ADVANCES AND PERSPECTIVES IN FARM ANIMAL LEARNING AND COGNITION

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ADVANCES AND PERSPECTIVES IN FARM ANIMAL LEARNING AND COGNITION

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A dwarf goat in front of an automated visual learning device.
Image by the Leibniz Institute for Farm Animal Biology (FBN).

Research on animal learning and cognition has so far mainly focused on a few prominent model species, including primates, corvids and dogs. For years, comparative psychologists and ethologists have been suggesting that more animal species should be considered in comparative cognitive science. The abundance and accessibility of livestock offer an opportunity, not merely to extend the comparative approach, but also to deepen our knowledge of the mental lives of farm animals. Such approaches also help to assess the needs of farm animals, in order to improve their welfare. In recent years, scientific interest in different aspects of farm animal psychology, including emotionality, personality and cognitive capacities, has been on the rise, proving that farm animals have sophisticated cognitive skills to comprehend and cope with their environment.

As knowledge of how farm animals perceive and interact with their physical and social environments is crucial for animal welfare, the aim of this Research Topic is to promote investigations of farm animal cognitive capacities and their implications for animal welfare-related issues. We have therefore collected original research and review articles, as well as opinion and perspective papers that are distributed among the two hosting magazines, *Frontiers in Veterinary Science* (section Animal Behavior and Welfare) and *Frontiers in Psychology* (section Comparative Psychology). The published articles present state-of-the-art research on farm animal learning and cognition, highlight future perspectives in this research area and pinpoint shortcomings and limitations in interpreting current findings. They offer new cross-disciplinary frameworks (e.g. links between affective states and cognition) and discuss the applied implementation of these findings (e.g. cognitive enrichment). These contributions will increase our understanding of the cognitive mechanisms that enable farm animals to effectively interact with their environment and pave the way for future cross-disciplinary endeavors.

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EDITORIAL
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Editorial: Advances and Perspectives in Farm Animal Learning and Cognition

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Keywords: animal welfare, animal behavior, livestock, human-animal interactions, enrichment

Editorial on the Research Topic

Advances and Perspectives in Farm Animal Learning and Cognition

The welfare of farmed animals is of major concern for society and food production (1–3). Of increasing relevance for understanding welfare is the knowledge on how farm animals perceive and deal with their physical and social environment. This information is crucial for applied ethology as it allows management practices to be adjusted to suit the animals' specific behavior and needs. The current Research Topic comprises 10 articles presenting state of the art research on farm animal learning and cognition. It includes novel and innovative empirical research, highlights the current state of farm animal cognition as well as its limitations, and discusses findings considering future interdisciplinary approaches and applications.

Three review articles summarize our present knowledge of different aspects of learning and cognition in farm animals and critically discuss their interpretation and potential for implementation. Nawroth et al. reports on the existing research on cognitive abilities of ungulate livestock species, focusing on a distinct set of cognitive capacities in the physical and social domain. They conclude that while research on livestock species is still underrepresented, the current findings indicate that ungulate livestock possess sophisticated mental capacities. They emphasized the importance of gaining a better understanding of how livestock species interact with their physical and social environments, as this information can be applied to improve housing and management conditions and to evaluate the use and treatment of animals in farming systems. From an ethical perspective, they also discuss whether animal cognition and the connection to animal welfare matters from the perspective of the animal.

Rørvang et al. critically evaluated the evidence for social learning in horses and the learning mechanisms involved. They conclude that many reported findings for social learning can be explained by relatively simple mechanisms such as social facilitation or stimulus and local enhancement, rather than by more complex phenomena such as emulation or true imitation (4). They state that, to date, there is no convincing evidence for true social learning in horses and discuss why the attribution of high-level social-learning abilities may even be maladaptive in horses.

A wide range of cognitive tests have been adapted or developed for the use in farm animals. The focus of most studies has been on variation of test performance at the species level; between- and within-individual differences of the same species remain largely unexplored. Bushby et al. summarized the contribution of factors such as choice of cognitive test, sex, early life environment, rearing conditions or personality to individual variation in cognitive outcomes. Further, the impact of such factors in recent farm animal studies is presented together with a framework on how to account for them statistically with special focus on experimental design and analytical techniques.

Inter-individual differences (5) in learning and cognitive performance of farm animals have also been a topic of strong interest in most of the five empirical studies that are covered in this Research Topic. Differences arising during early ontogeny, such as via differences in birth weight, were

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examined by Roelofs et al. They report the effects of low birth weight of piglets on post-weaning reference and working memory as well as learning flexibility. Their results show that pigs with low birth weight show a slightly impaired cognitive performance which goes along with higher long-term stress level in these individuals in comparison to pigs with normal birth weight.

Another factor rarely addressed in the investigation of individual differences in cognitive performance of farm animals is the prenatal stress to which mothers are exposed. To address this issue, Vas et al. examined how stress in pregnant goats, induced by reduced space allowance, affects their offspring's performance in a test on object permanence. In contrast to their initial hypothesis, they found that a higher prenatal maternal cortisol level was correlated with better performance by offspring on the most difficult task of the test.

Other cognitive traits might be important to consider, too, when talking about individual differences. The relationship between impulsive behavior and aggression in humans and animals might also have important implications for farm animal husbandry and welfare. Zebunke et al. adapted the "Marshmallow Test" to study impulse control in pigs. They found that piglets show impulse control, and that this is most strongly shown for rewards differing in quality rather than quantity. A broader understanding of impulse control might help in adapting husbandry conditions to the needs of individuals, especially in relation to social behavior, tail biting, and maternal behavior.

Intense selection for production traits is another factor that might account for differences in learning and cognition of farm animals. Dudde et al. investigated how laying performance and phylogenetic origin affect learning performance in laying hens. They hypothesized that there might be a trade-off between egg yield and cognitive performance in terms of the energy which is available. In contrast to their initial hypothesis, their results indicate that high performing laying hens performed better in a visual discrimination task compared to moderate productive hens in a feeding-rewarding context.

Another emerging area of research is how emotion and cognition are intertwined when it comes to interacting with conspecifics. Bellegarde et al. investigated if sheep can perceive the emotional valence displayed on the faces of conspecifics and how this valence affects their ability to discriminate between images of the same individual in different emotional contexts. They showed that sheep were able to differentiate between different emotional expressions of other sheep.

Two perspective articles outline future directions and potential implementations of basic research findings regarding the cognitive capacities of farm animals. Baciadonna et al. referred to the actual debate on how positive and negative emotions might spread by emotional contagion in farm animals (6) and argue in favor of future research on the mechanisms of how emotions in livestock are shared and how to use empathic responses to promote better welfare.

Finally, Lee et al. showcased how to take basic research into an applied setting. They developed a framework based on the cognitive activation theory of stress (CATS) and the cognitive evaluation of the environment in terms of predictability and controllability by an animal and used it in a case study to determine welfare outcomes of new technologies, here virtual fencing.

In conclusion, the contributions in this Research Topic will increase our understanding of how farm animals use their cognitive abilities to interact with their environment and aim to pave the way for new cross-disciplinary endeavors.

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

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REFERENCES

1. Buller H, Blokhuis H, Jensen P, Keeling L. Towards farm animal welfare and sustainability. *Animals*. (2018) 8:81. doi: 10.3390/ani8060081
2. Weary D. Understanding and responding to public concerns regarding farm animal welfare. *J Anim Sci*. (2018) 96:5. doi: 10.1093/jas/sky404.011
3. von Keyserlingk M, Weary D. Farm animal welfare: maintaining public trust and social acceptability. *J Anim Sci*. (2018) 96:507. doi: 10.1093/jas/sky404.1108
4. Heyes CM. Social learning in animals: categories and mechanisms. *Biol Rev*. (1994) 69:207–231. doi: 10.1111/j.1469-185X.1994.tb01506.x
5. Finkemeier M-A, Langbein J, Puppe B. Personality research in mammalian farm animals: concepts, measures, and relationship to welfare. *Front Vet Sci*. (2018) 5:131. doi: 10.3389/fvets.2018.00131
6. Reimert I, Bolhuis JE, Kemp B, Rodenburg TB. Indicators of positive and negative emotions and emotional contagion in pigs. *Physiol Behav*. (2013) 109:42–50. doi: 10.1016/j.physbeh.2012.11.002

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REVIEW
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Farm Animal Cognition—Linking Behavior, Welfare and Ethics

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Farm animal welfare is a major concern for society and food production. To more accurately evaluate animal farming in general and to avoid exposing farm animals to poor welfare situations, it is necessary to understand not only their behavioral but also their cognitive needs and capacities. Thus, general knowledge of how farm animals perceive and interact with their environment is of major importance for a range of stakeholders, from citizens to politicians to cognitive ethologists to philosophers. This review aims to outline the current state of farm animal cognition research and focuses on ungulate livestock species, such as cattle, horses, pigs and small ruminants, and reflects upon a defined set of cognitive capacities (physical cognition: categorization, numerical ability, object permanence, reasoning, tool use; social cognition: individual discrimination and recognition, communication with humans, social learning, attribution of attention, prosociality, fairness). We identify a lack of information on certain aspects of physico-cognitive capacities in most farm animal species, such as numerosity discrimination and object permanence. This leads to further questions on how livestock comprehend their physical environment and understand causal relationships. Increasing our knowledge in this area will facilitate efforts to adjust husbandry systems and enrichment items to meet the needs and preferences of farm animals. Research in the socio-cognitive domain indicates that ungulate livestock possess sophisticated mental capacities, such as the discrimination between, and recognition of, conspecifics as well as human handlers using multiple modalities. Livestock also react to very subtle behavioral cues of conspecifics and humans. These socio-cognitive capacities can impact human-animal interactions during management practices and introduce ethical considerations on how to treat livestock in general. We emphasize the importance of gaining a better understanding of how livestock species interact with their physical and social environments, as this information can improve housing and management conditions and can be used to evaluate the use and treatment of animals during production.

Keywords: animal ethics, animal behavior, animal welfare, enrichment, human-animal interactions, livestock, physical cognition, social cognition

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INTRODUCTION

Farm animal welfare assessment approaches have shifted from original concepts such as the five freedoms (1, 2) to more animal-centered concepts that also include the animals' needs (3), affective states (4, 5) and inter-individual differences (6). All these concepts emphasize the importance of having detailed knowledge on the cognitive capabilities of livestock [i.e., their ability to acquire, process, store and use information (7)] to better understand their behavior and to avoid exposing them to poor welfare conditions, such as those induced by stressful management practices. Thus, cognitive research on farm animals has the potential to highlight mismatches between current husbandry practices and adaptive abilities of livestock.

In recent decades, research on the cognitive capacities of non-human animals has gained increasing attention. Most work has focused on humans' closest relatives, i.e., primates in general and great apes in particular (8); additionally, in terms of convergent cognitive evolution, research has included corvids (9) and canids (10). However, compared to the amount of cognitive research that has been conducted on the aforementioned species, studies on the cognitive capabilities of farm animals are relatively underrepresented (11). Given the number of livestock animals kept under husbandry conditions worldwide, this lack of research is even more surprising.

The aim of this review article is to highlight advances in the field of farm animal cognition and identify their potential implications for livestock management and practices. First, we outline a distinct set of physico- and socio-cognitive processes. We then present a comprehensive overview on farm animal cognition research conducted on cattle, horses, pigs, and small ruminants. Our purpose is to offer a structural account of the content and value of these processes that can help researchers further explore the different cognitive mechanisms in a variety of farm animals to gain a more comprehensive profile of species-specific psychological traits. We then discuss the implications of these findings on issues concerning animal welfare (while focusing on enrichment and human-animal interactions) and ethics (comprising welfare ethics and ethical considerations beyond animal welfare). Finally, we offer directions for future research that will help to further our understanding of farm animal cognition and help us to adapt management practices that better meet their psychological needs.

We structured this review mainly in line with the classification of cognitive capacities used by Shettleworth (7). She broadly categorized cognitive mechanisms into two domains: physical and social cognition. We focused on a particular set of cognitive capacities in both domains, which we assume to have a major impact on how farm animals are able to interact with their physical and social environment (see **Tables 1**, **2** for an overview).

Physical Cognition

The term "physical cognition" refers to an organism's understanding of objects and their various spatial and causal relationships. For most animal species, the most important problem they face is related to locating and obtaining food. Thus, many important cognitive skills have evolved in the context of foraging (8).

Categorization is the ability to group items based on common features and to respond similarly to them. This trait enables an organism to group together objects and events based on physical, associative or relational similarities, and it provides the basis for higher cognitive processing (67). In complex environments, the ability to assign food types to categories by relying on certain relevant criteria could considerably reduce cognitive demand and thereby increase foraging efficiency. Being able to categorize positive and negative stimuli might also enhance adaptations to new environments, which could reduce the impact of stressors such as food acquisition in novel environments or handling during transport.

Numerical ability refers to the capacity to discriminate between two distinct quantities (e.g., 6 vs. 4 rewards or 3 vs. 1 conspecifics), regardless of the size and shape of these objects/subjects (68). Several mechanisms, such as "subitizing" or "approximate number system," can explain this phenomenon (69). The ability to assess the quantity of food or members of a group likely affects the predictability of the environment (e.g., group number) and the ability to adapt to stressors (e.g., group cohesion).

Object permanence refers to the notion that objects are perceived as separate entities that continue to exist even when they are out of the sight of the observer (70). Following the trajectory of a previously seen but then hidden object is highly adaptive in the context of foraging and also helps to avoid predation. As husbandry systems involve a variety of barriers and walls, the degree of how well object permanence is developed plays a crucial role in being able to predict upcoming events in farming environments.

Reasoning (inferences) implies the establishment of an association between a visible and an imagined event (71). The correct solution to a problem should be selected by excluding other potential alternatives even if only indirect information, such as the absence of a cue, is available. However, inferential reasoning can only be assumed if the subject exhibits adequate behavior, without explicit training. Otherwise, the role of learning mechanisms cannot be excluded (72, 73). The ability to reason about events likely impacts the predictability of husbandry environments.

Tool use, i.e., the ability to dynamically manipulate and to use an inanimate object (or animate subject) to reach a goal, is a topic that has gained interest in non-human cognitive research in recent decades and has been found in several animal taxa (74, 75). Goals can be manifold, i.e., the acquisition and gathering of food and water, grooming, self-defense, or recreational use. Animals that regularly use tools can be provided with a higher diversity (and complexity) of enrichment items (e.g., arbitrary anthills for chimpanzees).

Social Cognition

Conspecifics are physical objects that must be located and identified, but they also create additional cognitive problems that are not present in the world of inanimate objects (8). For example, living in groups requires the discrimination

TABLE 1 | Overview on various physico-cognitive capacities, their description, evidence in different farm animals, and their implications.

Cognitive trait	Description	Cattle	Horses	Pigs	Small ruminants	Implications
Categorization	Ability to group items based on common features	+ (12)	+ (13, 14)	n/a	+ (15) (16)	Adaptation to novel stressors (food acquisition, handling)
Numerical ability	Discrimination and judgment of distinct quantities	n/a	+ (17, 18)	n/a	n/a	Perceived predictability of environment (group number) and adaptation to stressors (group cohesion)
Object permanence	Notion that objects continue to exist when they move out of the visual field	n/a	(+) (19, 20)	± (21) (+) (22)	+ (23)	Perceived predictability of environment (housing)
Reasoning/Inferences	Establishment of an association between a visible and an imagined event	n/a	n/a	+ (24)	+ (goats) (25) – (sheep) (25)	Perceived predictability of environment (housing); Complexity of cognitive enrichment
Tool use	Manipulation of objects to reach a goal	n/a	n/a	n/a	n/a	Complexity of cognitive enrichment

+, positive results; (+), indirect positive results; ±, inconclusive results; –, negative results; n/a, no studies available.

and recall of conspecifics, either at the individual or group level. Being a social animal also may require additional forms of intelligence, such as manipulating the behavior of others (76). Moreover, another individual might behave spontaneously on its own. Thus, the ability to infer the motivations and desires of others can be advantageous in terms of lowering the level of uncertainty in predicting the behavior of others.

Individual discrimination and recognition of conspecifics and heterospecifics are considered complex processes that have evolved to facilitate social behaviors. Discrimination refers to the ability to differentiate between two identities (e.g., individual conspecifics or heterospecifics, such as humans) using cues inherent to these individuals. Individual recognition requires the ability to remember and recall other individuals (77). An essential feature of individual recognition in humans is that it is cross-modal, which means the trait enables the matching of current sensory cues of identity with stored information about that specific individual from other modalities (78). Being able to recognize conspecifics after short- or long-term periods of separation reduces aggressive behavior and injuries, while being able to correctly identify familiar handlers likely reduces stress during management practices.

Communication with humans can be crucial for domestic animals in terms of acquiring information from the environment (79). Several non-human animals have been shown to use human-given cues, such as a pointing gesture, when confronted with a task where they have to make a choice between two or more potential alternatives of where to find hidden food. In turn, communication can also be directed toward humans. When confronted with a problem they cannot solve themselves, children as well as some non-human animals use gazing behaviors (such as i.e., alternating their gaze between the problem and a human) as a form of referential and intentional communication when interacting with humans (80–82). Thus, the ability of livestock to communicate with humans can impact on management practices due to improvements in handling routines.

Another crucial part of social cognition involves *social facilitation and/or learning* [for a distinction see (83), from here on referred to as "social learning"]. These processes can occur through observational conditioning, local/stimulus enhancement, emulation and/or imitation (84). Social learning occurs when a subject's behavior is influenced by observing the behavior of other individuals, and it often arises when individual learning is costly, e.g., in terms of predation risk or offspring foraging behavior (85, 86), and is not limited by mechanistic constraints (87). Social learning can occur horizontally (i.e., from peer to peer) or vertically (i.e., from parents to offspring, but also from unrelated adults to young in general). It is apparent that acquiring new information in husbandry systems (e.g., where to find food) through social learning has huge advantages, e.g., reduced stress and increased food intake.

An individual's knowledge of the perceptual states of others, which is comprised by the ability to *attribute attention* or take the perspective of another individual, can be summarized under the so-called "Theory of Mind" or "Theory of Mind"-like abilities (88). Attributing attention to conspecifics or handlers can increase the predictability of future interactions and events (conspecifics: competition for access to resources; handlers: management practices).

Finally, the domain of social cognition also involves questions regarding the cognitive foundations of morality, e.g., actions involving the welfare of others, such as *prosociality* (89) and *fairness* [including inequity aversion and third-party punishment (90)]. The capacity for prosocial behavior can be used to promote positive mental states and well-being in social farm animals (91) while it also raises ethical questions regarding the use of animals in general.

TABLE 2 | Overview on various socio-cognitive capacities, their description, evidence in different farm animals, and their implications.

Cognitive trait	Description	Cattle	Horses	Pigs	Small ruminants	Implication
Discrimination and recognition of conspecifics	Differentiating and recalling other individuals	+ (12, 26)	+ (27, 28)	+ (29, 30)	+ (31, 32)	Group cohesion Reduction of aggressive behavior
Discrimination and recognition of humans	Differentiating and recalling handlers	+ (33)	+ (34, 35)	+ (36, 37)	+ (38)	Stockmanship (fear response to familiar/unfamiliar humans)
Communication with humans (Human → Animal)	Use of human communicative cues, such as a pointing gesture	n/a	+ (19, 39)	+ (22, 40)	+ (41, 42)	Management and stockmanship during handling and transport
Communication with humans (Animal → Human)	Expression of communicative behaviors, such as gaze alternations between a human and an object	n/a	+ (43)	n/a	+ (44, 45)	Signaling of needs
Social learning (vertical)	Information transfer from parents to offspring	± (46)	+ (47)	+ (48, 49)	+ (50)	Access to resources and avoidance of harm
Social learning (horizontal)	Information transfer from peer to peer	± (51, 52)	± (53, 54)	+ (55)	– (56, 57)	Group organization and access to resources
Social learning (from humans)	Information transfer from humans	n/a	± (58, 59)	n/a	+ (60)	Adaption to new environments
Attributing attention	Attending to signs of attention in conspecifics or humans (i.e., head direction or eye visibility)	n/a	+ (61)	± (62–65)	+ (42, 66)	Predictability of events/actions/ interactions; perceived access to resources
Prosocial behavior	Behavior that benefits other individuals and their welfare	n/a	n/a	n/a	n/a	Ethical implications
Fairness (inequity aversion, third party punishment)	Behavior regarding the outcome of decision as equal and just toward oneself and others	n/a	n/a	n/a	n/a	Ethical implications

+ positive results; (+), indirect positive results; ±, inconclusive results; –, negative results; n/a, no studies available.

COGNITIVE STUDIES IN LIVESTOCK ANIMALS

Cattle

Physical Cognition

Previous research in cattle has primarily focused on their learning ability rather than on their understanding of physical properties of their environment (92, 93). Cattle associate locations with the quantity and quality of food that is found there (92), and they adjust their foraging patterns to take advantage of this knowledge, which indicates context-dependent decision making. They also appear to have a categorization process for social stimuli: Cattle categorize individuals into "familiar" and "unfamiliar" subjects (12). However, no studies regarding number discrimination, object permanence, reasoning or tool use are available for this species.

Social Cognition

Cattle have social recognition abilities, which include individual recognition (77). There is also indirect evidence of discrimination based on familiarity, which is one of the simpler categories of social recognition. For example, intense fighting between cattle frequently occurs when groups of unfamiliar individuals are mixed at abattoirs, e.g., heifers were less aggressive to familiar members than to unfamiliar animals (94). In their herd, subordinate cattle will generally avoid more dominant animals, which suggests they have the ability to recognize familiar animals that have been previously associated with positive or negative experiences (95). Individuals within a herd also prefer the presence of social partners with whom they have already maintained close proximity and direct social grooming (94). Similarly, dam and offspring form strong social bonds and are able to recognize each other even within a large herd (96). Categorizing individuals based on familiarity, social status and genetic relatedness is important for social cohesion in cattle; moreover, these skills decrease aggression within a group and help categorize individuals as kin and non-kin.

Operant conditioning techniques have been used to test cattle in terms of their ability to discriminate and categorize individuals. Cattle easily discriminate among familiar conspecifics using visual, olfactory and auditory modalities (97), and they can be trained to discriminate between conspecifics using only olfactory cues (98). Cattle appear to use their sense of vision to discriminate between conspecifics, as altering their vision ability resulted in an increase in the frequency of aggressive interactions (99). Coulon et al. (12) tested the ability of cattle to visually discriminate between heads (including face views) of familiar and unfamiliar conspecifics represented as 2D images using a food-rewarded instrumental conditioning procedure. Eight out of the nine heifers succeeded in discriminating between images of familiar and unfamiliar conspecifics; furthermore, they could instantly differentiate between a new pair of images of familiar and unfamiliar conspecifics, suggesting cattle have a categorization process for social stimuli. In addition, Coulon et al. (12) argued that images of conspecifics were treated as representations of real individuals. Indeed, they observed that heifers were more attracted to images of familiar conspecifics than to images of unfamiliar conspecifics. In addition, heifers expressed different emotional reactions when confronted with these two types of stimuli. Heifers rewarded for images of unfamiliar conspecifics pointed their ears backwards more frequently (which is a behavior common during the confrontation with an unfamiliar and potentially threatening stimuli), and they showed less forward pointing ears (indicating less positive expectations) when they directed their attention to unfamiliar images compared to heifers that were rewarded for identifying familiar conspecifics (100). Using the same methodology, heifers visually discriminated their own species from other animal species (26) and kin-related conspecifics from non-kin conspecifics (101). It appears that the heads of conspecifics are sufficient for social discrimination in cattle. Several authors have shown that cattle can discriminate between humans using various criteria (102), such as a portion of their face or their body (33) or the color of the clothes of unfamiliar people (103).

