship between "Inhibitory control" and "Positive affective state" was looked at within each cohort separately, it was non-significant in Cohort 1 ($t = 0.061$, estimate $= 0.001$, SE $= 0.016$; $p = 0.95$, $N = 66$), Cohort 2 ($t = 0.91$, estimate $= 0.007$, SE $= 0.008$, $p = 0.37$, $N = 46$), and Cohort 3 ($t = -1.19$, estimate $= -0.04$, SE $= 0.005$, $p = 0.41$, $N = 52$). Thus, in younger chicks, inhibitory control was positively linked to negative affective state and negatively linked to positive affective state.

How Inhibitory Control Linked to Affective States in Older Chicks

In older chicks (i.e., ≈5 weeks old), "Inhibitory control" did not link to either "Negative affective state" ($t = -0.55$; estimate $= <$-0.001, SE $= 0.004$, $p = 0.59$, $N = 58$) or "Positive affective state" ($t = 0.73$; estimate $= 0.002$, SE $= 0.004$, $p = 0.79$, $N = 0.93$).

How Inhibitory Control Linked to Affective States in Adults

In adults (i.e., ≈28 weeks old), "Inhibitory control" did not link to either "Negative affective state" ($t = -1.62$; estimate = -0.001, SE $= 0.001$, $p = 0.11$, $N = 96$) or "Positive affective state" ($t = -0.59$; estimate $= 0.002$, SE $= 0.003$, $p = 0.56$, $N = 70$).

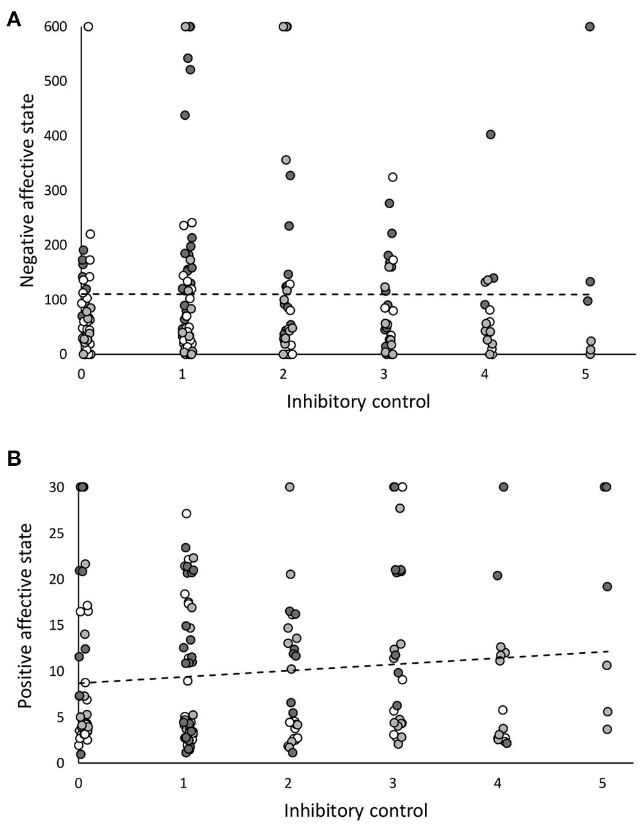

FIGURE 1 | The relationship between inhibitory control and affective state in younger red junglefowl chicks (aged around 2.5 weeks old, $N = 180$). Plots show the weak, yet significant relationships between inhibitory control and **(A)** negative affective state, **(B)** positive affective state. Inhibitory control is the number of trials, out of 5, in a detour task, in which an individual uses a learnt detour to obtain a reward from the centre of a transparent tube (a higher measure indicates better inhibitory control). Negative affective state was measured as latency to return to standing in a tonic immobility test (a higher measure indicates a more negative affective state). Positive affective state was measured as latency to approach an ambiguous cue between a learnt rewarded and a learnt unrewarded cue in a cognitive judgement bias test (a lower measure indicates a more positive affective state, observe the reversed nature of this). Each data point represents an individual bird, and the data are from all cohorts (Cohort 1 = white, Cohort 2 = mid grey, Cohort 3 = dark grey) and both sexes pooled. Dotted lines are trendlines.

DISCUSSION

We here investigated whether inhibitory control linked to affective states in red junglefowl at three different ages (younger chicks, older chicks, and adults, thus two different developmental stages, chicks and adults) and in both males and females. Our measures of affective states were not correlated. Regarding relationships between inhibitory control and affective states, we found that, in younger chicks, poorer inhibitory control was linked both to a less negative affective state (significantly, yet weakly) and, somewhat stronger, to a positive affective state (again significantly, though still weak). We found no links between inhibitory control and either positive or negative affective states in older chicks, or adults. Finally, we found no sex differences in relationships between inhibitory control and affective states, at any age.

Our measure of negative affective state did not correlate with our measure of positive affective state. This indicates that our affective state measures are two (relatively) independent measures, which capture separate aspects of affective states. This supports the concept that affective states are not unilinear, that is, that positive affective state cannot simply be considered the opposite of negative affective state, or vice versa (33, 71). To put it another way, optimism is not simply a lack of fearfulness and fearfulness is not simply a lack of optimism. Thus, by using multiple tests of affective state here, we could get at both positive and negative affective states and so explore the effects of inhibitory control on affective states at a broader level, than if we had only focused on one aspect of affective state. We recommend future researchers to do likewise. An interesting avenue for future research could be to explore a more complete description of the aspects of affective state in animals.

Our measures of inhibitory control, negative affective state, and positive affective state, were not consistent over the transition from chickhood to adulthood. On the other hand, inhibitory control was found to be moderately consistent over a shorter time span (between younger and older chicks). That measures were not consistent between chicks and adults is not unexpected, as red junglefowl undergo two major developmental changes, during this transition, which could potentially result in changes to their behaviour and/or cognition. First, becoming fully independent from their mother [at around 10–12 weeks of age, (60, 61)] and second, sexual maturation [at around 24–25 weeks of age, (62, 63)]. That our measure of inhibitory control was consistent over a shorter time span suggests that this captures individual variation, at least to some extent. We could not here explore temporal consistency in negative, or positive affective state, over shorter time spans, as we only took one measure of these per developmental stage (i.e., as chicks or as adults). Previous studies, on this species, have found negative affective state to be moderately consistent over time, within chicks (58) and adults (57), and positive affective state to be weakly consistent over time (and mainly driven by environmental effects), within chicks (34, 56). We acknowledge that, as temporal consistency in our measures varied (from low to moderate), and we used single, rather than repeated, measures of behavioural variation in our analyses, we here used unpartitioned phenotypic correlations to investigate among-individual patterns. This approach, though common practise in animal behaviour research, is problematic. While behavioural correlations may reflect among individual correlations, this should not be assumed outright (72, 73). Thus, further research able to partition variation of within and between individual patterns is needed to determine whether the patterns we observed here, reflect patterns found on the between individual level.

For the current work, we made two hypotheses. First, if inhibitory control linked to affective states via risk seeking vs. risk avoidance, and individuals with a poorer inhibitory control would be expected to have less negative, more positive, affective states. Second, if inhibitory control linked to affective states due to poorer inhibitory control having negative consequences, individuals with poorer inhibitory control were predicted to have more negative, less positive, affective states. Our results for younger chicks offer support for our first hypothesis, and not for our second hypothesis, in that we found a link between poorer inhibitory control and less negative, more positive, affective states. Thus, in younger chicks, poorer inhibitory control appeared to be associated with better welfare, if welfare is indicated by more positive, less negative affective states. In older chicks and adults, we found no connections between inhibitory control and affective states. Our second hypothesis was partly based on previous findings that individuals with poorer inhibitory control are less able to adapt to changing situations. However, our birds were not exposed to many changing situations in which being less able to adapt could have been expected to influence affective states. This is based on that (besides from Cohort 2, i.e., younger chicks tested in 2017), our birds did not experience changing situations in terms of where they were housed, or who they were housed with. Further, all birds were carefully habituated to the main change of situation they regularly experienced (i.e., taking part in testing) to reduce this causing stress. We also based our second hypothesis on previous findings that individuals with poorer inhibitory control are more likely to display behaviours that can have negative connotations [e.g., (25–27)], and that stress can worsen inhibitory control (28, 29). Our methods may have prevented such behaviour and stress from influencing affective states in our birds. First, we did not observe birds behaving in ways similar to described in previous work [e.g., feather pecking, vent pecking (26, 27, 49)]. Moreover, we tried to avoid stressing our birds as much as possible. Studies are needed to further explore and directly tests potential relationships between inhibitory control and affective states. This can be done, for example, by manipulating affective states (e.g., through providing enrichment, or stressors) and measuring how this affects inhibitory control. Such studies are currently scarce. So far, exposure to enrichment or stressors seem to result in poorer inhibitory control (28, 29, 40, 74, 75), though more research is needed to test the generality of this. As well as exploring how affective states directly affect inhibitory control, we encourage studies which aim to better understand how inhibitory control links to known welfare issues, for example, feather pecking. Feather pecking is a major welfare issue for chickens (27), which are one of the world's most intensively farmed animals [reviewed by (47, 48)]. The relationship between feather pecking and inhibitory control is currently unclear as, while the idea that feather pecking results from higher impulsivity has some support [e.g., (26, 27)], this is not always the case [e.g., (76)]. More research is therefore needed to disentangle the relationship between feather pecking and inhibitory control, as well as to explore how inhibitory control relates to other welfare issues.

We here assessed negative affective state with a tonic immobility test and positive affective state with a cognitive judgement bias test. While these tests are both well-established (7, 77) and can be used in a variety of species (7, 54, 55, 77, 78), they have their potential drawbacks. The reaction seen in tonic immobility test is thought to have developed as a defensive reaction to a predator attack (79, 80). Based on this, the tonic immobility test could be assumed to mimic a predator attack and, consequently, to be stressful and/or fear inducing to animals that experience it. Thus, the tonic immobility test itself could have negative implications for welfare if used frequently. The cognitive judgement bias test, while not intrinsically stressful, can be costly in terms of time, as animals need to be trained to reach a learning criterion before they can be tested. There are, therefore, incentives to develop less stressful and simpler ways to assess affective states. Especially useful would be single tests that can assess both positive and negative affective states simultaneously. That, in younger chicks, measures from the detour task associated with measures from both the tonic immobility test and the cognitive judgement bias test could imply that a detour task could, in some cases, function as such a test. However, we would advise against this. First, the relationships we found between inhibitory control and affective states were weak.

Furthermore, various factors, besides inhibitory control and affective states, may affect an individual's performance in a detour task. These include variation in food motivation, differences in how individuals are trained for the detour (81), and learning ability [as individuals can learn to improve inhibitory control over time (18, 40)]. To reduce the influence of these factors, we used a reward with high motivation for both chicks and adults. Further, we ensured that all birds were taught the detour in the same way and the number of trials birds had in this test was kept low (to avoid effects of learning). Another controversial aspect of the detour task is that its results do not necessarily correlate with other measures of inhibitory control [e.g., (82, 83)]. This, however, does not necessarily imply that detour tasks do not measure inhibitory control, but rather that inhibitory control is a complex construct consisting of distinct aspects (82, 83), one of which the detour task captures. Nevertheless, we acknowledge that we here explored the relationship between a particular aspect of inhibitory control and affective states, and that, to determine relationships between inhibitory control and affective states, in general, a battery of inhibitory control tasks should perhaps be used, to capture a broader picture of inhibitory control.

By collecting data from younger chicks, older chicks, and adults we were able to explore whether relationships between inhibitory control and affective states differed over ontogeny. Our results suggested that they were (since patterns were detected in younger chicks, but not in older chicks or adults). We acknowledge that we may have found links between inhibitory control and affective states in younger chicks only, because our sample size for younger chicks was larger than our sample size for older chicks and adults. However, finding differences over ontogeny in relationships between inhibitory control and affective states in red junglefowl, could be expected. Fowl are known to differ as they age in other aspects of behaviour and cognitive performance, including relationships between these [e.g., (57–59)]. These changes in behaviour and cognitive performance may be due to social and physiological changes which occur during maturation from chicks to adults [reviewed in (57)]. However, how, and why, social and physiological changes may affect behaviour or cognition is not fully understood. That younger chicks which were more impulsive in a detour task were also showed more fearful responses in a tonic immobility test and were less optimistic in a cognitive judgement bias test indicates that these chicks may have had a proactive-reactive behavioural syndrome. More proactive individuals are typically less fearful, more impulsive, and more optimistic, than more reactive individuals (84–87). That measures of impulsivity, fearfulness and optimism did not correlate in older chicks or adults suggests that this syndrome may fade as birds age. Another reason why relationships between inhibitory control and affective states change over ontogeny could be because individuals, including red junglefowl, can improve their inhibitory control over time (18, 40). Overall, our results suggest that age can affect the relationship between inhibitory control and affective states (both negative and positive), and we encourage future research to investigate the generality of this further. This could be done, for example, by measuring how individuals' inhibitory control relates to affective states at multiple points during their lives.

Previous work, in other species, has shown that both inhibitory control and affective states can differ between sexes (43, 44). Therefore, the relationship between inhibitory control and affective states could also be expected to differ between sexes, especially species which can show sex differences in behaviour, such as fowl (57, 88). Nevertheless, we did not observe sex differences in relationships between inhibitory control and affective states in the red junglefowl used in this study. Earlier studies have also found a lack of, or only weak, sex differences in aspects of behaviour [e.g., tonic immobility (57)] or cognition [e.g., learning speed in discrimination and spatial learning tests (64)] in this species. Regardless, we still encourage future studies on relationships between cognition and affective states to investigate sex differences in these relationships where possible.

Overall, we here show that, based on behavioural correlations, inhibitory control seems to link to both positive and negative affective states, and thus in turn, that inhibitory control, an aspect of cognition, may have implications for welfare. However, the nature of links we observed between inhibitory control and affective states varied over ontogeny. Such links may also differ between sexes, though we found no evidence of that here. Overall, this study, along with other recent studies [e.g., (11, 12)], suggests that individual variation in cognition can link to affective state, knowledge which in turn could help us to improve the welfare of animals [e.g., (89–91)].

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Linköping Ethical Committee, Linköping District Court (ethical permit numbers 50-13 and 288-2019).

AUTHOR CONTRIBUTIONS

LG came up with the idea for the study. LG and HL designed the study. LG collected data together with members of the Løvlie Group (see acknowledgements) and analysed it based on communication with HL. LG wrote the manuscript with input from HL and CC. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.872487/full#supplementary-material

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Judgment Bias During Gestation in Domestic Pigs

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In humans and rats, changes in affect are known to occur during pregnancy, however it is unknown how gestation may influence mood in other non-human mammals. This study assessed changes in pigs' judgment bias as a measure of affective state throughout gestation. Pigs were trained to complete a spatial judgment bias task with reference to positive and negative locations. We tested gilts before mating, and during early and late gestation, by assessing their responses to ambiguous probe locations. Pigs responded increasingly negatively to ambiguous probes as gestation progressed and there were consistent inter-individual differences in baseline optimism. This suggests that the pigs' affective state may be altered during gestation, although as a non-pregnant control group was not tested, an effect of learning cannot be ruled out. These results suggest that judgment bias is altered during gestation in domestic pigs, consequently raising novel welfare considerations for captive multiparous species.

Keywords: pregnancy, gestation, cognitive bias, affective state, information processing, pig

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BACKGROUND

Research investigating the links between pregnancy, affect and cognition is most often carried out with a human-centric focus with studies typically using case studies and cohorts. In humans, changes in affective state during pregnancy are common and alterations in levels of anxiety, depression and cognitive ability have been demonstrated in humans and rodents (1–3). These changes are often linked to the large and rapid hormone fluctuations that occur during the gestational period (4, 5). Where human subjects cannot be used, rodent models are often employed to experimentally investigate how factors such as diet, enrichment or stress can influence behavior during gestation (6–8). To infer anxiety and depressive-like behaviors, lab-based behavioral tests, such as a forced swim or open-field test are often used (9). These studies are conducted under laboratory conditions and are generally aimed at modeling human gestation, rather than investigating how gestation may impact on the rodent itself. Results from both human and rodent studies are varied, however most show that affective state is altered throughout gestation [for review see (2)] and it is clear that pregnancy impacts maternal affective state.

Understanding an animals' affective state better enables us to understand their subjective experience, both positive and negative, and is a key component of animal welfare (10). Affective state can influence and alter cognitive processes, such as judgment (11, 12), which may then be used to infer and understand an animals' affective state. Cognitive bias or judgment bias is the influence of affect on information processing, with more content individuals likely to make positive assumptions about ambiguous stimuli, and vice versa (13). Judgment bias tests have been used to assess changes in affective state in a range of species, including pigs, dogs, honeybees and European starlings (14–17). Research typically focuses on the impact of external stimuli on judgment bias;

this is likely to act via alteration to the internal, physiological environment ultimately resulting in changes in behavior and judgment bias (11, 18, 19). As such, we would expect internal stimuli, such as physiological changes, would also impact judgment bias directly even in the absence of external influences. Pregnancy is one of the biggest physiological changes a mammal may experience, involving major hormonal and cognitive adjustments (20, 21), yet little is known of how information processing and affective state may change in relation to gestation in animals.

The domestic pig *(Sus scrofa domesticus)* has been used as a human model in a wide range of medical research such as infectious disease (22), nutritional (23) and neurological studies (24). Pigs allow for longer lifespan studies and are more anatomically and physiologically similar to humans than other laboratory species, such as rodents (25, 26). More commonly, pigs are farmed around the globe for meat production. Modern intensive farming systems have been designed to produce food as quickly and cost efficiently as possible, and research is continually ongoing to understand how animal welfare can be optimized within these systems. Despite many studies on the behavioral and welfare needs of sows during gestation (27–30), only two studies used a specific judgment bias task to assess affective state in gestating sows. These studies focused on using judgment bias as a welfare indicator in gestating sows however, did not investigate how gestation itself influenced judgment bias (31, 32). More recently another study showed that gestating gilts that were classified as “friendly” visited an electronic sow feeder more often than individuals that were classified as “fearful” (33). The authors hypothesized that this feeding behavior may be similar to a judgment bias task and that the friendly individuals may have been more optimistic. However, again this study did not investigate how gestation itself influenced judgment bias.

We investigated how gestation may alter judgment, and therefore affective state, in domestic pigs. We compared within-individual affective state, as measured by a spatial judgment bias test, before mating, and during early and late gestation. We hypothesized that within-individual judgment bias would be more pessimistic during gestation than prior to mating, leading to an increase in latency to approach ambiguous cues throughout gestation. This is the first study to our knowledge to investigate the possible impact of gestation on judgment bias in domestic pigs.

METHODS AND MATERIALS

This work was carried out between July and October 2015 (replicate one) and between January and July 2017 (replicate two) on a pig farm in the UK.

Animal Housing and Husbandry

20 gilts (primiparous female pigs; $N = 10$ for each replicate) were selected based on age and time until first mating. Using gilts allowed for training time before gestation, as there is limited time between pregnancies once a sow has begun breeding. The average age of all 20 pigs on day one of training was 241.7 (SD: 15.93) days. Replicate two contained one Duroc and three Landrace pigs, the breed of all other individuals was Large White. Pigs were housed in pens of five or six animals, each pen (4.67 × 5.35 m) contained a sheltered sleeping area with straw bedding (2.70 × 4.67 m) and a run partially exposed to outdoor elements, such as wind and natural light (2.65 × 4.67 m). A standard lactating sow ration was fed once a day before mating and throughout gestation; there was continuous access to water and natural lighting. During the course of the study the animals remained within the same groups and pens to keep the external environment as controlled as possible throughout. The study pigs were able to interact with pigs in the pen next door via the gate and animals in the Neighboring pens may have been moved/changed. Due to involvement in a separate study, replicate one pigs received Regumate® (containing a steroidal progestin) orally with feed 23 days before planned estrus to allow for Synchronized farrowing. As of June 2020, no previous research was found investigating possible effects of Regumate® on affective state or behavior of pigs. Due to this research taking place on a working farm, it was not possible to test a separate non-pregnant control group and each pig was used as its own control.

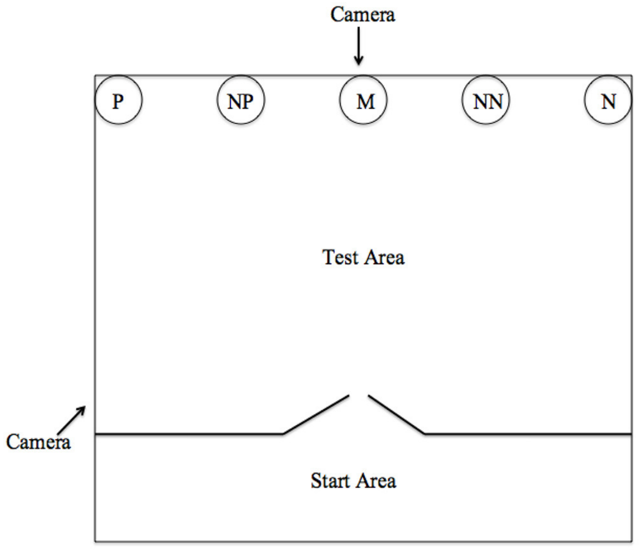

FIGURE 1 | Experimental set up for the judgement bias test with positive (P), near positive (NP), middle (M), near negative (NN), and negative (N) locations. The figure shows the locations for an individual trained to expect a positive reward in the left corner and to avoid the right. Only one bowl was present in one location at a time.

Judgement Bias

The training and testing area (**Figure 1**) used comprised of a testing room (3.72 × 5.26 m) and a starting room (3.72 × 1.79 m). All pigs were habituated to the test area in groups for two to three sessions, and then individually for a maximum of seven sessions to habituate the pigs to eating from a bowl which was initially placed in the center of the test area. Following this, individuals were trained to associate the bowl in two opposite corner locations with a positive (*P*) and a negative (*N*) outcome. When in the P location, the bowl contained a small amount of chocolate raisins (replicate 1) or sugar-coated chocolates (replicate 2) and when it was in the N location, the bowl contained unpalatable food (bitter tasting coffee beans) to discourage the pigs from approaching this location. The pigs were trained to discriminate between these reference locations by first only receiving positive trials and then later interspersing negative trials. Latency to reach the bowl was recorded using video cameras and was then used as a metric to assess whether each individual had learned the discrimination. Each trial was 30 s in duration. Correct responses were recorded when the subject approached and touched their nose to the bowl during the positive (P) trials; during negative (N) trials, a correct response was recorded when the individual did not approach the bowl within 30 s. The location of P and N was counterbalanced across individuals. For both replicates a criterion of 70% correct responses in the last 20 trials was required before moving onto the testing phase. Per individual, forty-four training trials were conducted during replicate one and sixty-two for replicate two. Replicate two required more training trials due to the pigs being slower to differentiate between the positive and negative locations, though had a higher rate of meeting the criterion by the end of training. Five pigs from replicate one failed to meet this criterion and were removed from the study. Two pigs from replicate 2 did not meet this criterion. The analysis represents only those 13 that met the learning criterion.

Each testing session comprised two sets of nine trials carried out on the same day, involving five different bowl locations; the three intermediate ambiguous probes: near positive (NP), middle (M) and near negative (NN), interspersed with P and N reference locations (e.g. P, N, M, P, N, NP, P, N, NN). Only one bowl was in the arena during each trial. The ambiguous probes were placed in predetermined equidistant positions (0.74 m) and were not reinforced (i.e., they were left empty). They were presented in a pseudo-randomized order and interspersed among training trials. All "during gestation" testing sessions were preceded by five "reminder" training trials the day before testing. Each pig was tested three times: before gestation (1–2 weeks before mating); early gestation (4 weeks after mating); and late gestation (10–11 weeks after mating). One pig in replicate two was not tested before gestation and was only tested in the early and late test phases.

Statistical Analysis

All data were analyzed in R version 3.4.1 using general linear mixed effects models with the *lmer* function in the package *lme4* (34). The response variable was natural logged to ensure that the residuals conformed to the assumptions of normality. To test the effects of gestation time on judgment bias, the response variable was *log time taken to approach* the presented probes; fixed explanatory effects were *probe location*, coded as a continuous variable from positive (1) to negative (5) with ambiguous locations at points 2, 3, and 4; and *gestation time* coded as a factor with three levels (pre, early and late gestation). *Probe location squared* was included as initial data exploration suggested curvature in the fits. Interactions between *gestation time* and *probe location* and *probe location squared* were also included.

TABLE 1 | Statistical model details.

Model	Random slope	Random intercept
1	1	Gestation time: Pig ID
2	1	Replicate/Gestation time: Pig ID
3	Location	Gestation time: Pig ID
4	Location	Replicate/Gestation time: Pig ID
5	Location^2	Gestation time: Pig ID
6	Location^2	Replicate/Gestation time: Pig ID
7	Location + location^2	Gestation time: Pig ID
8	Location + location^2	Replicate/Gestation time: Pig ID

Random models with fixed slopes (models 1 and 2) or slopes allowed to vary across probe location (models 3–8), with experimental replicate included (models 2, 4, 6, and 8) or not (models 1, 3, 5, and 7).

To find the most appropriate structure for the random model, we compared eight models: two intercept only models and six combinations of random intercept and slope models such that random intercepts were fitted for each pig at each experimental timepoint (or for each pig independent of experimental replicate), with variation allowed between gestation times and the shape of the curve was allowed to vary between pigs (**Table 1**).

The Akaike Information Criteria (AIC) values for all models were compared using the *model.sel* function in the *MuMIn* package (35). In each case the residuals of the final minimal model were visually assessed for deviations from normality. For the final models, predicted fits were produced using the *predict* function in base *R*. R^2 values for each model were calculated using the *r.squaredGLMM* function in the *MuMIn* package (35). For every model, the general pattern of results was robust, with the different random models only affecting the predictions very slightly. The best model is reported in the main text, and the corresponding figure for the other model where AIC comparison had delta <2 is reported as supplementary information.

RESULTS

Judgment Bias

The pigs' responses to ambiguous locations in the judgement bias test changed throughout gestation (**Tables 2**, **3**; **Figure 2**). Pigs consistently approached the positive probe quickly and the negative probe slowly (or not at all), getting generally slower during gestation (**Figure 2**). However, whilst the mean speed of approach was fairly linear between positive and negative pre- and early gestation (**Figures 2A,B**), by late gestation, pigs showed a shift toward pessimism, such that the positive probe continued to be approached quickly but ambiguous probes were approached more slowly (**Figure 2C**).

All models retained all interactions and gave qualitatively similar results. The best model was model 1, where the intercept was allowed to vary for each pig at each gestation time (**Table 1**). However, the result for model 2, where the intercept was

TABLE 2 | Table of candidate LMERs.

Model	*df*	AIC$_c$	Δ AIC$_c$	w	r^2 (*F* only)	r^2 (*F* + *R*)
1	12	215.2	0.00	0.580	0.751	0.805
2	11	216.9	1.72	0.245	0.748	0.806
3	16	219.7	4.50	0.061	0.751	0.805
5	13	219.8	4.57	0.059	0.751	0.805
7	13	221.2	6.01	0.029	0.750	0.818
4	22	222.2	7.02	0.017	0.738	0.810
6	16	224.1	8.89	0.007	0.740	0.817
8	16	227.5	12.35	0.001	0.729	0.833

*Table of candidate LMERs explaining time to approach the probe in relation to the interaction between the location of the presented probe and the gestation time for pigs that reached the 70% learning criterion only (n = 13). Each model retained all fixed terms (Location*Gestation time+Location^{2*}Gestation time) with only the random model varying. Model corresponds to the random model listed in **Table 1**, AIC$_c$, corrected Akaike Information Criteria values; Δ AIC$_c$, difference in AIC$_c$ values between the best model (lowest AIC$_c$) and the given model; w, Akaike weights; r^2 (F only), r^2 for the fixed model only, r^2 (F + R) r^2 for the fixed plus random model.*

TABLE 3 | Results of the best supported statistical models.

	Model 1			Model 2		
Term	**DF**	***F***	***P***	**DF**	***F***	***P***
Location	1, 141	62.96	<0.001	1, 141	62.96	<0.001
Gestation time	2, 168	2.03	0.134	2, 167	2.04	0.133
Location^2	1, 141	9.57	0.002	1, 141	9.57	0.002
Location: gestation time	2, 141	6.07	0.003	2, 141	6.07	0.003
Location^2: gestation time	2, 141	6.16	0.003	2, 141	6.16	0.003

Minimum adequate linear mixed effects model for the effects of probe location and gestation time on the time taken for pigs to approach the probe under testing, for pigs that reached the 70% learning criteria only (n = 13). The results equate to the best supported random models.

allowed to vary for each pig at each gestation time, within each replicate, was equally well supported (delta AIC <2; **Table 2**, **Supplementary Figure 1**).

DISCUSSION

In livestock species, judgment bias tasks are typically used to assess the impact of external factors, for example environmental enrichment (36) or stocking density (37). However, internal factors, such as the large physiological changes associated with gestation, also have the potential to influence affective state and therefore judgment bias. The aim of this study was to assess judgment bias in domestic pigs throughout gestation. It was hypothesized that the gilts would be more pessimistic during gestation than prior to mating, as indicated by an increase in latency to approach the ambiguous cues. Our results suggest this to be the case, with the gilts taking longer to approach the ambiguous locations in the later stage of gestation than before mating which indicates that judgment bias changed as gestation progressed. This was most apparent at the middle and most ambiguous location (**Figure 2**) and suggests the pigs were more pessimistic during the late gestational stage. Crucially, the latency to reach the positive location did not vary markedly throughout gestation, highlighting that changes, such as impaired locomotion or an increase weight, did not affect the gilts' response latencies to the other four locations (**Figure 2**). This also shows that the gilts were highly motivated by the reward, even though they were not feed-restricted. Thus, these results suggest increased pessimism during the late stage of gestation, despite the fact that the immediate external environment remained constant. This may infer that, alongside external factors, internally-driven factors can also influence judgment bias and affective state in domestic pigs. Although this result should be interpreted in light of the pigs being their own control and no separate control group being tested.

In this study, a spatial go/no-go judgment bias test was used as this type of task has been successfully used with pigs previously (14, 31, 32, 36). Previous judgment bias studies with livestock species have shown that a change of bias can occur in response to a change in external factors, such as enrichment (36) or handling (38). Recent studies by Horback and Parsons (31, 32) also used a spatial go/no-go task and found that group housed gestating sows displayed both positive and negative biases despite having the same external conditions. Interestingly, the sows' behavioral traits influenced judgment bias however, these studies were not specifically focusing on the effect of gestation on judgment bias, and therefore it is unclear if the stage of gestation may also play a role in these bias differences. The possibility that pigs' judgment bias may change from a positive to a more negative state during the late stage of gestation suggests that the pigs' welfare needs may change too. This highlights the importance of considering the impact of large physiological changes, such as gestation, on animal welfare. This study may have implications not only for the welfare of farmed animals that experience gestation, but also for research into affective state during gestation in other captive multiparous mammalian species, including how this may impact cumulatively across the life course on their health and welfare. For example, in humans, multiparous women appear to be more at risk and have a different pattern of anxious or depressive symptoms compared to primiparous women (39, 40). In humans, hormone fluctuations and other physiological changes throughout pregnancy are often correlated with changes in mood and affective state (4, 5). Pigs are frequently used as models for humans in medical and pharmaceutical studies (22, 23, 41, 42), so it is possible that a change in affective state during gestation may be caused by comparative mechanisms, however, further research is required to validate this.

Alongside this interesting result, there are some limitations to take into consideration. Previous studies have shown that multiple testing time points can result in an increase in pessimistic responses (43, 44) and this increase in latencies during the later testing phases is similar to what was found in this study. As it was not possible to test a non-pregnant control group, this effect of learning cannot be ruled out. However, the effects of gestation represent a plausible driver for the changes in affect we report as previous research in rodents and humans has shown that mood and affective state can vary throughout gestation (1–3), with negative mood more likely to be present during the first and third trimester in humans

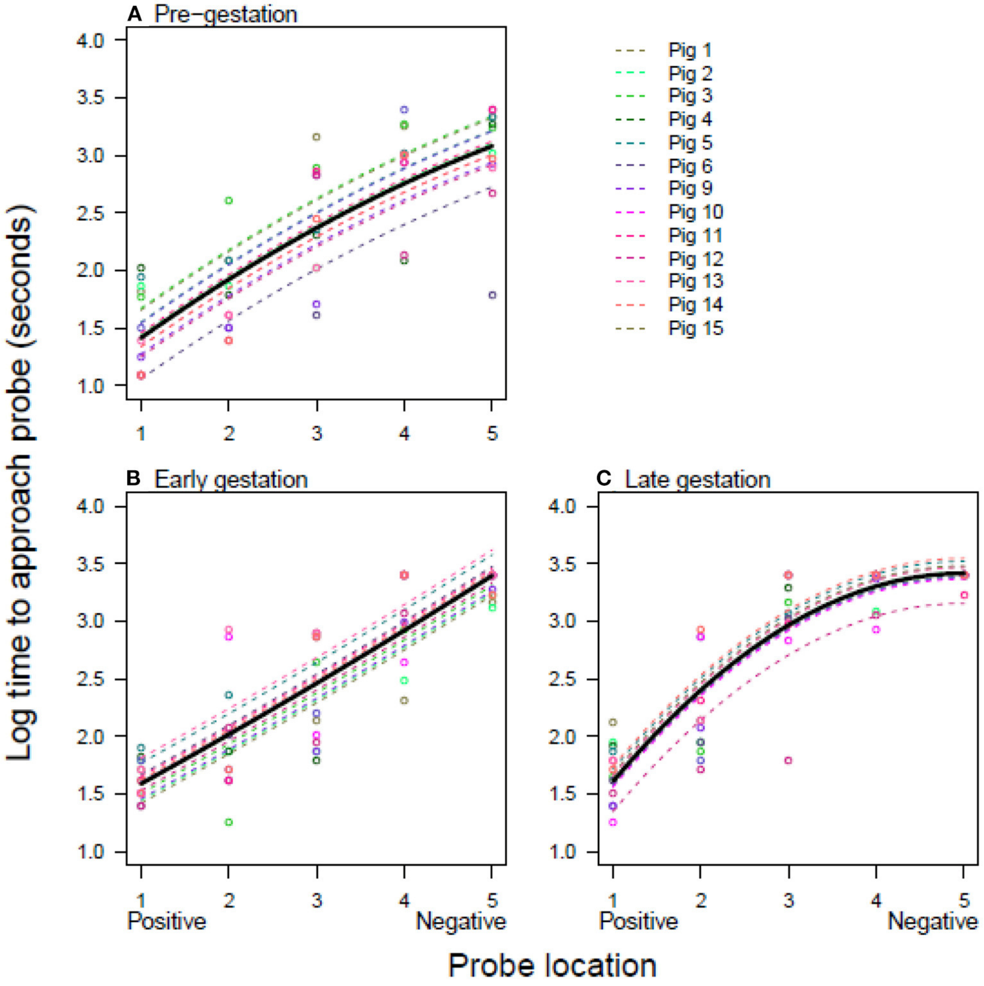

FIGURE 2 | The time to approach each location at three stages of gestation. Log time taken to approach each location for pigs at three different stages of the pig's 16-week gestational period; **(A)** pre-gestation, **(B)** early gestation (5 weeks), and **(C)** late gestation (10–11 weeks). The open circles are raw data points and the lines are model predictions from the minimal adequate model fixed to the level of experimental replicate 1. Results from model 1 are shown, where the intercept is allowed to vary for each pig at each gestation time. Pigs 1–5 are from replicate 1 and pigs 6–15 are from replicate 2.

(45, 46). Future studies should consider the role of learning by including a non-gestating control group, and whether ambiguous trial locations should be rewarded or un-rewarded (47). There were also some differences between replicates, such as one replicate receiving Regumate®, and different rewards being used. Despite this, the effect of replicate on the data was marginal, showing that the change in judgment bias over the course of gestation was robust and not influenced by these differences between replicates.

In conclusion, this study suggests that judgment bias in farmed domestic pigs may change with stage of gestation, inferring that internally driven stimuli may directly affect judgment bias without external influence. This study raises novel welfare considerations for captive primiparous, and possibly multiparous, species and provides a basis for future research into the effect of gestation on judgment bias in non-human animals.

DATA AVAILABILITY STATEMENT

The data and R code is available from the Open Science Framework database: https://osf.io/32wgy/.

ETHICS STATEMENT

The University of Lincoln, College of Science Ethics Committee approved this study (COSREC189; COSREC262). The animal owners provided written informed consent for participation of their animals in this study.

AUTHOR CONTRIBUTIONS

EB carried out data collection. LC conceived of the study. SC carried out statistical analysis. All authors assisted with study design and coordination, drafting the final manuscript, and gave final approval for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.881101/full#supplementary-material

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BRIEF RESEARCH REPORT
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Training of Motion Control May Not Improve Tool-Manipulation Ability in Rats (*Rattus norvegicus*)

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In recent times, previous studies have reported the manipulation of tools by rats and degus in controlled experimental settings. However, a previous study reported that only one out of eight experimentally naïve rats could manipulate a rake-shaped tool according to the position of a food reward without prior experience of obtaining the reward with the tool before the test. The present study aimed to improve the training of rats and investigate rodents' ability to manipulate tools according to food position. Stricter criteria were employed when training the rats to promote the rats' monitoring of their own tool manipulation. Additional training was introduced to give them the opportunity to learn that the reward moved closer to them by pulling an object connected to the reward. The present study showed that only one of eight rats could manipulate a tool according to the position of the reward without prior experience of obtaining the reward with the tool or perceiving that part of the tool came in contact with the reward, as the previous study showed. The change in training did not enhance the rats' tool-manipulation ability according to the food position. These procedures should be conducted in a wider variety of animals to investigate whether the training in motion control can promote the subjects' effective tool-use behavior.

Keywords: rats, rodents, tool-use behavior, tool-manipulation, motion control

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INTRODUCTION

Tool-use behavior in non-human animals has been investigated mainly in primates and birds (Bentley-Condit and Smith, 2010). The reason is that these animals have relatively high visual acuity, so it is easier to test their tool-use behavior than it would be in low-visual-acuity animals (Shumway, 2008). St Amant and Horton (2008, p. 1203) proposed the definition of tool-use: "Tool-use is the exertion of control over a freely manipulable external object (the tool) with the goal of (1) altering the physical properties of another object, substance, surface, or medium (the target, which may be the tool user or another organism) via a dynamic mechanical interaction, or (2) mediating the flow of information between the tool user and the environment or other organisms in the environment." In non-human primates, previous studies have reported that monkeys could be trained to manipulate a rake-shaped tool to obtain food beyond their reach in situations in which they could not obtain the food by pulling the tool perpendicularly to themselves via a step-by-step protocol [e.g., Japanese macaques (*Macaca fuscata*): Yamazaki et al., 2009; common marmosets (*Callithrix jacchus*): Yamazaki et al., 2011]. The distance between the rake and reward was gradually extended in the training used in these previous studies. In birds, a Goffin's cockatoo (*Cacatua goffiniana*) spontaneously manufactured a stick tool from a European larch and used it to rake food beyond its reach, but the other two cockatoos did not use tools to obtain the food (Auersperg et al., 2012).

Many researchers have attempted to shed light on the evolutionary processes of physical causal understanding by conducting tool-use tasks with a variety of animal species (Bentley-Condit and Smith, 2010). Primates, including humans, emerged through divergence of evolutionary processes from a common ancestor of mammals to rodents (Krubitzer, 2009). Thus, investigations of tool-use behavior in rodents, which have shared evolutionary processes with humans, are essential to shed light on the evolutionary processes of physical causal understanding in humans.

Recent studies have investigated tool-use behavior in relatively low-visual-acuity rodents (Prusky et al., 2002), including rats (*Rattus norvegicus*) (Nagano and Aoyama, 2017a,b; Nagano, 2019a,b, 2021) and degus (*Octodon degus*) (Okanoya et al., 2008; Kumazawa-Manita et al., 2013) in controlled experimental settings. Previous studies on rats have reported that these rodents manipulated a rake-shaped tool based on the position of the food reward placed beyond their reach after undergoing tool-use training (Nagano and Aoyama, 2017b; Nagano, 2019b). In this test, the rats could use only the position of the reward in relation to the rake as a cue to manipulate the rake effectively, and could not obtain the reward just by pulling the rake perpendicularly to themselves because the rake was placed at the center of the experimental apparatus, and the reward was randomly placed on either the left or right side of the rake. However, the rats could manipulate the rake in the direction of the reward by using a strategy similar to that learned during training because they had the experience of obtaining the reward by using tools (Nagano and Aoyama, 2017b; Nagano, 2019b). In contrast, Nagano (2021), who used the same test implemented in the two previous studies (Nagano and Aoyama, 2017b; Nagano, 2019b), reported that a rat manipulated the rake according to the reward position without prior experience of obtaining the reward with the tool or perceiving that a part of the tool came in contact with the reward that might cause it to move. However, only one out of the eight rats could manipulate the rake according to the position of the reward (Nagano, 2021). Thus, it could not be concluded that rats have a primitive ability to manipulate tools according to food position. In the main training (rake-manipulation training) by Nagano (2021), the rats were trained to move the rake laterally over successively greater distances without any food reward placed on the experimental apparatus. This training differed from the previous studies (Nagano and Aoyama, 2017b; Nagano, 2019b), particularly in that it promoted the manipulation of the rake in the lateral direction. Additionally, the rats were trained to obtain a reward on the apparatus directly with their paws or mouth (food-obtaining training).

The test mentioned above, where the rake was placed at the center and the food reward placed on either the left or right side of the rake, followed the food-obtaining training. Nagano (2021) suggested improving the methods for rake-manipulation and food-obtaining training as follows: the rake-manipulation training should employ stricter criteria to promote the rats' monitoring of their own manipulation of the rake, and the rats should be offered the experience of pulling something (i.e., thread sewn into the food reward in the present study) to obtain the reward. This incorporates the suggestions of Connolly and Dalgleish (1989) and Ramsey et al. (2021) that visual monitoring is necessary for tool-manipulation. Moreover, Nagano (2021) did not confirm whether their low visual acuity was the cause of their low performance in the test. In the study, the rats could obtain the reward just by moving the rake laterally over a certain distance in the training (Nagano, 2021). Therefore, it is possible that the rats could not manipulate the rake in the direction of the reward due to the awkwardness of their paws movements in the test of this previous study, not due to their low visual acuity (Nagano, 2021). Therefore, further confirmation is required to discount the possibility that most of the rats could not manipulate the rake based on the position of the reward due to their low visual acuity.

The present study aimed to improve the training techniques applied in Nagano (2021) as well as to investigate the effect of this enhanced training technique on the ability of rodents with low-visual acuity to manipulate tools according to food position without prior experience of obtaining rewards using the tool. In the rake-manipulation training in the present study, stricter criteria for success were employed to promote the rats' monitoring than those in Nagano (2021). Focusing on both the motion of their own paws and the criteria that exist in the external environment may promote them to pay attention to the relationship between their own motion and the external objects (the tool and food rewards) in the tool-use situations. In addition, another kind of training was introduced instead of the food-obtaining training, in which the rats were trained to pull a thread fastened to a food reward or a thread fastened to no reward. One of the purposes of this training was to promote the rats to pay attention to the position of the reward in the positional discrimination test. The rats never perceived that one object collided with another object and sent it into motion in the food-obtaining training. I hypothesized as follows: if trainings of motion control improve the tool-manipulation ability in rats, better performances by the rats would be observed in this test than in those of Nagano (2021).

A raking tool was used for the behavioral task for rats in the present study because the previous studies have reported that degus and rats could use rake-shaped tools (Kumazawa-Manita et al., 2013; Nagano and Aoyama, 2017a,b; Nagano, 2019a,b, 2021), and the animals in the present study were expected to learn to use the raking tool in a shorter period because it is easier for them to handle the tool with their forelimbs. Tool-use behavior in rats in the wild has never been reported (Bentley-Condit and Smith, 2010), and spontaneous tool-use behavior has never been observed in the experimental settings (Nagano and Aoyama, 2017a,b; Nagano, 2019a,b, 2021). However, rats are skillful in using their forelimbs dexterously to pull strings to obtain a food (Blackwell et al., 2018). The movement consists of alternating forelimb movements in which a limb is advanced to grasp a string and withdraw it toward the body in order to retrieve a food reward. The movements of aim, advance, grasp, pull and push are associated with hand shape changes including collect, overgrasp, grasp and release (Blackwell et al., 2018). Irvine et al. (2010) have also reported that rats handle objects with their forelimbs spontaneously and dexterously.

METHODS

Animals

Eight experimentally naïve three-month-old male Brown-Norway rats (subject numbers: BN57–BN64; Shimizu, Kyoto, Japan) were individually housed in wire cages. On the last day of free feeding, the rats weighed an average of 273.38 g (*SD* = 6.93). During training and testing, rats were maintained at around 85% of their free feeding weight. However, all rats could gain approximately 10 g/month. The animal room was maintained under a 12-h light/dark cycle (light phase: 8:00–20:00). All training and testing sessions were conducted during the light phase. All procedures and treatments were approved by the Doshisha University Animal Experiment Committee (protocol number: A17051), and were conducted in accordance with guidelines established by the Doshisha University Ethics Review Committee.

Apparatus

Experiments were performed in an experimental box identical to the one used in the previous study on rats (Nagano, 2021) (see **Supplementary Material** for details). The sliding doors were mounted on the front of the box. One of two kinds of sliding doors (one without a hole and one with a square hole) was always placed in front of the box. The door had a square hole in its upper portion and was used to offer rewards to the rats by hand. An experimental board, on which the tool and reward were presented, was set in front of the door. Black lines were drawn in a square lattice on the board.

The tool was identical to the one used in the previous study (Nagano, 2021) (**Figure 1A**, see **Supplementary Material** for details). The rake-shaped tool had a rectangular blade and a wire handle. In addition, two kinds of threads (one with a reward and one without a reward) were used in the training immediately before the test (**Figure 1B**, see **Supplementary Material** for details). Three-hundred and four threads were used for each kind of thread (608 threads in total). For threads with a reward, one end of each strand was sewn to a piece of cereal, and the other end was tied to a gem clip. For threads without a reward, one end of each strand was tied into a knot without a reward, and the other end was tied to a clip.

Procedure

Training

The training consisted of rake-pulling training, rake-manipulation training, and thread-pulling training (**Figure 2**, see **Supplementary Material** for details). Each daily experimental session consisted of 40 trials, with the exception of the thread-pulling training, for which a session consisted of 38 trials. A piece (one-eighth to one-sixth) of chocolate-flavored loop cereal was used as a food reward in each trial.

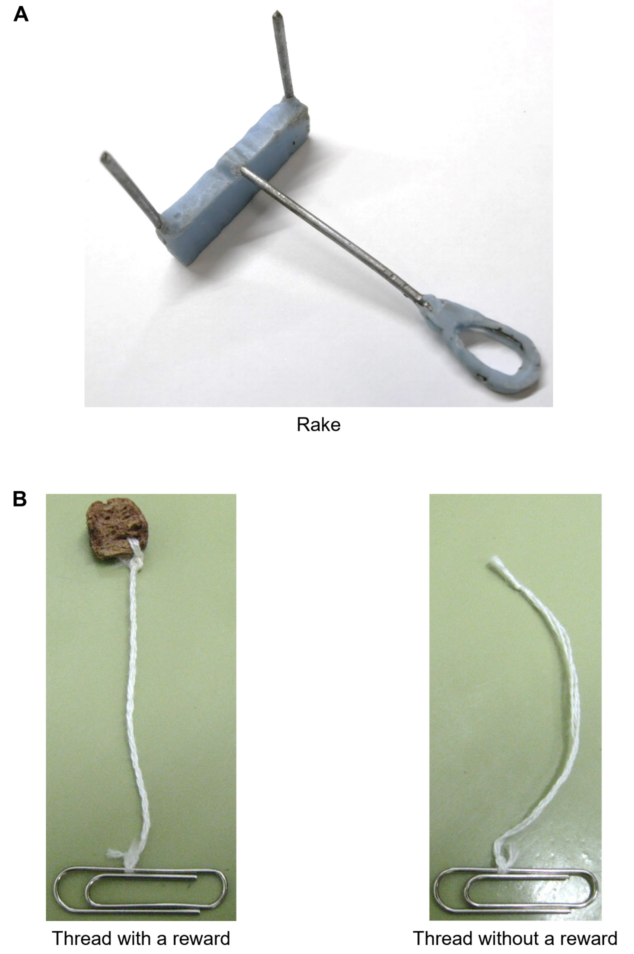

FIGURE 1 | Rake and threads. **(A)** Rake used in the rake-pulling training, rake-manipulation training, and positional discrimination test. **(B)** Examples of threads with a reward and without a reward used in the thread-pulling training. One end was sewn to the reward for threads with a reward or tied into a knot for threads without a reward. The other end of both types of threads was tied to a gem clip.

Rake-Pulling Training

The rake-pulling training procedure in the present study was similar to that in Nagano (2021). The experimenter presented the rake-shaped tool on the experimental board without any food reward, and the rats first learned to pull the rake to the end of the experimental board (i.e., toward the experimental box). The rake was alternately placed on either side.

This training was divided into eight phases (**Figures 2**, **3**, and see **Supplementary Material** for details), and the criterion for reward obtainment gradually became stricter. At the beginning of Phase 1, the experimenter placed the rake so that the distance from the door of the experimental box was 0 cm, and offered a

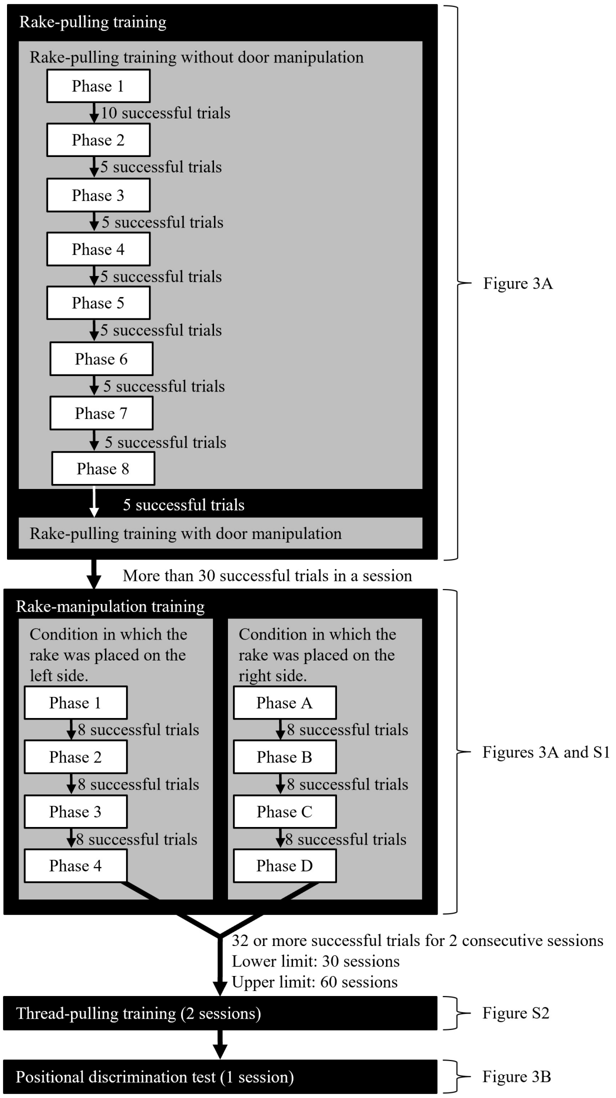

FIGURE 2 | Flowchart of the rake-pulling, rake-manipulation, and thread-pulling trainings and the positional discrimination test. Each daily experimental session consisted of 40 trials, except the thread-pulling training, which had 38 trials.

reward when the rat touched the rake with either its paw, nose, or mouth (successful trial). The experimenter offered a reward by hand through the small hole in the sliding door in successful trials (**Supplementary Video 1**). In Phases 2 to 8, the distance between the blade of the rake and the door was increased by 1.0 cm in each phase (**Figure 3A**).

Rake-Manipulation Training

In the rake-manipulation training, the rats were trained to move the rake laterally within fixed ranges (**Figure 2** and **Supplementary Figure 1**). In Nagano (2021), a trial was considered a success when the rat pulled the rake so that either of the wires glued to the rake blade touched the door while the wire of the rake blade passed over a criterion line in each phase. In the present study, the criterion for a successful trial was made stricter by narrowing the range that the rake blade had to be brought to. Apart from this alternation, the used procedure followed that in Nagano (2021).

In this training, the same procedure was used as in the rake-pulling training, except that the criterion for a successful trial became stricter. The criterion ranges were set to 5.0-mm intervals to the right of the rake when the rake was placed on the left side of the experimental board from the rat's perspective (**Supplementary Figure 1A**). The criterion ranges in each phase became narrower toward the center of the board every time the rat attained each criterion (**Supplementary Figure 1**). The four criterion ranges corresponded to the four phases: 25.0 mm for Phase 1 and 10.0 mm for Phase 4. This arrangement was mirrored when the rake was placed on the right side of the experimental board, where criterion ranges for Phases A to D corresponded to those for Phases 1 to 4, respectively (**Supplementary Figure 1B**). In each phase, once the rat pulled the rake so that either of the wires glued to the rake blade touched the door while the wire of the rake blade that was close to the center of the board was positioned within the criterion range, the experimenter retrieved the rake and offered the rat a reward through the hole in the door (successful trial, **Supplementary Video 2**); when the wire of the rake blade closer to the center of the board did not fall within the criterion range, the experimenter retrieved the rake without offering a reward (failed trial, **Supplementary Video 3**). Thus, the rake and reward were never presented simultaneously. Through this procedure, the rats were trained to laterally manipulate within gradually narrower ranges. The rake-manipulation training continued until the rat achieved the criterion of the last phase under both arrangement conditions (Phases 4 and D) and succeeded in 32 or more trials for two consecutive sessions.