There is evidence of social facilitation/learning in cattle. For example, calves can learn where to graze from their dam (46). If a cow is located in an area with high food resources, this information is transferred to other members of the social group by social facilitation (51). In addition, conditioned aversions have been eliminated following exposure to non-averted social companions in cattle (104). Although heifers provided with a trained demonstrator did not learn an operant task faster, they spent more time near the training box (52). Boissy and Le Neindre (105) also found that the degree of familiarity between heifers did not affect their social learning abilities in relation to an operant task.

To the best of our knowledge, there have been no studies on social learning from humans or the use of human-given cues in cattle. In addition, no studies have focused on more complex socio-cognitive phenomena, such as prosocial behavior or inequity aversion, in cattle.

Horses

Physical Cognition

Horses can learn to choose items in a choice task based on the shared characteristics of these items (e.g., filled item vs. items with an opening), indicating that they are using categorization skills when confronted with problem-solving tasks (13, 14).

Several investigations have evaluated the ability of horses to discriminate between different quantities. For example, Uller and Lewis (18) showed a spontaneous preference of horses to choose the higher quantity of plastic apples regardless of the total volume. In this study, the horses spontaneously chose the higher quantity in the 1 vs. 2 and in the 2 vs. 3 tasks, but they failed to discriminate between 4 vs. 6 apples. In the study by Henselek et al. (106), horses failed to discriminate pairs that differed in the number of edible and inedible objects (i.e., apple slices and small wooden blocks). On the other hand, Petrazzini (107) found that a horse could discriminate pairs of dots when they were presented under uncontrolled conditions (i.e., the ratio of surface was similar to the ratio of number) and controlled conditions (i.e., equal surface area over whole stimulus) in a 1 vs. 4 task, but the horses failed in the higher ratio of 2 vs. 4 in the controlled condition. She assumed that if discrimination becomes more difficult, horses also tend to use cues other than numerical cues. In each of the mentioned experiments, the positive stimulus was the one with the higher quantity of items. The fact that the horses in these studies failed to discriminate higher quantities or higher ratios could lead to the presumption that the animals based their decisions on approximate estimations. Gabor and Gerken (17) found that horses are able to discriminate the quantity of abstract symbols. Three horses could discriminate various ratios (1 vs. 2; 2 vs. 3; 3 vs. 4; 4 vs. 5), and one horse was able to transfer the performance to mixed geometrical symbols.

No research has directly investigated the ability of horses to track hidden objects (i.e., object permanence); however, results from experiments that have used hiding containers (18) have indirectly provided evidence for at least rudimentary developed object permanence skills. No studies regarding reasoning or tool use are available for this species.

Social Cognition

Horses are able to differentiate between conspecifics (27, 108) and humans using different modalities (34), and can identify subjects based on familiarity (109, 110). Previous research has shown that horses are also able to recognize conspecific and heterospecific individuals across two modalities (i.e., the individual visual and auditory cues must match) (28, 35, 111).

Horses are known to react to subtle behavioral cues from conspecifics and humans (112). Surprisingly, the general body posture used when approaching a horse did not appear to have an influence. However, the speed of the human's approach was more influential (113). In contrast to these findings, Proops and McComb (61) and Krueger et al. (20) found that horses differed in their approach behavior to humans based on the level of attention that was provided by an experimenter. The horses also showed higher obedience levels when a human was giving them attention (110). Several studies have reported that horses are able to use human-given cues, such as a pointing gesture, to locate a hidden reward. However, it seems that the pointing finger must be close to the baited location, as performance dropped toward levels equal to random chance when pointing was administered from a distance (19, 39, 114–116). In contrast to the use of human pointing gestures, horses failed to interpret the head direction of a human experimenter (19, 116), but they were able to use the head direction of a depiction of a conspecific to infer the location of a hidden reward (117). Horses appear to wait for humans to solve a task to obtain food instead of trying to solve the task themselves (118). They also show human-directed behavior when confronted with an inaccessible food reward (43), and they frequently gazed at an experimenter who was positioned near the reward. In addition, horses also considered the attentional stance of the experimenter during the task, depending on whether the experimenter was turned toward or away from them.

Horse owners often think that abnormal or stereotypic behaviors are learned through observation (119); however, several studies have shown that horses do not perform better after watching a demonstrator horse both in a simple operant conditioning task (120) or in a discrimination task (121–123). Krueger and Heinze (124) found that experimental horses copied specific following behaviors toward humans when a dominant conspecific followed the path of a human handler. However, studies in horses on imitation of complex behaviors have shown inconclusive results so far, which may be due to the lack of appropriate experimental designs (125). Recently, Ahrendt et al. (126), Rørvang et al. (54), and Burla et al. (58) showed that horses do not learn an instrumental or spatial task through social observation. In contrast, a study by Krueger et al. (53) found evidence for social learning in horses using an instrumental task. However, this was restricted to young, low-ranking and more exploratory horses who were learning from older group members. Additionally, test horses learned the same instrumental task faster than control horses when they were frequently exposed to a human demonstrator who was solving the instrumental task (59). When mares were habituated to the exposure of a human experimenter or unfamiliar and potential frightening objects, their foals showed less fear reactions in standardized fear tests or in approaching unfamiliar humans, indicating the social transfer of information from mother to offspring (47, 127).

No studies on the evaluation of more complex socio-cognitive phenomena, such as prosocial behavior or inequity aversion, are available.

Pigs

Physical Cognition

Pigs as omnivorous animals exhibit high foraging flexibility that is reflected in their dietary spectrum (128). Therefore, it should be of no surprise to find cognitive capacities that increase their ability to exploit food sources, either in relocating previously known food patches or in finding new ones.

Young pigs have been found to understand that once hidden, objects do not cease to exist, but had problems following more complex movements of hidden objects (21). Albiach-Serrano et al. (40) presented domestic pigs with a series of tasks that spanned the physico- and socio-cognitive domains. One of these tasks involved the presentation of a slighted board that covered a hidden reward, and another task involved a baited cup that was shaken to produce a rattling noise. Subjects had to infer the position of the reward by interpreting the causal relationships between the reward and the board/cup, i.e., the inclination of the board or the noise that was generated by shaking the cup. Although pigs could solve the tasks, it was unclear whether they simply relied on stimulus enhancement cues (i.e., the slope of the board and the shaking movement or noise of the cup). Nawroth and von Borell (24) repeated the latter task that used a shaking bucket with a modified setup. Here, pigs were tested in their ability to use indirect visual and auditory stimuli (i.e., the absence of visual or acoustic cues) by choosing between two potential hiding locations. Pigs used indirect visual cues and, to some degree, indirect auditory cues, i.e., the absence of food by lifting one bowl or the absence of noise during the shaking of the bowl, to infer the location of the hidden reward. Again, the experimental design could not exclude the possibility that pigs were simply avoiding the non-rewarded location and relied on learned contingencies. However, these results provide support that pigs can rapidly adapt to new foraging situations.

Although it was demonstrated that pigs were able to differentiate between different amounts of food (129, 130), more complex studies that evaluated numerical competence should be conducted to investigate the cognitive mechanisms involved in this process. In addition, no studies involving categorization abilities or tool use have been conducted with pigs.

Social Cognition

Pigs are highly gregarious animals and thus establish stable social hierarchies. This requires good discriminatory abilities to differentiate between group members and between familiar and non-familiar individuals. Studies found pigs were able to distinguish unfamiliar from familiar conspecifics (131); additionally, pigs could differentiate familiar individuals using visual, auditory or olfactory cues alone (29, 30). However, 2D head cues were insufficient for pigs to discriminate between familiar conspecifics (132); thus, features other than head cues may be more salient for pigs. For example, studies on the ability of pigs to visually discriminate between humans showed that pigs mainly relied on the body height and upper torso of the human (36, 37, 133).

Nawroth et al. (65) used an approach that focused exclusively on the differentiation among the attentive states of humans. Juvenile pigs had to choose between two unfamiliar persons, while only one human focused attention on the test subject; the test conditions varied, and it was assumed that only the attentive human would provide food immediately or would provide food at all. While the subject performance in the choice task was poor and the results were inconclusive, two approach styles were distinguished during decision making. Here, pigs chose the attentive person more often when they approached non-impulsively (i.e., changed direction of or paused during approach), which was not the case when subjects chose impulsively (i.e., went straight to one person).

An object choice task is another well-known test used to investigate heterospecific communicative abilities. Here, subjects must choose between two potential baiting locations, of which only one contains a reward. To find the reward, a human administers different types of cues (e.g., pointing or gazing) toward the baited location (134). Given that pigs, unlike dogs, are not domesticated for companionship but are commonly raised as meat stock, their human social environment is often less demanding than that of dogs, and this might hamper their inclination to rely on human-given cues. This was partly supported by the findings of Albiach-Serrano et al. (40), who reported inconclusive results in the use of human-given cues (e.g., pointing, head orientation) by pigs. In contrast, Nawroth et al. (22) provided evidence that even very young pigs were able to use a variety of pointing cues and were also able to utilize the body and head orientation of a human experimenter to locate a hidden reward. However, pigs could have learned the gestures rapidly or, in terms of the pointing gestures, relied on stimulus enhancement. Thus, it is not clear whether the pigs were able to comprehend the referential and intentional nature of the human-given cues or whether they used learned contingencies to solve the task [see (134)].

Only a few studies have shown that pigs seem to be capable of social learning, either vertically (48, 49) or horizontally (55). However, in most examples, learning was directly related to food cues and could have been acquired through direct snout-snout interactions rather than visual observation. Based on their foraging ecology, it would be advantageous to not only learn what to eat but also learn how to acquire und process particular food sources. Recently, it has been demonstrated that juvenile Kunekune pigs learned how to manipulate objects (i.e., open a door) to receive a reward from related adult individuals (49). However, there have not been any studies on horizontal social learning that involves problem-solving or object manipulation for pigs.

Pigs are highly competitive foragers, and they rely on patchily distributed food sources. Therefore, it is unsurprising that dominant pigs readily start to scrounge on subordinate individuals of the group by following them to food patches they have discovered (135). In terms of this exploitation, it seems adaptive to be aware of the presence and attentive states of other individuals. Indeed, research suggests that pigs are able to attribute attentive states toward other individuals. Using an informed forager paradigm (136), Held et al. (63) found that the approach time to a baited container of a subordinate but knowledgeable pig depends on the body position of a dominant but ignorant conspecific. Overall, the subordinates were more likely to show food-directed behavior when the chances of arriving at the food source ahead of their exploiters were higher. Intriguingly, subordinates adjusted their foraging behavior based on whom they were foraging with, i.e., counter-exploitation behavior was only observed with dominant subjects that had already scrounged on the subordinates in previous foraging trials (137). In another study, Held et al. (62) allowed pigs to follow two companion pigs, of which one was able to see the baiting of food and the other was not. Most pigs did not follow their companions, likely to avoid competitive and aggressive behavior. Nonetheless, out of ten pigs, two subjects followed their conspecifics, and one of them followed the "knowing" individual significantly more often than the "unknowing" individual. These studies suggest that pigs use body cues to discriminate between the different attentive states of conspecifics and that they, to some degree, might be able to interpret the visual perspective of others. No studies have focused on more complex socio-cognitive phenomena, such as prosocial behavior or inequity aversion, in pigs.

Small Ruminants

Physical Cognition

Goats and sheep are both small ruminants that preferably graze/browse on grass and herbs; thus, being able to categorize food stimuli should be useful to these animals during foraging. For example, sheep have been shown to use species-based categorization when selecting their diet. Sheep generalized their aversion among species and classes of plants into distinct categories (15, 138). Experiments under husbandry conditions have also shown that goats have more abstract learning and categorization abilities. By using an automated learning device, Langbein et al. (139) investigated "learning to learn" or "learning set" formation in dwarf goats. The performance of the animals improved when the animals were tested using a series of visual discrimination tasks, and the results indicated that the goats started to develop a "learning set." In addition, dwarf goats can form open-ended categories based on similarities in the visual appearance of artificial symbols, while individuals are also able to generalize these categories across new symbols (16). Using a similar experimental setup, individual goats were found to be capable of learning the oddity concept. When presented with an odd stimulus and three identical non-odd stimuli on the automated learning device, these animals consistently chose the odd stimulus after the initial training (140).

Small ruminants possess a sophisticated understanding of their physical environment. When confronted with a task in which a reward was hidden in one of two opaque containers that then switched positions, the goats showed moderate to high success rates in finding the reward (23). Individual goats, but not sheep, can also infer the location of a reward through exclusion. When provided with a choice between two containers (while only one was baited with a reward), goats and sheep were able to use direct information (i.e., the presence of food) from the baited container in the choice task. However, only goats, but not sheep, used indirect information (i.e., the absence of the reward) from the empty container to infer the presence of the reward in the baited container (25). Due to the different feeding preferences of goats (low-fiber feeders; dietary browsers) compared to sheep (high-fiber feeders; dietary grazers), goats might prefer and forage more selectively than do sheep. This higher flexibility may have led to the avoidance of a potential, but empty, food location in goats but not in sheep. In fact, an earlier study by Hosoi et al. (141) indicated that goats avoided high-fiber food when they were offered the option to feed on low-fiber food, but sheep did not. No studies have investigated number discrimination or tool use for either goats or sheep.

Social Cognition

Goats and sheep live in fission-fusion societies with stable dominance hierarchies (142, 143); thus, it should be highly advantageous for them to remember and recognize familiar group members. When presented with pairs of face images or vocalizations, sheep were able to discriminate between different species (including humans), breeds and sexes of their same breed. The sheep learned to distinguish between individual adult sheep faces, but breed and social familiarity influenced the level of discrimination performance (144, 145). Sheep behavioral and neural activities also indicated they remembered faces of familiar conspecifics after more than 2 years, which suggests sheep have a high capacity for learning and memory (31); moreover, that 2D images of conspecific faces seemed to be represented as a 3D equivalent of the real-life individual. Additional evidence for this was reported in a recent experiment where sheep recognized a familiar handler when the face of this handler was presented as a 2D image in a discrimination task (38).

Goats can differentiate among conspecifics using visual and/or acoustic cues (32, 146, 147). For example, Keil et al. (32) showed that goats discriminated between familiar and unfamiliar conspecifics, even when their heads were not visible. Surprisingly, there have been no investigations to determine how goats discriminate between humans.

There is broad popular interest in the relationship between humans and small ruminants, specifically on how small ruminants react to being observed by humans (e.g., the movie "The Men Who Stare at Goats"). Indeed, human gaze appeared to alter the behavior of domestic sheep compared to situations where there was no human eye contact (148): Sheep glanced at the gazing human more often and showed higher levels of activity. Nawroth et al. (42, 149) found that goats differed in their anticipatory behavior depending on a human's attentive state. For instance, when an inaccessible reward was positioned in front of a goat, an experimenter engaged in different postures that resembled different levels of attention toward the subject (e.g., back turned toward the subject or eyes closed). The anticipatory behavior of goats increased as the experimenter gave more attention to the subject, while alert behavior ("standing alert") was most prominent when the experimenter was present but not giving the test subject any attention. These results indicated that the goats adapted their behavior based on the head and body orientation, but not the eye visibility, of the experimenter as a means of being given a reward. The results related to the body orientation were confirmed in a different experiment that used a choice paradigm; specifically, goats could choose to beg for food from either an attentive or inattentive person (i.e., body turned toward subject vs. body turned away). However, the head orientation of humans did not affect the choice behavior of goats (66).

Nawroth et al. (42) and Kaminski et al. (41) investigated the ability of goats to use various human-given cues in an object-choice task to locate a hidden reward. The authors found that goats were able to utilize human pointing gestures, but goats could not interpret the head or gaze direction of a human to find the hidden reward. In contrast to the negative findings regarding the human head and gaze direction, goats could follow the gaze direction of conspecifics into distant space (41), which is an extremely important trait in terms of predator detection (150). Goats also showed human-directed behavior in the form of frequent gaze alternations toward humans when they were confronted with an inaccessible food reward (44, 45), which was similar to what has been found in dogs and horses (43, 81). Here, as well, goats considered the attentional stance of the experimenter and altered their use of gaze alternations during the task depending on whether the experimenter was turned toward or away from them.

In small ruminants, vertical information transfer between individuals (e.g., social learning by offspring from adults) is important for the development of foraging skills (50, 151). For example, lambs can learn how to use an artificial teat from knowledgeable lambs that where transferred into their group (152). Baciadonna et al. (56) tested goats in a foraging task where they had the opportunity to follow another goat in a Y-maze or to rely on their own experience where to find a reward. Goats relied more on personal information than on social information when both types were available and conflicted with each other. Briefer et al. (57) investigated social problem-solving abilities of goats using a complex two-step foraging task in which subjects had to first pull a rope and then lift a lever to receive access to food. Goats quickly learned the task on an individual basis. However, goats that observed a demonstrator goat first did not learn the task faster compared to goats that did not see a demonstration. This indicated that the goats relied on individual experience rather than on social experience in this particular task. In contrast to these previous findings, human demonstration improved goats' performance in a spatial problem-solving task (60). Goats that experienced a human demonstrator detouring a V-shaped hurdle solved the detour faster compared to goats that did not receive a demonstration. No studies are available regarding more complex socio-cognitive phenomena, such as prosocial behavior or inequity aversion.

IMPACT ON WELFARE PRACTICES

General knowledge of how farm animals perceive and address their physical and social environment is of interest for improving housing and management practices; it can also be used in future studies in the different fields of applied ethology (64, 153–155).

Enrichment

Livestock housing conditions are often structurally simple and offer limited possibilities to exhibit species-appropriate behavior (156, 157). These limitations can lead to boredom and frustration, which promotes the appearance of abnormal behavior, especially that which is related to stress and reduced welfare (158, 159). One way to decrease the level of boredom and frustration in livestock is to enhance the biological relevance of the housing conditions of farm animals; this can be done through the provision of a variety of new structures, items and challenges that are related to the animals' needs and natural behavioral repertoire (157). This so-called environmental enrichment is supposed to elicit a higher degree of behavioral diversity by increasing the physical and social complexity of the livestock environment (160, 161). Providing specific cognitive enrichment, e.g., artificial challenges associated with rewards, should, through positive reinforcement and the associated control and predictability of the environment, evoke positive affective states in livestock and improve their wellbeing (162–164). A detailed understanding of the cognitive capacities of farm animals, and especially their understanding of the physical properties of their environment, will provide help to design proper forms of structural and cognitive enrichment (165–168).

The integration of different types of cognitive enrichment into the housing of farm animals has received little attention; to date, approaches have been based on the instrumental or operant learning skills of subjects. For example, using a computerized feeding device ("call-feeding station," CFS), piglets were required to recognize an individual sound signal and then to operate a button at an increasing fixed ratio to receive a food reward (169).

Animals learned the task, which was taught using a combination of classical and operant conditioning, within a short time period. After several weeks of receiving food via the CFS, piglets showed less stress during feeding and evoked longer lasting positive emotions (170) compared to the control animals; moreover, the piglets displayed less abnormal behavior and showed reduced signs of fear in the context of being faced with a challenging environment (169). When goats were successively confronted with several different visual discrimination tasks through the use of a computer-based learning device that was integrated into the home pen, their heart rates initially increased but then decreased as the goats showed increased learning performance in consecutive tasks (171). This indicated that the goats had been exposed to a challenging task that induced positive eustress (172). It also appeared that goats seemed to seek challenges; for example, goats continued to operate the rewarding learning device even when an identical reward was available without the requirement of additional cognitive effort (173). This behavioral pattern is linked to the concept of contrafreeloading (174) and indicates that successfully coping with a cognitively challenging device or procedure could have intrinsic reinforcing properties beyond the extrinsic reward itself. Further evidence of this has been provided in experiments on heifers and beagles, who showed greater positive excitement after learning an operant task than did control animals who did not have to solve the task themselves. This excitement that accompanies success is believed to be related to positive affective states in non- human animals (175, 176).

Regardless of which device or procedure is used to cognitively challenge the animals, the device or procedure must be modified regularly to be remain challenging, e.g., by changing the rewarded cue or the entire task (177). Otherwise, the animals will develop routine-like behavior, and the device will no longer be challenging and enriching. On the other hand, to develop appropriate challenges that do not overstrain the animals, we need to have detailed knowledge on the species-specific problem-solving abilities of livestock animals.

As described, the first attempts to integrate cognitively challenging tasks into housing to promote cognitive enrichment have focused on operant conditioning tasks. In the future, in addition to relying on learning, it may be important to also rely on physico-cognitive traits (such as categorization abilities or making inferences) to pave the way for new opportunities on how to integrate changing challenges into the housing environments of animals (**Table 1**). However, the limited availability of solid evidence of the physico-cognitive capacities discussed in this review demonstrates how little we actually know about the problem-solving abilities of farm animals and their perception of their physical environment.

Transfer

When farm animals are transferred to new environments during ontogenesis and confronted with new devices, e.g., automatically delivered food or water or offered comfort, they often need time to acclimate to these new conditions and learn how to use the devices (178). During these situations, it might be highly beneficial to rely on the mechanisms related to social or observational learning from experienced conspecifics (or humans) who act as demonstrators; this may facilitate the adaptation process to the novel housing conditions. To achieve this, we must identify the distinct and species-specific mechanisms of social learning in farm animals (see **Table 2**). In sheep, social learning plays an important role in the transmission of diet preferences (179). Housing dairy calves in social groups results in increased weaning weights compared with calves that have been individually housed; this result is likely due to the increased intake of dry matter, which is often attributed to social learning or social facilitation during feeding (180, 181). In lambs, learning to suckle from an artificial teat was facilitated when an experienced partner was in the group compared to the control group that did not have a demonstrator. Experimental lambs sniffed or sucked the teat more often than did the lambs in the control group (152). Future research should identify which potential mechanisms, e.g., social facilitation, stimulus and local enhancement, or observational conditioning (86, 182), enable farm animals to use information from conspecifics or heterospecifics.