Thread-Pulling Training

In the thread-pulling training, the rats were trained to pull the thread with or without a reward with their paws or mouth (**Figure 2**, **Supplementary Video 4**). The first purpose of the training was to offer the rats opportunities to learn that the reward perceived through the sliding door was identical to the reward obtained by the rat in the experimental box, and that the reward was moved toward the rats by pulling an object (the gem clip tied by the embroidery thread). The second purpose of the training was to confirm that they could identify the position of the reward placed at a distance similar to that in the subsequent test. The thread was never removed from the reward on the experimental board, and therefore the rats were not meant to realize that any hard object (part of the tool) was in contact with the reward at all. The sliding door without holes was used. The daily experimental sessions consisted of 38 trials.

Positional Discrimination Test

After the thread-pulling training, the positional discrimination test was performed for one session according to a procedure similar to that used in previous studies on rats (Nagano and Aoyama, 2017b; Nagano, 2019b, 2021) (**Figure 2**). In this test, the experimenter examined whether the rats could manipulate the

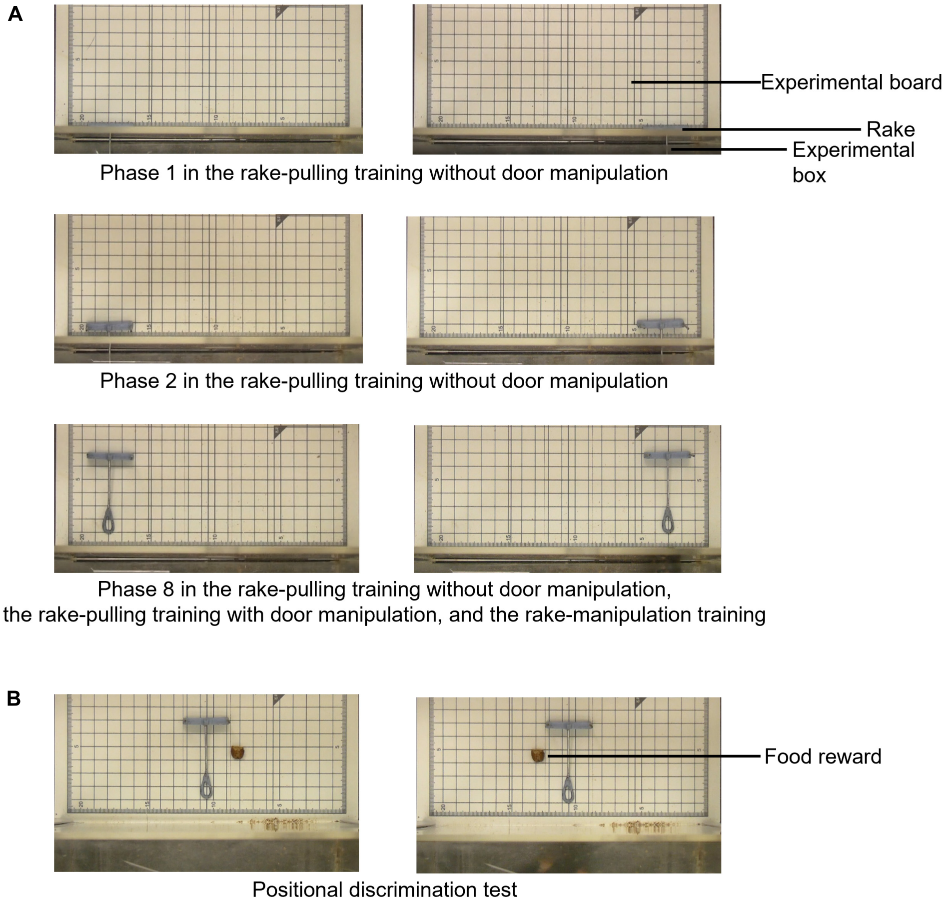

FIGURE 3 | Arrangement of the rake and reward during the rake-pulling training with or without door manipulation, thread-pulling training, and positional discrimination test. **(A)** Arrangement in Phases 1, 2, and 8 of the rake-pulling training without door manipulation, rake-pulling training with door manipulation, and rake-manipulation training. Only the rake was placed on the left or right side of the experimental board, and the reward was not placed on the board during the training. The rake's position in Phase 8 of the rake-pulling training without door manipulation and rake-pulling training with door manipulation was the same as that during the rake-manipulation training. **(B)** Arrangement during the positional discrimination test. The rake was placed at the center of the experimental board, and the reward was placed on the left or the right side of the rake.

rake laterally in relation to the position of food, even when the tool and food were presented simultaneously for the first time. The rake was placed at the center of the experimental board, and the reward was placed on either the left or right side of the rake (**Figure 3B**). The rats could obtain the reward when they pulled the rake in relation to the position of the reward until they pulled the rake to the position of the reward in the vertical direction (**Supplementary Video 5**). The rats were required to move the rake laterally before pulling it to obtain the reward.

Data Analyses and Statistical Methods

Rat behavior was analyzed using video records from the training and the positional discrimination test. The statistical analyses were performed using SPSS Statistics version 25.0. The criterion for statistical significance was set at $p < 0.05$.

The manipulation direction of the rake was analyzed using the method used in previous studies on rats (Nagano and Aoyama, 2017b; Nagano, 2019b, 2021). In this test, when the rat manipulated the rake toward the reward, it was recorded as a correct-direction trial. In contrast, when the rat manipulated the rake away from the reward, it was recorded as an incorrect-direction trial. These determinations were based on whether the intersection point of the blade and the handle was on the left or right side of the center line of the experimental board when the rat pulled the rake 2.0 cm (i.e., to the horizontal line contacting the reward). The correct-direction rate is a behavioral index that would enable the detection of trials in which the subject understood the appropriate direction to move the tool to obtain the reward, but does not successfully manipulate the rake because of insufficient motor ability (Nagano and Aoyama, 2017b). Trials in which the rat did not pull the rake, stopped pulling it before pulling it 2.0 cm toward itself, or flipped the rake out of reach before pulling it 2.0 cm toward itself were excluded from this analysis (BN57: seven trials; BN58: 18 trials; BN59: 19 trials; BN60: one trial; BN61: one trial; BN62: zero trial; BN63: one trial; BN64: zero trial).

In addition, the position of each rat's nose was analyzed when it first touched the rake with the left or right paw in each trial based on the video recordings from the positional discrimination test. This analysis was conducted to investigate whether the position of the rat in relation to the rake and the reward influenced their correct-direction rate by using the method used in previous studies on rats (Nagano and Aoyama, 2017b; Nagano, 2019b, 2021). For this analysis, the first column on the experimental board was divided into 21 areas (Areas 1–21) based on the squares on the board (**Supplementary Figures 3**, **4**), and the position of the rat's nose was recorded after the trial began. The position of each rat's nose was determined from the video frame at the moment the rat first touched the rake.

To analyze the relationships between the position of rats' noses and the correct-direction rates in the positional discrimination test, the first column (Areas 1–21) of the experimental board was divided into the area to the left (Areas 1–10) and right (Areas 12–21) of the rake handle, with Area 11 at the center (**Supplementary Figures 3**, **4**). The number of trials in which the rat's nose was in the left or right side area at the time of the first touch on the rake was calculated for each trial. Moreover, when the reward was placed left of the rat's view (20 out of 40 trials), if the rat's nose was in the left side area, then it was recorded as an ipsilateral trial. Under the same conditions, if the rat's nose was in the right side area, then it was recorded as a contralateral trial. Similarly, when the reward was placed right of the rat's view (20 out of 40 trials), if its nose was in the right side area, then it was recorded as an ipsilateral trial; but if the rat's nose was in the left side area, then it was recorded as a contralateral trial. Trials in which rats' noses were located in the center (Area 11) of the board when they first touched the rake were not considered ipsilateral or contralateral.

For the rake-manipulation training, the daily success rates were calculated by dividing the number of trials in which each rat moved the rake laterally within the criterion range within 60 s (number of successful trials) by the total number of trials (40 trials per day).

In the thread-pulling training, the average thread-pulling rates per session were calculated for the threads with and without a reward separately. The thread-pulling rates were calculated by dividing the number of trials in which each rat pulled the thread (with or without a reward) to the position in which the reward or knot entered in the box (number of thread-pulling trials) by the total number of trials in each thread condition (19 trials per session). A two-way analysis of variance (ANOVA) was performed with thread type and session as within-subject factors, followed by simple main effect analyses. In addition, the average thread-contacting rates per session were calculated for the threads with a reward and threads without a reward separately. The thread-contacting rates were calculated by dividing the number of trials in which the rat touched the rake with its paws or mouth within 60 s (number of thread-contacting trials) by the total number of trials in thread condition (19 trials per session). A two-way ANOVA was performed with thread type and session as within-subject factors followed by simple main effect analyses.

The success rate was also calculated for each rat in the positional discrimination test by determining whether the rats manipulated the rake toward the reward (in the correct-direction) or not (in the incorrect-direction), and comparing the number of trials in which the rake was moved in either direction (40 trials). The correct-direction rate was calculated by dividing the number of trials in which each rat manipulated the rake toward the reward (number of correct-direction trials) by those in which the rat manipulated the rake in either direction. The correct-direction trials included trials in which the rat manipulated the rake in the correct-direction but failed to obtain the reward. Using binomial tests, the number of correct- and incorrect-direction trials was compared for each rat. In the binomial tests, the null hypothesis was that the correct-direction rate was 50%. In addition, data analysis showed whether each rat manipulated the rake in the correct-direction from the beginning of the session in the test. The daily sessions (40 trials) were divided into eight blocks to calculate the average correct-direction rate of rake manipulation, with each block consisting of five trials. Trials 31–35 in one rat (BN58) and Trials 26–40 in another (BN59) were excluded from this analysis because the individuals never pulled the rake in these trials.

Ipsilateral trials were calculated for each rat to analyze the relationship between the position of the rats' noses and the correct-direction rates in the positional discrimination test. The number of ipsilateral and contralateral trials was compared for each rat using two-tailed binomial tests.

RESULTS

Results from the rake-pulling, rake-manipulation, and thread-pulling training are described in **Supplementary Material**.

Positional Discrimination Test

The success rates in the positional discrimination test were low in all the rats (5.0–40.0%, **Figure 4A**). One of the eight rats (BN64) manipulated the rake in the correct-direction significantly more frequently than in the incorrect-direction (BN57–BN63: *n. s.*, BN64: $p < 0.01$, binomial tests, **Figure 4B**). In addition, the performance of the one rat (BN64) that manipulated the rake in the correct-direction the most did not show a trend toward improvement in its correct-direction rate within a session (**Supplementary Figure 8**).

The position of each rat's nose the first time it touched the rake with the left or right paw in each trial was analyzed in the positional discrimination test (**Supplementary Figures 3**, **4**). The purpose of this analysis was to investigate the possibility that one rat (BN64) just moved to a position closer to the reward immediately before pulling the rake, and therefore initially had the rake close to itself; this may have resulted in a correct-direction rate above the chance level (50%) during the test. For instance, perhaps the rat used a strategy of moving closer to the reward (left side) based on the position of the rake handle to try to obtain the reward with its paws and manipulated the rake in the left direction (correct-direction). One of eight rats (BN64) positioned its nose on the side of the handle of the rake opposite to the reward rather than to the same side as the reward (BN57–BN63: *n. s.*; BN64: $p < 0.01$, binomial tests, **Supplementary Table 1**), and the rat manipulated the rake in the correct-direction significantly more frequently than in the incorrect-direction.

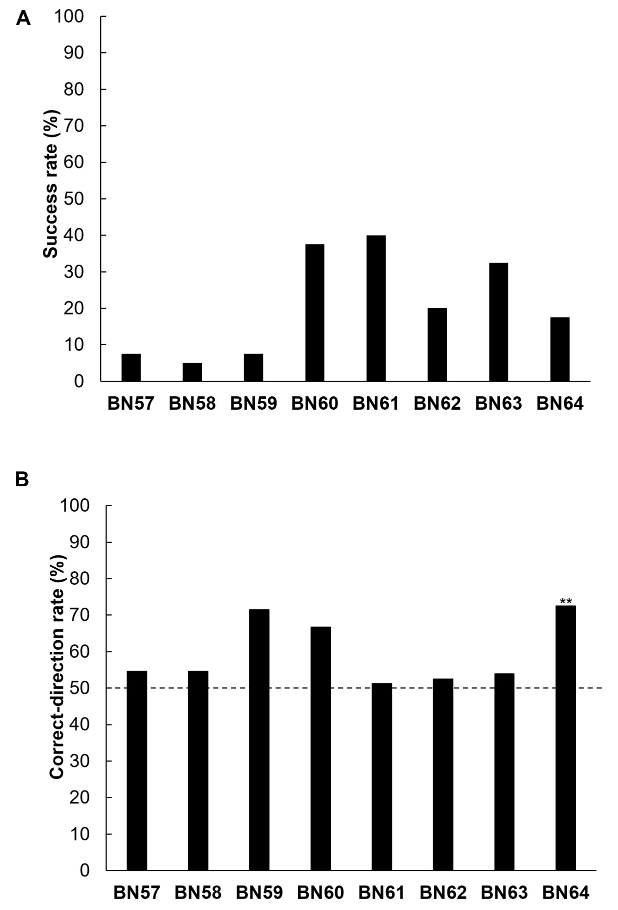

FIGURE 4 | Individual (BN57–BN64) performance in the positional discrimination test. **(A)** Individual success rates. **(B)** Individual correct-direction rates. The broken line indicates the level of chance (** $p < 0.01$).

DISCUSSION

The findings of the present study, in which the modified training procedure was applied, were found to be similar to that of Nagano (2021). The present study showed that one of the eight rats could manipulate a tool according to the position of the food reward without prior experience of obtaining the reward with the tool or perceiving that part of the tool came in contact with the reward. The hypothesis of this study, that the training of motion control improves the tool-manipulation ability in rats, was not supported. The correct-direction rate did not improve within a session after several trials of learning that manipulating the rake in the appropriate direction yielded a reward. In addition, the position of one rat's (BN64) nose when it first touched the rake was not ipsilateral to the side of the experimental board on which the reward was placed, confirming that the rat did not resort to the simple strategy of moving closer to the reward to try and grab it with its paws before attempting to use the rake to obtain the reward. The rat positioned its nose on the side of the handle of the rake opposite the reward, and a similar tendency was observed in previous rat studies (Nagano and Aoyama, 2017b; Nagano, 2021).

However, it is possible that the rat (BN64) could manipulate the rake in the direction of the reward by chance. Only this rat may have had a behavioral tendency to manipulate the rake in the direction of the reward for some reason (e.g., an innate behavioral tendency to manipulate objects toward foods). It would not be possible that the other seven rats could not manipulate the rake in the correct-direction because, due to their low visual acuity, they could not identify the position of the reward presented on the experimental board (Prusky et al., 2002). In the thread-pulling training, the rats pulled the threads with a reward significantly more often than the thread with no reward. To the best of our knowledge, only the present study and Nagano (2021) have indicated that some animals can manipulate tools according to the position of the target without prior experience of perceiving that one object collided with another object and sent it into motion in controlled experimental settings.

There were some limitations in the present study. It may be possible that the abilities of rodents to manipulate tools appropriately can be detected by conducting the experiments under environmental conditions that match to their biological needs. Rats are nocturnal animals (Norton et al., 1975), but the experiment in the presented study was conducted during the light phase due to the rules of the shared animal room with other researchers in the research institution. Testing the nocturnal rodents during the dark phase would be needed to detect their cognitive abilities more appropriately in future studies (Balcombe, 2010). Moreover, only male rats were used as the subjects in the present studies, as were the previous rat studies (Nagano and Aoyama, 2017a,b; Nagano, 2019a,b, 2021). To testify about the generality of the relationship between the motion control and tool-manipulation, testing with female rats would be needed in future studies. Alternatively, sex differences in tool-use may be observed in rats like capuchin monkeys (*Sapajus libidinosus*) (Falótico et al., 2021). In addition, the experimenter always manipulated the door with her left hand to avoid subconsciously giving the rats cues about the position of the food reward in the positional discrimination test. It may be possible that this procedure created the rats' side bias, and they manipulated the rake in the same direction in the most of trials.

The changes that the present study made to the experimental procedures used in Nagano (2021) did not enhance the detection of the rodents' ability to manipulate tools according to food position. It is possible that the tool-manipulation monitoring is not an important factor for tool manipulation in rodents. The procedures in the present study should be conducted in a wider variety of animals to investigate whether tool-manipulation monitoring promotes the subjects' effective tool-use behavior. Moreover, it can be examined whether the subjects can perceive the reward placed at the same position as the test by conducting the thread-pulling training immediately before the test. In addition, the appropriate distance between the subject and the reward can be determined by applying a procedure similar to the thread-pulling training technique adopted in the present study. Therefore, the procedure in the present study is valuable for investigating tool-use behavior in animals with relatively low visual acuity, such as rats, as well as in animals with relatively high visual acuity.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://osf.io/3x8tz/?view_only=f5c4b90614bd496e8ba6ddd3ed26207a.

ETHICS STATEMENT

The animal study was reviewed and approved by Doshisha University Animal Experiment Committee.

AUTHOR CONTRIBUTIONS

AN designed and performed the experiments, made the experimental apparatus, analyzed the data, wrote the manuscript, and prepared the figures.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2022.931957/full#supplementary-material

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Personality traits affect learning performance in dwarf goats (*Capra hircus*)

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A wide range of species exhibit time- and context-consistent interindividual variation in a number of specific behaviors related to an individual's personality. Several studies have shown that individual differences in personality-associated behavioral traits have an impact on cognitive abilities. The aim of this study was to investigate the relationship between personality traits and learning abilities in dwarf goats. The behavior of 95 goats during a repeated open field (OF) and novel object test (NO) was analyzed, and two main components were identified using principal component analysis: *boldness* and *activity*. In parallel, the goats learned a 4-choice visual initial discrimination task (ID) and three subsequent reversal learning (RL) tasks. The number of animals that reached the learning criterion and the number of trials needed (TTC) in each task were calculated. Our results show that goats with the lowest learning performance in ID needed more TTC in RL1 and reached the learning criterion less frequently in RL2 and RL3 compared to animals with better learning performance in ID. This suggests a close relationship between initial learning and flexibility in learning behavior. To study the link between personality and learning, we conducted two analyses, one using only data from the first OF- and NO-test (*momentary* personality traits), while the other included both tests integrating only animals that were stable for their specific trait (*stable* personality traits). No relationship between personality and learning was found using data from only the first OF- and NO-test. However, stability in the trait *boldness* was found to have an effect on learning. Unbold goats outperformed bold goats in RL1. This finding supports the general hypothesis that bold animals tend to develop routines and show less flexibility in the context of learning than unbold individuals. Understanding how individual personality traits can affect cognitive abilities will help us gain insight into mechanisms that can constrain cognitive processing and adaptive behavioral responses.

KEYWORDS

discrimination learning, personality, cognition, open field test, novel object test, serial reversal, goats

Introduction

A wide range of species exhibit time- and context-consistent interindividual variation in behaviors, such as activity, exploration, boldness, aggressiveness and sociability, which are all traits related to an individual's personality (1–5). More than 100 years ago, Pavlov was the first to suggest that canine personality could also be a marker for differential performance in associative learning (6). Regardless, potential relationships between animal personality and interindividual differences in cognitive abilities have been relatively understudied in nonhuman animals (7, 8); however, in the last decade, they have attracted new interest across a wide range of taxa, while the causes and strengths of this variation are still under discussion (9).

Proximate factors (attention, selectivity, persistence, and experience) represent behavioral mechanisms that have a major impact on individual learning and cognitive performance (10). Current frameworks that determine behavioral phenotypes that are stable across time and context, such as animal personality (1, 11), temperament (12), coping style theory (13) and behavioral flexibility (14), which are all interrelated (15) have been hypothesized to be functionally related to individual differences in learning and were used to explain consistent within-species interindividual variation in cognitive abilities such as learning and memory. In this way, personality may affect performance in cognitive tasks in that individual variation in attention and encounter rates of environmental stimuli act to either facilitate or inhibit learning (6). It is assumed that natural selection could have parallel effects on cognition and animal personality simultaneously, leading to a correlation between cognition and various personality traits, as both follow a continuum between fast and slow phenotypes (16). The link between variation in personality and cognition forms the basis of the cognitive style hypothesis and is centered on a speed-accuracy trade-off that assumes that individuals may apply different cognitive styles (fast or accurate) based on personality or coping style (7, 17). Proactive individuals, which tend to be bolder, more active, neophilic and fast explorers, learn at higher speeds but at the cost of accuracy. They are likely to become entrenched in a previously learned strategy and thus are less flexible when confronted with new challenges compared to reactive individuals, who are characterized as being rather shy, neophobic, and slow explorers (12, 13, 18). Furthermore, proactive animals are more rigid and fast in decision-making, while reactive animals are thought to be more attentive to environmental changes and stimuli. Attention to environmental cues and cognitive ability are positively related to behavioral flexibility and the ability of an individual to adjust to an ever-changing environment (6, 19). In contrast to much empirical work demonstrating a relationship between personality and learning abilities in different species (20–22), some recent authors cast doubt on a general parallelism between behavioral and cognitive dispositions, as some studies failed to show this link (8, 9, 23–25). For instance, while fast-exploring great tit males (*Parus mayor*) showed more flexible learning abilities than slow-exploring males, female slow explorers outperformed fast explorers, showing a sex dependency in the relationship between personality types and learning (26). Two different zebrafish (*Danio rerio*) strains classified as proactive and reactive were able to learn and recall the fearful association of an associative fear conditioning task. While both coping style strains showed no differences in memory, reactive zebrafish acquired fear memory at a significantly faster rate than proactive zebrafish (27). Another study on narrow-striped mongooses (*Mungotictis decemlineata*) tested the relationship between learning performance, social information and individual differences in boldness: learning performance of seven wild female groups (of two to six individuals each) was tested with an artificial feeding box using a demonstrator-observer paradigm. Bold individuals as well as individuals in groups with demonstrators were faster in learning the task, while individuals without a demonstrator learned the task more slowly, indicating an interaction between personality traits, use of social cues and learning performance (28). In male African striped mice (*Rhabdomys pumilio*), proactive individuals (measured by boldness, activity and exploration) performed better than reactive males in two learning tests: a string-pulling task to obtain food and a door-opening task to reach the nest (29). On the other hand, in guinea pigs and female guppies (*Poecilia reticulata*) exploration, sociability and boldness were not intercorrelated with learning performance (30, 31), again indicating that relationships between personality traits and learning are quite diverse in a number of different species.

In recent years, interest in individual differences in a variety of taxa has increased, including in livestock research (reviewed by 15). There is mounting evidence that these differences play an important role in various contexts in pigs (32–34), cows (35–37), horses (38–40), chickens (41, 42) and goats (43, 44). General knowledge about the cognitive capabilities of livestock (i.e., their ability to acquire, process, store and use information) is of great interest, as cognitive capacities have a major impact on how they are able to interact with their environment (45). Farm animals see themselves confronted with a multitude of challenges (i.e., automated feeding regimes, inflexible housing conditions, rigid management practices) during their lifetimes that have very specific and individual cognitive requirements. To date, numerous studies have focused on quantifying and understanding species-specific cognitive abilities and learning skills in farm animals. For instance, in a visual discrimination task, pigs were able to discriminate between two visual stimuli, and even 1/3 performed well in the reversal task (46). In an acoustic discrimination task, pigs were trained to visit the feeding site after discriminating an individual acoustic signal to obtain a food reward (47). In horses, observers were allowed to watch demonstrators opening a feeding apparatus: While young and lower ranking horses learned the task, older and higher ranking horses did not, showing an age and rank dependency in learning (48). Chickens were able to complete visual occlusion, have biological motion perception and were able to discriminate object and spatial representations, to name a few (reviewed by 42). Meyer and colleagues used an automated learning device that presents artificial symbols *via* a screen and found that goats have clear categorization capacities (49). They were also successful in object permanence tasks (50) and were seeking cognitive challenges by choosing to work for a reward even if they have the possibility of getting it for free, named "contrafreeloading" (51, 52). In playback experiments, it has been shown that goats were able to recall offspring vocalizations for at least 1 year (53, 54). Training a series of visual discrimination tasks showed that goats were able to improve their performance in a learning-to-learn process and develop a learning set (55). In summary, a wide range of cognitive tests has been applied in farm animals to assess a range of cognitive traits. However, differences in cognitive ability between and within individuals of the same species across repeated measurements or across different experimental conditions have also been noted but the causes of these differences remain largely unexplained (7, 8, 45). To date, there is only limited knowledge about a phenotypic link between individual personality and learning capacities in farm animals.

In the current study, we investigated the potential relationship between specific personality traits and learning performance in female dwarf goats. A 4-choice visual discrimination task and three subsequent reversal learning tasks were applied to characterize the learning abilities of the goats. Reversal learning paradigms are commonly used to test behavioral flexibility, a type of phenotypic plasticity that can influence how animals cope with environmental changes. To investigate the relationship between the consistency of certain personality traits and learning, first only data from the first personality test and then the combined data from both personality tests were used separately to test the respective effect on learning performance.

First of all, we expected a relationship between learning and reversal learning in that good learners in the visual discrimination task would need more trials to reach the learning criterion in the reversal learning task and vice versa. This is based on the findings of other studies showing that fast learners exhibited lower behavioral flexibility and, therefore, performed worse in a reversal learning paradigm compared to slow learners (56).

Second, we expected no interrelation between the personality traits deriving only from the first personality test and discrimination learning or reversal learning. On the other hand, taking consistent personality traits into account (data from both personality tests), we expected the more active and bold individuals to disproportionately achieve the learning criterion in the discrimination task faster while inactive and unbold individuals would perform better in the reversal learning tasks, in line with studies regarding behavioral flexibility in the context of coping style (7, 57).

Animals, materials and methods

Animals and housing

The experiments were conducted with 108 juvenile female Nigerian dwarf goats (*Capra aegagrus hircus*) bred from a line at the Leibniz Institute for Farm Animal Biology (FBN, Dummerstorf, Germany). Due to our breeding program, two experimental runs were conducted per year. From birth to weaning (Figure 1), goats were housed in mixed groups consisting of three to five adult females and their male and female kids. The pens (12 m^2) were littered with wheat straw as bedding material and contained a hayrack and a round feeder to deliver concentrate (800 g to 1,000 g twice per pen, Vollkraft, Mischfutterwerk GmbH, Güstrow, Germany). Hay and water were provided *ad libitum*. After weaning (seven weeks of age), female goat kids were kept in groups of up to 16 animals per group in pens as described above, with an additional climbing rack as an enrichment activity. All animals were ear tagged and wore a collar with a responder (Urban, Wuesting, Germany) for individual recognition at the free accessible automated learning device. The operating principle of the learning device has been described in detail elsewhere (49). In the following weeks, the kids were shaped to the learning device. During this time, we conducted repeated OF- and NO-tests (Figure 1). At 12 weeks of age, kids were kept in groups of up to eight animals per group in pens as described above. As the number of goat kids was different in every lambing season, runs one and two of the study were conducted with 32 kids each, run three with 14 kids and run four with 30 kids. From here on they will be referred to as goats.