Human—Animal Interactions

To improve handling practices under farm management conditions, it is important to know how livestock perceive and interact with humans. Based on this knowledge, applied research can be better adjusted to assess how subtle human behavioral changes can have rewarding or adverse effects on livestock behavior (183). During recent decades, it has been shown that different farm animal species can discriminate between individual humans (see **Table 2**), and animals may use individual humans to predict positive or negative events that are routinely involved in housing and management (184, 185). Mini pigs that were positively reinforced by their handlers over several weeks discriminated between the familiar keeper and a stranger in a Y-maze test using auditory, visual and olfactory cues (133). Similarly, cattle have been shown to discriminate between a handler who reinforced an operant action and a handler who did not (102). A differential reaction towards humans has been observed in sheep; for example, lambs handled by an unfriendly handler generalized their fear responses toward familiar and unfamiliar humans, while gently treated lambs discriminated between familiar and unfamiliar humans (186). Horses have also been shown to generalize their experiences with positive and negative stockpersons from one human to another (187). This gradual variation in the abilities of different livestock species to differentiate between individual humans based on their attitude toward the animals might have profound implications for animal housing and management.

Next, to avoid negative impacts, it is also important to identify and implement rewarding human-animal interactions (183). Studies on tactile human-animal interactions have demonstrated that there is potential to identify relevant stress-reducing behavior by stockpersons during handling and transport processes (188–191). For example, direct interactions between farm animals and their handlers (e.g., gentle touching or stroking) resulted in the animals having reduced stress and fear of humans (192–194); therefore, this improved the ease of handling, productivity and immune response (195–197).

Furthermore, various farm animal species follow human-given communicative cues and differ in their behavior based on whether a human gives them attention or not (see **Table 2**). Although most of these experiments included (previous) positive feedback from humans, animals will also likely show different responses based on the attentional stance of a human in more aversive settings, e.g., routine handling practices (148). Some livestock species, such as goats and horses (43, 44, 118), have been shown to also engage in communication efforts directed at humans (**Table 2**). Animals used behaviors, such as gaze alternations, to direct the attention of a human toward a problem that the animal could not solve themselves. In terms of applying this to the farm, the skilful reading of these cues could lead to the improved detection of livestock needs. Advanced communication between livestock and humans does exist and using it in an applied setting might help decrease stress during handling and better meet the needs of the animals. However, no relevant research on this topic has been conducted yet.

ETHICAL CONSIDERATION

The question about how we *should* treat farm animals based on their complex social, cognitive, and emotional capacities is a question of philosophy, and more specifically, of animal ethics (198). Several capacity-oriented approaches exist, and these, in one way or another, link the moral status of animals to their abilities (199). However, the role of such abilities and the weight they are assigned will vary based on the different normative frameworks of these theories (200). Two important primary approaches should be separated, as they lead to implications that partly overlap but are also profoundly different in their nature and impact. The moral implications of (farm) animal cognition can first be assessed by *welfare ethics* [understood as an interdisciplinary endeavor among welfare scientists, biologists, veterinarians, and philosophers (201–203)]. Second, one can complete such an assessment by applying *ethical theories that go beyond welfare* [e.g., (204, 205)].

Welfare Implications

It has been recognized that the links between cognition and welfare are important from an economic perspective in terms of its relation to production success (64). However, from the perspective of animal ethics, it can also be asked whether animal cognition and this connection to animal welfare matters *from the perspective of the animals*.

On the one hand, the nature of animal minds with regard to their capacity to feel pain and other adverse feelings can form the basis for an ethical account of experiential well-being in animals (199). On the other hand, experiential well-being is at the core of one of the most important and most recognized principles in animal ethics, i.e., the *principle of non-maleficence* (206). This principle asks us *not to cause extensive unnecessary harm to others without their consent*, which is a claim that can be specified into several sub-rules. Among them is, most importantly, the rule to *provide for the basic physical and psychological needs* of animals that are under human care (206). This means that welfare ethics establishes an argument that connects physical and psychological needs with welfare and connects welfare with a normative value.

If animal ethics is concerned with animal welfare and welfare is indeed "solely [dependent] on the mental, psychological and cognitive needs of the animals concerned" (207), then the range of connections among capacities, needs and welfare must be considered. For example, learning and memory capacities are assumed to have an impact on the capacity of an animal to cope with housing conditions; thus, these capacities can impact the welfare of the animal (64, 208). Similarly, we might argue that their abilities to recognize and remember conspecifics and to understand the mental states of others (such as their perception and motivations, **Table 2**) have an impact on the richness and quality of their social life. Such abilities could be important pre-requisites for (or building blocks of) more complex social interactions like empathetically motivated helping behavior or cooperation. The same will be true for animals' general prosocial tendencies and their understanding of fairness. Capacities like these are currently at the center of philosophical, psychological and biological debates, and may even be related to the question whether animals possess the ability to act morally themselves. They will increasingly attract scholarly attention and spur interdisciplinary debates (91, 209–212). Rowlands (212) for example, suggested a de-intellectualized approach to the moral abilities of animals: according to his theory animals can be regarded as moral subjects if their behavior is motivated by moral emotions like empathy.

Beyond Welfare

However, some ethical problems cannot be fully captured by welfare approaches. If good welfare was the only important ethical premise, then we could potentially instrumentalize, objectify, ridicule, or even kill animals as we like—as long as we did it painlessly. The question is if doing so still constitutes kinds of harms that occur even if the animals do not immediately suffer. In humans, at least, we clearly assume that objectification for example does damage to a human's dignity even if the person herself may not perceive it that way. Therefore, many ethicists meanwhile employ concepts such as respect and dignity in animal ethics as well (213), and develop approaches based on considering the animals' capabilities (205), integrity (214), or rights (215), Such accounts bear the potential to argue beyond the claim of welfare.

In such theories, the complex social and cognitive capacities of animals can play a more direct role in terms of moral qualities. Nussbaum, for example, argued that each species has a set of capabilities which are intrinsically valuable, meaning that behavior based on these capabilities is a value *in itself* and does not just have an instrumental value (205). Carrying out such capabilities is essential to the flourishing of members of that species. Pro-social care behavior falls in this category of capabilities. However, carrying out pro-social care behavior in housing systems that isolate and restrain animals might be impossible. The same might be true if social animals are frequently separated and regrouped according to productivity and reproductive state. In dairy cows, for example, long-term familiarity has an effect on the intensity of social relationships (216). Evidence from other species has suggested that animals have a higher probability of engaging in caring and helping behaviors when they are familiar with the other subject (217). Thus, dairy cows in standard husbandry systems might be restricted to impoverished relationships and social engagement. If the only possible relationships these animals can establish are short-term relationships and if they frequently lose their preferred social partners, this might be considered a welfare issue. However, it could be more than that. If complex capacities in the realm of prosociality, such as caring or helping behaviors, are capabilities that are inherently valuable, then it constitutes a much broader ethical problem that we have established husbandry systems that systematically prevent the animals from developing and maintaining such capacities [for a discussion related to this topic, see (218)].

In contrast to the welfare approach, the animal rights approach asserts that most animals we use as farm animals are *subjects-of-a-life*, i.e., a status for which a range of cognitive, emotional and social capacities are paramount. As such subjects, these animals deserve some basic inviolable rights (215). To build on this idea, biologists and rights philosophers have proposed the claim that animals whose cognitive capacities have high similarities with those of humans should at least be afforded a right to life and freedom and should not be tortured (204). Until now, such claims have been focused on animals that are obviously cognitively complex, e.g., great apes and cetaceans. Future cognition research will increasingly reveal whether the abilities of farm animal species should be interpreted in a substantially different way. The abilities of farm animals might in fact sufficiently resemble the capacities recognized in apes and dolphins and deserve similar moral relevance. Thus, with proceeding research, we can expect more ethical discussions, and some of them will continue to challenge the rather narrow focus of welfare ethics.

RECOMMENDATIONS FOR FUTURE RESEARCH AND CONCLUSIONS

Farm animal cognition is a relatively new, but growing, field of research. It provides an excellent opportunity for interdisciplinary work that combines research on animal cognition and animal welfare (48, 208). For instance, paradigms such as the judgement bias test first emerged in human psychological research; now this paradigm is an established test paradigm in applied ethology (219). Similar transfers of other test paradigms are likely to follow and will provide exciting new insights into the minds of farm animals. Indeed, the increased implementation of experimental designs used in human/primate psychological research is highly recommended to improve our understanding about how livestock perceive and interact with their environment.

The attribution (or lack of attribution) of certain cognitive capacities in farm animals is not only relevant for providing adequate welfare but also for consumer choices (220). For example, the tendency to not eat a specific kind of meat increases as more human-like cognitive capacities are attributed to a particular livestock species (221). In contrast, the "dumbing" down of farm animals leads to less moral concern in terms of eating these species (222). Current evidence only scratches the surface of farm animal cognitive capacities, but it already indicates that livestock species possess sophisticated cognitive capacities that are not yet sufficiently acknowledged in welfare legislation. Thus, the recognition of farm animal cognition plays—and will continue to play—a vital role in consumer attitudes as well as in ethical theory.

In this article, we reviewed the evidence on a variety of cognitive traits in farm animals. Certain traits, such as the ability to form categories or the differentiation among individuals, have been thoroughly investigated. However, we identified a lack of research on a diverse set of physico-cognitive capacities (such as numerosity discrimination and object permanence). This knowledge in particular is of key interest to better understand how farm animals perceive their physical environment; this information will improve our design of husbandry environments and enhance the development of management practices.

Finally, we want to emphasize that especially for research on farm animals it is important to know what they are not capable of; this helps us to avoid exposing these animals to stressful situations (83). For instance, the degree to which subjects are able to mentally travel in time is highly relevant to how they anticipate positive or negative future events (208). The "file drawer effect," i.e., negative findings remain unpublished because they are not novel or exciting enough (223), is thus likely to massively hamper progress on how to adequately address welfare issues.

AUTHOR CONTRIBUTIONS

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

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REFERENCES

1. Farm Animal Welfare Council. *The Five Freedoms*. Surbiton: Farm Animal Welfare Council (2013).
2. Wathes C. Guarding the welfare of farm animals. *Vet Rec.* (2010) 167:583–4. doi: 10.1136/vr.c4785
3. Špinka M. How important is natural behaviour in animal farming systems? *Appl Anim Behav Sci.* (2006) 100:117–28. doi: 10.1016/j.applanim.2006.04.006
4. Désiré L, Boissy A, Veissier I. Emotions in farm animals: a new approach to animal welfare in applied ethology. *Behav Process.* (2002) 60:165–80. doi: 10.1016/S0376-6357(02)00081-5
5. Mendl M, Burman OHP, Paul ES. An integrative and functional framework for the study of animal emotion and mood. *Proc R Soc B* (2010a) 277:2895–904. doi: 10.1098/rspb.2010.0303
6. Forkman B, Furuhaug IL, Jensen P. Personality, coping patterns, and aggression in piglets. *Appl Anim Behav Sci.* (1995) 45:31–42. doi: 10.1016/0168-1591(95)00601-N
7. Shettleworth SJ. *Cognition, Evolution, and Behavior*. Oxford: Oxford University Press (2010).
8. Tomasello M, and Call J. (1997). *Primate Cognition*. 1st ed. New York, NY: Oxford University Press.
9. Emery NJ. Cognitive ornithology: the evolution of avian intelligence. *Phil Trans R Soc B* (2006) 361:23–43. doi: 10.1098/rstb.2005.1736
10. Kaminski J, Nitzschner M. Do dogs get the point? A review of dog–human communication ability. *Learn Motiv.* (2013) 44:294–302. doi: 10.1016/j.lmot.2013.05.001
11. Shettleworth SJ. The evolution of comparative cognition: Is the snark still a boojum? *Behav Process.* (2009) 80:210–7. doi: 10.1016/j.beproc.2008.09.001
12. Coulon M, Baudoin C, Heyman Y, Deputte B. Cattle discriminate between familiar and unfamiliar conspecifics by using only head visual cues. *Anim Cogn.* (2011) 14:279–90. doi: 10.1007/s10071-010-0361-6
13. Hanggi EB. Categorization learning in horses (*Equus caballus*). *J Comp Psychol.* (1999) 113:243–52. doi: 10.1037/0735-7036.113.3.243
14. Hanggi EB, Ingersoll JF. Long-term memory for categories and concepts in horses (*Equus caballus*). *Anim Cogn.* (2009) 12:451–62. doi: 10.1007/s10071-008-0205-9
15. Ginane C, Dumont B. Do grazing sheep use species-based categorization to select their diet? *Behav Process.* (2010) 84:622–4. doi: 10.1016/j.beproc.2010.01.022
16. Meyer S, Nürnberg G, Puppe B, Langbein J. The cognitive capabilities of farm animals: categorisation learning in dwarf goats (*Capra hircus*). *Anim Cogn.* (2012) 15:567–76. doi: 10.1007/s10071-012-0485-y
17. Gabor V, Gerken M. Shetland ponies (*Equus caballus*) show quantity discrimination in a matching-to-sample design. *Anim Cogn.* (2014) 17:1233–43. doi: 10.1007/s10071-014-0753-0
18. Uller C, Lewis J. Horses (*Equus caballus*) select the greater of two quantities in small numerical contrasts. *Anim Cogn.* (2009) 12:733–8. doi: 10.1007/s10071-009-0225-0
19. Proops L, Walton M, McComb K. The use of human-given cues by domestic horses, *Equus caballus*, during an object choice task. *Anim Behav.* (2010) 79:1205–9. doi: 10.1016/j.anbehav.2010.02.015
20. Krueger K, Flauger B, Farmer K, Maros K. Horses (*Equus caballus*) use human local enhancement cues and adjust to human attention. *Anim Cogn.* (2011) 14:187–201. doi: 10.1007/s10071-010-0352-7
21. Nawroth C, Ebersbach M, von Borell E. A note on pigs' knowledge of hidden objects. *Arch Anim Breed.* (2013a) 56:861–72. doi: 10.7482/0003-9438-56-086
22. Nawroth C, Ebersbach M, von Borell E. Juvenile domestic pigs (*Sus scrofa domestica*) use human-given cues in an object choice task. *Anim Cogn.* (2014a) 17:701–13. doi: 10.1007/s10071-013-0702-3
23. Nawroth C, von Borell E, Langbein J. Object permanence in the dwarf goat (*Capra aegagrus hircus*): perseveration errors and the tracking of complex movements of hidden objects. *Appl Anim Behav Sci.* (2015b) 167:20–6. doi: 10.1016/j.applanim.2015.03.010
24. Nawroth C, von Borell E. Domestic pigs' (*Sus scrofa domestica*) use of direct and indirect visual and auditory cues in an object choice task. *Anim Cogn.* (2015) 18:757–66. doi: 10.1007/s10071-015-0842-8
25. Nawroth C, von Borell E, Langbein J. Exclusion performance in dwarf goats (*Capra aegagrus hircus*) and sheep (*Ovis orientalis aries*). *PLoS ONE* (2014b) 9:e93534. doi: 10.1371/journal.pone.0093534
26. Coulon M, Deputte B, Heyman Y, Delatouche L. Visual discrimination by heifers (*Bos taurus*) of their own species. *J Comp Psychol.* (2007) 121:198–204. doi: 10.1037/0735-7036.121.2.198
27. Krueger K, Flauger B. Olfactory recognition of individual competitors by means of faeces in horse (*Equus caballus*). *Anim Cogn.* (2011) 14:245–57. doi: 10.1007/s10071-010-0358-1
28. Proops L, McComb K, Reby D. Cross-modal individual recognition in domestic horses (*Equus caballus*). *Proc Natl Acad Sci USA* (2009) 106:947–51. doi: 10.1073/pnas.0809127105
29. McLeman MA, Mendl M, Jones RB, White R, Wathes CM. Discrimination of conspecifics by juvenile domestic pigs, *Sus scrofa*. *Anim Behav.* (2005) 70:451–61. doi: 10.1016/j.anbehav.2004.11.013
30. McLeman MA, Mendl MT, Jones RB, Wathes CM. Social discrimination of familiar conspecifics by juvenile pigs, *Sus scrofa*: development of a non-invasive method to study the transmission of unimodal and bimodal cues between live stimuli. *Appl Anim Behav Sci.* (2008) 115:123–37. doi: 10.1016/j.applanim.2008.06.010
31. Kendrick KM, da Costa P, Leigh E, Hinton MR, Peirce JW. Sheep don't forget a face. *Nature* (2001) 414:165–6. doi: 10.1038/35102669
32. Keil NM, Imfeld-Mueller S, Aschwanden J, Wechsler B. Are head cues necessary for goats (*Capra hircus*) in recognising group members ? *Anim Cogn.* (2012) 15:913–21. doi: 10.1007/s10071-012-0518-6
33. Rybarczyk P, Koba Y, Rushen J, Tanida H, de Passillá AM. Can cows discriminate people by their faces? *Appl Anim Behav Sci.* (2001) 74:175–89. doi: 10.1016/S0168-1591(01)00162-9
34. Stone SM. Human facial discrimination in horses: can they tell us apart? *Anim Cogn.* (2010) 13:51–61. doi: 10.1007/s10071-009-0244-x
35. Proops L, McComb K. Cross-modal individual recognition in domestic horses (*Equus caballus*) extends to familiar humans. *Proc R Soc B* (2012) 279:3131–8. doi: 10.1098/rspb.2012.0626
36. Koba Y, Tanida H. How do miniature pigs discriminate between people? The effect of exchanging cues between a non-handler and their familiar handler on discrimination. *Appl Anim Behav Sci.* (1999) 61:239–52. doi: 10.1016/S0168-1591(98)00192-0
37. Koba Y, Tanida H. How do miniature pigs discriminate between people? Discrimination between people wearing coveralls of the same colour. *Appl Anim Behav Sci.* (2001) 73:45–58. doi: 10.1016/S0168-1591(01)00106-X
38. Knolle F, Goncalves RP, Morton AJ. Sheep recognize familiar and unfamiliar human faces from two-dimensional images. *R Soc Open Sci.* (2017) 4:171228. doi: 10.1098/rsos.171228
39. McKinley J, Sambrook TD. Use of human-given cues by domestic dogs (*Canis familiaris*) and horses (*Equus caballus*). *Anim Cogn.* (2000) 3:13–22. doi: 10.1007/s100710050046
40. Albiach-Serrano A, Bräuer J, Cacchione T, Zickert N, Amici F. The effect of domestication and ontogeny in swine cognition (*Sus scrofa scrofa* and *S. s domestica)* *Appl Anim Behav Sci.* (2012) 141:25–35. doi: 10.1016/j.applanim.2012.07.005
41. Kaminski J, Riedel J, Call J, Tomasello M. Domestic goats, *Capra hircus*, follow gaze direction and use social cues in an object choice task. *Anim Behav.* (2005) 69:11–8. doi: 10.1016/j.anbehav.2004.05.008
42. Nawroth C, von Borell E, Langbein J. "Goats that stare at men": dwarf goats alter their behaviour in response to human head orientation, but do not spontaneously use head direction as a cue in a food-related context. *Anim Cogn.* (2015a) 18:65–73. doi: 10.1007/s10071-014-0777-5
43. Malavasi R, Huber L. Evidence of heterospecific referential communication from domestic horses (*Equus caballus*) to humans. *Anim Cogn.* (2016) 19:899–909. doi: 10.1007/s10071-016-0987-0
44. Nawroth C, Brett JM, McElligott AG. Goats display audience-dependent human-directed gazing behaviour in a problem-solving task. *Biol Lett.* (2016b) 12:20160283. doi: 10.1098/rsbl.2016.0283
45. Langbein J, Krause A, Nawroth C. Human-directed behaviour in goats is not affected by short-term positive handling. *Anim Cogn.* (2018) 21:795–803. doi: 10.1007/s10071-018-1211-1