Behavioral testing

To obtain the respective personality traits, we conducted repeated OF- and NO-tests. Repetitions were performed after 14 days as Takola et al. (58), who conducted a meta-analysis including 115 studies, found that repeatability of responses to novel objects was significant an greater in short-term studies than in long-term studies. In both tests, each goat stayed alone in an arena for 5 min. For both tests, we used an arena (3 × 4.8 m) with opaque walls (2 m high) in a different part of the barn, which was separated from the home pens by two doors. The arena was divided into 12 identical segments (1 × 1.2 m), which were indicated by white lines on the floor. A fully enclosed start box (1 × 1 × 1 m) was connected to the arena and had a

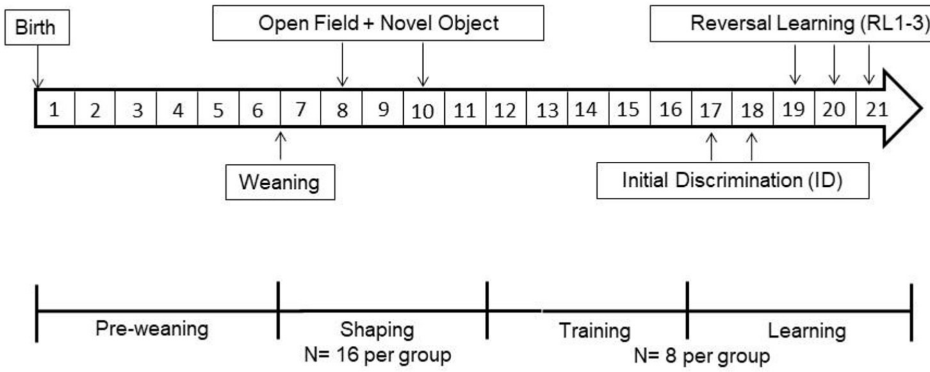

FIGURE 1
Timeline of the experimental procedure test sequence, housing condition and age of the dwarf goats (given in weeks).

guillotine door. For the first NO-test, a traffic cone, and for the second test, a medicine ball was used as the object. To ensure that the object could not be displaced by the focal animal, the object was fixed with a chain hanging from the ceiling. All tests were video recorded (Panasonic WV-CP500). For both tests and both test repetitions, we recorded 82 behavioral measures for each subject coded with Observer XT (Version 12, Noldus Information Technology, The Netherlands). The OF- and NO-tests were conducted on two consecutive days, the same for the repetitions. Further details concerning the testing procedure and the recorded behavioral measures were described by Finkemeier et al. (44).

Visual discrimination learning

Shaping

Over 6 weeks, we shaped the goats stepwise to the learning device. The learning device was integrated into the home pen with free access for all goats 24/7. We used drinking water as a reward (30 ml for each correct choice), which was only accessible at the learning device. For detailed information regarding the shaping procedure, see Langbein et al. (59). In short, we started with a float switch hanging in a water bowl with a button directly above the switch. By pressing the button, the goats could add 30 ml of water to the bowl. After 1 week, the float switch was removed, and we installed first one, later two buttons 20 cm above the bowl, one of which had to be pressed to fill water in the bowl. The reward button was changed weekly and at the end of the shaping phase, daily. At the end of the shaping phase, all goats were able to push the buttons and ensure their water demand independently. According to the veterinary and food control government, the daily water demand of dairy goats ranges between 1.5 and 4 L and based on our experience from previous learning experiments in dwarf goats, we can expect between 23.7 and 28.5 number of drinking actions (30 ml per action), which equals daily water consumption between 0.83 and 0.99 L (51).

Training

After completing shaping, goats were regrouped to up to eight animals per group into identically equipped pens, including the learning device. The learning device consisted of a 15-inch LCD screen (resolution of 640 x 480 pixels) behind a transparent acrylic panel. The screen was divided into four virtual sectors. In each sector, we displayed a different symbol (size $\sim 7\ \text{cm}^2$). To allow the goats to choose one of the symbols, four press buttons were mounted on the acrylic panel, one button beside each sector. Figure 2 gives a schematic overview of the compartment with the learning device. For a detailed description of the training procedure, see Langbein et al. (59).

During the first week, the goats were presented with a white screen, and all four buttons were rewarded. Next, the goats were presented with the first two training sets (Figures 3A,B). The symbol to learn was marked by a frame. The position of the symbols changed randomly after each trial. Each set was presented for 14 days. Similar to the shaping phase, goats were rewarded with 30 ml water for each correct choice. The device was accessible 24/7 for all goats. Even animals that did not learn the task were able to obtain enough water by increasing the number of trials at the device. At the end of training (60), all goats were able to use the device properly and reached the defined learning criterion (please see the *Data analysis and statistics* section for further details).

Initial discrimination learning and reversal learning

To investigate the impact of personality on the learning performance of the goats, a new 4-choice discrimination task was presented (Figure 3C). This initial discrimination task (ID)

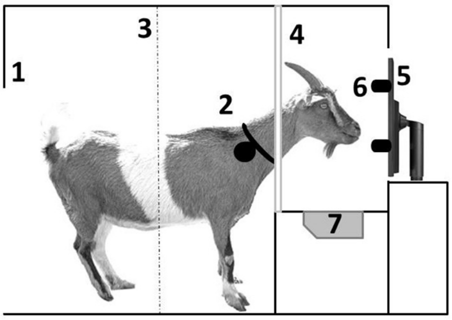

FIGURE 2
Lateral view of a goat inside the compartment with learning device: 1 = entrance (there is only space for one goat at a time at the device to avoid observational learning by the pen mates); 2 = collar with responder for individual recognition at the device; 3 = light beam indicating when a goat leaves the device; 4 = yoke to put only the head through; 5 = computer screen; 6 = buttons to choose a stimulus; 7 = water bowl for reward dispense.

was trained for 14 days. To investigate the effect of personality on the flexibility of learning behavior, three reversal-discrimination tasks were subsequently presented (RL1-3). The same set of symbols was used as in ID, but in each reversal task, a different one of the three previously unrewarded stimuli was now the stimulus to be discriminated (Figures 3D–F). All other conditions were identical to Training.

Ethical note

All animal care and experimental procedures were performed in accordance with the German welfare requirements for farm animals and the "Guidelines for the treatment of animals in behavioral research and teaching" (61). All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Ministry of Agriculture, Environment and Consumer Protection of the federal state of Mecklenburg-Vorpommern, Germany (Ref. 7221.3-2-005/14).

Data preparation

From 108 tested goats, we excluded those for whom technical issues concerning the learning device occurred (e.g., technical failure of the software and hardware of the learning device and/or loss of transponder for individual identification of the animal.). Finally, a total of 95 goats were included in all further analyses.

The learning performance of the goats was characterized by two steps: First, we analyzed whether the animals reached the learning criterion in ID and in RL1-3, respectively and, if so, we analyzed how many trials they needed to do so (trials to criterion, TTC). Given the four-symbol choice task where chance responding to a given symbol is 25%, the criterion for a statistically significant level ($p < 0.05$) of correct responding was determined according to the Binomial test when $n = 20$ and $p = 0.25$, to be 46% of correct choices in at least two consecutive blocks of 20 trials (learning criterion). This reflects the same learning success compared to the learning criterion of 75% in similar studies applying the common two-choice design (55).

Based on the TTC, we calculated the respective quartiles for the learning performance in ID (Table 1) to test its impact on learning performance in RL1-3. Consequently, Q1 indicated animals with the highest learning performance (lowest number of TTCs) in ID, and Q4 indicated the animals with the lowest learning performance (highest number of TTCs) in ID.

The personality traits *boldness* and *activity* were calculated by conducting a principal component analysis (PCA) for the initial OF- and NO-tests and their respective repetitions (please see 44 for further details). We selected 11 behavioral measures (see Supplementary Material) out of 82 identical for both test periods, which were not affected by season and body weight and/or are commonly used in animal personality studies (44, 62, 63). Using these measures, we calculated one PCA for each test repetition (PCA1+PCA2). We found two main PCs in PCA1 (overall MSA = 0.7). The first PC (PC1a; eigenvalue = 3.5) consists of four measures with loadings above 0.7 and below−0.7 describing the interaction with the novel object. The second PC (PC1b; eigenvalue = 2) consists of two measures with loadings above 0.7 and below −0.7 describing active-like behavior. We labeled PC1a "*boldness*" and PC1b "*activity*." The two PCs explained 32 and 18.2% of the variation in the data, respectively. In PCA2 (overall MSA = 0.71), we found two similar main principal components, with the first describing the interaction with the novel object (PC2a; eigenvalue = 3.7) and the second describing active-like behavior (PC2b; eigenvalue = 1.3), explaining 34% and 12% of the variation in the data, respectively. Finally, we calculated PC scores (from −3 to +3) for each personality trait for each animal in each of the two test periods, where each individual score was calculated from the standardized original data and the respective loadings of each PC separately for PCA1 and PCA2. These scores were used for any further statistical analysis.

We tested the impact of the personality traits on learning performance and characterized *momentary* and *stable* personality traits using either the first or both OF- and NO-tests as the basis for the calculations. Using the personality scores resulting from the PCA1 (*activity* and *boldness* scores from −3 to +3), the respective quartiles were calculated to test the impact of a *momentary* personality trait on cognitive performance (see Table 1 for *activity* and *boldness* quartiles). Conversely, to investigate whether consistency in these personality traits had an effect on learning performance, we selected animals that

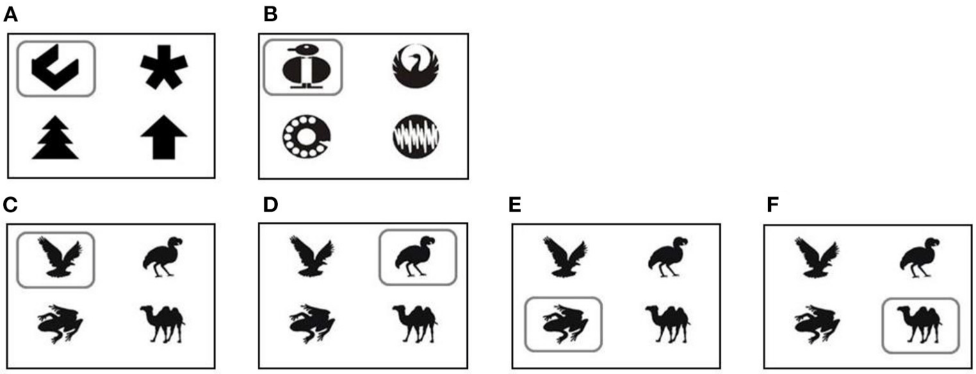

FIGURE 3
Symbol sets for training: training set 1 **(A)**; training set 2 **(B)**. Symbol sets during learning and reversal learning tasks: Initial discrimination learning task [ID, **(C)**] and the three reversal learning tasks: reversal learning 1 [RL1, **(D)**]; reversal learning 2 [RL2, **(E)**]; reversal learning 3 [RL3, **(F)**]. The rewarded symbols are marked with a square (for illustration purposes only). The position of the different stimuli changed after each individual choice.

showed stable *activity* and *boldness* scores in the repeated OF- and NO-tests (= *stable* personality traits). Stability was met if the scores from the first and second OF- and NO-tests were similar (both either positive or negative) for each personality trait (including a protection zone of ± 0.5 from the zero line).

Statistical analysis

All statistical analyses were conducted in SAS® 9.4 (SAS Institute Incorporated, Cary, North Carolina, USA). The data were evaluated by various analyses of variance (ANOVAs) using the GLIMMIX procedure.

Relationship between learning performance and reversal learning

To measure the impact of learning performance in ID, allocated among the four quartiles, on the overall achievement of the learning criterion during RL1-3, we used the variance function for the binomial distribution (binary data) and the logit link function in the statistical model. For more detailed analyses of learning, we ran a model with TTC in RL1-3 as our response variable and evaluated the effect of learning performance in ID (allocated to the respective quartiles, see above), task (RL1, RL2, RL3) and their interaction, which were fitted as fixed effects. Both models contained motherID, pens and season nested in replicates as random effects.

Impact of a momentary personality traits on cognitive performance

The impact of the quartiles of *activity* (A1-A4; from very inactive to very active) and *boldness* (B1-B4; from very unbold to very bold) in PCA1 as fixed factors was tested on the number of animals reaching the learning criterion and on the TTC in RL1-3. In each model, motherID, pen and season nested in replicates were included as random effects.

Impact of stable personality traits on learning performance

To investigate whether consistency in specific personality traits had an effect on learning performance, we selected animals that showed stable *activity* and *boldness* scores in the repeated OF- and NO-tests. Out of 95 animals, 32 animals showed a stable value in *activity* (16 active, 16 inactive), and 42 animals showed a stable value in *boldness* (16 bold, 26 unbold) across the repeated OF- and NO-tests. The selected animals were then assigned to the groups *stable in activity* (inactive or active) or *stable in boldness* (unbold or bold), which were used as fixed effects on the TTC in ID and RL1-3 in the statistical model. In these models, motherID, replicates and season were included as random effects.

Least squared means (LSMs) and their standard errors (SEs) were computed for the fixed effects in all models, and pairwise differences in the LSM were tested using the Tukey–Kramer correction. All analyses included the animal as a repeated factor in the random statement, and mean differences with $p < 0.05$ were considered significantly different.

Results

Relationship between learning performance and reversal learning

A significant effect of learning performance in ID (Q1–Q4) was found on the number of goats that reached the learning

TABLE 1 Calculation of the respective quartiles concerning. (A) the learning performance (number of TTC) in the initial discrimination task (ID); (B) the activity scores based on the results of the principal component analyses of the first open field/novel object test (44); and (C) the boldness scores based on the results of the principal component analyses of the first open field/novel object test. Respective lower and higher limits as well as the category description and number of animals per quartile are indicated.

Quartile label	Lower limit	Higher limit	Description	*n* (animals)
(A) Quartile limits based on the number of TTCs				
Q1	20	20	"High performer"	24
Q2	40	80	"Good performer"	22
Q3	100	200	"Poor performer"	24
Q4	220	1,700	"Low performer"	24
(B) Quartile limits based on activity scores				
A1	−3	−0.5248	"Very inactive"	24
A2	−0.5249	0.0459	"Inactive"	23
A3	0.046	0.6414	"Active"	24
A4	0.6415	3	"Very active"	24
(C) Quartile limits based on boldness scores				
B1	−3	−0.9164	"Very unbold"	24
B2	−0.9165	−0.0655	"Unbold"	24
B3	−0.0656	0.7794	"Bold"	23
B4	0.7795	3	"Very bold"	24

criterion in RL2 ($F_{3,41} = 4.56$, $p < 0.01$) and RL3 ($F_{3,41} = 5.05$, $p < 0.01$), while a tendency was found concerning RL1 ($F_{3,41} = 2.48$, $p < 0.1$). In other words, 46% of the low performers in ID (Q4) were not able to learn the first reversal task ($n = 11$). This effect was significant for the second (71%; $n = 17$) and third reversal tasks (79%; $n = 19$). Goats with the lowest learning performance in ID (Q4) reached the learning criterion in RL2 less frequently compared to goats with only poor learning performance in ID (Q3) ($p < 0.01$) and also tend to reach the learning criterion less frequently compared to goats with good (Q2, $p < 0.1$) and very good (Q1, $p < 0.1$) learning performance in ID. Additionally, low performers in ID (Q4) reached the learning criterion in RL3 less frequently compared to all other groups of learning performance in ID (Q1-Q3), as shown in Figure 4A (Q1 and Q3: $p < 0.05$, Q2: 0.01). Regarding the animals that successfully reached the learning criterion in RL1, RL2 or RL3, we found an effect of learning performance in ID on TTC in the reversal tasks ($F_{3,145} = 5.21$, $p < 0.001$). Pairwise comparisons indicate that low performers (Q4) needed significantly more TTC than the other learning groups (Q1-Q3) in RL1 ($p < 0.01$, Figure 4B), while this difference is not apparent in RL2 and RL3 (all $p > 0.1$). Furthermore, we found a significant effect of task on TTC ($F_{2,145} = 20.3$, $p < 0.001$) in that the animals significantly reduced their TTC in RL3 compared to RL1 and RL2 ($p < 0.001$, respectively) that was mainly apparent in Q4 regarding the TTC in RL3 compared to RL1 ($p < 0.05$). However, the interaction between learning performance in the ID and RL tasks revealed no significant effect.

Impact of momentary personality traits on learning performance

The statistical analysis indicated no significant effects of *activity* (A1-A4) or *boldness* (B1-B4), based on only the first conducted OF- and NO-tests, on the number of goats that reached the learning criterion during RL1-3. Furthermore, *activity* and *boldness* did not show any significant effects on the TTC either during ID nor during RL1-3.

Impact of stable personality traits on learning performance

We found no significant effects of *stability* in *activity* or *boldness* on the number of goats that reached the learning criterion in RL1-3. Regarding TTC, *stability in activity* (inactive vs. active) was not found to have a significant effect on TTC in ID or RL1-3 (Figure 5A). In contrast, *stability in boldness* (unbold vs. bold) significantly affected TTC in RL1 ($F_{1,7} = 11.36$, $p < 0.05$). As shown in Figure 5B, bold animals exhibited a lower learning performance (higher number of TTCs) in RL1 than unbold animals. This effect was not observed in RL2 and RL3.

Discussion

Our study investigated the potential relationship between specific personality traits and individual learning performance

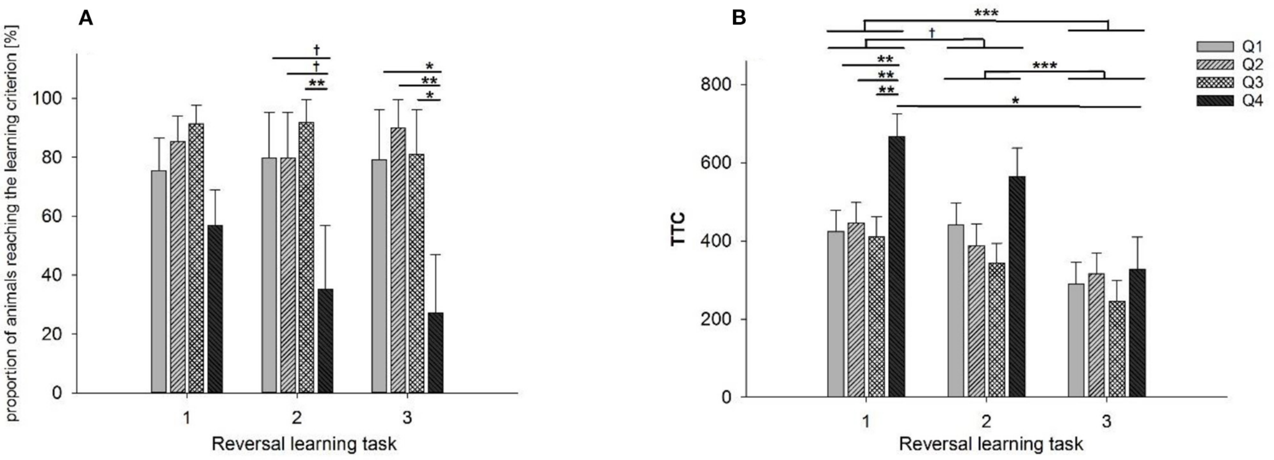

FIGURE 4
Performance of different groups of goats in the reversal learning task: **(A)** proportion of goats that reached the learning criterion during reversal tasks RL1-RL3. **(B)** Number of trials to reach the learning criterion (TTC) in reversal tasks RL1-RL3. The groups Q1-Q4 indicate the learning performance during the initial discrimination task (ID): Q1: TTC in ID = 20, Q2: TTC in ID = 40–80, Q3: TTC in ID = 100–200, Q4: TTC in ID>220. Data are presented as least squared means and standard errors (LSM ± SE); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

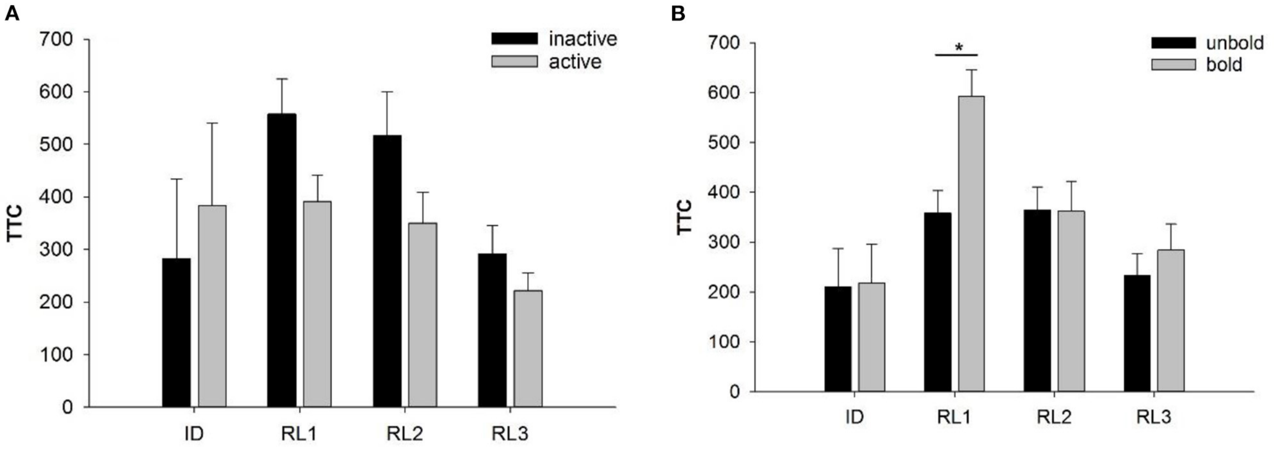

FIGURE 5
Performance of goats with stable personality scores in activity **(A)** or boldness **(B)** in the reversal learning tasks: **(A)** number of trials to reach the learning criterion in the reversal tasks RL1-RL3 of goats assigned to the inactive (black bars) vs. active groups (gray bars). **(B)** Number of trials to reach the learning criterion in the reversal tasks RL1-RL3 of goats assigned to the groups unbold (black bars) vs. bold (gray bars). Data are presented as least squared means and standard errors (LSM ± SE); *$p < 0.05$.

by using a visual discrimination paradigm. Goats learned to associate a visual stimulus with a reward in the initial 4-choice visual discrimination task (ID) presented 24/7 using an automated learning device and were able to reverse their associations in a serial reversal task (RL1-3). Their performance in the three consecutive RL's depended on their learning success in the ID in that low performers in the ID also performed low in the RL's. Taking specific personality traits (measured once) into account, we did not find any evidence of a relationship between *activity* and/or *boldness* and learning performance, either in the discrimination task or in the reversal tasks. However, including only the animals that were *stable* for personality trait *activity* and/or *boldness* (measured twice), no evidence for any link between *activity* and learning performance was found, while *boldness* revealed a significant link to learning performance in the first reversal task in that unbold individuals outperformed bold individuals in RL1.

The learning performance of the goats in the three consecutive reversal tasks depended on their learning success in the initial learning task. A higher proportion of goats performing low in the initial discrimination task did not achieve the learning criteria in the reversal tasks compared to the goats that learned the initial discrimination task better. This effect is not as obvious in the first reversal task but reaches significance in the second and third reversal tasks. Additionally, the low-performing goats that were able to reverse the initially learned association needed more trials to solve the reversal task, which was significant at least in RL1. This indicates a relationship between visual discrimination learning and reversal learning in goats in the sense that the learning speed in the visual discrimination task would predict the learning performance in a reversal task regarding low-performing learners. Unexpectedly, goats who performed low in the initial discrimination task also performed low in the reversal task. This finding contrasts other learning studies showing that good learners (high learning speed) seem to be poor reversal learners (low learning speed), which has been demonstrated in guppies (64) and parrots (65). Discrimination learning underlies the ability to associate a stimulus with appetitive (or aversive) stimuli, whereas reversal learning involves the capability of extinguishing a previously learned association to form a new association. This is thought to be more cognitively challenging, as previously learned cues must be deleted before a new association can be formed. Reversal learning tasks are frequently used as a measure of behavioral flexibility, as they require the subjects to flexibly adjust their behavior when the reward-related contingencies that they have previously learned are reversed (66). The clear difference between low performers and the other three learning groups in learning performance may indicate less flexibility and greater perseverance in low performers once they understood the initial discrimination task. One possible explanation comprises the role of the individual stress level during learning. A range of studies have demonstrated that elevated corticosterone levels affect learning performance across taxa, although the degree to which learning is affected and whether exposure results in benefits or decrements depends on the extent and timing of corticosterone exposure (67–69). The low-performing individuals in our study may be more stressed compared to the other three groups of learners due to the stress originating from the learning task itself or from external factors such as dominance or rank within the group. These factors have already been found to show a relationship to learning performance and might have impaired learning performance in the initial discrimination task. To shed light on this issue, and as we did not investigate the individual stress level in our study, future research should take into account the stress responses of animals in such learning tests. However, the learning performance of the other three learning groups in the discrimination task did not have an effect on reversal learning; thus, we did not find a negative correlation, as has been shown in red junglefowl (70). The learning groups might differ in several aspects associated with performance, such as motivation or physical strength. We also take into account differences in general learning ability, as some animals might be more cognitively impaired than others, which is likely to explain the differences observed in discrimination and reversal learning. Furthermore, goats seem to be able to improve their performance across three reversals by progressively reducing their TTC, indicating that they are getting better at switching to the alternative stimulus. Similar serial reversal learning experiments, in which the alternation of training to criterion and contingency reversal was repeated several times, have revealed that rats, pigeons, frogs and goats improve their performance over successive reversals in a learning-to-learn process (71–73). In our study, we found that low performers were able to improve their TTC across the reversal tasks, but we must note that goats that did not achieve the learning criterion at all in RL1-3 fell out of scope.

Taking specific personality traits into account, we did not find any evidence of a relationship between the two *momentary* personality traits *activity* and *boldness* (measured once) and learning performance either in the discrimination task or in the reversal tasks. We might have expected, based on previous studies concerning personality measures and learning/cognition, that bold and/or active individuals learn to associate a cue with a reward faster than unbold and/or inactive individuals (7, 74) but perform lower in reversal learning (56, 75, 76). A possible explanation for the lack of a link between personality traits and learning success in this analysis may be that only a single measurement of personality was integrated in the analysis. A recent review stated that personality measured just once cannot provide enough information about all aspects of personality differences (77). This is easily comprehensible, as animal personality refers per definition to the repeatable part of an individual's behavior (12, 78), and a behavior measured only once probably reflects mostly the within-individual rather than the among-individual component (77, 79). Among studies that have investigated the relationship between personality and cognition in nonhuman animals, some conducted a behavioral test only once to characterize specific personality traits and often failed to show interrelations between personality and learning, while it is recommended to measure personality traits across time and contexts by applying several repetitions of different tests to find a connection between personality and learning capabilities (28, 31, 80). For example, Christensen et al. (81) found that behavior toward a novel object (labeled exploration) correlated with learning performance in a visual discrimination task in horses. More exploratory horses were more successful in a two-choice visual discrimination task (81). Similar to our study, the NO-test was conducted repeatedly, indicating that relationships between cognitive performance and personality can be found when personality traits are confirmed to be stable over time because they are measured at least twice.

In our study, the behavior of the individuals was observed in two tests that were independent from each other, and two separate PCA were conducted. In a following step, we used the data from both personality tests, including only the animals that were stable for personality trait *activity* and/or *boldness*, to test whether stability in a specific personality trait would have an effect on learning performance. We found no evidence for any link between *activity* and learning, either in ID or in RL, whereas *boldness* revealed a significant link to learning performance in RL1. By exhibiting a higher level of activity and exploration, active personality types sample their environment more rapidly, albeit more superficially. As a consequence, these animals should learn novel tasks more quickly but at the cost of accuracy and responsiveness to changes in the meaning of cues (6, 13, 57). Caused by their superficial sampling and inaccuracy, they were thought not to perceive the change in cue meaning during reversal learning and thus would make a greater number of mistakes when associating the symbols with the reward. In contrast, inactive types were thought to have a reduced learning speed but reveal more learning flexibility through their greater attention toward cue relevancy, increasing their accuracy under variable environmental conditions. We therefore expected active individuals to successfully reach the learning criterion in the discrimination task more rapidly and that inactive individuals would outperform active individuals in the context of reversal learning tasks. In testing these predictions, we found that *activity* did not have any influence on learning success in the goats. Similar results were reported by Chung et al. (17), where active ("fast") lizards did perceive a change in cue meaning (other than expected) and were not less accurate than their inactive ("slow") conspecifics when tested in a two-phase associative task. In general, several terms in the context of animal personality research have referred to partially overlapping concepts (82), and traits such as exploration, boldness (and sociability) are most commonly used in the context of animal personality. Only a few studies find a direct relationship between activity and learning success, and if so, they reveal different results (17, 74, 83). This may be due to varying definitions and measurements of activity. While some studies measure activity as the number and rate of transitions between squares (17; lizard) or swim distance (83; guppies), others use locomotion behavior that results in a change in body position in space as an indicator for activity (74; cavies). This impairs the general comparability across experimental studies, measurement methods and statistical analyses. There is some evidence that links between activity and learning abilities are highly task dependent. For example, fearful and active individuals perform better in an avoidance task, whereas the authors did not find such a relationship in a backwardss-forwards task in horses (84). It is therefore possible that activity might reveal a predisposition to react to specific stimuli involved in learning.

However, we found a significant impact of the *stable* (repeatedly measured) personality trait *boldness* on learning performance in RL1 (unbold individuals outperform bold individuals) but not in ID. This partly reflects the general assumption that bold or proactive individuals are better at learning a discrimination task, but unbold or reactive individuals are better at adapting to changes in an already learned task reflecting a higher behavioral flexibility (7, 57, 76, 85–87). This association has been shown in studies with great tits (26) and red junglefowls (88), while others fail to show this relationship (31). Differences in the readiness to approach a novel stimulus have been described as risk-taking behavior, novelty-seeking, proactivity or boldness in different studies across different species and have been shown to be a distinguishing factor between individuals differing in coping patterns or personality traits (13, 89–91). It is assumed that bold/proactive animals are more successful in forming routines during learning, which results in being less able to react flexibly in adapting their behavior to changing environmental conditions (20, 92, 93). Interestingly, the difference in learning success only refers to the first reversal task and disappeared in RL2 and RL3, as bold individuals were able to improve their learning performance. We assume that they learned to form a new routine of the concept of reversal, strengthening the hypothesis that bold individuals are fast learners in the sense that they quickly form routines. This interpretation fits with Cockrem's (92) classification of personalities in that proactive individuals may perform better in environments that are constant or predictable compared to reactive individuals who perform better in unpredictable conditions. Reactive individuals may tend to generalize a formerly learned rule (i.e., "one of the symbols is rewarded") more quickly being able to shift the general rule to a new symbol. On the other hand, fast explorers may learn more about the absolute properties of the stimulus in the visual discrimination task and thus fail to classify new symbols immediately "correctly." Once the reward reversal was no longer new (RL2 and RL3), proactive individuals learned that the general concept changes and were able to form a new routine. Even so, it is unclear why and how learning more or less flexibly is related to how individuals react to a novel object in the context of a standardized behavioral test. At least, novelty seems to play an important role in driving these processes, as differences between individuals refer to both the novel object and the novel (first) reversal learning task. In most studies, the trait boldness refers to behavioral reactions to novel stimuli and situations and is often measured in a NO- or novel human test (reviewed by 8). However, boldness has also been used in predator-dependent contexts showing a relationship to learning performance, whereas boldness measured in a NO-test did not, especially when measured only once (reviewed by 9). Furthermore, boldness concepts may refer to an individual's reactions to risky situations (12, 94), while exploration is often used in the context of an individual's reactions to new situations (12). The differences between a new and/or risky situation are not easily detectable and depend to a large amount on the type of experimental setup and recorded parameters (12, 18, 82).

To address our findings, the performance of the goats in the three reversal tasks depended on their learning success in the initial discrimination task in that low performers in the initial discrimination task also performed low in the reversal tasks. We found evidence in the current study that there is a relationship between learning and *boldness* in goats: bold individuals show reduced behavioral flexibility, as shown in the first reversal task; however, bold animals were able to adapt in the subsequent reversals once a new routine was formed.

Conclusion

To conclude, in the present study, one personality trait, *boldness*, proved to have substantial consequences on learning performance in a reversal task. Our findings show that we should take personality bias into account when conducting learning or cognitive experiments and underline the importance of the repeated measurement of individual personality traits. However, the explanation for the observed interrelations between personality traits and learning performance is currently unclear and requires further empirical and theoretical investigation. To understand whether intrinsic differences in learning exist across individuals, we need to investigate the extent to which learning and personality traits covary and identify the mechanisms that can constrain cognitive processing and adaptive behavioral responses.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://osf.io/wudj7/?view_only=ed372f7bdded4ff2a46901a0bdc15081.

Ethics statement

The animal study was reviewed and approved by Committee for Animal Use and Care of the Ministry of Agriculture, Environment and Consumer Protection of the federal state of Mecklenburg-Vorpommern, Germany (Ref. 7221.3-2-005/14).

Author contributions

JL conceptualized the study. M-AF and AK collected and analyzed the data and did the statistical analysis together with AT. M-AF, AK, JL, and BP contributed to the conception of the manuscript. M-AF, AK, and JL wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.916459/full#supplementary-material

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Horses wait for more and better rewards in a delay of gratification paradigm

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Self-control, defined as the ability to forgo immediate satisfaction in favor of better pay-offs in the future, has been extensively studied, revealing enormous variation between and within species. Horses are interesting in this regard because as a grazing species they are expected to show low self-control whereas its social complexity might be linked to high self-control abilities. Additionally, self-control may be a key factor in training and/or coping with potentially stressful husbandry conditions. We assessed horses' self-control abilities in a simplified delay of gratification test that can be easily implemented in a farm setting. In Experiment 1, we gave horses (N = 52) the choice between an immediately available low-quality reward and a delayed high-quality reward that could only be obtained if the horse refrained from consuming the immediate reward. Different experimenters (N = 30) that underwent prior training in the procedures, tested horses in two test phases either with their eyes visible or invisible (sunglasses). Twenty horses waited up to the maximum delay stage of 60 s while all horses performed worse in the second test phase. In Experiment 2, we improved the test procedure (i.e., one experimenter, refined criterion for success), and tested 30 additional horses in a quality and quantity condition (one reward vs. delayed bigger reward). Two horses successfully waited for 60 s (quality: N = 1, quantity: N = 1). Horses tolerated higher delays, if they were first tested in the quantity condition. Furthermore, horses that were fed hay *ad libitum*, instead of in a restricted manner, reached higher delays. Coping behaviors (e.g., looking away, head movements, pawing, and increasing distance to reward) facilitated waiting success and horses were able to anticipate the upcoming delay duration as indicated by non-random distributions of giving-up times. We found no correlations between owner-assessed traits (e.g., trainability and patience) and individual performance in the test. These results suggest that horses are able to exert self-control in a delay of gratification paradigm similar to other domesticated species. Our simplified paradigm could be used to gather large scale data, e.g., to investigate the role of self-control in trainability or success in equestrian sports.

KEYWORDS

self-control, inhibitory control, delay of gratification, horses, coping behavior, error times

Introduction

Being able to wait for something with a better outcome instead of going for an immediate but inferior outcome is advantageous in multiple situations (Beran, 2008). For example, while foraging it might pay to wait until sufficient gum has extruded from the tree before consuming it or to wait until the prey is in a favorable position before launching the attack (Stevens, 2014). But also, in social interactions it might prove beneficial for subordinate individuals to wait until the dominant ones have left the food resource before starting to feed (Johnson-Ulrich and Holekamp, 2020). The ability or capacity to invest more effort into obtaining a more valuable outcome instead of selecting a less valuable outcome has been termed self-control (see Beran, 2015 for a review). Self-control is essential in improving decision-making processes as it facilitates goal-directed behavior and ultimately future planning (Santos and Rosati, 2015). The ability of self-control is one aspect of inhibitory control, which additionally encompasses response or motor inhibition (regulation of impulsive motor actions) and cognitive inhibition (ability to control conditioned responses; Miller et al., 2019a). Self-control is generally assumed to be more cognitively demanding than response inhibition as it involves an additional decision component. Indeed, past research has shown that individual self-control abilities are linked to success in later life in humans (Shoda et al., 1990; Moffitt et al., 2011; but see Watts et al., 2018). Also, in chimpanzees, self-control is linked to other measures of general intelligence (Beran and Hopkins, 2018). This indicates that self-control plays an important role in cognitive processing of information.

While self-control is certainly beneficial in natural contexts [e.g., mate choice (Sozou and Seymour, 2003); foraging (Stevens and Stephens, 2010)], it might be equally important for domesticated animals that are no longer facing foraging decisions or mate choice but are subjected to various situations that most non-domesticated species would never experience in the wild. These situations include prolonged social isolation, frequently encountering unfamiliar individuals, living in barren and captive environments, and being subjected to procedures that might be stressful to the animals, to name just a few examples. In particular, farm animals are often kept in housing conditions that do not resemble their natural habitat (i.e., barren environments that limit expression of natural behaviors and induce frustration) and thus require flexible behavioral responses to cope with these conditions (e.g., Mason et al., 2013). Specifically, in these conditions, self-control is potentially beneficial as it allows individuals to flexibly adapt to their environment by optimizing decision-making processes in tempting and/or conflicting situations. It has been hypothesized that domesticated animals were selected for tamer and less aggressive behavior (Price, 1999), which might be linked to enhanced inhibitory control abilities in certain situations. Indeed, it has been shown that aggressiveness is linked to inhibitory control in a way that aggressive individuals often exhibit elevated impulsivity (e.g., hamsters: Cervantes and Delville, 2007; rats: Coppens et al., 2014; dogs: Gobbo and Zupan Šemrov, 2022). Furthermore, recent research found that inhibitory control is linked to emotional states in young chicken (Garnham et al., 2022). Accordingly, individual differences in inhibitory control might directly affect animal welfare, for example, explaining why some individuals cope with environmental or social conditions while others fail to do so. Individuals with better self-control might be less prone to develop stereotypical behaviors because they can restrain themselves from engaging in impulsive actions. Additionally, inhibitory control might play an important role during human-animal interactions. Animals with better inhibition might be less reactive in stressful situations and, thus, perceived as easier to handle. Self-control, in particular, might enhance attention in a training setting as the animals can better focus on the human signals instead of being tempted by the later reward.

Enormous variation in self-control abilities across animal species have been reported consistently. Accordingly, multiple hypotheses have been proposed as an attempt to explain the observed variation. These hypotheses are based on physiological explanations (Mayack and Naug, 2015; Miller et al., 2015), metabolic rate and longevity (Stevens and Mühlhoff, 2012; Stevens, 2014), brain size (MacLean et al., 2014) but also foraging ecology (Stevens et al., 2005) and social complexity (Amici et al., 2008; Lucon-Xiccato et al., 2022). However, considering that domesticated species show a decreased brain size (Kruska, 2007) and have an altered metabolic state depending on selection purposes (Rauw et al., 2017), some hypotheses might be difficult to test in domesticated species. Nevertheless, a handful of domesticated species have been tested in delay of gratification paradigms to assess their self-control abilities. On a group level, dogs waited between four to 25 times longer (Leonardi et al., 2012; Brucks et al., 2017b; Range et al., 2020) compared to pigs (Zebunke et al., 2018; Krause et al., 2021) and chicken (Abeyesinghe et al., 2005); however, domesticated animals generally exhibited rather low self-control abilities compared to other non-domesticated species (e.g., long-tailed macaques: Pelé et al., 2010; cleaner wrasse: Aellen et al., 2021). While certainly differences in experimental procedures and paradigms are accountable for some variation (Susini et al., 2021), more data on domesticated species' self-control abilities, in particular of farm animals, is needed in order to better understand whether and how domestication affected self-control abilities.

In addition to this species-level variation, individual differences in inhibitory control abilities are frequently reported in studies. For example, sex (e.g., Brandão et al., 2019), age (e.g., Krause et al., 2021), food motivation (Meier et al., 2017; van Horik et al., 2017), social rank (Johnson-Ulrich and Holekamp, 2020), individual body conditions [i.e., hunger levels (Mayack and Naug, 2015)], but also the social environment

(Lucon-Xiccato et al., 2022) can affect individual inhibition capacities. For example, chicken reared in a cognitively enriched environment exhibited poorer inhibitory control than chicken reared in a standard environment (Ryding et al., 2021). Consequently, to capture a species' inhibitory control abilities, a large sample size is needed. Furthermore, it needs to be considered that not all measures of inhibitory control are necessarily tapping into the same behavioral construct (e.g., Bray et al., 2013; Marshall-Pescini et al., 2015; Brucks et al., 2017a); accordingly, comparative conclusions should only be drawn if either the same experimental paradigm is employed or if multiple different tests are used.

Horses (*Equus caballus*) have not been tested for their self-control abilities so far, even though they represent an interesting model species in this regard. Firstly, horses are generalist herbivores and can find food in rather evenly distributed patches (e.g., Salter and Hudson, 1979). This feeding ecology potentially requires only very little self-control as horses need to make only few decisions during foraging compared to carnivore or frugivore species that face resources with quickly changing availability and quality (Stevens et al., 2005). Nonetheless, horses show distinct resource preferences based on macronutrient and protein content (van den Berg et al., 2016) and sample from different foraging patches before making a choice (Devenport et al., 2005; Goodwin et al., 2007). Data on self-control in grazing species is missing so far. Secondly, horses live in complex social organizations that require high levels of social flexibility (Krueger, 2008; Maeda et al., 2021). According to the social complexity hypothesis, species living in complex social organizations that necessitate repeated interactions with various different partners possess enhanced inhibitory control abilities since they need to inhibit social behaviors in various situations (Amici et al., 2008; Johnson-Ulrich and Holekamp, 2020; but see Lucon-Xiccato et al., 2022). And thirdly, horses are domesticated species and high levels of inhibitory control are likely favorable for handling and training. In particular, self-control could be important in a training setting, as it might allow animals to better attend to human signals, and the prospect of appraisal (in case of correct responses), instead of focusing on immediate rewards. Whether domestication *per se* affected inhibitory control abilities (Marshall-Pescini et al., 2015; Gatto et al., 2018; Brucks et al., 2019), and self-control in particular (Range et al., 2020), is not clear; however, data of domesticated animals is particularly interesting for testing the links between self-control and animal welfare. As hypothesized also elsewhere (e.g., Langbein, 2018; Zebunke et al., 2018), individuals with better inhibition abilities might be better equipped for coping with stressful conditions, such as overcrowded housing, and lack of environmental stimulation. Especially horses are subjected to various housing conditions and training techniques that can affect trainability, handleability, and rideability (König von Borstel, 2013).

Various different experimental paradigms have been developed to test self-control abilities across animal species (see Miller et al., 2019a for a review). Broadly, these paradigms can be divided into two categories depending on the delivery mode of the rewards. In accumulation tasks, food items are delivered, either automatically via a remotely controlled device (Evans and Beran, 2007) or by an experimenter (Hillemann et al., 2014), one item at a time with a fixed interval between items until the subject starts to consume the accumulated food items. In exchange tasks, the subject is handed a less valuable reward and after a certain delay, this reward can be exchanged for a more valuable reward (e.g., Leonardi et al., 2012; Auersperg et al., 2013). Depending on the morphology of the animal species, this task is potentially inducing more or less temptation. For example, while a monkey can hold the food item with his/her hands, a dog would be required to hold the reward with his/her mouth and thus taste organ. Accordingly, the exchange task has been modified for some species to circumvent this potential confounding effect. For example, in dogs (Brucks et al., 2017b) and wolves (Range et al., 2020), rewards were delivered on retractable containers instead of handing the reward directly to the animals' mouth. And recently, another experimental paradigm has been established, the so-called rotating tray task, in which the rewards are placed on a disk and rotate within reach of the animals without overt involvement of humans (e.g., Bramlett et al., 2012; Miller et al., 2019b; Brucks et al., 2021). While these paradigms certainly represent standardized tests for a laboratory setting, they are difficult to implement in an applied context as they require either larger apparatuses (e.g., rotating tray) or extensive training to familiarize the animals with the required action for obtaining the more valuable reward (e.g., exchange task).

In the current study, we aimed at establishing a simplified version of the exchange task that could be easily implemented in non-standardized environments (e.g., barns, stables, and meadows) and applied also by lay persons. Horses were given a choice between a less preferred immediate reward and a highly preferred but delayed reward presented on the experimenter's hands in front of the horse. Considering that this experimental paradigm involves close and direct social interactions with an experimenter, the gaze of the experimenter could add an additional social inhibition component that could increase the horses' success in the task. For example, dogs behave in a more inhibited way in a food context depending on whether the experimenter's eyes are visible or not (Call et al., 2003). Also, horses are sensitive to human social cues (i.e., body orientation, gestures) (e.g., Proops and McComb, 2010) and potentially also gaze directions (Birke et al., 2011). Accordingly, we tested whether horses perform differently depending on whether the experimenters' eyes are visible in Experiment 1. Based on the results of Experiment 1, we aimed at refining the protocol to allow a better comparison of horses' self-control abilities with other species. Consequently, we adopted similar procedures as used in other studies, in terms of reward types, delay stages, criteria for success, and also tested horses in a more standardized setting. In Experiment 2, we tested a new population of horses in two conditions, a quality (less preferred reward vs. highly preferred reward) and a quantity (one reward vs. multiple reward items) condition. To find out whether certain behaviors facilitated waiting success, we coded the horses' behavior during the test. Furthermore, to explore whether individual horse characteristics (i.e., nervousness, trainability, patience, and food motivation) are linked to self-control and whether horse owners can assess their horses' self-control, we asked the horse owners to fill in a questionnaire.

Horses are a grazing species and thus potentially require little self-control during foraging but also live in a complex social environment that potentially requires enhanced self-control abilities. Accordingly, two mutually exclusive hypotheses can be derived: if horses show good self-control abilities this could be seen as support for the social complexity hypothesis; however, a lack of self-control would support the feeding ecology hypothesis. Furthermore, we hypothesize that distraction behaviors emitted during the waiting period facilitate waiting success. Accordingly, horses that show more distraction behaviors are expected to be more successful in delaying gratification than horses showing fewer of these distraction behaviors.

Experiment 1

Methods

Subjects and housing

We tested 56 privately-owned horses of various breeds in a delay of gratification paradigm. Four horses did not complete testing due to health-related issues (*N* = 2) and frustration/aggression during the test (*N* = 2), one horse developed aggressive behaviors during the course of the second phase of the test, accordingly, data from the first test phase could still be collected from this horse. Thus, in total, 52 horses (29 F/23 M; age: 15.1 ± 7.0 years, range: 3.5–30.5 years) of various breeds (see **Supplementary Data 1** for individual characteristics) were included in the analyses. The horses were kept in group-housing conditions (*N* = 32) or in individual boxes with daily access to outdoor areas (*N* = 20). Hay was provided either *ad libitum* (*N* = 20) or in a restricted manner (3–5× a day; *N* = 32).

The tests were conducted by thirty different experimenters (3 M/27 F) that were familiar with the horses (e.g., owner of horse, rider of horse, horse from same social group as own horse). Each experimenter tested two horses. Due to the exclusion of four horses, data from four experimenters that tested only one horse were present in the data set. To ensure reliability between the different experimenters, they were required to complete a training session at the beginning of data collection. This training session included reading the detailed procedures and providing a video sequence of performing the food preference test as well as the training phase (see description below). The study coordinator (DB) checked all videos and gave feedback in case that the procedure was not performed correctly. Only when the procedures were applied accurately, the experimenters were allowed to start data collection.

Experimental procedures

Each horse was tested individually in a box or paddock. The horses were either free to move or tied to the wall in case no box was available. In case that a door from a box or a stranded wire (not electrified) was used to separate the horse and experimenter it was ensured that the horses could easily reach across the barrier with their head and neck. The test areas were chosen to minimize distractions during the test. The experimenters stood in front of the horse at a distance of 1–2 m (depending on the horse's size) and were instructed to behave passively during the test (i.e., no verbal commands or gestures).

The food rewards were stored on the ground behind the experimenter and out of reach for the horses. Instead of presenting the food items on containers or a rotating tray as in previous studies, we presented the food items on the experimenter's hands in front of the horse but out of reach (similar to e.g., Leonardi et al., 2012; Auersperg et al., 2013). Food was presented on open hand palms on each side of the body (approximately 40 cm distance between hands) at the height of the experimenter's hip (see **Supplementary Video**). The distance between both hands and the horse's head was between 20 and 30 cm at the beginning of each trial ('start position'). This distance was maintained in a dynamical way, i.e., if a horse reached forward with extended head, the experimenter could take a step back to avoid that the horses reached the delayed food reward before the respective delay-time was over.

Food preference test

To find a highly preferred food reward and a less preferred but still consumable food reward for each horse, we conducted a food preference test at the beginning of the experiment. Horse owners were asked about their horses' preferences and to validate these suggestions, the horses were repeatedly offered both reward types simultaneously. To ensure that the horses would consume the less preferred reward consistently, if no better reward was offered, the horses were presented with one piece of the owner-suggested low-value reward (LVR) at a time. This was repeated for a total of 10 trials. If the horse readily consumed each piece of the reward type, it was used as LVR in the subsequent test. For all of the horses in Experiment 1, hay (either as a loose bundle or as cobs) was used as LVR.

Once the LVR was determined, the horses were presented with a choice between the LVR and a high value reward (HVR; e.g., carrot, apple, and banana; see **Supplementary Material** for

HVRs per horse). The experimenter visibly placed one piece of the HVR on one hand and a small bundle of hay (=LVR) on the other hand. Both hands (with open palms) were presented to the horse for 3 s before both hands were simultaneously stretched forward within the horses' reach. The horses were allowed to select and consume one of the rewards while the experimenter retracted the hand holding the food reward that was not selected (see **Supplementary Video**). The horses' choice was noted and the next trial was started. To prevent horses from developing a side preference, the sides of the HVR and LVR were alternated between each trial.

Twenty trials were conducted per session. If a horse selected the HVR in 16 out of the 20 trials (one-sided binomial test: $p = 0.001$), he/she reached the criterion and the HVR was used in the subsequent test. In case that a horse did not reach the criterion, the food preference test was repeated for a total of three sessions. If no preference was shown, a different reward type was used as HVR. If a horse did not reach the criterion within six sessions in total, she/he was excluded from the study. Some horses developed side preferences and the experimenter performed 15 trials with food only on the non-preferred hand to counteract this preference. Following this step, another food preference session was conducted.

Training

In the training phase, the horses were familiarized with the test procedure and the concept of gaining access to the HVR only if the LVR is not consumed. For each horse, it was randomly determined on which hand the less preferred and highly-preferred reward was positioned and these sides were kept constant throughout the study. To ensure that the horses were paying attention to the test, the experimenter initiated a trial only if the horse was looking toward the experimenter. If a horse was not attentive (e.g., head turned away), the experimenter called the horse by her/his name and made an attention-getting sound that the horse was familiar with (e.g., clicking with tongue or whistling). In case that this was not successful, the same procedure was repeated twice; however, if a horse was still not attentive, the test was terminated and repeated at a later point in time.

At the beginning of each trial, both reward types were presented on the hand palms for 3 s in the start position. The hand holding the LVR was now stretched out within reach of the horse while the hand holding the HVR remained in the start position out of reach (see **Supplementary Video**). If the horse did not consume the LVR, the hand with the HVR was also stretched forward after 1 s had passed. If the horse consumed the LVR, the hand holding the HVR was instantly closed and withdrawn. In total, 15 trials were conducted per session. Horses reached training criterion, if they refrained from eating the LVR and instead waited for the HVR in at least twelve of the 15 trials (one-sided binomial test: $p = 0.004$). If this criterion was not reached, another session was conducted. In case of horses not reaching this criterion within six sessions they were excluded from the experiment.

Test phase

Horses that reached the training criterion entered the test phase, in which the delay between the immediately available LVR and the delayed HVR was increased in a stepwise manner depending on each horse's success. As before, both reward types were presented simultaneously at the beginning of each trial on the predetermined hands ('start position') for 3 s before stretching the hand holding the LVR within the horse's reach while the hand holding the HVR remained in the start position and hence out of reach (see **Supplementary Video**). Both hands remained motionless throughout the trial duration. If the horse did not consume the LVR, the HVR was made available by stretching the hand forward after the delay had passed. If a horse consumed the LVR, the hand holding the HVR was closed and withdrawn. The next trial started after an inter-trial interval of 5–10 s, once the horse had finished chewing.

Per session 15 trials were conducted. Up to three sessions were performed per day with at least a 5-min break in-between sessions (see **Table 1** for overview). Each test was video recorded. If a horse waited for the HVR in at least three out of 15 trials within a session, he/she proceeded to the next delay stage. The delay between LVR and HVR was increased in a stepwise manner depending on the horses' success, starting at 2 s, then 5, 10, 15, 20, 25, 30, 40, 50, and up to a maximum of 60 s. If a horse did not reach this criterion, the session was repeated. A maximum of six sessions was conducted per delay stage and if a horse did not reach the criterion within these six sessions, the test was terminated.

The horses were tested until they gave up waiting or reached the maximum delay stage of 60 s. All horses were tested in two test phases: (1) *eyes visible*: the experimenter directly gazed at the horse during the whole delay duration; (2) *eyes invisible*: the experimenter wore sunglasses during the test, thus, shielding the eyes. Horses were randomly assigned to start with either of the two test phases (eyes visible first: $N = 25$; eyes invisible first: $N = 31$) and switched to the respective second phase (at the 2 s delay) once they gave up waiting or once they had reached the maximal delay stage of 60 s.

Analyses

For each test session, the number of choices for the HVR (=waiting) as well as number of trials waiting for the LVR (=not waiting) was noted. Based on this data the maximum delay stage (last delay stage that was successfully completed) was extracted for each horse. If a horse did not pass the training criterion, it was excluded from the analyses ($N = 4$). If a horse did not pass the 2 s delay stage in the test phase, a maximum delay of 0 s was entered. If a horse successfully passed all

TABLE 1 Overview and order of training and test procedure (for a horse assigned to the eyes visible-first test group).