46. Howery LD, Provenza FD, Banner RE, Scott CB. Social and environmental factors influence cattle distribution on rangeland. *Appl Anim Behav Sci.* (1998) 55:231–44. doi: 10.1016/S0168-1591(97)00054-3
47. Christensen JW. Early-life object exposure with a habituated mother reduces fear reactions in foals. *Anim Cogn.* (2016) 19:171–9. doi: 10.1007/s10071-015-0924-7
48. Oostindjer M, Bolhuis JE, Mendl M, Held S, van den Brand H, Kemp B. Learning how to eat like a pig: effectiveness of mechanisms for vertical social learning in piglets. *Anim Behav.* (2011) 82:503–11. doi: 10.1016/j.anbehav.2011.05.031
49. Veit A, Wondrak M, Huber L. Object movement re-enactment in free-ranging Kune Kune piglets. *Anim Behav.* (2017) 132:49–59. doi: 10.1016/j.anbehav.2017.08.004
50. Glasser TA, Ungar ED, Landau SY, Perevolotsky A, Muklada H, Walker JW. Breed and maternal effects on the intake of tannin-rich browse by juvenile domestic goats (*Capra hircus*). *Appl Anim Behav Sci.* (2009) 119:71–7. doi: 10.1016/j.applanim.2009.02.028
51. Bailey DW, Howery LD, Boss DL. Effects of social facilitation for locating feeding sites by cattle in an eight-arm radial maze. *Appl Anim Behav Sci.* (2000) 68:93–105. doi: 10.1016/S0168-1591(00)00091-5
52. Veissier I. Observational learning in cattle. *Appl Anim Behav Sci.* (1993) 35:235–43. doi: 10.1016/0168-1591(93)90139-G
53. Krueger K, Farmer K, Heinze J. The effects of age, rank and neophobia on social learning in horses. *Anim Cogn.* (2014) 17:645–55. doi: 10.1007/s10071-013-0696-x
54. Rørvang MV, Ahrendt LP, Christensen JW. Horses fail to use social learning when solving spatial detour tasks. *Anim Cogn.* (2015) 18:847–54. doi: 10.1007/s10071-015-0852-6
55. Figueroa J, Solà-Oriol D, Manteca X, Pérez JF. Social learning of feeding behaviour in pigs: effects of neophobia and familiarity with the demonstrator conspecific. *Appl Anim Behav Sci.* (2013) 148:120–7. doi: 10.1016/j.applanim.2013.06.002
56. Baciadonna L, McElligott AG, Briefer EF. Goats favour personal over social information in an experimental foraging task. *PeerJ* (2013) 1:e172. doi: 10.7717/peerj.172
57. Briefer EF, Haque S, Baciadonna L, McElligott AG. Goats excel at learning and remembering a highly novel cognitive task. *Front Zool.* (2014) 11:20. doi: 10.1186/1742-9994-11-20
58. Burla JB, Siegwart J, Nawroth C. Human demonstration does not facilitate the performance of horses (*Equus caballus*) in a spatial problem-solving task. *Animals* (2018) 8:96. doi: 10.3390/ani8060096
59. Schuetz A, Farmer K, Krueger K. Social learning across species: horses (*Equus caballus*) learn from humans by observation. *Anim Cogn.* (2017) 20:567–73. doi: 10.1007/s10071-016-1060-8
60. Nawroth C, Baciadonna L, McElligott AG. Goats learn socially from humans in a spatial problem-solving task. *Anim Behav.* (2016a) 121:123–9. doi: 10.1016/j.anbehav.2016.09.004
61. Proops L, McComb K. Attributing attention: the use of human-given cues by domestic horses (*Equus caballus*). *Anim Cogn.* (2010) 13:197–205. doi: 10.1007/s10071-009-0257-5
62. Held S, Mendl M, Devereux C, Byrne RW. Behaviour of domestic pigs in a visual perspective taking task. *Behaviour* (2001) 138:1337–54. doi: 10.1163/156853901317367627
63. Held S, Mendl M, Devereux C, Byrne RW. Foraging pigs alter their behaviour in response to exploitation. *Anim Behav.* (2002a) 64:157–66. doi: 10.1006/anbe.2002.3044
64. Held S, Mendl M, Laughlin K, Byrne RW. Cognition studies with pigs: livestock cognition and its implication for production. *J Anim Sci.* (2002b) 80:E10–17. doi: 10.2527/animalsci2002.0021881200800ES10003x
65. Nawroth C, Ebersbach M, von Borell E. Are juvenile domestic pigs (*Sus scrofa domestica*) sensitive to the attentive states of humans? - The impact of impulsivity on choice behaviour. *Behav Process.* (2013b) 96:53–8. doi: 10.1016/j.beproc.2013.03.002
66. Nawroth C, McElligott AG. Human head orientation and eye visibility as indicators of attention for goats (*Capra hircus*). *PeerJ* (2017) 5:e3073. doi: 10.7717/peerj.3073
67. Zentall TR, Galizio M, Critchfied TS. Categorization, concept learning, and behavior analysis: an introduction. *J Exp Anal Behav.* (2002) 78:237–48. doi: 10.1901/jeab.2002.78-237
68. Pepperberg I. Grey parrot numerical competence: a review. *Anim Cogn.* (2006) 9:377–91. doi: 10.1007/s10071-006-0034-7
69. Feigenson L, Dehaene S, Spelke E. Core systems of number. *Trends Cogn Sci.* (2004) 8:307–14. doi: 10.1016/j.tics.2004.05.002
70. Jaakkola K. Do animals understand invisible displacement? A Critical Review. *J Comp Psychol.* (2014) 128:1–15. doi: 10.1037/a0035675
71. Premack D. Cause/induced motion: intention/spontaneous motion. In: Changeux JP, Chavaillon J, editors. *Origins of the Human Brain*. Oxford: Oxford University Press (1995). p. 286–309.
72. Heyes CM. Anecdotes, training, trapping and triangulating: do animals attribute mental states? *Anim Behav.* (1993) 46:177–88. doi: 10.1006/anbe.1993.1173
73. Penn DC, Povinelli DJ. Causal cognition in human and nonhuman animals: a comparative, critical review. *Annu Rev Psychol.* (2007) 58:97–118. doi: 10.1146/annurev.psych.58.110405.085555
74. Bentley-Condit V, Smith EO. Animal tool use: current definitions and an updated comprehensive catalog. *Behaviour* (2010) 147:185–32A. doi: 10.1163/000579509X12512865686555
75. Vaesen K. The cognitive bases of human tool use. *Behav Brain Sci.* (2012) 35:203–18. doi: 10.1017/S0140525X11001452
76. Barton RA, Dunbar RIM. Evolution of the social brain. In: Whiten A, Byrne RW, editors. *Machiavellian Intelligence II*. Cambridge: Cambridge University Press (1997). p. 240–63. doi: 10.1017/CBO9780511525636.010
77. Tibbetts, EA, Dale J. Individual recognition: it is good to be different. *Trends Ecol. Evol.* (2007) 22:529–37. doi: 10.1016/j.tree.2007.09.001
78. Adachi I. Cross-modal representations in primates and dogs: a new framework of recognition of social objects. *Interact Stud.* (2009) 10:225–51. doi: 10.1075/is.10.2.07ada
79. Bradbury JW, Vehrencamp SL. *Principles of Animal Communication.* Sunderland, MA: Sinauer Associates, Inc. (1998).
80. Marshall-Pescini S, Colombo E, Passalacqua C, Merola I, Prato-Previde E. Gaze alternation in dogs and toddlers in an unsolvable task: evidence of an audience effect. *Anim Cogn.* (2013) 16:933–43. doi: 10.1007/s10071-013-0627-x
81. Miklósi Á, Kubinyi E, Topál J, Gácsi M, Virányi Z, Csányi V. A simple reason for a big difference: wolves do not look back at humans, but dogs do. *Curr Biol.* (2003) 13:763–6. doi: 10.1016/S0960-9822(03)00263-X
82. Savalli C, Ades C, Gaunet F. Are dogs able to communicate with their owners about a desirable food in a referential and intentional way? *PLoS ONE* (2014) 9:e108003. doi: 10.1371/journal.pone.0108003
83. Rørvang MV, Christensen JW, Ladewig J, McLean A. Social learning in horses—fact or fiction? *Front Vet Sci.* (2018) 5:212. doi: 10.3389/fvets.2018.00212
84. Huber L, Range F, Voelkl B, Szucsich A, Virányi Z, Miklosi A. The evolution of imitation: what do the capacities of non-human animals tell us about the mechanisms of imitation? *Phil Trans R Soc B* (2009) 364:2299–309. doi: 10.1098/rstb.2009.0060
85. Galef BG, Laland KN. Social learning in animals: empirical studies and theoretical models. *Bioscience* (2005) 55:489. doi: 10.1641/0006-3568(2005)055[0489:SLIAES]2.0.CO;2
86. Heyes CM. Social learning in animals: categories and mechanisms. *Biol Rev.* (1994) 69:207–31. doi: 10.1111/j.1469-185X.1994.tb01506.x
87. Truskanov N, Shy R, Lotem A. Context-specific learning and its implications for social learning. *Behav Ecol.* (2018) 29:1046–55. doi: 10.1093/beheco/ary078
88. Call J, Tomasello M. Does the chimpanzee have a theory of mind? 30 years later. *Trends Cogn. Sci.* (2008) 12:187–92. doi: 10.1016/j.tics.2008.02.010
89. Horner V, Carter JD, Suchak M, de Waal FBM. Spontaneous prosocial choice by chimpanzees. *Proc Natl Acad Sci USA* (2011) 108:13847–51. doi: 10.1073/pnas.1111088108
90. Brosnan SF, De Waal FBM. Monkeys reject unequal pay. *Nature* (2003) 435:297–9. doi: 10.1038/nature01963
91. Rault JL. Be kind to others: prosocial behaviours and their implications for animal welfare. *Appl Anim Behav Sci.* (2019) 210:113–23. doi: 10.1016/J.APPLANIM.2018.10.015

92. Bailey DW, Sims PL. Association of food quality and locations by cattle. *J Range Manag.* (1998) 51:2–6. doi: 10.2307/4003555
93. Howery LD, Bailey DW, Ruyle GB, Renken WJ. Cattle use visual cues to track food locations. *Appl Anim Behav Sci.* (2000) 67:1–14. doi: 10.1016/S0168-1591(99)00118-5
94. Bouissou MF, Andrieu S. Etablissement des relations préférentielles chez les bovins domestiques. *Behaviour* (1978) 64:148–57. doi: 10.1163/156853978X00486
95. Bouissou MF, Andrieu S. Etablissement des relations de dominance-soumission chez les bovins domestiques. *Biol Behav.* (1977) 2:97–107.
96. Le Neindre P. Influence of cattle rearing conditions and breed on social relationships of mother and young. *Appl Anim Behav Sci.* (1989) 23:117–27. doi: 10.1016/0168-1591(89)90012-9
97. Hagen K, Broom DM. Cattle discriminate between individual familiar herd members in a learning experiment. *Appl Anim Behav Sci.* (2003) 82:13–28. doi: 10.1016/S0168-1591(03)00053-4
98. Baldwin BA. Ability of goats and calves to distinguish between conspecific urine samples using olfaction. *Appl Anim Ethol.* (1977) 3:145–50. doi: 10.1016/0304-3762(77)90023-2
99. Cummins KA. Olfactory and visual cues. Individual recognition and social aggression in lactating cows. *J Dairy Sci.* (1991) 74:301.
100. Lensink J, Leruste H, Tillie M. L'Observation du troupeau bovin : Voir, interpréter, agir. *Fr Agric.* (2006). Available online at: https://www.unitheque.com/Livre/france_agricole/Agri_production/L_observation_du_troupeau_bovin-58428.html
101. Coulon M, Baudoin C, Abdi H, Heyman Y, Deputte BL. Social behavior and kin discrimination in a mixed group of cloned and non cloned heifers (*Bos taurus*). *Theriogenology* (2010) 74:1596–603. doi: 10.1016/j.theriogenology.2010.06.031
102. Taylor A, Davis H. Individual humans as discriminative stimuli for cattle (*Bos taurus*). *Appl Anim Behav Sci.* (1998) 58:13–21. doi: 10.1016/S0168-1591(97)00061-0
103. Munksgaard L, de Passillé A, Rushen J, Ladewig J. Dairy cows' use of colour cues to discriminate between people. *Appl Anim Behav Sci.* (1999) 65:1–11.
104. Ralphs MH, Olsen JD. Adverse influence of social facilitation and learning context in training cattle to avoid eating larkspur. *J Anim Sci.* (1990) 68:1944–52. doi: 10.2527/1990.6871944x
105. Boissy A, Le Neindre P. Social influences on the reactivity of heifers: implications for learning abilities in operant conditioning. *Appl Anim Behav Sci.* (1990) 25:149–65. doi: 10.1016/0168-1591(90)90077-Q
106. Henselek Y, Fischer J, Schloegl C. Does the stimulus type influence horses' performance in a quantity discrimination task? *Front Psychol.* (2012) 3:504. doi: 10.3389/fpsyg.2012.00504
107. Petrazzini ME. Trained quantity abilities in horses (*Equus caballus*): a preliminary investigation. *Behav Sci.* (2014) 4:213–25. doi: 10.3390/bs4030213
108. Péron F, Ward R, Burman O. Horses (*Equus caballus*) discriminate body odour cues from conspecifics. *Anim Cogn.* (2013) 17:1007–11. doi: 10.1007/s10071-013-0717-9
109. Lemasson A, Boutin A, Boivin S, Blois-Heulin C, Hausberger M. Horse (*Equus caballus*) whinnies: a source of social information. *Anim Cogn.* (2009) 12:693–704. doi: 10.1007/s10071-009-0229-9
110. Sankey C, Henry S, André N, Richard-Yris MA, Hausberger M. Do Horses have a concept of person? *PLoS ONE* (2011) 6:e18331. doi: 10.1371/journal.pone.0018331
111. Lampe J, Andre J. Cross-modal recognition of human individuals in domestic horses (*Equus caballus*). *Anim Cogn.* (2012) 15:623–30. doi: 10.1007/s10071-012-0490-1
112. Pfungst O. Clever Hans (the Horse of Mr. von Osten): a contribution to experimental, animal, and human psychology. *J Philos Psychol Sci Methods* (1911) 8:663. doi: 10.2307/2012691
113. Birke L, Hockenhull J, Creighton E, Pinno L, Mee J, Mills D. Horses' responses to variation in human approach. *Appl Anim Behav Sci.* (2011) 134:56–63. doi: 10.1016/j.applanim.2011.06.002
114. Lovrovich P, Sighieri C, Baragli P. Following human-given cues or not? Horses (Equus caballus) get smarter and change strategy in a delayed three choice task. *Appl Anim Behav Sci.* (2015) 166:80–8. doi: 10.1016/j.applanim.2015.02.017
115. Maros K, Gácsi M, Miklósi Á. Comprehension of human pointing gestures in horses (*Equus caballus*). *Anim Cogn.* (2008) 11:457–66. doi: 10.1007/s10071-008-0136-5
116. Proops L, Rayner J, Taylor AM, McComb K. The responses of young domestic horses to human-given cues. *PLoS ONE* (2013) 8:e67000. doi: 10.1371/journal.pone.0067000
117. Wathan J, McComb K. The eyes and ears are visual indicators of attention in domestic horses. *Curr Biol.* (2014) 24:R677–9. doi: 10.1016/j.cub.2014.06.023
118. Lesimple C, Sankey C, Richard Ma, Hausberger M. Do horses expect humans to solve their problems? *Front Psychol.* (2012) 3:306. doi: 10.3389/fpsyg.2012.00306
119. McGreevy PD, Cripps PJ, French NP, Green LE, Nicol CJ. Management factors associated with stereotypic and redirected behaviour in the Thoroughbred horse. *Equine Vet J.* (1995) 27:86–91. doi: 10.1111/j.2042-3306.1995.tb03041.x
120. Lindberg A, Kelland A, Nicol C. Effects of observational learning on acquisition of an operant response in horses. *Appl Anim Behav Sci.* (1999) 61:187–99. doi: 10.1016/S0168-1591(98)00184-1
121. Baer KL, Potter GD, Friend TH, Beaver BV. Observation effects on learning in horses. *Appl Anim Ethol.* (1983) 11:123–9. doi: 10.1016/0304-3762(83)90121-9
122. Baker AEM, Crawford BH. Observational learning in horses. *Appl Anim Behav Sci.* (1986) 15:7–13. doi: 10.1016/0168-1591(86)90017-1
123. Clarke JV, Nicol CJ, Jones R, McGreevy PD. Effects of observational learning on food selection in horses. *Appl Anim Behav Sci.* (1996) 50:177–84. doi: 10.1016/0168-1591(96)01071-4
124. Krueger K, Heinze J. Horse sense: social status of horses (*Equus caballus*) affects their likelihood of copying other horses' behavior. *Anim Cogn.* (2008) 11:431–9. doi: 10.1007/s10071-007-0133-0
125. Hanggi EB. The thinking horse: cognition and perception reviewed. In: *AAEP Proceedings*, Lexington, KY (2005). p. 246–55.
126. Ahrendt LP, Christensen JW, Ladewig J. The ability of horses to learn an instrumental task through social observation. *Appl Anim Behav Sci.* (2012) 139:105–13. doi: 10.1016/j.applanim.2012.03.004
127. Henry S, Hemery D, Richard MA, Hausberger M. Human–mare relationships and behaviour of foals toward humans. *Appl Anim Behav Sci.* (2005) 93:341–62. doi: 10.1016/j.applanim.2005.01.008
128. Stolba A, Wood-Gush DGM. The behaviour of pigs in a semi-natural environment. *Anim Sci.* (1989) 48:419–25.
129. Held S, Baumgartner J, Kilbride A, Byrne RW, Mendl M. Foraging behaviour in domestic pigs (*Sus scrofa*): remembering and prioritizing food sites of different value. *Anim Cogn.* (2005) 8:114–21. doi: 10.1007/s10071-004-0242-y
130. Melotti L, Thomsen LR, Toscano MJ, Mendl M, Held S. Delay discounting task in pigs reveals response strategies related to dopamine metabolite. *Physiol Behav.* (2013) 120:182–92. doi: 10.1016/j.physbeh.2013.08.009
131. Mendl M, Randle K, Pope S. Young female pigs can discriminate individual differences in odours from conspecific urine. *Anim Behav.* (2002) 64:97–101. doi: 10.1006/anbe.2002.3040
132. Gieling ET, Musschenga MA, Nordquist RE, van der Staay FJ. Juvenile pigs use simple geometric 2D shapes but not portrait photographs of conspecifics as visual discriminative stimuli. *Appl Anim Behav Sci.* (2012) 142:142–53. doi: 10.1016/j.applanim.2012.10.018
133. Tanida H, Nagano Y. The ability of miniature pigs to discriminate between a stranger and their familiar handler. *Appl Anim Behav Sci.* (1998) 56:149–59. doi: 10.1016/S0168-1591(97)00095-6
134. Miklósi Á, Soproni K. A comparative analysis of animals' understanding of the human pointing gesture. *Anim Cogn.* (2006) 9:81–93. doi: 10.1007/s10071-005-0008-1
135. Held S, Mendl M, Devereux C, Byrne RW. Social tactics of pigs in a competitive foraging task: the "informed forager" paradigm. *Anim Behav.* (2000) 59:569–76. doi: 10.1006/anbe.1999.1322
136. Coussi-Korbel S. Learning to outwit a competitor in mangabeys (*Cercocebus torquatus torquatus*). *J Comp Psychol.* (1994) 108:164–71. doi: 10.1037/0735-7036.108.2.164
137. Held SDE, Byrne RW, Jones S, Murphy E, Friel M, Mendl MT. Domestic pigs, *Sus scrofa*, adjust their foraging behaviour to whom they are foraging with. *Anim Behav.* (2010) 79:857–62. doi: 10.1016/j.anbehav.2009.12.035

138. Ginane C, Dumont B. Do sheep (*Ovis aries*) categorize plant species according to botanical family? *Anim Cogn.* (2011) 14:369–76. doi: 10.1007/s10071-010-0371-4
139. Langbein J, Siebert K, Nürnberg G, Manteuffel G. Learning to learn during visual discrimination in group housed dwarf goats (*Capra hircus*). *J Comp Psychol.* (2007) 121:447–56. doi: 10.1037/0735-7036.121.4.447
140. Roitberg E, Franz H. Oddity learning by African dwarf goats (*Capra hircus*). *Anim Cogn.* (2004) 7:61–7. doi: 10.1007/s10071-003-0190-y
141. Hosoi E, Swift DM, Rittenhouse LR, Richards RW. Comparative foraging strategies of sheep and goats in a T-maze apparatus. *Appl Anim Behav Sci.* (1995) 44:37–45. doi: 10.1016/0168-1591(95)00572-A
142. de la Lama GCM, Mattiello S. The importance of social behaviour for goat welfare in livestock farming. *Small Rumin Res.* (2010) 90:1–10. doi: 10.1016/j.smallrumres.2010.01.006
143. Stanley CR, Dunbar RIM. Consistent social structure and optimal clique size revealed by social network analysis of feral goats, *Capra hircus*. *Anim Behav.* (2013) 85:771–9. doi: 10.1016/j.anbehav.2013.01.020
144. Kendrick KM, Atkins K, Hinton MR, Broad KD, Fabre-Nys C, Keverne B. Facial and vocal discrimination in sheep. *Anim Behav.* (1995) 49:1665–76. doi: 10.1016/0003-3472(95)90088-8
145. Tate AJ, Fischer H, Leigh AE, Kendrick KM. Behavioural and neurophysiological evidence for face identity and face emotion processing in animals. *Phil Trans R Soc B* (2006) 361:2155–72. doi: 10.1098/rstb.2006.1937
146. Briefer EF, Padilla de la Torre M, McElligott AG. Mother goats do not forget their kids' calls. *Proc R Soc B* (2012) 279:3749–55. doi: 10.1098/rspb.2012.0986
147. Pitcher BJ, Briefer EF, Baciadonna L, McElligott AG. Cross-modal recognition of familiar conspecifics in goats. *R Soc Open Sci.* (2017) 4:160346. doi: 10.1098/rsos.160346
148. Beausoleil NJ, Stafford KJ, Mellor DJ. Does direct human eye contact function as a warning cue for domestic sheep (*Ovis aries*)? *J Comp Psychol.* (2006) 120:269–79. doi: 10.1037/0735-7036.120.3.269
149. Nawroth C, von Borell E, Langbein J. "Goats that stare at men"—revisited: do dwarf goats alter their behaviour in response to eye visibility and head direction of a human? *Anim Cogn.* (2016c) 19:667–72. doi: 10.1007/s10071-016-0957-6
150. Gómez JC. Species comparative studies and cognitive development. *Trends Cogn Sci.* (2005) 9:118–25. doi: 10.1016/j.tics.2005.01.004
151. Thorhallsdottir AG, Provenza FD, Balph DF. Ability of lambs to learn about novel foods while observing or participating with social models. *Appl Anim Behav Sci.* (1990) 25:25–33. doi: 10.1016/0168-1591(90)90066-M
152. Veissier I, Stefanova I. Learning to suckle from an artificial teat within groups of lambs: influence of a knowledgeable partner. *Behav Process.* (1993) 30:75–82. doi: 10.1016/0376-6357(93)90013-H
153. Broom DM. Cognitive ability and awareness in domestic animals and decisions about obligations to animals. *Appl Anim Behav Sci.* (2010) 126:1–11. doi: 10.1016/j.applanim.2010.05.001
154. Mendl M, Held S, Byrne RW. Pig cognition. *Curr Biol.* (2010b) 20:R796–8. doi: 10.1016/j.cub.2010.07.018
155. Nawroth C. Invited review: socio-cognitive capacities of goats and their impact on human-animal interactions. *Small Rumin Res.* (2017) 150:70–5. doi: 10.1016/j.smallrumres.2017.03.005
156. Averós X, Brossard L, Dourmad, J.-Y., de Greef KH, Edge HL, Edwards SA, et al. A meta-analysis of the combined effect of housing and environmental enrichment characteristics on the behaviour and performance of pigs. *Appl Anim Behav Sci.* (2010) 127:73–85. doi: 10.1016/j.applanim.2010.09.010
157. Newberry RC. Environmental enrichment: increasing the biological relevance of captive environments. *Appl Anim Behav Sci.* (1995) 44:229–43. doi: 10.1016/0168-1591(95)00616-Z
158. Mason G, Clubb R, Latham N, Vickery S. Why and how should we use environmental enrichment to tackle stereotypic behaviour? *Appl Anim Behav Sci.* (2007) 102:163–88. doi: 10.1016/j.applanim.2006.05.041
159. Wemelsfelder F. The concept of animal boredom and its relationship to stereotyped behaviour. In: Lawrence AB, Rushen J, editors. *Stereotypie Behavior: Fundamentals and Applications to Animal Welfare*. Wallingford: CAB International (1993). p. 65–95.
160. Carlstead K, Shepherdson DJ. Alleviating stress in zoo animals with environmental enrichment. In: Moberg GP, Mench JA, editors. *The Biology of Animal Stress. The Biology of Animal Stress:Basic Principles and Implications for Animal Welfare*. Oxon, NY: CAB1 Publishing (2000). p. 337–54. doi: 10.1079/9780851993591.0337
161. van de Weerd HA, Day JEL. A review of environmental enrichment for pigs housed in intensive housing systems. *Appl Anim Behav Sci.* (2009) 116:1–20. doi: 10.1016/j.applanim.2008.08.001
162. Dantzer R. Can farm animal welfare be understood without taking into account the issues of emotion and cognition? *J Anim Sci.* (2002) 80:E1–9. doi: 10.2527/animalsci2002.0021881200800ES10002x
163. Greiveldinger L, Veissier I, Boissy A. Emotional experience in sheep: predictability of a sudden event lowers subsequent emotional responses. *Physiol Behav.* (2007) 92:675–83. doi: 10.1016/j.physbeh.2007.05.012
164. Manteuffel G, Langbein J, Puppe B. From operant learning to cognitive enrichment in farm animal housing: bases and applicability. *Anim Welf.* (2009) 18:87–95.
165. Kalbe C, Puppe B. Long-term cognitive enrichment affects opioid receptor expression in the amygdala of domestic pigs. *Genes Brain Behav.* (2010) 9:75–83. doi: 10.1111/j.1601-183X.2009.00536.x
166. Meyer S, Langbein J, Puppe B. Kognitive Umweltanreicherung bei Zoo- und Nutztieren - Implikationen für Verhalten und Wohlbefinden der Tiere. *Berl Munch Tierarztl Wochenschr.* (2010) 12:446–56. doi: 10.2376/0005-9366-123-446
167. Puppe B, Ernst K, Schön PC, Manteuffel G. Cognitive enrichment affects behavioural reactivity in domestic pigs. *Appl Anim Behav Sci.* (2007) 105:75–86. doi: 10.1016/j.applanim.2006.05.016
168. Zebunke M, Langbein J, Manteuffel G, Puppe B. Autonomic reactions indicating positive affect during acoustic reward learning in domestic pigs. *Anim Behav.* (2011) 81:481–9. doi: 10.1016/j.anbehav.2010.11.023
169. Ernst K, Puppe B, Schön PC, Manteuffel G. A complex automatic feeding system for pigs aimed to induce successful behavioural coping by cognitive adaptation. *Appl Anim Behav Sci.* (2005) 91:205–18. doi: 10.1016/j.applanim.2004.10.010
170. Zebunke M, Puppe B, Langbein J. Effects of cognitive enrichment on behavioural and physiological reactions of pigs. *Physiol Behav.* (2013) 118:70–9. doi: 10.1016/j.physbeh.2013.05.005
171. Langbein J, Nürnberg G, Manteuffel G. Visual discrimination learning in dwarf goats and associated changes in heart rate and heart rate variability. *Physiol Behav.* (2004) 82:601–9. doi: 10.1016/j.physbeh.2004.05.007
172. Selye H. *Stress in Health and Disease.* Boston, MA: Butterworth-Heinemann (1976).
173. Langbein J, Siebert K, Nürnberg G. On the use of an automated learning device by group-housed dwarf goats: do goats seek cognitive challenges? *Appl Anim Behav Sci.* (2009) 120:150–8. doi: 10.1016/j.applanim.2009.07.006
174. Osborne SR. The free food (contrafreeloading) phenomenon: a review and analysis. *Anim Learn Behav.* (1977) 5:221–35. doi: 10.3758/BF03209232
175. Hagen K, Broom DM. Emotional reactions to learning in cattle. *Appl Anim Behav Sci.* (2004) 85:203–13. doi: 10.1016/j.applanim.2003.11.007
176. McGowan RTS, Rehn T, Norling Y, Keeling LJ. Positive affect and learning: exploring the "Eureka Effect" in dogs. *Anim Cogn.* (2014) 17:577–87. doi: 10.1007/s10071-013-0688-x
177. Meehan CL, Mench JA. The challenge of challenge: can problem solving opportunities enhance animal welfare? *Appl Anim Behav Sci.* (2007) 102:246–61. doi: 10.1016/j.applanim.2006.05.031
178. Baymann U, Langbein J, Siebert K, Nürnberg G, Manteuffel G, Mohr E. Cognitive enrichment in farm animals-the impact of social rank and social environment on learning behaviour of dwarf goats. *Berliner und Muenchener Tierarztl Wochenschrift* (2007) 120:89–97. doi: 10.2376/0005-9366-120-89
179. Chapple RS, Wodzicka-Tomaszewska M, Lynch JJ. The learning behaviour of sheep when introduced to wheat. II Social transmission of wheat feeding and the role of the senses. *Appl Anim Behav Sci.* (1987) 18:163–72. doi: 10.1016/0168-1591(87)90190-0
180. Chua B, Coenen E, van Delen J, Weary DM. Effects of pair versus individual housing on the behavior and performance of dairy calves. *J Dairy Sci.* (2002) 85:360–4. doi: 10.3168/jds.S0022-0302(02)74082-4
181. Costa JHC, Costa WG, Weary DM, Filho LCPM, von Keyserlingk MAG. Dairy heifers benefit from the presence of an experienced companion when learning how to graze. *J Dairy Sci.* (2015) 99:562–8. doi: 10.3168/jds.2015-9387

182. Laland KN. Social learning strategies. *Anim Learn Behav.* (2004) 32:4–14. doi: 10.3758/BF03196002
183. Hemsworth P. Human–animal interactions in livestock production. *Appl Anim Behav Sci.* (2003) 81:185–98. doi: 10.1016/S0168-1591(02)00280-0
184. Brajon S, Laforest JP, Bergeron R, Tallet C, Devillers N. The perception of humans by piglets: recognition of familiar handlers and generalisation to unfamiliar humans. *Anim Cogn.* (2015) 18:1299–316. doi: 10.1007/s10071-015-0900-2
185. Fureix C, Jego P, Sankey C, Hausberger M. How horses (*Equus caballus*) see the world: humans as significant "objects." *Anim Cogn.* (2009) 12:643–54. doi: 10.1007/s10071-009-0223-2
186. Destrez A, Coulon M, Deiss V, Delval E, Boissy A, Boivin X. The valence of the long-lasting emotional experiences with various handlers modulates discrimination and generalization of individual humans in sheep. *J Anim Sci.* (2013) 91:5418–26. doi: 10.2527/jas.2012-5654
187. Sankey C, Richard-Yris MA, Leroy H, Henry S, Hausberger M. Positive interactions lead to lasting positive memories in horses, *Equus caballus*. *Anim Behav.* (2010) 79:869–75. doi: 10.1016/j.anbehav.2009.12.037
188. Jago J, Krohn C, Matthews L. The influence of feeding and handling on the development of the human–animal interactions in young cattle. *Appl Anim Behav Sci.* (1999) 62:137–51. doi: 10.1016/S0168-1591(98)00219-6
189. Lürzel S, Windschnurer I, Futschik A, Waiblinger S. Gentle interactions decrease the fear of humans in dairy heifers independently of early experience of stroking. *Appl Anim Behav Sci.* (2016) 178:16–22. doi: 10.1016/j.applanim.2016.02.012
190. Probst JK, Spengler Neff A, Leiber F, Kreuzer M, Hillmann E. Gentle touching in early life reduces avoidance distance and slaughter stress in beef cattle. *Appl Anim Behav Sci.* (2012) 139:42–9. doi: 10.1016/j.applanim.2012.03.002
191. Waiblinger S, Boivin X, Pedersen V, Tosi MV, Janczak AM, Visser EK, et al. Assessing the human-animal relationship in farmed species: a critical review. *Appl Anim Behav Sci.* (2006) 101:185–242. doi: 10.1016/j.applanim.2006.02.001
192. Boissy A, Bouissou MF. Effects of early handling on heifers' subsequent reactivity to humans and to unfamiliar situations. *Appl Anim Behav Sci.* (1988) 20:259–73.
193. Schmied C, Boivin X, Waiblinger S. Stroking different body regions of dairy cows: effects on avoidance and approach behavior toward humans. *J Dairy Sci.* (2008) 91:596–605. doi: 10.3168/jds.2007-0360
194. Stewart M, Shepherd HM, Webster JR, Waas JR, McLeay LM, Schütz KE. Effect of previous handling experiences on responses of dairy calves to routine husbandry procedures. *Animal* (2013) 7:828–33. doi: 10.1017/S175173111200225X
195. Breuer K, Hemsworth PH, Barnett JL, Matthews LR, Coleman GJ. Behavioural response to humans and the productivity of commercial dairy cows. *Appl Anim Behav Sci.* (2000) 66:273–88. doi: 10.1016/S0168-1591(99)00097-0
196. Caroprese M, Napolitano F, Albenzio M, Annicchiarico G, Musto M, Sevi A. Influence of gentling on lamb immune response and human-lamb interactions. *Appl Anim Behav Sci.* (2006) 99:118–31. doi: 10.1016/j.applanim.2005.08.023
197. Lürzel S, Münsch C, Windschnurer I, Futschik A, Palme R, Waiblinger S. The influence of gentle interactions on avoidance distance towards humans, weight gain and physiological parameters in group-housed dairy calves. *Appl Anim Behav Sci.* (2015) 172:9–16. doi: 10.1016/j.applanim.2015.09.004
198. Garner R. *Animal Ethics*. Cambridge: Polity Press (2005).
199. Palmer C. *Animal Ethics in Context*. New York, NY: Columbia University Press (2010).
200. Benz-Schwarzburg J, Knight A. Cognitive relatives yet moral strangers? *J Anim Ethics* (2011) 1:9–36. doi: 10.5406/janimalethics.1.1.0009
201. Fraser D. Animal ethics and animal welfare science: bridging the two cultures. *Appl Anim Behav Sci.* (1999) 65:171–89. doi: 10.1016/S0168-1591(99)00090-8
202. Jones RC. Science, sentience, and animal welfare. *Biol Philos.* (2013) 28:1–30. doi: 10.1007/s10539-012-9351-1
203. Rollin BE. The inseparability of science and ethics in animal welfare. *J Agric Environ Ethics* (2015) 28:759–65. doi: 10.1007/s10806-015-9558-7
204. Cavalieri P, Singer P. *The Great Ape Project: Equality Beyond Humanity*. New York, NY: St. Martin's Griffin (1993).
205. Nussbaum MC. Beyond "compassion and humanity": justice for nonhuman animals. In: Sunstein CR, Nussbaum MC, editors. *Animal Rights: Current Debates and New Directions*. New York, NY: Oxford University Press (2004). p. 299–320.
206. DeGrazia D. *Taking Animals Seriously: Mental Life and Moral Status*. Cambridge: Cambridge University Press (1996). doi: 10.1017/CBO9781139172967
207. Duncan IJH, Petherick JC. The implications of cognitive processes for animal welfare. *J Anim Sci.* (1991) 69:5017–22. doi: 10.2527/1991.69125017x
208. Mendl M, Paul ES. Do animals live in the present? Current evidence and implications for welfare. *Appl Anim Behav Sci.* (2008) 113:357–82. doi: 10.1016/j.applanim.2008.01.013
209. Andrews K, Monsó S. Animal moral psychologies. In: Doris J, Vargas M, editors. *The Oxford Handbook of Moral Psychology*. New York, NY: Oxford University Press (in press).
210. Bekoff M, Pierce J. *Wild Justice: The Moral Lives of Animals*. Chicago, IL: University of Chicago Press (2009). doi: 10.7208/chicago/9780226041667.001.0001
211. Monsó S. Morality without mindreading. *Mind Lang.* (2017) 32:338–57. doi: 10.1111/mila.12146
212. Rowlands M. *Can Animals Be Moral?* New York, NY: Oxford University Press (2012). doi: 10.1093/acprof:oso/9780199842001.001.0001
213. Cataldi SL. Animals and the concept of dignity: critical reflections on a circus performance. *Ethics Environ.* (2002) 7:104–26. doi: 10.2979/ETE.2002.7.2.104
214. Rutgers B, Heeger R. Inherent worth and respect for animal integrity. In: Dol M, Fentener Van Vlissingen M, Kasanmoentalib S, Visser T, Zwart H, editors. *Recognizing the Intrinsic Value of Animals: Beyond Animal Welfare*. Assen: Van Gorcum Ltd. (1999). p. 41–51.
215. Regan T. *The Case for Animal Rights*. Berkeley, CA: University of California Press (2004).
216. Gutmann AK, Špinka M, Winckler C. Long-term familiarity creates preferred social partners in dairy cows. *Appl Anim Behav Sci.* (2015) 169:1–8. doi: 10.1016/j.applanim.2015.05.007
217. Cronin KA. Prosocial behaviour in animals: the influence of social relationships, communication and rewards. *Anim Behav.* (2012) 84:1085–93. doi: 10.1016/j.anbehav.2012.08.009
218. Monsó S, Benz-Schwarzburg J, Huber L. Morality in animals: what it means and why it matters. *J Ethics* (2018) 22:283–10. doi: 10.1007/s10892-018-9275-3
219. Baciadonna L, McElligott A. The use of judgement bias to assess welfare in farm livestock. *Anim Welf.* (2015) 24:81–91. doi: 10.7120/09627286.24.1.081
220. Spence C, Osman M, McElligott AG. Theory of animal mind: human nature or experimental artefact? *Trends Cogn. Sci.* (2017) 21:333–43. doi: 10.1016/j.tics.2017.02.003
221. Ruby MB, Heine SJ. Too close to home. Factors predicting meat avoidance. *Appetite* (2012) 59:47–52. doi: 10.1016/j.appet.2012.03.020
222. Bastian B, Loughnan S, Haslam N, Radke HRM. Don't mind meat? The denial of mind to animals used for human consumption. *Pers Soc Psychol Bull.* (2012) 38:247–56. doi: 10.1177/0146167211424291
223. Rosenthal R. The file drawer problem and tolerance for null results. *Psychol Bull.* (1979) 86:638–41. doi: 10.1037/0033-2909.86.3.638

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REVIEW
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Social Learning in Horses—Fact or Fiction?

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Prima facie, the acquisition of novel behaviors in animals through observation of conspecifics seems straightforward. There are, however, various mechanisms through which the behavior of animals can be altered from observing others. These mechanisms range from simple hard-wired contagious processes to genuine learning by observation, which differ fundamentally in cognitive complexity. They range from social facilitation and local enhancement to true social learning. The different learning mechanisms are the subject of this review, largely because research on learning by observation can be confounded by difficulties in interpretation owing to the looming possibility of associative learning infecting experimental results. While it is often assumed that horses are capable of acquiring new behavior through intra-species observation, research on social learning in horses includes a variety of studies some of which may overestimate the possession of higher mental abilities. Assuming such abilities in their absence can have welfare implications, e.g., isolating stereotypical horses on the assumption that these behaviors can be learned though observation by neighboring horses. This review summarizes the definitions and criteria for the various types of social transmission and social learning and reviews the current documentation for each type in horses with the aim of clarifying whether horses possess the ability to learn through true social learning. As social ungulates, horses evolved in open landscapes, exposed to predators and grazing most of the day. Being in close proximity to conspecifics may theoretically offer an opportunity to learn socially, however anti-predator vigilance and locating forage may not require the neural complexity of social learning. Given the significant energetic expense of brain tissue, it is likely that social facilitation and local enhancement may have been sufficient in the adaptation of equids to their niche. As a consequence, social learning abilities may be maladaptive in horses. Collectively, the review proposes a novel differentiation between social transmission (social facilitation, local, and stimulus enhancement) and social learning (goal emulation, imitation). Horses are undoubtedly sensitive to intra-species transfer of information but this transfer does not appear to satisfy the criteria for social learning, and thus there is no solid evidence for true social learning in horses.

Keywords: cognition, equine, local enhancement, social facilitation, social learning, social transmission, training, welfare

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INTRODUCTION

Individual behavior can be altered in various ways, some of which involve actual learning mechanisms, while others such as social facilitation rely on a particular behavior being contagiously triggered by a similar behavior in others (e.g., flight responses in horses or yawning in humans). Conversely, learning of novel behaviors can occur through individual or social learning. Individual learning refers to the animal acquiring new behavior by the trial-and-error processes of associative learning, that is learning by its own experience. In contrast, the contemporary understanding of social learning is that the animal attains new behavior after observing a conspecific performing the behavior (1). At least some forms of social learning are likely to entail higher mental abilities such as insight as they require the animal to see and remember the behavior, transfer the behavior to its own behavioral repertoire, and subsequently perform it (2). In theory, social learning is related to group-living because living in close proximity to conspecifics offers an opportunity to watch and learn (3, 4). Several authors have also emphasized this connection [e.g., (5, 6)], although more recent studies have reassessed the theory and found conflicting results, possibly due to interspecific differences in learning [e.g., (7)].

As group-living animals, it is often assumed that horses are capable of acquiring new behavior through observation of conspecifics (8–10), but solid evidence of true social learning in horses is lacking. Research on social learning in horses includes a variety of studies, some of which may over-estimate the mental abilities of horses. This review critically assesses these studies and reveals that the number of demonstrations provided by the demonstrator horse varies to the extent that one could reasonably argue that the observer horses learned by *associative* learning rather than by *social* learning. Importantly, over-estimating the mental abilities of horses is not only a scientific challenge but may also have welfare consequences. The anecdotal assumption that horses can learn so-called "vices" via observation of conspecifics is often used as an argument to keep stereotypic horses in social isolation to prevent the abnormal behavior from spreading (11, 12). This assumption, however, has never been confirmed in either experimental (13, 14) or in epidemiological studies (15). Additionally, horse trainers may assume that naïve horses are able to observe and learn from older, well-trained horses (9, 16). Although horses undoubtedly are sensitive to transfer of emotional states (17) they are less likely to learn specific behaviors from conspecifics. Both over- and underestimating the mental capabilities of horses can have significant welfare implications as this has been used to justify punishment in some training systems. Training methods based on a flawed understanding of equine learning processes may to some extent explain differences in horse training methodologies [reviewed in (18)].

Dennett (19) pointed out that assuming without solid proof that animals have insight into their instinctive behaviors amounts to an unacceptable rejection of the null hypothesis. With regard to the implication of higher mental abilities in animals where such abilities may not be within the cognitive realm of the subject species, a similar precautionary principle should be applied.

This review aims to assess the evolutionary basis for higher mental abilities in horses, including their ability to learn new behavior from observation of conspecifics. We revisit studies on social learning in horses and discuss the extent to which the available results may reflect social influence on individual learning, rather than true social learning.

DEFINITIONS OF SOCIAL COGNITIVE MECHANISMS

> "In no case is an animal activity to be interpreted in terms of higher psychological processes if it can be fairly interpreted in terms of processes which stand lower in the scale of psychological evolution and development" (20).

Lloyd Morgan's Canon is a fundamental tenet in cognitive science. Morgan was reacting to interpretations of animal behavior he found excessively anthropomorphic and described cases in which behavior that may, at first, seem to involve higher mental processes could in fact be explained by simple associative learning. He used the example of how his dog skillfully opened the garden gate, which could easily be interpreted as an insightful act by someone seeing only the final behavior. Morgan had, however, recorded the series of approximations by which the dog gradually learned the response and could demonstrate that no insight was required to perform the behavior.

In line with Lloyd Morgan's observations, humankind's general fascination with the cognitive abilities of animals may sometimes lead to conclusions that ascribe higher mental processes to animals than are actually necessary to perform a specific behavior. For example, doubts were raised on imitation as the underlying mechanism for two of the most well-known examples of apparently imitative behavior transmission: potato washing in Japanese macaques (21) and milk bottle opening in tits (22). Reinforcement by human caretakers may have interfered with the spread of potato-washing, which was found to be easily learned by monkeys (21). Similarly, Sherry and Galefs' (22) results demonstrated that experience with previously opened bottles was sufficient to establish milk bottle opening in birds, i.e., no observation of a demonstrator bird was necessary.

Since Edward Thorndike set out to investigate whether animals can "*from an act witnessed, learn to perform that act*" (23), a considerable amount of research has aimed at exploring the cognitive abilities of animals to learn via observation of conspecifics. The term ***social learning*** has been used in a broad and general way to label a wide range of cognitive processes. Some of these only include mere social influence on individual learning, e.g., local enhancement, while others such as the more complex processes of imitation and goal emulation require higher mental abilities (24, 25). To avoid the misleading implication of higher mental abilities across the gamut of so-called social learning, we suggest a more precise taxonomy. We suggest that social influence on individual learning is clearly distinct from true observational learning and should instead be labeled ***social transmission,*** i.e., a transfer of information between individuals that merely influences the likelihood of subsequent individual learning.

Henceforth, in this review we use the term ***social transmission*** to cover all processes that involve a more simple transfer of information and/or behavior between individuals of the same or different species, whereas ***social learning*** encompasses observational learning of novel behavior requiring more complex cognitive abilities. We use the term ***social learning*** to describe intra-species processes, i.e., learning from conspecifics, and the more general term ***observational learning*** to refer to inter-species processes, i.e., learning from observation of an individual of another species. In this dichotomy, ***social transmission*** includes the following terms:

- ***Social facilitation***, in which the behavior of a conspecific changes the *motivation* of the observer, resulting in the tendency of individual animals to do what other individuals are doing. This phenomenon can be considered a social influence on behavior but not as a form of learning, as it only leads to an increase (or decrease) in performance of an existing behavior (26, 27). Social facilitation is involved in the synchronization of various behaviors, such as feeding and resting behavior (28).
- ***Stimulus enhancement,*** where the observer becomes more likely to interact with stimuli of the *same physical type* as those with which the demonstrator interacts. The observer is therefore more likely to learn about the *consequences* of interacting with these types of stimuli through individual associative learning (1, 27, 29).
- ***Local enhancement,*** where the behavior of a demonstrator results in an increase in the *salience* of a particular stimulus or location. The observer's attention may be increasingly drawn toward previously irrelevant features, or the observer's motivation to investigate the stimulus or location may be increased. Any subsequent acquisition of the same motor behavior as the demonstrator will be accomplished by individual associative learning directed toward the newly salient part of the environment (30, 31).

In contrast, ***social learning*** requires higher mental abilities and includes:

- ***Goal emulation***, which refers to the reproduction of the *results* of a model's behavior, rather than the reproduction of the precise *behavior* that produced those results. The observer sees the movement of the objects involved and then gains new insight about their relevance to its own motivations (32).
- ***Imitation***, which describes situations where the observer *copies* the motor patterns of the demonstrator by some process of cross-modal matching (31). Imitation of non-vocal demonstrations requires an observer to match a visual representation of an observed motor input with its own proprioceptive control and regardless of the concurrent visual signal of its own behavior (25). Thus, imitation requires a certain cognitive sophistication. It is notable that many of the classic field observations of apparent imitation could be explained as examples of associative learning, mediated by local or stimulus enhancement or even explicit human reinforcement (25).
- ***Program-level imitation***, which refers to the most cognitively complex expression of observational learning. It involves a *sequence of copied movements* that are observed and imitated (33). Program-level imitation evolved for the rapid acquisition of complex skills and is seen in animals such as mountain gorillas where the young learn how to prepare certain noxious plants for consumption.

The first convincing evidence of imitation in animals came from studies reporting that naive budgerigars (observers) that had watched a trained conspecific (a demonstrator) use either its foot or its beak to press a lever to obtain food tended to use the same appendage as had their respective demonstrators (34). Later, a number of other authors conducted "two-action experiments" and reported similar effects, e.g., rats pushed joysticks either to the right or left, depending on the act of the demonstrator (35) and chimpanzees either pulled or pushed artificial fruit to obtain rewards (36). These studies provide evidence that by observing an act some animals tend to produce that same act. However, Galef (24) argues that since the behaviors needed to perform the acts (stepping, pulling, pushing) were already present in the animals' behavioral repertoires, the two-action experiments only provide evidence that observing an act can increase the relative probability that an animal will express that act rather than others in its repertoire. Instead, Galef (24) argues, true imitation requires that the animals copy a completely novel behavior, which was not previously in its behavioral repertoire.

Whereas, social learning in terms of imitation of motor patterns may play an important role in the acquisition of new skills in some species, it is noteworthy that social transmission in a broader context has a variety of functions, e.g., in relation to acquisition of information about the environment and the acquisition of social behavior (31). In this context, local or stimulus enhancement followed by associative learning may be a more efficient way of acquiring skills in many circumstances. As we shall discuss in the next section, local enhancement appears to be more biologically relevant to horses.