Order	Phase	Choice	Delay	Sessions	Trials	Criteria
1	*Food Preference Test*	LVR vs. HVR	–	Until criterion reached or max. 6	20	Choice for HVR in at least 16 trials
2	*Training*	LVR vs. HVR after 1 s	1 s	Until criterion reached or max. 6	15	Waiting for HVR in at least 12 trials
3	*Test – eyes visible*[a]	LVR vs. HVR after Xs	2, 5, 10, 15, 20, 25, 30, 40, 50, and 60 s	Until criterion reached or max. 6 per delay stage	15	Waiting for HVR in at least 3 trials
4	*Test – eyes invisible*[a]	LVR vs. HVR after Xs	2, 5, 10, 15, 20, 25, 30, 40, 50, and 60 s	Until criterion reached or max. 6 per delay stage	15	Waiting for HVR in at least 3 trials

[a]The order of the two test phases was counterbalanced across horses. Once a horse had reached her/his max. delay stage, the next phase started at the 2 s delay.

delay stages up to the maximum delay stage, 60 s was entered as maximum delay.

A second coder coded 15% of the videos. To assess inter-observer reliability, we calculated the intra-class correlation coefficient (ICC) using the 'irr' package (version 0.84.1; Gamer et al., 2019). Consistency between coders was very good [ICC (two-way, consistency): LVR choices: ICC = 0.949, $p < 0.001$].

To assess whether individual and environmental factors affect the maximum delay stage tolerated by horses, we fitted an ordinal mixed model [i.e., cumulative linear mixed model (CLMM) with a logit-link function (Agresti, 2002)]. Maximum delay (factor: 0, 2, 5, 10, 15, 20, 25, 30, 40, 50, and 60) was set as the response variable while age in months (numeric), sex (factor: F, M), test phase (factor: eyes invisible and visible), test order (numeric: 1 and 2), housing (factor: group and individual), and roughage feeding management (factor: *ad libitum* and restricted) were included as predictors. An interaction term between test phase and test order was included to assess whether starting with a particular test phase influenced the maximally tolerated delay time. To control for repeated testing of horses and the involvement of different experimenters, we included horse ID and experimenter ID as random effects.

As a means to avoid 'cryptic multiple testing' (Schielzeth and Forstmeier, 2009), the full model was compared to a conceptual null model (comprising only housing and sex as predictors). Significance was determined by dropping one predictor at a time from the full model and compared the results with the full model using the *drop1* function. Model comparison was done by utilizing a likelihood ratio test (Dobson and Barnett, 2018). Considering that each horse was tested only twice (i.e., once in each test phase), random slopes were not identifiable. Prior to fitting the model, we checked the distribution of age, which was symmetrical, and subsequently scaled age to a mean of 0 and a standard deviation of 1 to facilitate model convergence.

The model was fitted using R (version 4.0.2; R Core Team, 2021) using the function *clmm* of the package 'ordinal' (version 2019.12-10; Christensen, 2019). Model assumptions of the CLMM (i.e., proportional odds, collinearity, and model stability) were assessed (see **Supplementary File** for diagnostics). Confidence intervals (95%) of the estimates and fitted values were determined by a parametric bootstrap ($N = 1000$ bootstraps) using a function kindly provided by Roger Mundry. The data set included 95 observations from 48 horses (note that horses that failed the training criterion were excluded from the analyses).

Results

On a group level, the horses tolerated a maximum delay of 36.08 ± 22.85 s (mean ± SD; median: 40 s). Twenty horses (41.67%) reached the maximum delay stage of 60 s (see **Figure 1**).

The full-null model comparison revealed that the full model described the data significantly better than the conceptional null model (Likelihood Ratio Test: $\chi^2 = 16.02$, df = 5, $p = 0.007$).

The interaction between test phase and order did not reveal significance (see **Table 1**). Likewise, no effect of sex or housing on the maximally tolerated delay stage could be detected. Older horses tended to reach higher delay stages compared to younger horses. And horses that had access to hay *ad libitum* tolerated higher delays (45.63 ± 20.01 s) compared to horses fed hay in a restricted manner (29.15 ± 22.43 s; see **Table 2** and **Figure 2**). Since the interaction between test phase and order was not significant, we removed the interaction from the model to assess the main effects of the two variables (see **Supplementary Table 3** for the estimates of the reduced model). Accordingly, test phase (eyes visible, invisible) did not significantly affect the horses' performance (CLMM: −0.186 ± 0.436, $p = 0.669$), however, the horses waited only for shorter delays in the second test (33.60 ± 24.47 s) compared to the first test (38.52 ± 21.12 s; CLMM: −0.931 ± 0.459, $p = 0.038$; **Figure 1**).

Experiment 2

Methods

Subjects and housing

We tested 30 horses [19 M/11 F; age (mean ± *SD*): 16.1 ± 6.2 years, range: 5.4–27.8 years; see **Supplementary Data 1** for details] of different breeds. Some horses were

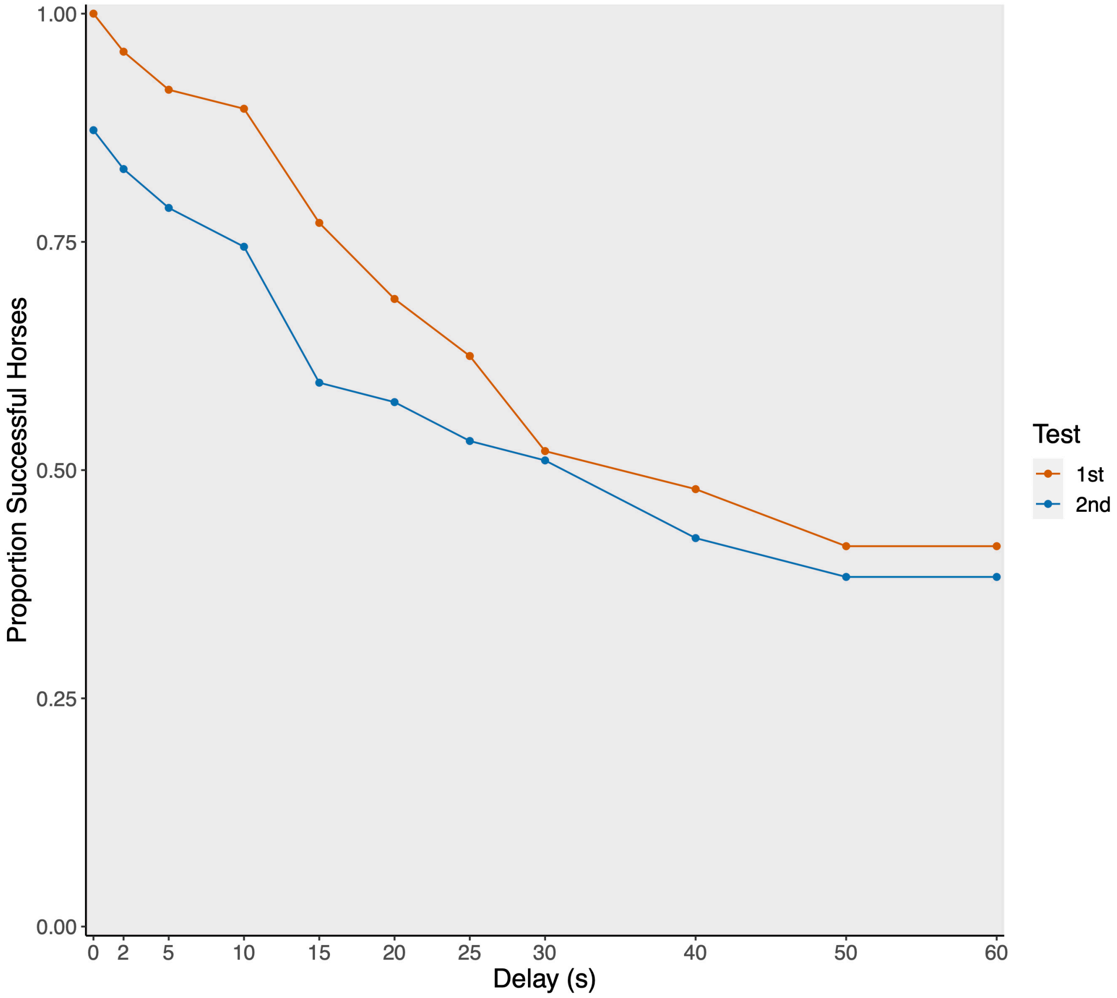

FIGURE 1
Proportion of successful horses per delay stage plotted separately for first (red; N = 52) and second (blue; N = 48) test.

privately-owned (N = 14) while other horses belonged to an equestrian center (N = 16). The horses were kept either in individual boxes with daily access to outdoor areas (N = 15) or in group-housing conditions (N = 15). To ensure an equal food motivation between horses that were fed hay *ad libitum* and horses that were fed hay in a restricted manner 2–3 times a day, all tests were conducted 0.5 h after the horses consumed their hay portion (restricted feeding) or at times of the day when the horses had not fed from the hay for at least 0.5 h (*ad libitum* feeding).

Owners were asked to fill in a questionnaire prior to starting the data collection. The questionnaire included questions related to (1) the horses' general trainability (How easily does your horse learn novel skills? How would you rate your horse's trainability?), (2) food motivation (How insistent is your horse when you have food in your bag? How food motivated is your horse?), and (3) coping abilities (How skittish is your horse? How patient is your horse? How susceptible to stress is your horse?). The questions could be answered on a 6-point Likert scale (see **Supplementary Data 3** for details).

Experimental procedure

The experimental setup and procedures were identical to those employed in Experiment 1; however, we made some small adjustments: All horses were tested individually in the same test-box (approx. 3 m × 3 m) with a nylon stable guard fixed to the door adjusted to chest height to ensure that all horses could reach across the box door independent of their height. To avoid visual distractions during the test, a fabric panel construction (1.85 m height) was set up around the test arena (see **Supplementary Video**).

The horses were tested in two conditions: a *quality condition* with the choice between an immediately available low-value reward (LVR) and a delayed high-value reward (HVR). And a *quantity condition*, in which the horses were given the choice between an immediately available low quantity reward (LQR; 1

TABLE 2 Effects of predictors on maximum delay duration based on CLMM with horse and experimenter as random effects (full model).

Term	Estimate	*SE*	lower CI	upper CI	Chisq	df	*P*-value
0| 2	−6.409	1.888	−11.599	−3.187			[2]
2| 5	−5.663	1.837	−10.499	−2.378			[2]
5| 10	−5.092	1.800	−9.453	−1.826			[2]
10| 15	−4.719	1.775	−9.145	−1.521			[2]
15| 20	−3.383	1.692	−7.530	−0.266			[2]
20| 25	−2.933	1.669	−6.909	0.208			[2]
25| 30	−2.509	1.650	−6.623	0.578			[2]
30| 40	−2.025	1.637	−6.032	1.097			[2]
40| 50	−1.520	1.630	−5.484	1.614			[2]
50| 60	−1.052	1.626	−4.771	2.133			[2]
Phase (eyes invisible)	1.309	2.558	−3.854	6.472			[2]
Order	−0.443	0.930	−2.480	1.326			[2]
Age[1]	0.846	0.477	−0.026	1.902	3.242	1	0.072
Sex (male)	0.648	0.820	−0.956	2.317	0.643	1	0.422
Feeding (restricted)	−2.239	0.873	−4.555	−0.733	7.391	1	**0.007**
Housing (individual)	0.094	0.886	−1.657	1.970	0.011	1	0.916
Phase (eyes invisible) × order	−1.006	1.697	−4.406	2.359	0.363	1	0.547

[1] Age was z-transformed to a mean of zero and standard deviation of one. Original variable: 176.71 ± 84.60 months.

[2] Not shown due to limited interpretability. Significant effects ($p < 0.05$) are highlighted in bold.

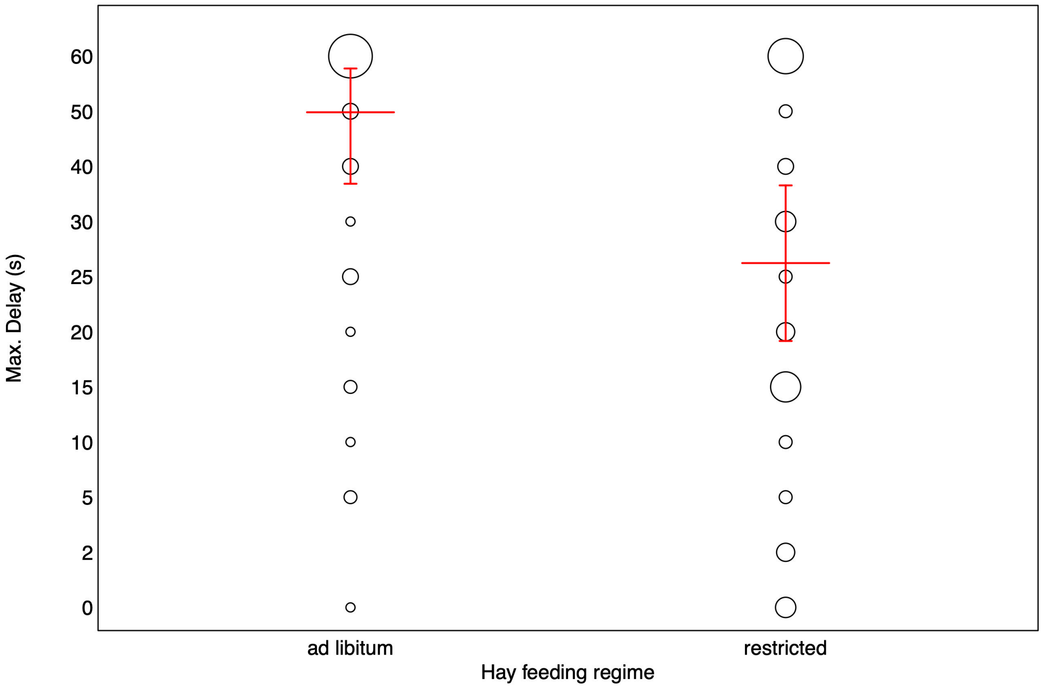

FIGURE 2
Maximum delay stages reached by horses fed hay *ad libitum* and restricted. Bubbles depict the frequency of maximally tolerated delay stages while the size of the bubbles corresponds to the number of horses [range: 1 (smallest bubble) – 23(largest bubble)]. The red horizontal bar depicts the fitted model and the error bars show the confidence limits for all other variables in the model centered to a mean of zero.

piece of reward) and a delayed high quantity reward (HQR; 5 pieces of reward). These quantity differences were selected as horses have been shown to successfully discriminate between even smaller quantities (Uller and Lewis, 2009; Petrazzini, 2014) (but see Henselek et al., 2012). Half of the trials in the quantity condition comprised the LVR reward (i.e., 1 vs. 5 pieces of LVR) and half of the trials the HVR (1 vs. 5 pieces of HVR). The order of LVR and HVR trials was semi-randomized with each trial type no more than twice in a row. Accordingly, two food preference tests were conducted, one for the quality condition and a separate preference test for the quantity condition [using the same criterion as in Experiment 1 (i.e., 16 out of 20 trials choice for HQR/HVR)] prior to starting with the respective condition (see **Supplementary Table 4** for timeline). Likewise, the training phase was conducted twice (once for each test condition). The horses were randomly assigned to start either with the quality condition ($N = 15$) or the quantity condition ($N = 15$) before switching to the respective other once they stopped waiting. In-between the two test conditions a break of 2 weeks was implemented.

And, horses were tested until they gave up waiting (i.e., not reaching criterion within 5 sessions) with no fixed upper delay limit. The order in which horses were tested was randomized per day and counterbalanced in total in a way that each horse was at least once tested first or last. Test sessions were performed on four consecutive days followed by a 3-day break. One female experimenter (AH) tested all horses wearing sunglasses throughout the test to minimize provision of subconscious mimic-based cues to the horse.

Analyses

Behavioral coding

The videos were coded using Solomon Coder (2015 by András Péter). The subject's choice (HVR or LVR) was coded as well as the latency to consume the reward. Furthermore, we analyzed the horses' behavior during the delay duration. Specifically, we coded the distance to the hand holding the LVR, the horses' attention, and other behaviors, such as oral manipulations, head movements, chewing, Flehmen, pawing, as well as reward-directed behaviors (see **Table 3** for detailed descriptions).

A second coder coded 10% of the videos and inter-observer reliability was calculated. Consistency between coders was good [ICC (two-way, consistency): LQR choices: ICC = 0.992, $p < 0.001$; looking away: ICC = 0.991, $p < 0.001$; head movements: ICC = 0.719, $p < 0.001$; large distance to food: ICC = 0.953, $p < 0.001$; pawing: ICC = 0.988, $p < 0.001$; chewing: ICC = 0.959, $p < 0.001$; sniffing LVR: ICC = 0.912, $p < 0.001$; pushing LVR: ICC = 0.810, $p < 0.001$; latencies to take food: all ICC > 0.842, all $p < 0.001$].

Statistical analyses

For each horse, the maximally tolerated delay stage was extracted. Furthermore, the behaviors recorded during the waiting duration (i.e., large distance, looking away, chew, oral manipulation, head movement, flehmen, and pawing) were summed up and subsequently divided by the total session duration to account for differences in length between sessions. Reward-directed behaviors were not included in the analysis, as these were observed only in a subset of horses ($N = 6$) and occurred rarely (median ± SD: 0.00 ± 0.08 proportion per test duration).

Similar to the analyses of Experiment 1, we wanted to find out whether individual characteristics affected individual performance in the test; accordingly, we included age, sex (factor: female and male), as well as housing (factor: individual and group) and feeding management (factor: restricted and *ad libitum*) as predictors. Furthermore, to assess whether horses performed better in the quality or quantity condition and whether the order of these tests matters, we included an interaction term between test condition (factor: quality and quantity) and test order (numeric: 1 and 2) into the model. To account for repeated testing of horses, horse ID was included as a random effect. Considering that each horse could reach only two maximum delay stages, no random slopes were identifiable. Prior to fitting the model, we z-transformed age to a mean of 0 and a standard deviation of 1.

Initially, we aimed at fitting a cumulative linear mixed model (CLMM) to model maximum delay as a factor; however, the proportional odds were strongly violated (see **Supplementary Table 6**). Consequently, we switched to fitting a generalized linear mixed model (GLMM1) with a poisson error distribution and a log-link function using the *glmer* function within the 'lme4' package (version 1.1–27.1; Bates et al., 2015). The same predictors were entered but maximum delay was included as a numeric variable. Model assumptions were checked prior to fitting the model (i.e., no zero-inflation was detectable and residuals of the random intercepts were symmetrical; see **Supplementary File** for additional diagnostics). We obtained confidence intervals via the *bootMer* function ($N = 1000$ bootstraps) within the lme4 package. The data set used for the maximum delay analyses consisted of 49 observations from 29 horses.

For ruling out that horses' performance was affected by satiation due to the high number of trials per session, we ran an additional binomial model (GLMM2) with success (binary: waiting/not waiting) as response variable and trial number (numeric: 1–15) as predictor. Horse ID was entered as random effect and trial number as random slope (see **Supplementary File** for details).

Verifying that the owners' answers to the questionnaire were indeed tapping into the same behavioral construct as intended, we ran correlations between all seven questions (see **Supplementary Data 3**). Variables that exhibited a high correlation, were averaged for further analyses. For assessing whether the owners' assessment of their horses' self-control abilities, general trainability, food motivation, coping abilities, and patience were linked to the individually reached maximum delay times, we ran Spearman correlations with a Bonferroni correction for multiple testing. For this

TABLE 3 Ethogram of coded behaviors.

Category	Variable	Description
Latency choice	Latency to consume LVR/HVR	Time from start of trial until horse either closed his/her lips around the LVR or until the delay time has passed
Attention	Look away	Head turned away in >45° angle from experimenter
Distance	Large distance	Horses' mouth is more than 0.5 m away from the hand holding the LVR
Other behaviors	Empty chew[a]	Horse chews without having food in his/her mouth
	Oral manipulation	Licking, nibbling or biting into barrier/box/door or own body parts
	Head movement	Any repeated movements with the head (i.e., horizontal and vertical movements or rotational movements)
	Flehmen	Lifting the upper lip, usually associated with a forward stretched neck
	Pawing	Repeatedly lifting one leg and scratching with the hoof on the ground
Reward-directed behaviors	Sniffing LVR	Sniffing on LVR without taking it into the mouth
	Pushing LVR	Pushing hand holding LVR away with mouth or head

[a] Only coded after 10 s of a trial had elapsed to avoid coding instances of horses still chewing the previous reward.

analysis, we used only the highest delay stage that each horse reached in the test.

To analyze how coping behaviors affected individual waiting success within a session, we fitted a logistic generalized linear mixed model (GLMM3). The response variable was entered as a two-column matrix with the number of successes (=choice HVR) and the number of failures (=choice LVR) per individual using the *cbind* function (Baayen, 2008). Since we were interested in the influence of coping behaviors as delays increased and whether horses used these coping behaviors differently across the two test conditions, we included two interaction terms between coping and delay, and between coping and test phase into the model. Furthermore, age and sex were included into the model as control variables. As random effects, we included horse ID and session number nested within horse ID.

To avoid overconfident model estimates and to maintain the type I error rate at 0.05, random slopes were included into the model (Schielzeth and Forstmeier, 2009). Delay (as a numeric variable) and coping behaviors were included as slopes for the intercept of session number nested in horse ID; and delay (as numeric variable) as well as an interaction between test phase (manually dummy coded and centered to a mean of 0) and coping behaviors were included as slopes for the intercept of horse ID.

The model was fitted using the *glmer* function with the 'lme4' package (version 1.1–27.1; Bates et al., 2015) with a binomial error distribution and logit-link function. After an exploratory analysis, we decided to set the 10 s delay stage as reference level for the delay variable as this seems to be a more biologically relevant stage than the 2 s delay (see **Supplementary File** for details). Confidence intervals were obtained via parametric bootstrapping (N = 1000 bootstraps) using the function *bootMer* within the lme4 package. The data set for this analysis consisted of 551 observations form 29 horses.

To assess whether the horses' success in the quantity condition differed between trials with the HVR and the LVR, we fitted an additional binomial GLMM (GLMM4) with the number of waiting success (=choice delayed option) and the number of failures (=choice immediate option) as a response matrix. As predictors, we included age (z-transformed to mean of 0 and standard deviation of 1), sex (factor: female and male) and an interaction term between reward type (factor: LVR and HVR) and delay stage (factor: 0, 2, 5, 10, 20, 30, 40, 60, and 80 s). To account for repeated testing of horses, we included two random intercept terms: horse ID and session number nested within horse ID. Delay (numeric) and reward type (factor) were included as random slopes for both random effects terms (see **Supplementary File** for model diagnostics).

Deciding early on in a trial whether it is worth waiting or not is generally seen as an indication for anticipation of the upcoming delay duration, as it does not pay to invest energy into a resource that is devaluated by a large delay (e.g., Pelé et al., 2010; Auersperg et al., 2013). To find out whether the horses gave up waiting at a random time point within each trial or rather at the beginning or end of a trial, we ran an analysis of the error times. The distribution of observed error times (=latency to consume LVR) was compared with the distribution of error times expected under the null hypothesis of a constant giving up chance during the trial. If horses can anticipate the upcoming delay duration, we would expect that horses decide early on in a trial whether the delayed reward is worth waiting for. Using a Kaplan–Meier survival analysis, we calculated the estimated probability to continue waiting at each time point that the horses gave up waiting. The error times (i.e., time point at which the LVR was consumed) as well as successful trials (i.e., successfully waiting for HVR) were entered as censored data. The survival probability (chance to wait longer than elapsed time within a trial) and the expected distribution (chance to wait under null hypothesis) were compared using an adjusted

Kolmogorov–Smirnov test (Haccou and Meelis, 1992). The analysis was run only for the delay stages above 5 s.

Results

Maximum delay

In the second experiment, seven horses did not pass training in the quality condition (25%) while all horses passed the training in the quantity condition. One horse was not food motivated and was excluded at the beginning of the study. An additional horse lost interest in the test and refused to participate at some point during the second test condition (the first test condition was still included in the analyses) and another horse did not pass the food preference test for the quality condition.

On a group level, the horses tolerated a delay of 13.35 ± 14.45 s (median: 10 s) in the quality and 15.07 ± 11.17 s (median: 10 s) in the quantity condition (see **Figure 3**).

Overall, the full model fitted the data significantly better than the null model (Likelihood ratio test: $\chi^2 = 11.11$, $p = 0.049$). Age did not significantly affect individual performance (see **Table 4**). Horses fed hay *ad libitum* tended to reach higher delay stages than horses fed hay in a restricted manner. Furthermore, we could detect a significant interaction between condition and test order. Horses that started with the quantity condition performed better in the quality condition compared to horses that started with the quality condition first (see **Table 4** and **Figure 4**).

We found no effect of trial number on success (GLMM: -0.008 ± 0.007, z-value $= -1.146$, $p = 0.252$), accordingly, horses were equally likely to wait in the beginning, middle, and end of a session (see **Supplementary File** for details).

The owners' assessment of their horses' self-control abilities was not related to the maximum delay stage that their horse reached (Spearman: $N = 29$, $r_s = 0.02$, $p = 0.903$). Furthermore, none of the other variables showed a significant correlation with the horses' performance in the test (see **Supplementary Data 3**).

Influence of distraction behaviors on waiting performance

The full model fitted the data significantly better than the conceptual null model with only sex and age as predictors (Likelihood ratio test: $\chi^2 = 130.42$, df = 17, $p < 0.001$). Horses that showed more coping behaviors were more successful, in particular during the delay stages of 20, 30, and 40 s compared to the 10 s delay stage (see **Table 5** and **Supplementary Figure 2**). During lower delay stages (i.e., 2 and 5 s), coping behaviors did not affect waiting success. For the high delay stages (60 and 80 s), coping behaviors were not linked to waiting success either. Coping behaviors did not differ between the two test conditions (see **Table 5**).

In the quantity condition, the full model containing the reward type – delay stage interaction did not explain the data better compared to the null model lacking the reward type term (LRT: $\chi^2 = 9.05$, df = 8, $p = 0.338$). Accordingly, the interaction between reward type and delay was not significant (LRT: $\chi^2 = 3.77$, df = 7, $p = 0.805$). To assess whether reward type affected waiting success as a main effect, we fitted a reduced model lacking the interaction term between reward type and delay stage; however, also as a main effect, reward type did not significantly affect waiting success (GLMM: -0.035 ± 0.202, $z = -0.174$, $p = 0.862$; see **Supplementary Table 11** for complete model results).

Error times

The majority of horses (21 out of 29 horses) gave up waiting at the beginning of trials instead of at random time points throughout the trials (see **Supplementary Data 2**). These early error times occurred more often during higher delay times than during lower times. Interestingly, the majority of horses gave up waiting earlier than expected by a constant giving up chance in the quality condition (i.e., in higher delay stages; 75% of horses) while only half of the horses gave up waiting significantly earlier than expected in the quantity condition (see **Supplementary Material**).