ADAPTIVENESS OF SOCIAL LEARNING IN HORSES

The study of the adaptive use of social and non-social information has the potential to increase our understanding of how animals interact with the social and physical environments in which they live (37, 38). How animals procure their food has been a significant driving force in the evolution of mental abilities in animals and one would expect different mental abilities to have evolved for various foraging niches (39). From this viewpoint, it follows that convergent evolution of mental abilities would arise in animals that occupy similar niches. Kendal et al. (40) point out that it is more advantageous and therefore adaptive for species that use complex foraging skills, such as cooperative predation and tool-use, to rely more on social information than individual learning. In particular, the dispatching of large and dangerous prey would be ameliorated by social learning. Nevertheless, herbivores are reported to socially learn to choose food items and avoid toxic foods from a very early age (41, 42). They also monitor the eating behavior of group members and minimize the risk of predation by choosing food patches closer to conspecifics (43).

Such behaviors in herbivorous animals, however, may be more parsimoniously explained by local enhancement and associative learning. It follows that when a particular food choice is reinforced, the animal will be more likely to choose similar foods. From this viewpoint, it seems unlikely that the evolution of herbivory would require cognitive capabilities greater than local enhancement. Accordingly, Marinier and Alexander (44) have shown that foals learn their mother's diet not by social learning or even social transmission, but by coprophagy. It is evident that the horse has circumvented the cognitive complexity and energetic costliness of social learning of at least some elements of foraging via a non-cognitive process. Similarly, Provenza et al. (45) demonstrated that sheep learn food aversions via a similar non-cognitive process, which can occur even under anesthesia.

An important consideration is that herbivory may not facilitate the evolution and maintenance of higher mental abilities because of its low energy yield compared to e.g., carnivory. Brains are energetically expensive (46) and it is likely that complex mental abilities, such as those required for true social learning compared to social transmission, may be an "unaffordable luxury" for an obligate herbivore. Accordingly, this would be true for any obligate herbivore regardless of phylogenetic affinity unless it was biologically adaptive such in the Gorilla whose young learn to render stinging plants edible partly by social learning (39). As studies of the behavior of ancestral equids are scarce, studies of wild and reintroduced breeds of equids provide insight to the environment in which equine cognitive abilities evolved. Wild equids have evolved in open landscapes, exposed to predators and with high fiber/low nutrient food. Living in such an environment, synchronizing activities may help individuals increase the benefits of group living, e.g., early detection of predators and subsequent flight responses as well as forage detection when the environment is patchy (47, 48). Generally, social learning is thought to be adaptive at intermediate rates of environmental variability, because in highly variable environments, social information could be outdated or have no fitness benefit in the new environment (49). In order for social learning to be adaptive, the cost-benefit ratio of socially acquiring new skills should outweigh that of individual associative learning.

Finally, homeothermy is expensive from an energetic point of view. So too are higher mental abilities (50, 51) which are generally associated with larger brains due to more neurons and neural activity. A larger brain with more neurons functions to enhance gathering, storing and integrating information (52), and facilitating acquisition of new and altered behavior patterns through cognitive processes (51). Accordingly, Martin (53) found a significant positive correlation between basal metabolic rate and brain mass in a balanced sample of 51 mammalian species. Isler and van Schaik (50) also showed that this correlation persisted while controlling for body size effects (including 347 mammalian species). In mammals, the energy costs of homeothermy are compensated through either increased energy intake or reduced allocation of energy to other biological functions such as growth, reproduction, locomotion, or digestion (50). Clearly, homeothermy should place a limitation on costly mental processes such as those required for social learning. Indeed, as pointed out by McLean (39), this restriction is largely true of grazing mammals across the taxa, from the dual standpoint of published data on the existence of higher mental abilities and the requirements of the herbivorous niche.

On the other hand, studies on species where foraging requires refined and precise skills show much clearer signs of animals being more likely to perform a behavior after observing a conspecific performing that behavior. Voelkl and Huber (54) for instance found significant differences between the two observer groups of common marmosets, who had observed a method for opening a container by either hands or the mouth. Individuals who observed the hand method, all used their hands when opening the container, whereas individuals observing the mouth method mostly used their mouth. There are several such reports from various monkey species, but also canine species appear able to learn from observation. For example, domestic dogs observing a trained dog opening a food container with the paw, also used their paw whereas naïve dogs were more likely to use their mouth (55). Social learning is in these cases advantageous because individual learning can be costly and the advantage of exploiting the expertise of others outweigh the biological cost of this ability [for more details, see (56)].

Optimal foraging in ungulates may require little more than following the movements of other conspecifics in order to detect the best forage while local enhancement may deliver the necessary transmission of learning from one horse to another obviating the need for more energetically costly mental abilities. Thus, from the viewpoint of the equine foraging ethogram and mammalian metabolic demands, it is clear that social transmission provides sufficient transfer of behavior from one horse to another without the need for complex social learning abilities.

SOCIAL LEARNING OR SOCIAL TRANSMISSION IN HORSES?

Despite the aforementioned potential maladaptiveness of social learning in horses, a few studies have suggested that horses possess the ability to learn via this process. In one study, horses had to open a box by pulling a rope with their mouth to obtain food rewards (57). Twenty-five horses watched a trained horse demonstrating the task and in a separate experiment, 14 horses were used as controls, i.e., no demonstrations and thus possibly less food cues on the rope. The authors concluded that 12 of the 25 observing horses learned to pull the rope. However, only 4 of these horses learned the task after 8 demonstrations, three by pulling with their mouth and one by pulling with a hoof. The remaining 8 horses needed between 14 and almost 80 demonstrations to learn to pull the rope. The authors further note that the majority of the 14 control horses rapidly lost interest in the task (i.e., stopped engaging in behavior directed toward the rope) and only two learned to pull the rope after about 80 trials. The results suggest that local enhancement cues from the demonstrations gave the observing horses a small advantage compared to the controls. Additionally, the 12 horses that solved the task used different techniques in order to achieve the goal of opening the feed container, with only 6 horses using the same behavior as the demonstrator. Considering the number of horses that learned to pull the rope and the low speed at which they learned the task, the results can be more accurately explained by local enhancement and associative learning rather than social learning.

Another study analyzed the extent to which horses could learn by observation to follow a person in a round pen using four different tests (58). In the first test, 12 horse pairs were included (one demonstrator and one observer horse in each pair). The demonstrator horses either followed ($n = 4$) or did not follow the person ($n = 8$) during the demonstration. Three of the observer horses observing the following behavior, expressed following behavior themselves when subsequently tested, whereas one horse did not. None of the 8 observer horses paired with a non-following demonstrator showed following behavior when tested. In the second test, eight "dominant" horses demonstrated the following behavior to eight horses, which had participated in the first experiment and had not expressed the following behavior when tested. In this second test, the 8 horses showed following behavior when tested. In the third test, a "dominant" horse observed a "subordinate" horse perform the following behavior, resulting in one horse showing the following behavior after the demonstration whereas 13 horses did not. Lastly, the fourth test paired 8 observer horses with 8 unfamiliar demonstrator horses, which resulted in no observer horses following. Based on these tests the authors conclude that "subordinate" observers copy the behavior of a familiar, "dominant" horse, and that "dominant" horses do not copy the behavior of a familiar, "subordinate" horse. Across the tests, however, only horses experienced in round pen training ($n = 3$) performed the following behavior and as none of the inexperienced horses performed the following behavior after having watched a demonstration, it is likely that no social learning took place. Additionally, although the authors mention familiarity in their conclusions, it is unclear how familiar each demonstrator and observer was to each other: The included 38 horses were kept in groups of 11, 6, 9, and 4 horses and an additional 6 in pairs and 4 solitarily. During the experiment, 14 horses were used as demonstrators and observers, 8 horses only as demonstrators and 15 horses only as observers but without mentioning familiarity or testing for a potential group effect.

Conversely, a number of studies have failed to show social learning in horses. In a study by Baer et al. (59) observer horses ($n = 8$) watched conspecifics perform a discrimination task for 5 days with 4 demonstrations per day. Observer and control ($n = 16$) horses were then tested daily for 14 days. The discrimination learning criterion was set at 7 out of 8 responses correct with at least 5 consecutively correct. Control and observer horses did not differ significantly, but from the data it can be suggested that an effect of prior observation could have been present if more horses were included. Baker and Crawford (60) investigated if horses ($n = 9$) learned the location of grain by watching another horse finding it in one of two feed buckets of similar color and shape (i.e., only location cues). No significant difference between test and controls ($n = 18$) occurred for both first and total correct choices, nor for time to reach the feed bucket with grain. The authors therefore concluded that no social learning had occurred. In another discrimination experiment, Clarke et al. (61) tested if observer horses could learn to choose between two differently colored and shaped buckets after having it demonstrated by a conspecific. Twelve of the 14 observer horses reduced their latency to approach the bucket area during a series of 10 trials implying local enhancement, but there was no significant difference between observer and control horses in the number of correct bucket choices.

Two studies investigated the ability of horses to learn an operant task of opening a feeding apparatus by observation of a trained conspecific. One study found no significant effect of prior demonstration, only that across treatments younger horses engaged in more investigatory behavior [$n = 18$; (8)]. The other study found that horses observing a demonstrator horse opening an apparatus spent more time near the apparatus, although they did not learn to open it more quickly than control horses [$n = 66$ across two experiments; (62)]. Again, these results indicate that local enhancement cues are responsible for transfer of information between horses, rather than actual social learning. Another approach to investigating social learning in horses was conducted by Rørvang et al. (63) testing if observer horses ($n = 11$) could learn a simple detour task (turning left or right) by watching a demonstrator horse making the correct turn to navigate around a fence. Observer horses did not perform better than control horses ($n = 11$) that did not see the route demonstrated. Although turning left or right is indeed within the behavioral repertoire of horses, the test horses in this study did not appear to benefit from social observation. Thus, compared to species such as rats and budgerigars where observing an act can increase the probability that the observer animal will express that act rather than others in its repertoire (24), horses do not appear to benefit from prior social observation to solve operant and detour tasks. This difference may relate to these species facing very different foraging challenges.

Other studies have explored the effects of habituated demonstrators in fear-eliciting situations. Christensen et al. (64) investigated if a calm companion influenced fear reactions of naïve horses, by pairing 18 naïve horses with either a habituated companion horse ($n = 9$) or a non-habituated companion horse ($n = 9$). When exposed to the fear-eliciting stimulus, the horses accompanied with a habituated companion reacted less (less fear-related behavior and lower heart rate), compared to horses paired with non-habituated companions. The reduced fear reactions were also present 3 days later when the horses were tested alone (without a companion), reflecting social facilitation in combination with associative learning. Using a different experimental set-up, Rørvang et al. (65) tested the effect of prior observation of a habituated demonstrator crossing a novel surface. The observer horses watched the demonstrator crossing the novel surface from a distance of ∼10 m. These observer horses ($n = 11$) had lower mean and maximum heart rates when subsequently having to cross the novel surface themselves, compared to control horses ($n = 11$), suggesting that social facilitation even occurs from a distance and with a short delay (10 s) between demonstration and test.

Studies of social learning in other domesticated ungulates support the results on social learning in horses. In cattle, Ralphs et al. (66) found that social facilitation causes naïve cattle to start eating novel and even previously avoided food items. Veissier (67) investigated if heifers were able to obtain food from a box by pressing a panel after observing a familiar conspecific doing so. Heifers observing the demonstrator were more attentive to the box and the panel but acquisition of the task did not improve. Thus, also in other herbivores such as cattle, local enhancement appears to be the underlying mechanism for exchange of information between individuals. In domestic pigs, Held et al. (68) reported that observer pigs found relocated food using fewer bucket investigations than expected by random search, after watching a demonstrator finding relocated food. Non-informed pigs were additionally able to exploit the knowledge by following behind the demonstrator pigs to the food source. Nicol and Pope (69) analyzed the extent to which pigs could acquire information from their siblings. No significant effect of observation on rewarded panel pressing was found, but pigs that had observed the demonstrators, spent more time facing the operant panels and directed more non-rewarded presses toward the operant panels compared to controls. Collectively these studies indicate an effect of social facilitation and local/stimulus enhancement on food acquisition in pigs. Additionally, in relation to food preferences, weaned piglets show a preference for a flavored feed following a 30 min social interaction with an experienced demonstrator (70), which could even override neophobia toward the feed. Thus, socially transmitted cues seem important for pig feeding behavior (71) possibly owing to their omnivorous foraging behavior (39), but nevertheless pigs do not seem to utilize actual social learning in their foraging behavior.

Horses and other domesticated ungulates are indeed sensitive to transfer of information from conspecifics and the underlying mechanisms appear to be social facilitation and local/stimulus enhancement, rather than true social learning. Notwithstanding however, there seems to be significant potential in exploring the role of the dam as a salient demonstrator to her foal in fear-eliciting situations (72, 73). Studies of this sort may help elucidate how innate fear reactions can be modulated through an appropriate maternal environment. We conclude that instead of resorting to unlikely explanations of social learning in horses in complex experimental situations, more parsimonious explanations should be sought that are consistent with the horse's evolutionary biology and the tenets of Occam's razor and Morgan's Canon.

CONCLUSION AND PERSPECTIVES

In this review we propose a differentiation between social transmission (social facilitation, local, and stimulus enhancement) and social learning (goal emulation, imitation). The latter appears to be more cognitively complex, and in order to avoid assuming such high mental abilities of horses and for the sake of clarity of terms, this differentiation is essential. Herbivory may not facilitate the evolution and maintenance of higher mental abilities and it is likely that complex mental abilities such as those required for true social learning compared to social transmission, may be an "unaffordable luxury" for an obligate herbivore regardless of ungulate phylogenetic affinities. Studies on social transmission and social learning in horses show that horses are undoubtedly sensitive to transfer of information between conspecifics, however the underlying mechanisms are most likely to be social facilitation and local enhancement, rather than true social learning. Horse trainers should therefore not expect horses to be able to learn new behavior from watching conspecifics. Instead, acknowledging that horses are adept at using social cues in terms of social facilitation and local enhancement can greatly benefit horse training, e.g., through the use of habituated companion horses for habituation of naïve horses to frightening situations.

AUTHOR CONTRIBUTIONS

JC, JL, and AM initiated the idea for this review and all authors contributed to the initial discussions of the subtopics of the review. MR wrote the first draft and all authors contributed in writing, proofreading, and fine-tuning the review for publication.

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REFERENCES

1. Heyes CM. Social learning in animals: categories and mechanisms. *Biol Rev.* (1994) 69:207–31. doi: 10.1111/j.1469-185X.1994.tb01506.x
2. Nicol CJ. Farm animal cognition. *Anim Sci.* (1996) 62:375–91. doi: 10.1017/S1357729800014934
3. Klopfer PH. Social interactions in discrimination learning with special reference to feeding behavior in birds. *Behaviour* (1959) 14:282–99. doi: 10.1163/156853959X00117
4. Klopfer PH. Observational learning in birds: the establishment of behavioral modes. *Behaviour* (1961) 17:71–80. doi: 10.1163/156853961X00376
5. Krebs JR, MacRoberts MH, Cullen JM. Flocking and feeding in the great tit, Parus major- an experimental study. *Ibis* (1972) 114:507–30. doi: 10.1111/j.1474-919X.1972.tb00852.x
6. Altmann SA. The monkey and the Fig. *Am Sci.* (1989) 77:256–63.
7. Lefebvre L, Palameta B, Hatch KK. Is group-living associated with social learning? A comparative test of a gregarious and a territorial columbid. *Behaviour* (1996) 133:241–61. doi: 10.1163/156853996X00134
8. Lindberg AC, Kelland A, Nicol CJ. Effects of observational learning on acquisition of an operant response in horses. *Appl Animal Behav Sci.* (1999) 61:187–99. doi: 10.,1016/S0168-1591(98)00184-1
9. Krueger K, Flauger B. Social learning in horses from a novel perspective. *Behav Process.* (2007) 76:37–9. doi: 10.1016/j.beproc.2006.08.010

10. Ninomiya S. Social leaning and stereotypy in horses. *Behav Process.* (2007) 76:22–3. doi: 10.1016/j.beproc.2006.09.018
11. McBride S, Long L. Management of horses showing stereotypic behaviour, owner perception and the implications for welfare. *Vet Rec.* (2001) 148:799–802. doi: 10.1136/vr.148.26.799
12. Henderson AJ. Don't fence me in: managing psychological well being for elite performance horses. *J Appl Anim Welf Sci.* (2007) 10:309–29. doi: 10.1080/10888700701555576
13. Cooper JJ, Albentosa MJ. Behavioral adaptation in the domestic horse: potential role of apparently abnormal responses including stereotypic behavior. *Livestock Prod Sci.* (2005) 92:177–82. doi: 10.1016/j.livprodsci.2004.11.017
14. Nagy K, Schrott A, Kabai P. Possible influence of neighbours on stereotypic behaviour in horses. *Appl Anim Behav Sci.* (2008) 111:321–8. doi: 10.1016/j.applanim.2007.06.006
15. Albright JD, Mohammed HO, Heleski CR, Wickens CR, Houpt KA. Crib-biting in US horses: breed predispositions and owner perceptions of aetiology. *Equine Vet J.* (2009) 41:455–8. doi: 10.2746/042516409X372584
16. Murphy J, Arkins S. Equine learning behavior. *Behav Process.* (2007) 76:1–13. doi: 10.1016/j.beproc.2006.06.009
17. Hall C, Randle H, Pearson G, Preshaw L, Waran N. Assessing equine emotional state. *Appl Anim Behav Sci.* (2018) 205:183-93. doi: 10.1016/j.applanim.2018.03.006
18. Brubaker L, Udell M. Cognition and learning in horses (*Equus caballus*): what we know and why we should ask more. *Behav Process.* (2016) 126:121–31. doi: 10.1016/j.beproc.2016.03.017
19. Dennett DC. *Kinds of Minds*. London: Orion Books Ltd. (1996).
20. Morgan CL (ed.). Other minds than ours. In: *An Introduction to Comparative Psychology, 2nd Edn.* London: Walter Scott (1903). p. 59.
21. Galef BG. The question of animal culture. *Human Nat.* (1992) 3:157–78. doi: 10.1007/BF02692251
22. Sherry DF, Galef BG. Cultural transmission without imitation: milk bottle opening by birds. *Anim Behav.* (1984) 32:937–8. doi: 10.1016/S0003-3472(84)80185-2
23. Thorndike EL. *Animal Intelligence*. New York, NY: Macmillan. (1911) (Reprinted in Darien, Connecticut: Hafner, 1970, 248:74–76).
24. Galef BG. Imitation and local enhancement: detrimental effects of consensus definitions on analyses of social learning in animals. *Behav Process.* (2013) 100:123–30. doi: 10.1016/j.beproc.2013.07.026
25. Galef BG. Laboratory studies of imitation/field studies of tradition: towards a synthesis in animal social learning. *Behav Process.* (2015) 112:114–9. doi: 10.1016/j.beproc.2014.07.008
26. Clayton DA. Social facilitated behavior. *Quart Rev Biol.* (1978) 53:373–91. doi: 10.1086/410789
27. Galef BG. Imitation in animals: history, definition, and interpretation of data from the Psychological Laboratory. In: Zentall TR, Galef BG, Editors. *Social Learning: Psychological and Biological Perspectives*. Hillsdale,NJ: Lawrence Erlbaum Associates. (1988) p. 3–28.
28. Ligout S. Chapter 8: Grazing behaviour. In: Mills DS, editor. *The Encyclopedia of Animal Behaviour and Welfare.* Wallingford: CABI International (2010). p. 562.
29. Spence KW. Experimental studies of learning and higher mental processes in infra-human primates. *Psycol Bull.* (1937) 34:806–50. doi: 10.1037/h0061498
30. Thorpe WH. *Learning and Instinct in Animals*. London: Methuen. (1963).
31. Nicol CJ. The social transmission of information and behaviour. *Appl Anim Behav Sci.* (1995) 44:79–98. doi: 10.1016/0168-1591(95)00607-T
32. Boesch C, Tomasello M. Chimpanzee and human cultures. *Curr Anthropol.* (1998) 39:591–614. doi: 10.1086/204785
33. Byrne RW. Imitation: the contributions of priming and program-level copying. In: Bråten S, editor. *Intersubjective Communication and Emotion in Early Ontogeny*. Cambridge: Cambridge University Press (1999). p. 228–45.
34. Dawson BV, Foss BM. Observational learning in budgerigars. *Anim Behav.* (1965) 13:470–4. doi: 10.1016/0003-3472(65)90108-9
35. Heyes CM, Dawson GR. A demonstration of observational learning in rats using a bidirectional control. *Quart J Exp Psychol.* (1990) 42:59–71.
36. Whiten A, Custance DM, Gomez J-C, Teixidor P, Bard KA. Imitative learning of artificial fruit processing in children (Homo sapiens) and chimpanzees (Pan troglodytes). *J Compar Psychol.* (1996) 110:3–14. doi: 10.1037/0735-7036.110.1.3
37. Rendell L, Fogarty L, Hoppitt WJE, Morgan TJH, Webster MM, Laland KN. Cognitive culture: theoretical and empirical insights into social learning strategies. *Trends Cogn Sci.* (2011) 15:68–76. doi: 10.1016/j.tics.2010.12.002
38. Thornton A, Malapert A. Experimental evidence for social transmission of food acquisition techniques in wild meerkats. *Anim Behav.* (2009) 78:255–64. doi: 10.1016/j.anbehav.2009.04.021
39. McLean AN. Cognitive abilities – the result of selective pressures on food acquisition? *Appl Anim Behav Sci.* (2001) 71:241–58. doi: 10.1016/S0168-1591(00)00181-7
40. Kendal RL, Coolen I, Laland KN. Adaptive trade-offs in the use of social and personal information. In: Dukas R, Ratcliffe JM, editors. *Cognitive Ecology II*. Chicago, IL: University of Chicago Press (2009). p. 249–71. doi: 10.7208/chicago/9780226169378.003.0013
41. Provenza FD, Pfister JA, Cheney CD. Mechanisms of learning in diet selection with reference to phytotoxicosis in Herbivores. *J Range Manage.* (1992) 45:36–45. doi: 10.2307/4002523
42. Glasser TA, Ungar ED, Landau SY, Perevolotsky A, Muklada H, Walker JW. Breed and maternal effects on the intake of tannin-rich browse by juvenile domestic goats (*Capra hircus*). *Appl Animal Behav Sci.* (2009) 119:71–7. doi: 10.1016/j.applanim.2009.02.028
43. Schrader AM, Brown JS, Kerley GIH, Kotler BP. Do free-ranging domestic goats show 'landscapes of fear'? Patch use in response to habitat features and predator cues. *J Arid Environ.* (2008) 72:1811–9. doi: 10.1016/j.jaridenv.2008.05.004
44. Marinier SL, Alexander AJ. Coprophagy as an avenue for foals of the domestic horse to learn food preferences from their dams. *J Theor Biol.* (1995) 173:121–4. doi: 10.1006/jtbi.1995.0049
45. Provenza FD, Lynch JJ, Nolan JV. Food aversion conditioned in anaesthetized sheep. *Physiol Behav.* (1994) 55:429–32. doi: 10.1016/0031-9384(94)90096-5
46. Deacon TW. Fallacies of progression in theories of brain-size evolution. *Intl J Primatol.* (1990) 11:193–236. doi: 10.1007/BF02192869
47. Giraldeau L-A, Caraco T. *Social Foraging Theory*. Princeton, NJ: Princeton University Press. (2000).
48. Rands SA, Cowlishaw G, Pettifor RA, Rowcliffe JM, Johnstone RA. The emergence of leaders and followers in foraging pairs when the qualities of individuals differ. *BMC Evol Biol.* (2008) 8:51. doi: 10.1186/1471-2148-8-51
49. Boyd R, and Richerson PJ. An evolutionary model of social learning. In: Zentall TR, Galef BG, editors. *Social Learning: Psychological and Biological Perspectives.* Hillsdale, MI: Erlbaum (1988). p. 29–48.
50. Isler K, van Schaik C. Metabolic costs of brain size evolution. *Biol Lett.* (2006) 2:557–60. doi: 10.1098/rsbl.2006.0538
51. Sol D. Revisiting the cognitive buffer hypothesis for the evolution of large brains. *Biol Lett.* (2009) 5:130–3. doi: 10.1098/rsbl.2008.0621
52. Lefebvre L, Sol D. Brains, lifestyles and cognition: are there general trends? *Brain Behav Evol.* (2008) 72:135–44. doi: 10.1159/000151473
53. Martin RD. Comparative aspects of human brain evolution: scaling, energy costs and confounding variables. In: Jablonski NG, Aiello LC, editors. *The Origin and Diversification of Language*. San Francisco, CA: University of California Press (1998). p. 35–68.
54. Voelkl B, Huber L. True imitation in marmosets. *Animal Behav.* (2000) 60:195–202. doi: 10.1006/anbe.2000.1457
55. Range F, Viranyi Z, Huber L. Selective imitation in domestic dogs. *Curr Biol.* (2007) 17:868–72. doi: 10.1016/j.cub.2007.04.026
56. Russon AE. Exploiting the expertise of others. In: Whiten, A, Byrne RW, editors. *Machiavellian Intelligence II: Evaluations and Extensions*. Cambridge: Cambridge University Press (1997). p. 174–206.
57. Krueger K, Farmer K, Heinze J. The effects of age, rank and neophobia on social learning in horses. *Anim Cogn.* (2014) 17:645–55. doi: 10.1007/s10071-013-0696-x
58. Krueger K, Heinze J. Horse sense: social status of horses (*Equus caballus*) affects their likelihood of copying other horses' behavior. *Anim Cogn.* (2008) 11:431–9. doi: 10.1007/s10071-007-0133-0
59. Baer KL, Potter GD, Friend TH, Beaver BV. Observation effects on learning in horses. *Appl Anim Ethol.* (1983) 11:123–9. doi: 10.1016/0304-3762(83)90121-9
60. Baker AEM, Crawford BH. Observational learning in horses. *Appl Animal Behav Sci.* (1986) 15:7–13. doi: 10.1016/0168-1591(86)90017-1