Discussion

We found that horses were able to wait for a delayed reward of better quality and quantity up to 60 s in a delay of gratification paradigm. Individual variation in self-control was consistently explained by hay feeding management in both experiments as horses having access to hay *ad libitum* reached higher delay stages than horses with restricted access to hay. We found no correlations between the behavioral traits assessed by the owners and the horses' success in the test. Horses that engaged in many distraction behaviors were more successful than horses that exhibited only few of these behaviors during the waiting time.

Individual variation in self-control

We observed great individual variation in self-control abilities amongst the horses. Some horses did not manage to pass the 2 s delay, whilst others successfully waited for 60 s. Older horses tended to reach higher delays in Experiment 1 but this effect was not replicated in Experiment 2. Also, sex did not explain individual differences; however, it needs to be noted that no horses below 3.5 years and no stallions were included in the study. The horses' housing conditions (group-living vs. individual boxes) did not account for the observed individual differences in self-control; however, the hay feeding regime was related to individual performance. In both experiments, we found that horses with permanent access to hay (*ad libitum* feeding) reached or tended to reach higher delay

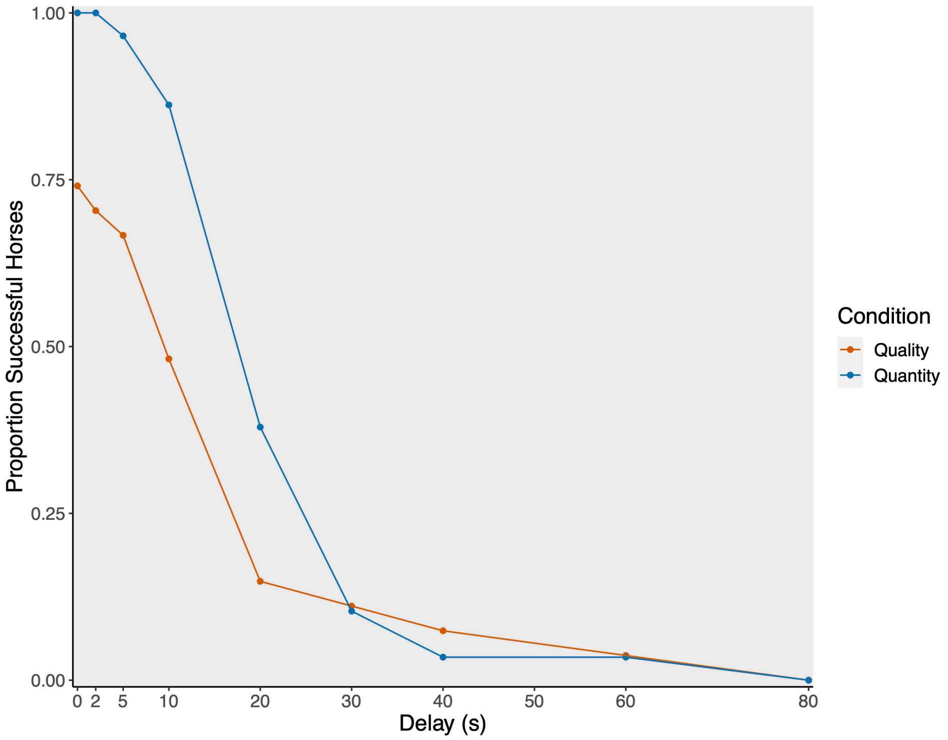

FIGURE 3
Proportion of successful horses per delay stage plotted separately for quality (red; *N* = 28) and quantity condition (blue; *N* = 29).

TABLE 4 Effects of condition, order, age, and feeding management on maximally reached delay durations (full model; GLMM1).

Term	Estimate	*SE*	Lower CI	Upper CI	Chisq	df	*P*-value
Intercept	0.980	0.484	−0.006	1.947			[2]
Condition (QUAN)	2.077	0.662	0.689	3.403			[2]
Order	0.946	0.314	0.296	1.578			[2]
Age[1]	0.073	0.096	−0.127	0.257	0.557	1	0.456
Feeding (restricted)	−0.431	0.227	−0.859	0.031	3.403	1	0.065
Condition (QUAN) × Order	−1.202	0.421	−1.994	−0.318	7.382	1	**0.007**

[1] Age was z-transformed to a mean of zero and standard deviation of one. Original variable: 197.06 ± 77.74 months.

[2] Not shown due to limited interpretability. Significant effects ($p < 0.05$) are highlighted in bold.

stages compared to horses with only restricted access to hay. While satiation during the course of sessions did not account for success, this effect might be due to two factors, on the one hand, unlimited access to hay might make horses generally more satiated, which in turn can facilitate self-control. On the other hand, general food availability might affect self-control abilities. If food is constantly available without any shortages (as in horses fed hay *ad libitum*), it might be valued differently and more risky foraging decisions for delayed options could be made. For example, honey bees show less self-control, if they are hungry (Mayack and Naug, 2015) and experiences of food shortage reduces self-control in children (Jackson et al., 2018). During food-shortages, immediate energy intake rather than waiting, and thus, increasing the risk of losing all of the available food, is likely more adaptive. While one study found no effect of satiation on self-control abilities in capuchins (De Petrillo et al., 2015), more studies investigating the effects of food availability and resulting relative food values on self-control are warranted. These results open up numerous novel research questions pertaining to the influence of food availability (i.e., hay feeding regime) on general learning capacities, cognitive performance, and emotional states in horses.

Horses that engaged in certain behaviors while waiting for the delayed reward were more successful. In particular, increasing the distance to the LVR, looking away, repetitive horizontal or vertical head movements, pawing, empty chewing, and nibbling of box or body, were related to a better performance in the test. All these behavioral patterns are related

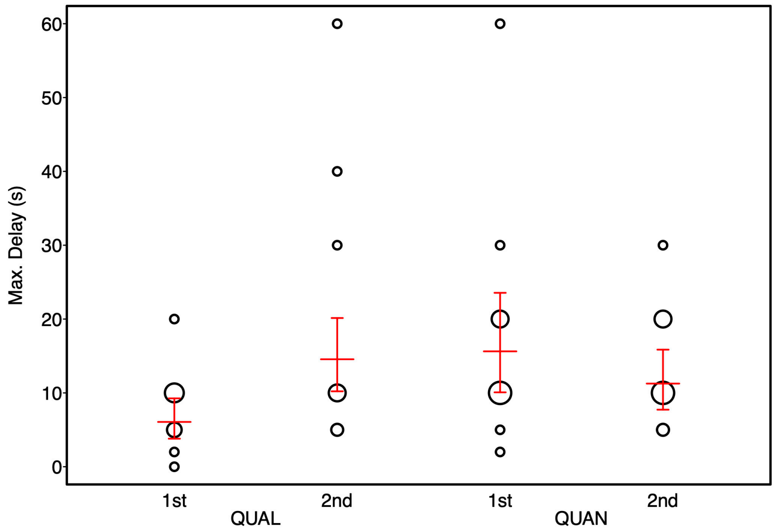

FIGURE 4
Maximum delay stages reached by horses in the quality (QUAL) and quantity (QUAN) test condition as a function of test order. Bubbles depict the frequency of maximally tolerated delay stages while the area of the bubbles corresponds to the number of horses (range: 1–7). The red horizontal bar depicts the fitted model and the error bars show the confidence limits for feeding management centered to a mean of zero.

TABLE 5 Effects of age, sex, amount of coping behaviors, test phase and delay on number of successful trials (GLMM3).

Term	Estimate	*SE*	Lower CI	Upper CI	Chisq	df	*P*-value
Intercept	−0.709	0.640	−1.894	0.518			[2]
Age[1]	0.130	0.390	−0.613	0.956	0.110	1	0.740
Sex (M)	0.054	0.777	−1.402	1.442	0.005	1	0.945
Coping behav[1]	1.304	0.288	0.727	1.980			[2]
Phase (Quan)	0.652	0.382	−0.080	1.441			[2]
Delay 2 s	2.361	0.29	1.805	2.897			[2]
Delay 5 s	1.462	0.194	1.101	1.842			[2]
Delay 20 s	−4.165	0.352	−4.886	−3.537			[2]
Delay 30 s	−7.874	0.718	−9.428	−6.656			[2]
Delay 40 s	−12.548	1.372	−13.937	−11.037			[2]
Delay 60 s	−15.747	3.461	−16.692	−14.395			[2]
Delay 80 s	−26.931	4.613	−27.802	−25.980			[2]
Cope:Phase	−0.314	0.316	−0.955	0.370	0.972	1	0.324
Cope:Delay 2 s	−0.023	0.212	−0.582	0.526	[3]	[3]	0.914
Cope:Delay 5 s	0.229	0.173	−0.191	0.645	[3]	[3]	0.185
Cope:Delay 20 s	0.553	0.185	0.026	1.084	[3]	[3]	**0.003**
Cope:Delay 30 s	0.864	0.243	0.138	1.734	[3]	[3]	**<0.001**
Cope:Delay 40 s	1.341	0.468	0.147	2.474	[3]	[3]	**0.004**
Cope:Delay 60 s	0.314	1.188	−0.963	1.539	[3]	[3]	0.791
Cope:Delay 80 s	1.924	1.367	0.920	3.262	[3]	[3]	0.159

Note that the 10 s delay stage was set as reference level for the delay variable.

[1] Variables were scaled to a mean of 0 and a standard deviation of one. Original variables (mean ± SD): age = 197.06 ± 77.74 months; proportion of coping behaviors per test duration = 0.40 ± 0.38.

[2] Not depicted due to limited interpretability.

[3] Likelihood ratio test for coping × delay: Chisq = 20.296, df = 7, $p = 0.005$. Significant effects ($p < 0.05$) are highlighted in bold.

to directing the attention away from the available LVR. To tease apart whether these behaviors are indeed a way to divert the attention away from the food reward or rather only represent individual differences in general activity or frustration, one would need to implement a control condition, in which food is present but inaccessible (similar to Evans and Beran, 2007). Interestingly, these behavioral patterns seem to be very similar across animal species from parrots (e.g., Auersperg et al., 2013;

Brucks et al., 2021), to canids (Range et al., 2020), cephalopods (Schnell et al., 2021), chimpanzees (Evans and Beran, 2007), and humans (Steelandt et al., 2012). We also observed reward-directed behaviors in horses (i.e., sniffing LVR and pushing LVR away); however, since these behaviors were recorded only very infrequently, we were not able to analyze them. Similar reward-directed behaviors have so far only been reported in chimpanzees (Evans and Beran, 2007), children (Steelandt et al., 2012) and in a gray parrot (Koepke et al., 2015) and were not consistently related to success.

Furthermore, we found that the horses exhibited non-random giving up times, especially during higher delay stages and more frequently in the quality condition compared to the quantity condition. Deciding whether a delayed reward is worth waiting should be made in the beginning of a trial to reduce the amount of effort invested into a reward that is temporally discounted by a delay. Especially, during higher delays it pays to make such waiting decisions early on in a trial. Similarly, also dogs (Leonardi et al., 2012; Brucks et al., 2017b), parrots (Auersperg et al., 2013; Schwing et al., 2017; Brucks et al., 2021), and primates (Dufour et al., 2007; Pelé et al., 2011) give up waiting earlier than expected during higher delay stages. Whether giving up times are purely explained by decisions about anticipated time or rather also by frustration about not receiving the delayed option, however, is difficult to infer.

Simplified delay of gratification paradigm and effects of test procedures on performance

To make the delay of gratification test more easily implementable in an applied setting, we simplified the test procedure in a way that no additional equipment is needed to conduct the test. Furthermore, since horses were not required to directly exchange food items but rather only refrain from consuming them, we could reduce the training to a minimum. Horses are very sensitive to various human social cues (e.g., Clever Hans; Pfungst, 1911; Proops et al., 2010) and since our simplified test design involved direct interactions with an experimenter we aimed at testing whether one of the potential cues emitted by the experimenter, namely the gaze, affected horses' performance in the test. For example, dogs refrain from taking food, if a human is directly gazing at them but are less inhibited, if the human closes the eyes (Call et al., 2003). Consequently, also horses might experience social inhibition when the experimenter directly gazes at them. We found that horses reached equally high delay stages independent of whether the experimenter's eyes were visible (i.e., directly gazing at the horse) or invisible (i.e., wearing sunglasses); accordingly, the presence of the experimenter's eyes did not induce social inhibition in horses. It needs to be noted that the experimenter could have provided also other subtle behavioral cues to the horses, such as changes in body tension or posture. To rule out effects of such subtle behavior, however, one would need to conduct the test in the absence of an experimenter, for example in an automated setting (e.g., Evans and Beran, 2007) or at hide the experimenter behind a barrier (e.g., Brucks et al., 2017b).

While the results from Experiment 1 showed that the simplified delay of gratification was feasible in a farm setting and resulted in enormous individual variation in performance, we wanted to ensure that the inclusion of multiple experimenters and the rather relaxed success criteria did not affect the horses' self-control abilities in our simplified test. Consequently, we refined the procedures in Experiment 2 for making the results more comparable to other studies.

Indeed, a comparison between the horses' performance in Experiment 1 and Experiment 2, revealed differences in the maximally tolerated delay [median exp. 1: 40 s, exp. 2 (quality): 10 s]. Even though individual variation is likely one explanation for the observed differences in self-control abilities between the two study populations, small modifications in the test procedure might also account for it. Firstly, only one female experimenter conducted all tests; thus, reducing the individual variation in the experimenter's behavior. Secondly, the test environment was more controlled as all horses were tested in the same box with a barrier adjusted to their height. And thirdly, we adjusted the criteria for success to reduce the occurrence of horses passing a delay stage by chance as horses were required to show a stable performance in consecutive sessions instead of passing the criteria only once. To avoid overtraining the horses due to the stricter criterion for success, we reduced the number of delay stages (i.e., fewer incremental steps) in Experiment 2. Furthermore, different reward types were used as LVR in Experiment 1 (hay) and Experiment 2 (lucerne), which could have affected horses' ability to wait. Indeed, horses needed fewer sessions to reach the criterion in the food preference test in Experiment 1 (mean: 1.18 sessions, range: 1–3) compared to Experiment 2 (mean: 2.39 sessions; range: 1–6); thus, indicating that lucerne was potentially valued higher compared to hay as LVR. These differences in self-control performance of horses in the two experiments indicates that rather small changes in procedure can greatly affect the outcome of studies. Furthermore, control conditions should be implemented to rule out that the horses rely on avoidance learning (i.e., always avoid reward on one side) instead of having a complete understanding of the task's contingencies. For example, the sides of rewards (left/right) could be randomly switched instead of keeping fixed sides, the order of rewards could be reversed (i.e., HVR first and LVR after delay), or both rewards could be of low quality. But, also the criteria for success, and the experimental paradigms (e.g., standard exchange task and rotating tray task) might affect individual self-control abilities. Certainly, the development of simpler experimental procedures opens up the possibility to collect data on a larger scale (e.g., using a citizen science approach), however, procedural caveats need to be considered carefully when designing such studies. Future studies need to investigate how such procedural differences affect individual self-control abilities.

Role of self-control in other behaviors

The horse owners' predictions about their horses' self-control abilities were not correlated with the actual performance in the test. This is interesting as past research has shown that owners are e.g., able to correctly predict their horses' reaction in behavioral tests (e.g., Ijichi et al., 2013); however, these behavioral tests did not involve any food rewards. Considering that most horse owners rely on negative reinforcement to train their horses instead of positive reinforcement using food rewards (McLean and Christensen, 2017), they might have only limited experience regarding their horses' behavior in the context of food. Self-control as assessed in the present study likely is linked very closely to food motivation (as also supported by the influence of the horses' hay feeding regimen, though contradicted by the missing link to owners' assessment of their horse's food motivation), and this is a different motivational axis compared to the motivations that need to be inhibited when, e.g., refraining from showing fear reactions to frightening stimuli or to show other strongly motivated behaviors in a training setting. Additionally, as suggested by McLean and Christensen (2017), training success may be affected by factors such as arousal, affective and attachment states. For example, arousal levels in the present training setting may have been different from those typically encountered for the participating horses in their regular training sessions with their owners. Generally, owners' assessment of their pets' behavioral tendencies should always be treated cautiously as pet owners might not be able to accurately predict their animals' behavior or might define behaviors differently compared to those assessed in experimental studies.

Individual self-control abilities were additionally not correlated with other behavioral traits that were rated by the owners, such as trainability, patience, food motivation, and reactions in stressful situations. In light of the findings that self-control abilities are linked to general intelligence (Moffitt et al., 2011; Beran and Hopkins, 2018), including learning performance (Schnell et al., 2021), we would have expected to observe better self-control in horses that were rated as more trainable and patient; however, this lack of a correlation between trainability and self-control might be due to two aspects. On the one hand, we did not test the horses' learning capacity or trainability but rather relied on the owners' assessment, and, on the other hand, it needs to established whether self-control is actually consistent in situations involving food (i.e., delay of gratification paradigm) and situations outside of the food context (i.e., training or handling).

Further investigations into the links between inhibitory control and trainability, but also general coping capacities, are warranted. Individual differences in inhibitory control abilities, and self-control in particular, might be responsible for differences in coping abilities and behavioral flexibility in captive animals. Considering that in humans, self-control can be improved by training (Murray et al., 2016; but see Friese et al., 2017), similar training regimes might be implemented in horse training to improve human-animal interactions and ultimately horse welfare.

Horses' self-control abilities in a comparative context

Considering that horses' foraging behavior requires only little self-control as resources are evenly distributed with slowly changing quality, and they face no delays to access the resource, we would have expected horses to show rather poor self-control in such a food-based delay of gratification paradigm. Contrary to our hypothesis, horses exhibited rather good self-control abilities on a group level. In Experiment 1, the horses waited on average for 36.1 s (median: 40 s), while in Experiment 2, the average of the maximally tolerated delay was 13.4 s (median: 10 s) in the quality condition and 15.1 s (median: 10 s) in the quantity condition. In both experiments, a number of horses successfully waited for 60 s, while, for example, dogs waited for up to 15 min (Leonardi et al., 2012; Brucks et al., 2017b), pigs up to 20 s (Zebunke et al., 2018), chicken up to 7 s (Abeyesinghe et al., 2005). Other non-domesticated species, however, tolerated much higher delay times [e.g., long-tailed macaques: 21 min (Pelé et al., 2010), cleaner wrasse: 480 s (Aellen et al., 2021), ravens: 640 s (Hillemann et al., 2014), and cuttlefish: 130 s (Schnell et al., 2021)]. Interestingly, horses were more successful in the quantity condition compared to the quality condition. All horses passed the food preference test and training in the quantity condition, while several horses failed to pass these pre-tests in the quality condition. Furthermore, starting with the quantity condition subsequently facilitated success in the quality condition but not vice versa. Previous research in other species has shown that many species are more willing to wait for rewards of better quality than quantity (e.g., cockatoos: Auersperg et al., 2013; corvids: Hillemann et al., 2014; dogs: Brucks et al., 2017b; pigs: Zebunke et al., 2018; and children: Miller et al., 2019b). Cleaner wrasse, which regularly encounter quantitative but only rarely qualitative differences in resources in a natural context, tolerated higher delays if the rewards differed in terms of quantity (Aellen et al., 2021). Accordingly, the ability of horses to wait for quantitative, but less so for qualitative rewards, might be linked to their foraging ecology; under natural conditions resources are more evenly distributed and do not differ as strongly in quality (Devenport et al., 2005) as for species with other foraging styles (e.g., dogs, parrots, and corvids). This aspect of horses' foraging ecology might make it more adaptive to pay attention to differences in quantity.

Other studies, however, suggest that social complexity is the main driver for the evolution of self-control abilities (Amici et al., 2008; Aellen et al., 2021; but see Schnell et al., 2021). Horses live in complex social environments (Krueger, 2008) and dominance regulates access to limited resources (Ingólfsdóttir and Sigurjónsdóttir, 2008); consequently, self-control in social interactions is certainly important as, for example, subordinate horses need to refrain from accessing limited resources in the presence of dominant horses. Accordingly, our results certainly lend some support for this hypothesis; however, since comparable data of self-control abilities in closely-related species is missing, it is difficult to draw strong conclusions. Equidae with their small variation in foraging ecology but differences in sociality (Linklater, 2000) definitely pose an interesting model taxon to further investigate the role of sociality in the evolution of self-control abilities.

Conclusion

Horses showed great individual variation in their self-control abilities ranging from 0 to 60 s. This variation is partly explained by food availability (i.e., hay feeding regime) and reward type (quality and quantity), but also engagement in distraction behaviors during the waiting period. Our study provides the first data on self-control abilities in a grazing species; thus, broadening our knowledge about underlying evolutionary forces driving the evolution of self-control across animal species. While we found no link between self-control and behavioral traits of horses outside of the test context, we hope that our study gives rise to further research questions related to horse welfare, such as understanding the role of self-control in coping behaviors and general trainability.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Animal Welfare Officer, University of Giessen (approval number: JLU_kTV_6_2022). We adhered to the Guidelines for the Treatment of Animals in Behavioural Research and Teaching (Animal Behaviour, 2020). Written informed consent for participation was not obtained from the owners because some owners conducted the study themselves (i.e., in Experiment 1) and the other owners were verbally informed and gave consent for their horses to participate in the study.

Author contributions

DB and UKvB designed the study. AH collected the data and scored the videos. DB analyzed the data and wrote the first draft of the manuscript. UB reviewed and edited the manuscript. All authors read and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2022.954472/full#supplementary-material

SUPPLEMENTARY FILE
Model diagnostics (i.e., model stability, tests for odds assumptions in CLMM) and outputs of reduced models.

SUPPLEMENTARY VIDEO
Video illustrating the food preference test, training and test procedure.

SUPPLEMENTARY DATA 1
Individual characteristics, housing and hay feeding regime of horses participating in Experiments 1 and 2.

SUPPLEMENTARY DATA 2
Results of Kaplan–Meier analyses of error times for each horse.

SUPPLEMENTARY DATA 3
Overview of questions (translated from German) asked in the questionnaire with scoring options and correlations between owners' answers.

SUPPLEMENTARY DATA 4
Raw data sets of Experiments 1 and 2.

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Brain gain—Is the cognitive performance of domestic hens affected by a functional polymorphism in the serotonin transporter gene?

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The serotonin transporter (5-HTT) plays an important role in regulating serotonergic transmission *via* removal of serotonin (5-HT) from synaptic clefts. Alterations in 5-HTT expression and subsequent 5-HT transmission have been found to be associated with changes in behaviour, such as fearfulness or activity, in humans and other vertebrates. In humans, alterations in 5-HTT expression have been suggested to be able to lead to better learning performance, with more fearful persons being better at learning. Similar effects of the variation in the 5-HTT on fearfulness have been found in chickens, and in this study, we investigated effects on learning. Therefore, we tested 52 adult laying hens, differing in their functional 5-HTT genotype (W/W, W/D and D/D) in an operant learning paradigm in three different phases (initial learning, reversal learning and extinction) and in a tonic immobility test for fearfulness. We found that the 5-HTT polymorphism affects the initial learning performance of laying hens, with homogeneous wild-type (W/W) hens being the slowest learners, and the most fearful birds. W/W hens, showed significantly more choices to solve the initial learning task (LME, $p=0.031$) and had the highest latencies in a tonic immobility test ($p=0.039$), indicating the highest fearfulness. Our results provide interesting first insights into the role of 5-HTT in chickens and its sensitive interaction with the environment. We further suggest that the 5-HTT gene can be an interesting target gene for future breeding strategies as well as for further experimental studies.

KEYWORDS

chicken cognition, learning, serotonin transporter, domestic chicken, reversal learning

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is an important hormone in the body as well as a key regulatory neurotransmitter in the brains of vertebrates (Lesch et al., 1996). The transmission of neural serotonin from the pre-to the postsynapse is controlled through a reuptake of 5-HT from the synaptic clefts by the serotonin transporter (5-HTT, abbreviation for the 5-HT transporter). Thus, serotonin transporters are crucially involved in the neural serotonin regulatory system. In humans, a polymorphism of the 5-HTT gene is associated with functional consequences in the emotional system (Lesch et al., 1996). Humans with an S-allele, i.e. a short allele, in comparison to the individuals with an L-allele, i.e. long allele, have a lower 5-HTT expression as a result of decreased 5-HTT gene promoter efficiency. This change contributes to behavioural modifications, such as increased anxiousness-like traits or the prevalence of the occurrence of depression (Lesch et al., 1996; Canli and Lesch, 2007). Similar patterns are also found in other mammalian species, such as monkeys (Lesch et al., 1997) and rodents (Brigman et al., 2010).

These effects of polymorphisms in the 5-HTT gene on behaviour have led the focus to similar effects in other mammals. The domestic chicken possesses a functional polymorphism in the 5-HTT gene (Phi-van et al., 2014), with a wild-type allele (W) and a deletion allele (D). In the deletion allele, four bases are deleted (5′-AATT-3′), and a single base (A → T) is exchanged (Phi-van et al., 2014) in comparison to the wild-type allele. Interestingly, the W allele of domestic chickens functionally resembles the human S allele in terms of lowered 5-HTT expression and increased fear-like behaviours (Phi-van et al., 2014; Krause et al., 2017; Phi Van et al., 2018). In line with this behaviour, the D-allele of domestic chickens shows 5-HTT and behavioural pattern expression comparable to the human L-allele. Domestic chickens with D-alleles, in comparison to homozygous W-allele chickens, show increased body mass and abdominal fat deposition (Phi-van et al., 2014; Kjaer and Phi van, 2016; Krause et al., 2017), increased feed uptake during ontogeny (Kjaer and Phi van, 2016), increased locomotor activity (Phi-van et al., 2014; Krause et al., 2019), and a lower level of fearfulness (Krause et al., 2017, 2019) but no differences in social-related behaviours (Krause et al., 2019).

One aspect, linked to the polymorphism at the 5-HTT gene in humans, has not yet been studied in domestic chickens. In humans, 5-HTT polymorphisms are thought to be linked to cognitive performance, such as learning (Lesch et al., 1997). The S-allele human carriers tend to show increased attention towards biological conditioned stimuli and hence might perform better in learning (Homberg and Lesch, 2011). This assumption is supported by other studies showing that humans carrying S-alleles perform better in decision-making and learning than homozygote L-allele carriers (Roiser et al., 2007; Strobel et al., 2007; Madsen et al., 2011; Karabeg et al., 2013). In line with these findings, mice and monkeys demonstrated a higher flexibility in learning when carrying shorter alleles in various reversal learning tasks using visual or auditorial cues (Brigman et al., 2010; Jedema et al., 2010; Harris et al., 2012). However, in rats, such differences were not revealed (Karabeg et al., 2013). Based on the strong indications of a role of 5-HTT polymorphisms in cognitive performance in mammalian species, we aimed to address the question of whether the domestic chicken polymorphism in the 5-HTT gene also has a functional impact on cognitive performance.