61. Clarke JV, Nicol CJ, Jones R, McGreevy PD. Effects of observational learning on food selection in horses. *Appl Anim Behav Sci.* (1996) 50:177–84. doi: 10.1016/0168-1591(96)01071-4
62. Ahrendt LP, Christensen JW, Ladewig J. The ability of horses to learn an instrumental task through social observation. *Appl Anim Behav Sci.* (2012) 139:105–13. doi: 10.1016/j.applanim.2012.03.004
63. Rørvang MV, Ahrendt LP, Christensen JW. Horses fail to use social learning when solving spatial detour tasks. *Anim Cogn.* (2015) 18:847–54. doi: 10.1007/s10071-015-0852-6
64. Christensen JW, Malmkvist J, Nielsen BL, Keeling LJ. Effects of a calm companion on fear reactions in naive test horses. *Equine Vet J.* (2008) 40:46–50. doi: 10.2746/042516408X245171
65. Rørvang MV, Ahrendt L, Christensen JW. A trained demonstrator has a calming effect on naïve horses when crossing a novel surface. *Appl Anim Behav Sci.* (2015) 171:117–20. doi: 10.1016/j.applanim.2015.08.008
66. Ralphs MH, Graham D, James LF. Social facilitation influences cattle to Graze Locoweed. *J Range Manage.* (1994) 47:123–6. doi: 10.2307/4002819
67. Veissier I. Observational learning in cattle. *Appl Anim Behav Sci.* (1993) 35:235–43. doi: 10.1016/0168-1591(93)90139-G
68. Held S, Mendl M, Devereux C, Byrne RW. Social tactics of pigs in a competitive foraging task: the 'informed forager' paradigm. *Anim Behav.* (2000) 59:569–76. doi: 10.1006/anbe.1999.1322
69. Nicol CJ, Pope SJ. Social learning in sibling pigs. *Appl Animal Behav Sci.* (1994) 40:31–43. doi: 10.1016/0168-1591(94)90085-X
70. Figueroa J, Solá-Oriol D, Manteca X, Pérez JF. Social learning of feeding behaviour in pigs: effects of neophobia and familiarity with the demonstrator conspecific. *Appl Animal Behav Sci.* (2013) 148:120–7. doi: 10.1016/j.applanim.2013.06.002
71. Oostindjer M, Bolhuis JE, Mendl M, Held S, van den Brand H, Kemp B. Learning how to eat like a pig: effectiveness of mechanisms for vertical social learning in piglets. *Anim Behav.* (2011) 82:503–11. doi: 10.1016/j.anbehav.2011.05.031
72. Henry S, Hemery D, Richard M-A, Hausberger M. Human–mare relationships and behaviour of foals toward humans. *Appl Anim Behav Sci.* (2005) 93:341–62. doi: 10.1016/j.applanim.2005.01.008
73. Christensen JW. Early-life object exposure with a habituated mother reduces fear reactions in foals. *Anim Cogn.* (2016) 19:171–9. doi: 10.1007/s10071-015-0924-7

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REVIEW
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Factors Influencing Individual Variation in Farm Animal Cognition and How to Account for These Statistically

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For farmed species, good health and welfare is a win-win situation: both the animals and producers can benefit. In recent years, animal welfare scientists have embraced cognitive sciences to rise to the challenge of determining an animal's internal state in order to better understand its welfare needs and by extension, the needs of larger groups of animals. A wide range of cognitive tests have been developed that can be applied in farmed species to assess a range of cognitive traits. However, this has also presented challenges. Whilst it may be expected to see cognitive variation at the species level, differences in cognitive ability between and within individuals of the same species have frequently been noted but left largely unexplained. Not accounting for individual variation may result in misleading conclusions when the results are applied both at an individual level and at higher levels of scale. This has implications both for our fundamental understanding of an individual's welfare needs, but also more broadly for experimental design and the justification for sample sizes in studies using animals. We urgently need to address this issue. In this review, we will consider the latest developments on the causes of individual variation in cognitive outcomes, such as the choice of cognitive test, sex, breed, age, early life environment, rearing conditions, personality, diet, and the animal's microbiome. We discuss the impact of each of these factors specifically in relation to recent work in farmed species, and explore the future directions for cognitive research in this field, particularly in relation to experimental design and analytical techniques that allow individual variation to be accounted for appropriately.

Keywords: cognition, refinement, individual, welfare, livestock, multilevel modeling

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INTRODUCTION

Understanding the cognitive capabilities of animals, how they may be affected by the environments in which we keep them, and the extent to which these changes can be used as an indicator of welfare, are increasingly of interest in the field of animal welfare. Cognition has been broadly defined as any process or mental action required to gain, process, and use information collected via experience, thought and senses (1). This includes functions such as attention, memory, social learning, associative learning, judgment, and reasoning, to name a few (2). Understanding the cognitive capabilities of animals thus allows us a window into the way in which an animal perceives and makes sense of the world around it. This is particularly important for farmed species, which there are large numbers of globally, and where the husbandry practices directly affect their welfare and have the potential to cause suffering (3, 4). There are numerous tests available that allow us to assess cognitive ability (**Table 1**) and multiple factors that we know are important to consider in applying these. Despite advances in this endeavor, assessing and interpreting cognition in non-human animals is challenging.

Part of the difficulty with studying cognition in farmed animal species is due to individual variation in cognitive abilities. The majority of animal cognition studies examine how individuals perform in cognitive tasks on average, using aggregated data (e.g., the mean number of trials correct, average latency to respond). However, individuals also show between- and within-individual variation in cognition across repeated measurements or across different experimental conditions (97). Variation in individuals' average performance may represent relatively consistent between-individual differences in cognitive styles, similar to animal personality (98). Variation in within-individual cognitive change (e.g., the rate of learning) may further indicate differences in cognitive flexibility, a posited mechanism of behavioral plasticity (97). Variation in residual within-individual change (i.e., the amount of variation around an individual's average performance) could be used as a measure of cognitive resilience in farmed animals [e.g., (99)]. In turn, factors such as age, sex, breed and personality may predict these facets of individual variation.

In this review, we explore the main causes of within- and between-individual variation in cognitive testing of livestock. By "causes," we mean factors that would result in individual variation all else being equal [e.g., (100)]. In section one, we give a brief overview of the types of cognitive tests available, including a table which shows the type of test and which cognitive function it has been used to assess. In section two, we then identify some key areas that can contribute to variation, including; sex, breed, personality, life stage, diet, mood, motivation, and gut microbiome. Following this, in section three, we highlight statistical methods for estimating different facets of individual variation and include a statistical example.

TYPES OF COGNITIVE TESTS

There are multiple ways to assess an animal's cognitive capabilities and in **Table 1** we list some of the most common tests used to investigate different aspects of cognition in farmed animal species. However, many require the use of more than one cognitive ability and tests are often adapted to suit the species or cognitive function of interest (101). Indeed, the notion of embodied cognition (102) highlights the connection and interaction of the brain with the body and the environment. The anatomical features of a species may allow it to perform better or worse on a given cognitive task than another species. Thus, to accurately measure cognition with cognitive tasks, it is necessary to ensure that the task is designed to suit the physical abilities of the species being tested. It should also be noted that, when exploring sources of variation in cognitive testing, the test itself could be a contributing factor. One example of this is side preference, which can develop in T-maze or Y-maze study designs, as shown in a variety of species including sheep (103), rats (104), and cows (105). In these cases, the learning outcomes of the task can be overshadowed by the animal's preference to occupy one side of the maze. Furthermore, farm animals often have not had an extensive period of regular positive contacts with humans, as may be the case for companion animals. Therefore, in order to conduct cognitive testing in these animals, habituation to the test set-up will be required to ensure that the responses measured are task specific and not affected by fear of either the experimenters or the testing situation. Regardless of the cognitive task employed, determining which factors are causing individual variation in cognition requires distinguishing between direct causal relationships and correlations.

FACTORS CONTRIBUTING TO INDIVIDUAL VARIATION IN COGNITIVE PERFORMANCE

Cognition and behavior are hierarchically organized and continuously interacting with endogenous (e.g., life stage) and exogeneous (e.g., the behavior of conspecifics, developmental environments) factors. This likely results in correlations between many different cognitive and behavioral variables [referred to as the "crud factor" in human psychology; (106)], as well as their significant associations with endogenous and exogenous variables, but these do not necessarily reflect direct causal relationships. As such, we interpret the "causes" of individual variation below as factors that result in variation between individuals who are equal in all other respects. This interpretation of causality follows Pearl's work (100) on distinguishing causal from associative relationships by determining which correlations in multivariate data disappear when all variables are conditioned on each other, i.e., finding conditional independence relations in the data [see (107) for an accessible review]. We also acknowledge that individual variation can emerge simply from the cumulative effects of unsystematic events occurring in the environment and/or in how individuals process information, even in genetically identical individuals [e.g., (108)]. This means that individual variation is to be expected a priori even when no obvious cause exists.

Development and Early Life

During prenatal and neonatal stages of growth, there is a period of rapid brain development, including cell birth, migration, dendritic outgrowth, programmed cell death, and synapse production. The brain is particularly vulnerable to perturbations during development (109) and both endogenous and exogenous factors occurring at this time have been shown to influence cognition in livestock animals (103, 110). Prenatal stress is well-known to have an effect on the developing brain and on programming of the hypothalamic-pituitary-adrenal (HPA) axis (111–113). These effects have consequences for stress reactivity, behavior, and cognition in offspring that can continue throughout life (114). For example, ewes exposed to stressful situations during late pregnancy produced lambs that showed increased levels of fear and a decreased ability to navigate a

TABLE 1 | An overview of cognitive tests that have previously been used in farmed species and the type of cognitive ability they assess.

Cognitive ability	Task	References
Spatial cognition	Learning distribution/position of baited locations	*Sheep:* (5, 6); *Cattle*: (7, 8); *Chickens*: (9–11); *Fish:* (12)
	Parallel arm maze	*Cattle*: (13); *Fish:* (14)
	Radial arm maze	*Pigs:* (15, 16); *Chickens*: (17, 18)
	Spatial maze with zones	*Sheep:* (19–21); *Pigs:* (22) *Cattle*: (23–25); *Fish*: (26, 27)
	T-maze	*Cattle:* (28); *Chickens*: (17, 29); *Fish:* (30, 31)
	Y-maze	*Chicken*: (17); *Sheep*: (32); *Cattle:* (33)
	Rotating enclosure	*Chickens:* (34, 35)
Memory	Holeboard spatial discrimination	*Pigs:* (36–39); *Chickens:* (29)
	Object recognition	*Pigs:* (40)
	Delayed match to sample	*Chickens:* (41)
	Devaluation foraging technique	*Chickens:* (42, 43);
	Delayed search task	*Chickens:* (44, 45)
	Two step foraging task	*Goats:* (46)
Social cognition	Foraging arena task	*Pigs:* (47, 48)
	Follow knowledgeable individual	*Pigs:* (49)
	Mirror task	*Pigs:* (50, 51) *Sheep:* (52)
	Y-maze	*Pigs:* (53–55); *Sheep*: (56, 57); *Chickens:* (58, 59); *Cattle:* (60)
	Social recognition test	*Pigs:* (61)
	Social recognition based on visual/olfactory cues–operant tasks	*Chickens*: (62); *Cattle:* (63, 64); *Sheep:* (65); *Chickens:* (59)
	Choice test	*Fish:* (66)
Social learning	Distance to aversive/gentle handler	*Cattle:* (67)
	Operant task	*Cattle:* (68)
	Food choice test	*Pigs:* (69); *Chickens:* (70)
	Object choice test	*Goats:* (71)
	T-maze	*Goats:* (72)
	Detour task	*Goats:* (73)
Inferential reasoning	Preferential looking paradigm choice test	*Goats:* (74)
	Object choice task	*Goats, pigs:* (75–77)
Discrimination learning	Image discrimination (visual discrimination)	*Pigs, Goats:* (55, 78, 79)
	Acoustic discrimination	*Pigs:* (80)
	Social discrimination (visual discrimination)	*Sheep:* (81)
Object permanence	Hidden reward object	*Pigs, Goats:* (82, 83)
	Perseveration error	*Goats:* (83)

(Continued)

TABLE 1 | Continued

Cognitive ability	Task	References
Classical conditioning	Clicker training	*Cattle:* (84)
	Eye blink response conditioning	*Sheep:* (85, 86)
	Trained to approach feed source with audio cues	*Cattle:* (87)
	Trace classical conditioning	*Chickens:* (88)
	Classical conditioning using light to signal arrival of food.	*Fish:* (89)
	Delay conditioning regime	*Fish:* (90)
Operant conditioning	Trained to approach feed source with audio cues	*Cattle:* (87, 91)
	Social contact motivation task	*Cattle:* (92)
	Nose wheel feeding task	*Pigs:* (93)
	Trained to urinate in a specific location	*Cattle:* (94)
Numerical understanding	Free-choice tests	*Chickens:* (95)
	Identification of trained rank-order target locations among identical alternatives	*Chickens:* (96)

maze, suggesting decreased spatial and working memory (21). Similarly, domestic chicks that experienced hypoxic conditions for 24 h during embryonic development had poorer performance in a bead discrimination task designed to test memory (115).

A further factor relating to development and cognition is an animal's origin litter. For example, Hernandez et al. (116) found that lambs from twins were more likely to change side preference in a two-armed maze test in comparison to singleton born lambs. The environment and experiences that an individual is subjected to during their developmental period are also influential. Calves fed using an enriched feeding method (instead of standard bucket feeding) showed decreased reactivity to novelty and, although initially they took longer to locate a reward, performed better in the reversal stage of a T-maze task (28).

Age and timing of weaning can also have implications for cognition and behavior. In livestock species, weaning often also involves separation from the mother, littermates and mixing into large groups of unfamiliar conspecifics in a new environment (117, 118). Weaning at earlier or later ages than the industry standard has been shown to influence stress (119) and behavior (120–123). Early weaning and material deprivation can significantly affect the brain and consequently cognition. For example, piglets that were weaned early at 10 days of age had decreased gene expression in the hippocampus (124). In other species, maternal deprivation can increase cell death in the brains of young rats (125) and can reduce neurogenesis in mice (126). Overall, these different life-stage factors can all influence cognitive function within an individual.

Sex

Of all the factors considered here that may influence cognition, sex is perhaps one of the most evolutionarily well-conserved (127). In cognitive testing of farmed species, there has not been the same drive to detect sex-related effects as there has been in clinical trials on laboratory animals, so observed differences between sexes are typically reported incidentally rather than explicitly investigated. For example, Erhard et al. (103) found that male sheep required fewer runs to learn and solve a reversal-learning task than females at 18 months old. Conversely, another study with a similar maze design found female sheep were quicker to learn and solve a reversal-learning task than males at 4 months of age (116). However, this finding was not present by 18 months and, as the authors suggest, may only have reflected differing maturation rates of male and female animals.

Although sex has rarely been explicitly tested in farm animal cognition studies, statistically significant differences have been identified between the sexes for many biological parameters in clinical trials. Of particular note for this review are the studies that have identified sex differences in stress-related psychiatric disorders, such as depression, generalized anxiety disorder, acute and chronic post-traumatic stress disorder, with a higher risk of development in females than in males (128–131). Stress-related disorders are linked closely with cognitive alterations, and differing levels of performance in learning and memory tests in particular. For example, exposure to an acute or repeated stressful event is associated with enhanced learning in a classical conditioning task in male rats, but impaired performance in females (132); though this is only the case in adult females with mature oestrous cycles (133). By contrast, the opposite effect has been found in spatial learning and memory tasks, where acute stress exposure impairs males' performance in a Y maze, but enhances female rats' performances regardless of their oestrous cycle (134). Similar effects have been shown in another memory test, the Morris water maze test (135, 136). Even without the stress exposure, there are clear male advantages in spatial working and reference memory in rats that transcend strain, age, environment, and testing protocol differences. However, mouse studies have found a different pattern—that females have an advantage in water maze tests, but males have a small advantage in radial maze tests (137). Of relevance to the cognitive bias testing paradigm, risk seeking behavior in humans tested using a computerized balloon analog risk task, showed clear sex differences. Following exposure to an acute stressor, risk avoidance increased in females but risk seeking increased in males (138).

One consideration with the measurement of sex differences is that it is typically included as a binary variable and used as a simple to measure, catch-all, umbrella indicator for what is in reality a host of non-discrete, underlying interacting complex systems. As suggested by Maney (139), sex should be viewed as a proxy for as-yet unknown factors that co-vary with it, such as hormonal differences, sex-linked genetics, or experience. Testing simply for the existence of a difference between the sexes may mask distributional differences in co-varying variables, resulting in false negative outcomes; as such, it may be more informative to consider the extent to which the sexes differ, rather than whether or not a significant difference exists.

Breed

The genetic composition of different livestock breeds has the potential to influence temperament, behavior, and cognition. The differences in cognitive task performance between different breeds may be due to factors that are not directly attributable to cognitive abilities *per se*. For example, temperament differences between breeds may alter the likelihood of an individual engaging with the task and/or influence their opportunities to be exposed to the stimulus (98). A study by Nordquist et al. (29) compared the responses of chickens breed for low mortality and a control breed/line in multiple cognitive tests, including the holeboard task and T-maze. Overall, chickens breed specifically for low mortality displayed lower levels of fearfulness than the control individuals. McBride et al. (52) found that Welsh mountain sheep spent more time looking and touching their self-image in a mirror than two other breeds of sheep. The authors suggest this may have been due to breed differences in exploration and social tendencies (52). Veissier et al. (68) found breed differences in an observational learning task in female cattle, with more Limousin heifers learning the task than Aubrac heifers in the same experiment. The difference in task success between the two groups appeared to be due to differences in fearfulness between the breeds, with Aubrac heifers spending more time trying to escape the experimental room, rather than engaging with the task. Kendrick et al. (57) found Dalesbred and Clun forest sheep differed in their performance on a vocal discrimination task. In this case, the authors suggested that this could be due to the differing habitats of the breeds. Hill sheep have better abilities in vocal discrimination tasks, possibly due to being more dispersed in their natural habitat than lowland sheep, thus requiring more reliance on the use of vocalization to discriminate individuals when widely dispersed.

Many studies investigating cognitive performance standardize for breed differences by using just a single breed. As such, there are relatively few studies that directly compare across breeds. Perhaps also due to publication bias, it is possible that such studies have been conducted but no significant results found, leaving few published studies with an absence of breed differences to draw upon as examples. One example of such a lack of difference is in Murphy et al. (140), who compared Göttingen miniature pigs and standard commercial breed pigs in a judgment bias task and found no difference in their abilities to discriminate between auditory cues associated with positive and negative outcomes.

At this time, it is not possible to draw firm conclusions about the role of breed in cognitive performance, simply because there have been so few studies published where this has been directly compared between breeds. However, drawing from the studies that have found a breed-related association with performance, the results suggest that cognition is influenced by an animal's evolutionary, ecological and developmental environment (102).

Personality

Animal personality is defined as moderately consistent individual differences in behavior across time and contexts (98, 141). A number of terms have been used to capture individuals' consistent patterns of behavioral, physiological and/or neuroendocrine profiles, including coping styles and behavioral syndromes. Personality research has traditionally focused on traits such as exploration, boldness, activity levels, sociability and aggressiveness (142). While moderately stable across time and contexts, personality also interacts with behavioral plasticity and predictability [i.e., within-individual change (143)].

Two common categorizations of personality types or coping styles are *proactive* and *reactive*. Proactive individuals are bolder and more exploratory than reactive individuals, allowing them to learn quickly in new situations but become relatively inflexible when previously learned rules change (144). By contrast, reactive individuals demonstrate greater behavioral flexibility than proactive individuals. A predominant hypothesis about the relationship between personality and cognition is that proactive individuals prioritize speed over accuracy in decision making (145). For instance, Nawroth et al. (79) report that goats scoring higher for exploration and sociability (consistent with proactive personality types) performed worse in tasks of object permanence and visual discrimination. Reactive laying hens also learned to associate a color-cue with a reward better than proactive hens (146). In fish, White et al. (27) found a negative correlation between boldness and learning to use cues to find hidden food in brook trout. Bensky et al. (147) report that bolder three-spined sticklebacks were quicker to learn a color discrimination task than shyer individuals, although no evidence was found for shyer individuals to perform better when the task was altered.