Complex forms of learning are in general quite well documented in domestic chickens (Krause et al., 2006; Marino, 2017; Dudde et al., 2018; Garnham and Løvlie, 2018). Thus, we aimed to characterise the cognitive abilities of domestic chickens with three different 5-HTT genotypes, homozygous wild-type W/W, homozygous deletion D/D and heterozygous genotype W/D, and to validate their differences in fearfulness using a tonic immobility test (Krause et al., 2019). To study their cognitive performance, we used an established automated operant learning paradigm for domestic chickens (Dudde et al., 2018), which included initial associative learning, followed by reversal learning and finally an extinction phase. In accordance with mammalian studies and the convergence of the human S-allele with the domestic chicken W-allele, we assume that domestic chickens with a homozygous wild-type allele W/W would perform better in the cognitive task and be more fearful compared to the homozygous deletion D/D birds, while heterozygous chickens should perform in the intermediate range.

Materials and methods

Animals and housing

Adult domestic hens (*Gallus gallus* forma domestica) with polymorphisms in the flanking region of the 5-HTT gene were used (Phi-van et al., 2014). The D-allele is characterised by the deletion of four nucleotides (5′-AATT-3′) and a nearby single nucleotide substitution (A → T) compared to the wild-type allele W (Phi-van et al., 2014; Phi Van et al., 2018). From these two alleles, three 5-HTT genotypes appeared: homozygous wild-type W/W, homozygous deletion-type D/D and heterozygous W/D. The hens with the three 5-HTT genotypes were obtained through a controlled breeding regime using W/W and D/D parents from the laboratory stock, returning to the genetic Lohmann Brown (Phi-van et al., 2014; Kjaer and Phivan, 2016; Krause et al., 2017; Phi Van et al., 2018). Briefly, 20 cockerels of each genotype were randomly intercrossed with 20 hens of the same genotype and with 10 hens of the other genotype. The hens of that breeding were marked with numbered wing tags and raised in identical littered pens until the experiments. For the experiment, we used 52 hens, 15 hens of the W/W, 19 hens of the W/D and 18 hens of the D/D alleles that had an age of 1.5 years at the start of the tests. The three genotypes of the hens are not linked to their phenotypic appearance, which enabled us to conduct all data collection blind with respect to the genotype of the animal.

The genotypes were revealed after completion of data collection for the analysis.

Approximately 12 weeks before the behavioural tests started, hens were randomly allocated to two identical compartments that were next to the room used for the behaviour test, all in the same stable. These litter floor compartments (each a size of 11 m^2) were equipped with perches, a box filled with sand for dustbathing, pick blocks and a group nest. The birds had *ad libitum* access to standard commercial food (15.5% crude protein, 5.2% crude fat, 3.4% crude fibre, and 12.8% crude ash; the three main ingredients were 35.7% wheat, 18.4% maize, and 17.3% soy) and to water. Light was provided for 14 h per day. To habituate the hens to the experimenters and the food reward of the test, the chickens were additionally fed wheat grains once a day by the experimenters.

Learning experiment setup

The experimental setup and the procedures of the learning experiment were similar to the experimental setup and the procedures described in Dudde et al. (2018). The procedures are briefly described in the following. Hens were trained and tested in different phases: (a) habituation, (b) screen training that consisted of three stages, and (c) the cognitive test phase that consisted of three learning stages: (i) initial associative learning, (ii) reversal learning, and (iii) extinction (see Table 1). The hens were tested during these phases in one out of four identical custom-built test boxes, which were located in a room adjacent to the home compartments of the hens. The test boxes (width, depth, height: 55 cm × 46.5 cm × 66 cm) with a touchscreen (height × wide: 19 cm × 25 cm), a speaker and a food reward-delivery system (foldable food trough (height × wide × depth: 1.5 cm × 4 cm × 8 cm)) are described in detail in Dudde et al. (2018). An in-house developed C++ software (Microsoft Visual Studio, 2010, Microsoft, Redmond, WA, United States; code can be provided upon request) controlled the complete electronic setup of the test box, such as light, sound, reward delivery, touchscreen and monitor. For additional observation of the hens, a video camera was installed at one side of each test box. During the experiments, the hens were not able to see each other from inside the boxes. For habituation, training and learning phases, hens were individually taken from their home compartments and gently placed into the test boxes. They were rewarded with wheat grains to which they had been familiarised in advance. At no time in the experiment were hens' food restricted prior to testing. The experience of success with only positive rewards was established in a previous study (Dudde et al., 2018). The time in the test box was increased during the habituation stage (see Table 1). The hens remained in the test box for a session that lasted up to a maximum of 20 min. However, if a hen made quicker decisions, she could decrease the time in the test box in that respective session, as each hen had to make 20 decisions per session (for details see Dudde et al. (2018)) or alternatively, the time in the test box ended after 20 min. If a hen did not finish one of the training phases (see details below) or one of the three learning phases within 20 daily sessions, the testing ended, and she was thus excluded from the further experiments (Dudde et al., 2018). To successfully solve each training phase, initial learning and reversal learning, hens needed 80% correct decisions out of at least ten decisions (Dudde et al., 2018). This learning criterion differs from the 50% chance level and is in accordance with other learning studies (Garner et al., 2006; Brust et al., 2014; Dudde et al., 2018). To successfully finish the extinction, hens needed to demonstrate no responses in 70% of at least ten trials (Dudde et al., 2018).

Habituation and training phases

During the habituation phase, hens were individually habituated in five consecutive sessions, with increasing time in the box from 5 to 20 min (Table 1). Thereafter, the training phase started, and hens were familiarised with pecks on a dot on the touch screen to receive a food reward (Table 1). During training, hens were asked to go through three training phases as described in detail in Dudde et al. (2018). Once a hen had successfully finished the training, she was tested in the initial associative learning phase.

Testing in three learning phases

The cognitive testing consisted of three phases: (i) initial associative learning, (ii) reversal learning, and (iii) extinction phase.

i.) Initial associative learning phase

For the initial associative learning, the hens needed to learn to differentiate between two simultaneously shown coloured bars, red and green, independent of the orientation of the bars (for details, see Dudde et al., 2018). Which colour was rewarded was alternatingly changed between subjects. To avoid side preferences, the presentation side of the rewarded bar was randomised on both sides within sessions (de Haas et al., 2017a,b). Pecking one of the bars was recorded as an active decision and as correct when the bar pecked was the rewarded bar and as incorrect when the bar packed was the unrewarded bar. Pecking the rest of the screen outside the bars, i.e. the black screen, was not recorded as a decision. If a hen made a correct decision, she received wheat grains for 5 s before the next trial appeared. Thereafter, a black screen was shown for 20 s (intercomponent time) before the two coloured bars reappeared with a randomised position and orientation. If a hen made a wrong decision, no reward was provided, and a black screen appeared for 5 s, followed again by 20 s of intercomponent time. Then, the previously shown bars appeared at the same position again. Hens solved the initial learning phase when they made 80% correct decisions of at least ten decisions within a session.

TABLE 1 Experimental phases and their characteristics.

	Level	Time	Stimulus	Task/reward for	Learning criteria
Habituation	0	One session	None	Stay 5 min in the box and eat wheat grain *ad libitum*	None
		One session	None	Stay 10 min in the box and eat wheat grain *ad libitum*	None
		One session	None	Stay 10 min in the box and eat wheat grain *ad libitum*, turning on and off of reward delivery system	None
		One session	None	Stay 15 min in the box and eat wheat grain only when reward system turns on, time to eat 20 s	None
		One session	None	Stay 15 min in the box and eat wheat grain only when reward system turns on, time to eat 5 s	None
Screen training	1	Individual	Circle	Peck on circle or no peck on circle within 30 s—rewarded	80% correct
	2	Individual	Circle	Peck three times on circle—rewarded	80% correct
	3	Individual	Circle	Peck five times on circle—rewarded	80% correct
Discrimination	4	Individual	Bars	Peck five times on correct symbol—rewarded	80% correct
Reversal	5	Individual	Bars	Peck five times on correct symbol—rewarded	80% correct
Extinction	6	Individual	Bars	No response, not rewarded	70% correct

ii.) Reversal learning phase

During reversal learning, the initially unrewarded colour was rewarded, and the initially rewarded colour was unrewarded. Everything else remained identical to the process described in the initial learning phase (Dudde et al., 2018). The reversal learning was successfully finished after 80% correct decisions of at least ten decisions within a session.

iii.) Extinction phase

In the extinction phase, none of the two bars were rewarded, and the extinction learning criterion was reached when a hen did not respond to any of the two symbols on the screen in 70% of a least ten trials within a session. If a hen did not peck, the symbols vanished after 20 s, followed by an intercomponent time of 20 s. If a hen pecked on one of the symbols on the touchscreen, the black screen appeared for an intercomponent time of 20 s (see as well Dudde et al., 2018, for details).

Stimuli used in the training and cognitive phases

During the training phase, we used as stimulus a grey circle (diameter: 2 cm, colour in red-green-blue (RGB) values: R = 224, G = 224, B = 224) that was shown on the touch screen. During the cognitive testing, we used a green bar (high × length: 10 mm × 40 mm, colour in RGB values: R = 20, G = 184, B = 29) and a red bar (high × length: 10 mm × 40 mm, colour in RGB values: R = 237, G = 28, B = 36) as stimuli, which were presented on a black screen and were all suited for the visual physiology of the hens (Osorio et al., 1999). These stimuli had already been used successfully in former experiments (Dudde et al., 2018). The screens were thin-film transistor (TFT) screens 12.1″ with super video graphics array (SVGA) 600 * 800 with an infrared (IR) frame for touch detection (IR Touch-kit 121.-A301, Citron GmbH, Augsburg, Germany).

Fear-related measures estimated by the tonic immobility test

We further measured a fear-related trait in advance using the tonic immobility paradigm to understand potential genotype-related links between fear-related behaviours (Krause et al., 2017, 2019) and cognitive performance. Therefore, all individuals were individually tested in a tonic immobility (TI) test approximately 8 weeks prior to the learning test. The TI is a robust measure for fearfulness in chickens (Gallup et al., 1971; Jones, 1986). A longer latency to rise reflects a higher level of fear (Gallup et al., 1971; Jones, 1986). The test was conducted as described earlier (Krause et al., 2019): briefly, hens were individually tested in an adjacent room using a V-shaped cradle. Birds were put on their backs into the cradle, and once they remained immobile for 10 s, the latency to rise was measured (maximal 600 s). Birds that did not remain immobile within three attempts were recorded with 0 s, which was only the case for a single bird (from the D/D genotype). Immediately after testing, birds were released into their respective home compartments.

Statistical analysis

To analyse the participation success of the hens from the different genotypes, we first compared the proportion of hens that met the criterion for each phase of the screen training (three phases) and cognitive tasks (three phases). Thus, hens solving all tasks successfully achieved 6 phases in total. These numbers were tested for survival curve differences using the Gehan-Wilcox test.

Thereafter, we compared the learning performance of the hens with respect to their 5-HTT genotype. We analysed the sum of active decisions they needed to fulfil the learning criteria of each level. Active decisions were counted as the number of correct and wrong decisions, whereby inactive trials with no decisions were not counted, in line with Dudde et al. (2018). The respective data processing was performed using a custom-written MATLAB script (Matlab and Statistics Toolbox Release, 2017). The residuals of the average number of active decisions per learning phase were tested for normal distribution with Shapiro–Wilk tests and homogeneity of variances with Levene's test. We analysed the respective data for each learning level with a linear mixed effects (LME) model. The active decisions at the respective level were analysed in linear mixed effects models with the explanatory factor 5-HTT genotype (3-level factor: W/W, W/D, D/D) and housing compartment as a random factor. For significant linear models, we calculated *post-hoc* pairwise *t*-test comparisons for genotypes. Body weight was analysed using an LME model as described above. In addition, body weights were correlated to the active choice from the three different learning levels to examine whether there was a linkage using Pearson correlations. Latencies from the tonic immobility test were not normally distributed even after transformation and thus analysed with the nonparametric Kruskal–Wallis test with regard to the 5-HTT genotype, as a pairwise *post-hoc* unpaired Wilcoxon test was used. Furthermore, tonic immobility test latencies were correlated with the active choice from the three different learning levels to examine whether there was a linkage using Spearman correlations.

All analyses were calculated with R 4.0.3 (R Core Team, 2019), the package nlme (Pinheiro et al., 2007) car (Fox, 2019) and survival (Therneau, 2020).

The raw data of the study are available from Dudde et al. (2022).

Ethical note

Animals were visually controlled daily for health status. The study was in accordance with the German Laws and has been approved by the respective regional authority, the Lower Saxony State Office for Consumer Protection and Food Safety (LaVes) (# 33.19–42,502–04-18/2993). The hens were housed as laying hens after the tests had ended, and the eggs were marketed.

Results

Success in participating throughout the experiment

The proportion of hens successfully participating in the experiment differed between the three 5-HTT genotypes. In particular, hens from the W/W genotype failed to achieve the learning criteria of the different learning levels throughout the experiment compared to hens from the D/D and W/D genotypes (Gehan-Wilcox test, $Chi^2 = 8.6$, df = 2, $p = 0.01$, see Figure 1).

Cognitive performance

(i.) Initial learning

The initial learning performance was significantly affected by the 5-HTT genotype of the hens (LME: factor genotype: $F_{2,20} = 4.14$, $p = 0.031$, Figure 2). The *post-hoc* pairwise comparison revealed that W/W differed significantly from W/D and D/D (both $p < 0.02$), whereas D/D and W/D did not differ from each other ($p = 0.73$). W/W hens needed more active decisions to reach the learning criteria compared to the other genotypes. Three WW hens, 12 W/D hens and 9 D/D hens were tested in initial learning.

ii.) Reversal learning and extinction

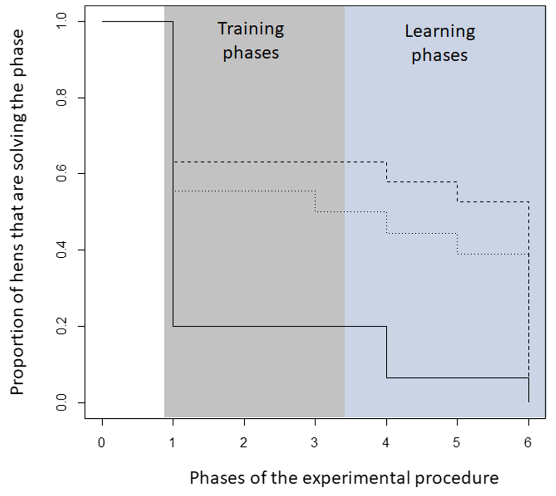

FIGURE 1
Proportion of hens that successfully passed the learning tasks of each level according to their genetic origin. In particular, hens from the W/W genotype failed to achieve the learning criteria of the different learning levels compared to hens from the D/D and W/D genotypes (Gehan-Wilcox test, Chi2=7.5, df=2 p=0.02). In the phases, the following number of hens took part in the respective phase: W/W (phase 1–6: 15,3,3,3,1,1); W/D (19,13,12,12,11,10); D/D (18,10,10,9,8,7).

In reversal learning, hens from all three 5-HTT genotypes did not differ in the number of active decisions needed to reach the respective learning criteria (LME: factor genotype, $F_{2,16} = 1.46$, $p = 0.26$, Figure 3A). Additionally, in the extinction, 5-HTT genotypes did not show differences in the number of active choices (LME: factor genotype, $F_{2,14} = 0.48$, $p = 0.63$, Figure 3B). There were 1 W/W, 11 D/W and 8 D/D hens tested in the reversal tests and one less of each W/D and D/D in the extinction, according to the learning criteria.

Body weight

The body weight of the hens from the three 5-HTT genotypes differed significantly from each other (LME: factor genotype, $F_{2,48} = 4.60$, $p = 0.015$; Figure 4). Pairwise *post-hoc* tests revealed that W/W differed from W/D and D/D (both $p < 0.038$), but D/D and W/D did not differ from each other ($p = 0.65$). On an individual level, body weight was not correlated with any of the three numbers of active choices from the cognitive performance (Pearson correlations, all three $r < -0.16$, all three $p > 0.26$).

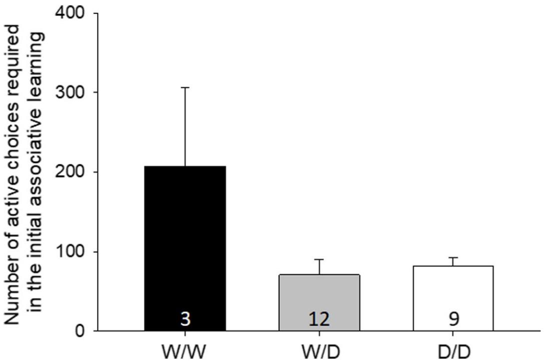

FIGURE 2
Mean number of active choices required in the initial learning [± standard error (SE)]. W/W hens needed more active decisions to reach the learning criteria compared to the other genotypes. The number in the bars indicates the number of hens that participated in the initial learning.

Fear-related measures estimated by the tonic immobility test

The latency in the tonic immobility test differed between the hens of the three 5-HTT genotypes (Kruskal-Wallis test, $X^2 = 6.48$, $df = 2$, $p = 0.039$; Figure 5). *Post-hoc* comparisons between the three 5-HTT genotypes showed that WW differed from DD ($p = 0.018$), while all other comparisons were not different (both $p > 0.12$). On an individual level, tonic immobility latency was not correlated with any of the three numbers of active choices from the cognitive performance (Spearman correlations, all three $r < 0.25$, all three $p > 0.24$).

Discussion

Hens with the 5-HTT genotypes W/D and D/D performed significantly better in the initial learning phase than hens with the W/W genotype. The D/D and W/D hens not only performed better in the learning phase but also participated in all three cognitive phases at a higher rate than the W/W hens and were also less fearful in a tonic immobility test than the W/W hens.

This result on cognitive performance is in contrast to our initial assumption that was derived from humans and other mammalian studies (Homberg and Lesch, 2011); however, the result is in line with our expectation with regard to fearfulness (Krause et al., 2019). W/W hens were the most fearful, participated less in the learning task and had worse results in this task. This functional effect of the 5-HTT genotype is interesting. The effect

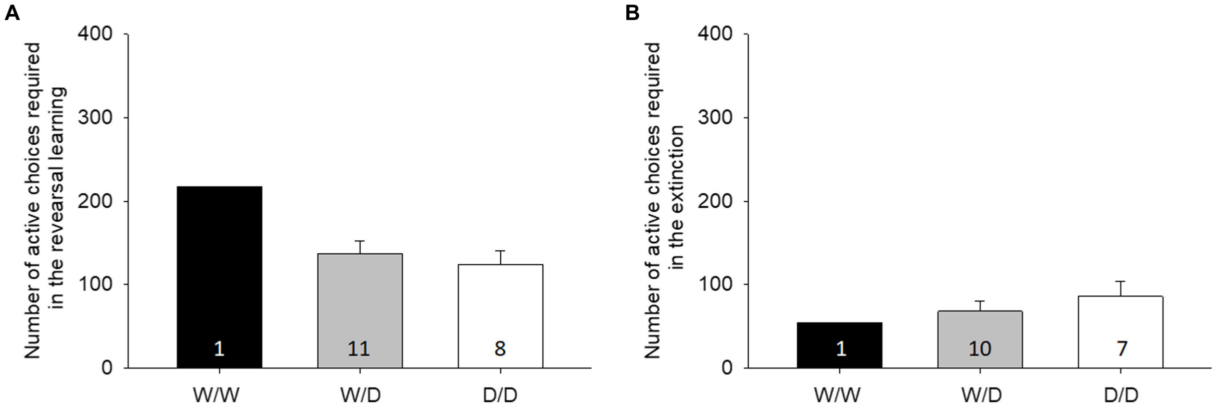

FIGURE 3
Mean number (± SE) of active choices required: **(A)** reversal learning, as well as **(B)** extinction learning, with no significant differences found between the three genotypes. The number in the bars indicates the number of hens that participated in the initial learning.

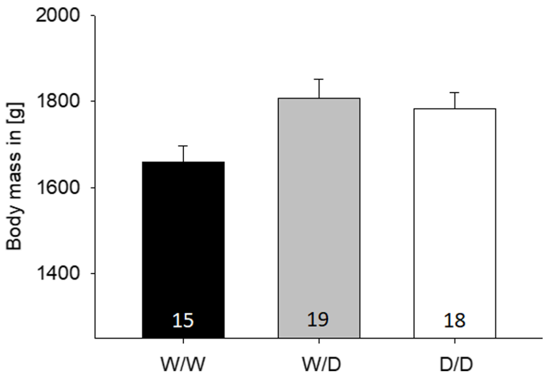

FIGURE 4
Mean body (±SE) mass in grams from the hens of the three 5-HTT genotypes, with the W/W being significantly lighter than the other two. The number in the bars indicates the number of hens that were measured.

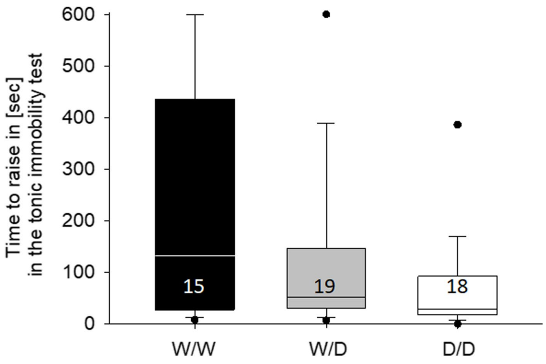

FIGURE 5
Median time in the tonic immobility test required by the hens of the three genotypes to rise up. W/W hens needed longer than the hens from the other genotypes. The number in the boxes indicates the number of hens that were measured.

may have been caused by several factors, which we discuss in the following.

One of the main factors affecting learning performance in this experiment can be the diverging fearfulness of the hens. The hens with the W/W genotype are more fearful than the other types (Krause et al., 2017, 2019), as shown in the tonic immobility test, where longer latencies to rise indicate a higher level of fearfulness (Gallup et al., 1971; Jones, 1986). This is an interesting difference from humans, where the genotype resembling the chicken W allele is the S allele, which is also linked to more fearful responses but is assumed to show more thoughtfulness towards learning tasks (Homberg and Lesch, 2011). In our experiment, an assumption for worse learning of D/D hens could be associated with the potentially stressful learning situation in the Skinner box, separated from the conspecifics, hence paying more attention to the environment but not to the actual learning task. Future studies should therefore also address the question of whether D/D and W/W hens differ in their coping style, e.g., reactive or proactive (Koolhaas et al., 1999, 2010), as such is also known to affect learning speed (Guillette et al., 2015). However, in contrast to humans, learning performance is not positively linked to fearfulness, but it might be that fear hinders chickens from training and learning. This idea also supports the finding that the participation success in training and testing over all experimental phases was significantly different between the genotypes and lowest in W/W hens, either reflecting their fearfulness or alternatively cognitive limitations.

Whether hens with the W/W genotype might be better at learning in a fear-free context might be considered in future studies. However, intrinsic fear cannot be compensated for by a fear-reducing environmental situation.

A further potential factor, which might theoretically have affected the learning performance of the hens in our study, is the different motivations for the food reward used in our test (Dudde et al., 2018). Previous studies, as well as this study, have shown that hens possessing the D/D genotype are heavier and feed more during certain juvenile stages in comparison to W/W hens (Kjaer and Phi van, 2016). Therefore, even adult D/D hens might have a higher food motivation in certain contexts and hence have a higher motivation to participate in the learning task. We cannot fully exclude effects caused by food motivation in this study design, although all hens were not food deprived prior to the study. Nevertheless, it could be interesting to design future experiments that use other than food rewards in cognitive tasks, e.g. social rewards, to avoid potential bias of food motivation.

Significant differences in cognitive performance were only detected in the initial learning, while during reversal, learning was solved by hens from all three 5-HTT genotypes similarly well. This finding has to be taken with a certain caution, as in the reversal and extinction phase, only a single hen from the W/W genotype participated in testing. Thus, whether the performance of the individuals from the three genotypes differs in these two phases cannot be robustly evaluated and may be further examined in future studies. Nevertheless, it is of great general interest that, although in the opposite direction as expected from mammalian studies, polymorphisms in the 5-HTT gene affect the initial learning of domestic chickens.

The results of the tonic immobility test show that W/W hens had the longest latencies to rise and thus the highest levels of fearfulness (Gallup et al., 1971; Jones, 1986). In addition to their help to potentially understand why W/W hens participated less and poorly in the learning test, the result itself is a nice replication of the findings of earlier studies (Krause et al., 2019). On the genotype group level, tonic immobility latencies were high in W/W hens as the number of active choices needed in the learning phases; however, no significant correlation between both parameters was found. Thus, there does not seem to be a strong association between the level of fearfulness and cognitive performance in individuals. No such correlation was found for body masses and cognitive performance; however, genotype-level differences in body masses replicate earlier studies (e.g. Phi-van et al., 2014; Krause et al., 2017, 2019).

From an applied animal science perspective, the polymorphism at the serotonin transporter gene is an interesting candidate for future commercial breeding strategies. Selecting for the deletion D-allele may lead not only to heavier hens but also to more robust and thus less fearful hens, which might be important for the mental welfare of the hens. However, thus far, the abundance of the D allele in commercial breeds investigated is quite low, e.g., approximately 7.5% in a brown layer strain (Krause et al., 2019).

Taken together, we found that polymorphisms in the serotonin transporter gene 5-HTT significantly affected the training and initial learning performance of laying hens. Genotypes related to less fearfulness perform better in the initial associative learning task, showing the impact of the 5-HTT polymorphism on cognitive performance in domestic chickens.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: https://doi.org/10.6084/m9.figshare.20237754.v1.

Ethics statement

The study was carried out in accordance to the German Laws and has been approved by the respective regional authority, the Lower Saxony State Office for Consumer Protection and Food Safety (LaVes;# 33.19–42502–04-18/2993).

Author contributions

AD, EK, LS, and LP contributed to conception and design of the study. AD and EK organised the experiments. EK and AO performed the statistical analysis. AD wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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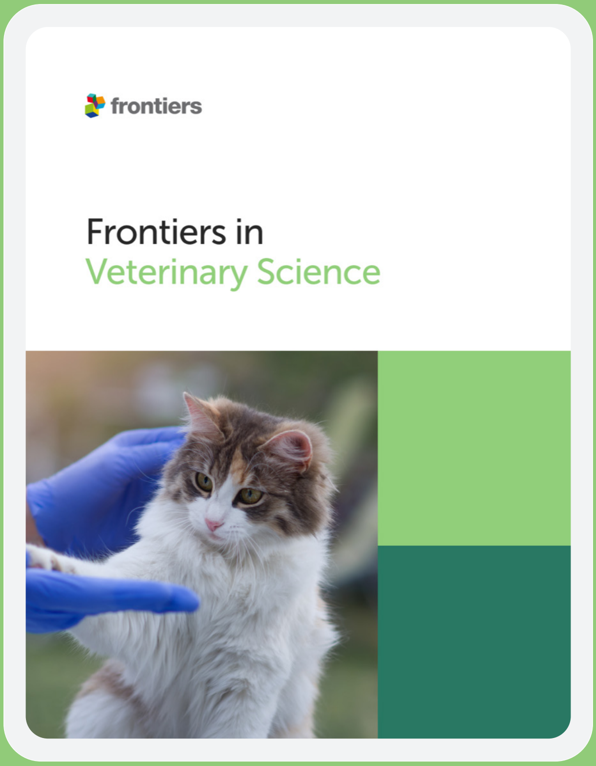

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