Griffin et al. (97) note that discerning robust relationships between an individual's personality and cognitive style will require tests of both cognitive abilities and personality traits to allow the full array of competing alternative hypotheses to be tested. This may lead to a multi-method multi-trait approach across both personality and cognition tests (148) to ensure the validity and robustness of relationships between personality and cognitive measurements.

Mood

Affective state and cognition are deeply intertwined, with cognition influencing affective state and affective state in turn influencing cognitive processes (149). Affective state can be categorized into emotion and mood. Emotions are short-lived mental states that arise in response to rewarding or punishing stimuli (150). Emotions change rapidly and contribute to within individual variability in test performance (151). Moods, on the other hand, are longer-term mental states that are not tied to a specific stimulus and are thought to be the result of the accumulation of affective experiences in the mid- to longer-term past (152, 153). Moods are more specific to the individual and may contribute to between individual variability on cognitive tasks. Mood can also interact with personality to affect cognitive processes. For example, more reactive individuals in a negative mood judge novel information more negatively than reactive individuals in a positive mood, whilst proactive individuals' mood did not affect their judgements (154).

Mood affects information processing by altering response thresholds to stimuli (152). This has most commonly been evaluated in situations of ambiguity and is linked to risk taking. In particular, the cognitive bias test has been widely applied with farm animals to investigate the effect of mood on decision making under ambiguity [for a review see Baciadonna and McElligott (155)]. These tests have been devised to assess emotional state rather than cognition, and thus they cannot directly answer questions on the effect of mood on cognition. In addition, the question of how emotion may affect other cognitive processes, such as social learning, spatial cognition, or working memory, has not been assessed in farm animals to our knowledge. Affective states influence a wide range of cognitive processes in humans, such as self-regulation, information processing and decision making [e.g., see Martin and Clore (156)]. This suggests that mood may also be a source of within-individual variation in performance in animals, in tests measuring cognitive abilities other than those involved in risk taking. However, it is difficult to disentangle the effects of the specific test set-up from the effects of mood alone. Such as in tests of visual discrimination of faces, an animal's ability may be affected by how aversive they find the stimulus, which in turn impacts their ability to attend to the stimulus for long enough to complete the task. For example, horses spent less time looking at agonistic conspecific faces than neutral or positive conspecific faces (157). Whilst Lee et al. (158) showed that less anxious sheep spent less time attending to a threat stimulus than anxious sheep. Thus, if mood affects how aversive a stimulus is to attend to, this could affect performance on cognitive tasks requiring a certain level of attention toward specific stimuli.

Both genetic predisposition and environmental factors cause variation in mood. Whilst the former can be partially controlled for by using individuals of the same breed and genetic line, controlling for the environment can be less reliable due to the stochastic nature of life. In addition, genetic × environment interactions can lead to further variability at the individual level (108). To complicate matters further, we do not completely understand how mood is generated, and thus cannot fully control for it in cognition studies. Eldar et al. (159) proposed the theory that mood is the cumulative result of differences in expectations and the obtained outcomes of recent experiences. Raoult et al. (151) did not find strong evidence for this theory in their review of 95 papers on cognitive bias and manipulations used to affect mood. However, further research is required with more precise and overt tests of the predictions from this theory, as the studies reviewed did not have the original aim of testing Eldar's theory. Future cognitive studies could also benefit from assessing mood alongside the specific cognition test in which they are interested, as this would provide valuable information on the contribution of differences in mood to the variability found in tests of cognitive abilities.

Motivation

In order that an animal completes a cognitive task, it must be motivated to engage and perform. Levels of motivation can differ both within and between individuals as a result of multiple factors: the reward type and timing, protocols used to induce motivation, and the inherent value of completing the task, all of which may be influenced by previous experience.

If correct trials are to be reinforced, the first consideration is the researcher's choice of reward. In farmed species, common rewards include access to food or conspecifics. When using food, providing a reward distinct from that of an animal's standard feed may increase motivation for some individuals and decrease it for others, dependent on individual preferences. For some non-livestock animals, it has been shown that using a preferred reward can increase motivation (160–162) but that preferences change over time [e.g., orangutans: Clay et al. (163)]. Therefore, depending on the length of the testing period, variation in task performance may reflect changes in an individual's reward preference.

When using appetitive rewards, the levels of pre-task satiety can influence the animal's willingness to participate. To induce motivation, animals may be food restricted prior to testing, such that access to food becomes more appealing. Blanket protocols are often applied across a study group (e.g., restrict test subjects to 70% of *ad libitum* intake or provide a set volume of food). It cannot be expected that each animal will respond equally to a fixed restriction, resulting in variability in levels of motivation and thus in perceived cognitive ability. If restrictions are not staggered, the first animal to be tested may be less motivated than the final individual, given the difference in total restriction time. Similarly, in cases where restriction protocols are not applied, the time since last feeding may also impact any appetitively rewarded trials. A final consideration is fluctuations in motivation over the course of a testing session. If a session requires many iterations of a task, the reward value may depreciate and, subsequently, trials carried out at the beginning of testing may not be comparable to those performed at the end.

As an alternative to food rewards, some social species may be rewarded socially by providing access to conspecifics—for example fish (30) and sheep (20) in maze-based tasks. Introducing conspecifics may mitigate some of the confounding factors of using appetitive rewards, but social reinforcers bring complications of their own. For example, motivation to gain access to a conspecific may be partially affected by social rank, as has been shown in non-human primates (164). Levels of motivation may also be influenced by the degree of contact offered as a reinforcer; calves were more motivated to perform an operant conditioning task for full contact with a conspecific, than for only contact with the head (92). Given that livestock animals can have preferences for certain group mates or familiar animals [e.g., cows: (165); sheep: (166, 167)] the identity of the "reward animal" is also of importance. Rewarding with a preferred or non-preferred individual could alter the perceived outcomes of the task. Interestingly, social interactions can also impact on the motivation to work for food rewards. Pedersen et al. (168) demonstrated that isolating a pig from its pen mate decreased the value of a food reward, highlighting the impact of social context on cognitive trial outcomes.

The type of task and the animal's perception of it may also influence motivation. Some tasks may have an inherent motivational value, regardless of any reward received for completion of a correct trial. For example, de Jonge et al. (169) found evidence of contra-freeloading in domestic pigs, meaning that the pigs preferred to work for food despite identical food being freely available. However, such a task may be cognitively stimulating to one animal, but not to another. It is also possible that motivation to perform may depend on living conditions. If a task is novel or enriching, an individual from a complex, enriched home environment may not find the task as rewarding as would an individual from a less stimulating home environment.

In all of the above examples, the researcher risks measuring motivation to engage with the task, rather than judging the cognitive ability of an individual. This confound is perhaps most important to consider when using latency to give a correct response as a measure of cognition, e.g., in maze completion.

Diet

Diet and access to food can influence cognitive function, for example feed restriction, which is known to impact on learning and some aspects of memory in rodents (170, 171). Feed restriction during gestation, even for a short period of time, can have lasting effects on behavior and cognition in offspring. A study by Erhard et al. (103) investigated the impact of temporary feed restriction during early gestation in Scottish Blackface sheep. For this study, a control group was compared with a treatment group whose mothers had their feed intake reduced by 50% for the first 95 days of gestation. Although there was no difference in the average birth weight of the control and the treatment groups, lambs that experienced prenatal feed restriction were more active than control animals in novel object, social isolation, physical restraint, and suddenness tests. This increase in emotional reactivity also affected performance in cognitive tasks with prenatal feed restricted individuals less likely to learn the first reversal task in the T-maze if they had high levels of locomotion during social isolation and novel object tests. Similarly, there is evidence that the early life diet of an individual can influence cognitive performance, which, as shown by some studies, has the potential to last into adulthood (39, 172). For example, Rytych et al. (173) found that severely iron deficient piglets could not acquire a spatial T-maze task. Similarly Antonides et al. (174) found that iron deficient piglets had reduced reference memory in a holeboard task in comparison to non-iron deficient piglets.

The nutritional content of feed and the time period of exposure can have a significant impact upon cognitive function. In the context of biomedical research, several cognition studies have been conducted using pigs to investigate the effect of the "Western"-style diet, comprising high energy, high fat, and high sugar levels. Both Val-Laillet et al. (175) and Clouard et al. (176) found that prenatal exposure to a "Western"-style diet improved both working and reference memory in piglets, in comparison to piglets on a standard diet. However, there was no effect on cognitive function in piglets fed this diet during the early postnatal period with no previous exposure during gestation (176). Although this diet is typically not applicable for livestock, it highlights the influence that nutrition can have. However, other dietary constituents in the mother's diet during gestation and lactation can impact upon offspring cognition. Examples of these include sialic acid which improved learning and memory (177, 178) whilst iron which was shown to impair reference memory in piglets (173).

Diet will inevitably vary between life stages, however it is worth noting that this can be a cause of cognitive variation, especially when comparing results between two studies of the same species. In addition, diet directly impacts the gut microbiome, which is also closely linked to the brain and cognitive function.

Gut Microbiome

The gut is inhabited by trillions of microbes (the microbiota) and the term "gut microbiome" refers to their genetic material and capabilities. Microbiomics is a rapidly developing field, as interest in the broader effects of diet continues to grow. For example, a search of the literature published over the last 10 years shows an increase from 16 papers in 2007 to 2,210 papers in 2017 using the search term "gut microbiome" (Web of Science). It is outside of the scope of this review to fully evaluate the links between the gut and brain, but some relevant ideas are discussed in this section.

The gut microbiome and the brain communicate bidirectionally via multiple suggested mechanisms, known collectively as the microbiome-gut-brain axis [see comprehensive reviews from Mayer (179) and Galland (180)]. These lines of communication substantiate the idea that the gut may influence cognitive function. Many studies of the microbiome-gut-brain axis and its relationship to cognition have focused on the context of aging, disease and/or neurodisability. For example, some researchers use cognitive function to measure the efficacy of an intervention or as an indicator of the neurological impairments associated with, for example, Alzheimer's (181), diabetes (182), and autism (183). Although these studies are not directly comparable to livestock, they give an indication of how differences in the gut microbiome, caused by illness or physiological disruption, may lead to variability in cognitive function.

In addition to disease syndromes, disruption of the gut microbiome is linked with stress [e.g., Bailey et al. (184); O'Mahony et al. (185); Jašarević et al. (186)], and stress has known impacts on cognition (187). It may therefore be considered that an animal suffering stress could perform poorly in a cognitive task, either as a direct neurological consequence of the stress, or via changes in the composition of the gut microbiota. Indeed, Weinstock (188) showed that male mice exposed to prenatal stress showed signs of cognitive deficits—this was attributed in part to the mother's vaginal microbiome, which in turn influences the offspring's gut microbiome (186, 189).

The complex relationships between gut health, diet, stress, illness, and cognition are further complicated by the fact that the gut microbiome does not remain stable throughout life and can be influenced by a variety of factors including birth conditions, diet, environment, disease and aging (190–192).

These multi-level interactions make it difficult to suggest that variability in livestock cognitive performance could be attributed to the microbiome, but it is nevertheless something important to consider as part of a wider system.

ACCOUNTING FOR INDIVIDUAL VARIATION

Accounting for individual variation in cognition is important in farmed animal species, and applied ethology in general, because we are often interested in how individuals experience their environment, not just a population. Aggregating and analysing data across individuals can lead to misleading conclusions when the goal is to understand individual-level processes. For example, not accounting for within-individual variation in cognitive change risks committing an ecological fallacy (193). That is, incorrectly inferring the form of within-individual processes (e.g., the relationship between stress and cognitive ability) from results pertaining to group-level, aggregated patterns of change. Extreme cases may lead to Simpson's paradox, where the relationships between variables at an aggregate level are the reverse of those relationships at lower levels of scale. In animal welfare science, evidence suggests that most behavioral variation is explained by individual variation rather than by higher-level factors such as groups or pens [e.g., chickens: (194)], so appropriately incorporating individual variation is key.

Animal cognition studies often record repeated measurements on individuals, which are then used to quantify summary measures of cognitive performance, such as the number of trials needed to learn a task or the average probability of responding correctly. However, this often precludes estimating between- and within-individual variation as a result of data aggregation across repeated measurements. In addition, when the goal is to quantify the relationship between individual variation in cognition, and endogenous and exogenous factors (such as those discussed above), researchers may be motivated to conduct a number of separate statistical analyses. Yet, conducting multiple analyses on the same data set can lead to increased chances of false positives. This is further complicated by the low sample size of animal cognition studies (195), which are not only at increased risk of Type II errors (i.e., not enough signal to reject the null hypothesis), but also Type I errors (i.e., incorrectly rejecting the null hypothesis), and errors of sign and magnitude (196). For example, a significant *p*-value in a small sample size study should not be taken as evidence of a robust effect (197).

Accounting for individual variation may further improve the reproducibility of studies. In recent years, the reproducibility of scientific findings has received increasing scrutiny, most notably in the psychological sciences (197) but also in a number of other areas [e.g., cancer biology; (198); economics: (199); artificial intelligence: (200)]. Conditions for irreproducibility include studies with low sample sizes and small true effect sizes for the relationships being investigated, along with questionable research practices such as data dredging or p-hacking (running analyses multiple times until a significant *p*-value is found) and poor research incentives (201). Studies of farmed animal cognition may also be at risk of irreproducibility due the small number of animals used, especially when potentially large systematic individual variation in cognitive performance is not accounted for, making across-study results inconsistent. Indeed, Voelkl and Wurbel (202) argue that a key condition for irreproducibility in pharmacological studies could be the lack of appropriate estimates of phenotypic variation, and argue that greater attention should be paid to quantifying phenotypic reaction norms.

In this section, we highlight how multilevel models can be used to investigate individual variation across repeated measurements. While multilevel models are neither the only approach for measuring variation nor particularly new (203), their use is increasingly encouraged as the state-of-the-art approach in accounting for variation across distinct clusters (e.g., individuals) in a range of disciplines [e.g., animal welfare: (204); ecology: (205); human evolutionary ecology: (206); psychology: (207); health: (208)]. This includes analysing individual variation in behavior (e.g., personality) and reaction norms in behavioral ecology (209), and individual variation in human cognition (210). Adopting multilevel models for assessing individual variation in cognition in farmed animals is a natural extension. Below, and in the Supplementary Materials (available on Github: https://github.com/ConorGoold/Bushby-et-al-individual-variation-cognition), we demonstrate how multilevel models may be applied to explore facets of individual variation in animal cognition.

Multilevel Models

Multilevel models extend the general linear model framework to account for variation across different groups or clusters, such as repeated measurements on individuals. Specifically, deviations for each clustering unit are estimated from the population-level intercept, slope and/or residual standard deviation parameters (known as "random effects"). These deviations are constrained by their own (usually normal) distribution, which improves the predictive ability of these models compared to non-multilevel models through the effects of *partial-pooling* (203). The deviations represent the amount of individual variation, which in turn can be predicted by a number of "cluster-level" predictor variables. For example, in studies of behavior, variation among individuals in the intercept parameter is used to operationally define animal personality, variation among individuals in the slope parameter across an environmental gradient defines behavioral plasticity, and variation among individuals in the residual variation captures behavioral predictability. Together, the analysis of these sources of variation is referred to *behavioral reaction norms* (211, 212). Variation in these parameters can, in turn, be predicted by individual-level predictor variables such as sex, age, or life stage. Behavioral repeatability is calculated using the intraclass correlation coefficient: the random intercept variance divided by the total model variance (213).

As an example case, imagine a reversal learning task, where we first teach individuals an initial contingency and then reverse this contingency to assess cognitive flexibility. The data are a series of binary (0/1 values, i.e., Bernoulli distributed) trials for each individual indicating whether they completed the task on each trial correctly or incorrectly. We may also be interested in whether individual variation is affected by some

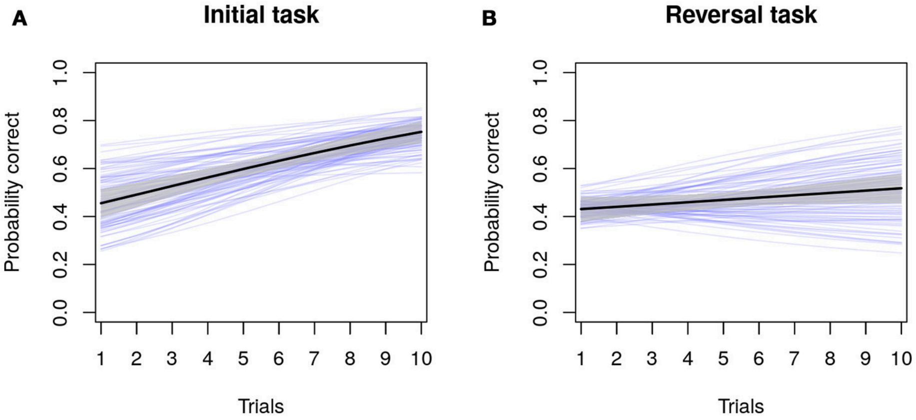

FIGURE 1 | Model results from a hypothetical study of individual variation in cognition. Dark black lines and gray areas show the population-average probability and its 89% credible interval of choosing a correct answer across trials in an initial learning task **(A)**, and reversal learning task **(B)**. Blue lines show estimates for each individual ($n = 100$), for which variance parameters and individual predictions can be directly compared.

independent variable, such as personality type (e.g., reactive or proactive), diet (e.g., Western vs. non-Western diet) or sex. To compare the different groups, one option is to summarize the data for each individual by the difference in the number of trials taken to learn the initial and reversal contingencies, and estimate the relationship between this summary measure and group (e.g., using an independent samples *t*-test). However, this analysis has a number of drawbacks. Firstly, it cannot distinguish between- from within-individual variation. Secondly, it splits the data analysis into multiple stages that may limit reproducibility (Gelman and Loken, unpublished manuscript). Thirdly, it requires defining a potentially arbitrary criterion to decide whether the task was learned by each individual, which could lead to throwing out data for those individuals not matching that criterion. For instance, only 29 out of 64 sheep in Erhard et al. (103) met the required learning criterion in a reversal learning T-maze task, meaning subsequent analyses were conducted on varying numbers of individuals while other data was dropped from the analysis. van Horik et al. (214) also discuss selection biases in participation rates of cognitive tests in pheasant chicks, which were dependent on sex, personality and body condition.

Figure 1 presents the results of a (Bayesian) multilevel logistic or Bernoulli regression model for our hypothetical example study. For those unaccustomed with fitting multilevel models, a formal description of this analysis is presented in the Supplementary Materials (available on Github: https://github.com/ConorGoold/Bushby-et-al-individual-variation-cognition), including an R script file for simulating the data, running the analyses (both Bayesian and frequentist approaches), and producing the figures. In **Figures 1A,B**, the black lines indicate the population average change in the probability of a correct choice, the gray shaded region illustrates the 89% Bayesian credible interval around the population average (i.e., the 89% most likely parameter values), and the thinner blue lines demonstrate the individual-level regression lines (the posterior means) for each individual ($n = 100$). **Figure 1A** demonstrates the probability correct across trials in the initial task and (**Figure 1B**) the probability correct across trials in the reversal task, with the average probability and the rate of learning being lower in the reversal task across individuals. As can be seen from the dispersion of the blue regression lines, there is individual variation in the parameters (both average probabilities and the rate of learning across trials). In the reversal learning task, some individuals' probabilities of responding correctly become worse across trials, despite the population-average slope being positive. From this model, the variance of the different random effect parameters can be extracted and compared directly using the Bayesian posterior distribution. Coefficients describing the linear relationship between individual-level predictor variables and individual variation in learning rates (see the Supplementary Material for further examples on Github: https://github.com/ConorGoold/Bushby-et-al-individual-variation-cognition), can also be investigated.

Multilevel models are flexible tools for a range of data types, including unbalanced designs and more complicated cases with multiple dependent variables, such as multilevel structural equation models or multilevel network models [e.g., (215)]. Practically, it is recommended to have at least 100 individuals to accurately estimate individual variation (216), although estimates of cluster-level variation in Bayesian multilevel models tend to be more accurate in small sample data sets than frequentist models using maximum likelihood estimation. In addition, an advantage of Bayesian estimation is that we can estimate the uncertainty (via Bayesian credible intervals) in cluster-level parameters (e.g., individual-level predictions), meaning estimates from smaller sample sizes may just be more uncertain rather than inaccurate. Fortunately, as demonstrated in the Supplementary Material (available on Github: https://github.com/ConorGoold/Bushby-et-al-individual-variation-cognition), fitting Bayesian models is becoming just as easy as frequentist models in common statistical software.

Finally, statistically accounting for individual variation is just one component needed to ensure reproducibility of scientific findings. Due to ethical and practical limitations, simply obtaining larger sample sizes in farmed animal cognition studies may not be realistic. Instead, researchers should consider pre-registering their studies to limit questionable (but often unconscious) research practices such as data dredging. Moreover, replication experiments and cross-lab collaboration efforts could help to confirm key hypotheses in the field [e.g., (217)] and make use of a larger number of subjects without increasing the sample size per study unnecessarily.

CONCLUSION

We have reviewed factors causing within- and between-individual variation in cognitive testing of farmed animal species and demonstrated how to account for individual variation using multilevel models. We emphasize the importance of taking into consideration other factors that could cause variation and the importance for accounting for individual variation to ensure the reproducibility of farm animal cognition and cognition studies in general.

AUTHOR CONTRIBUTIONS

EB, MF, CG, HG, LS, and LC contributed to writing and editing this manuscript. Supplementary material was provided by CG.

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REFERENCES

1. Shettleworth SJ. Modularity and the evolution of cognition. In: Heyes C, Huber L, editors. *The Evolution of Cognition.* Cambridge: MIT Press (2000). p. 43–60.
2. Duncan S, Barrett LF. Affect is a form of cognition: a neurobiological analysis. *Cogn Emot*. (2007) 21:1184–211. doi: 10.1080/02699930701437931
3. Dawkins MS. A user's guide to animal welfare science. *Trends Ecol Evol.* (2006) 21:77–82. doi: 10.1016/j.tree.2005.10.017
4. Dawkins MS, Donnelly CA, Jones TA. Chicken welfare is influenced more by housing conditions than by stocking density. *Nature* (2003) 427:342. doi: 10.1038/nature02226
5. Dumont B, Petit M. Spatial memory of sheep at pasture. *Appl Anim Behav Sci.* (1998) 60:43–53. doi: 10.1016/S0168-1591(98)00152-X
6. Edwards GR, Newman JA, Parsons AJ, Krebs JR. Use of cues by grazing animals to locate food patches: an example with sheep. *Appl Anim Behav Sci.* (1996) 51:59–68. doi: 10.1016/S0168-1591(96)01095-7
7. Ksiksi T, Laca EA. Cattle do remember locations of preferred food over extended periods. *Asian-Australas J Anim Sci.* (2002) 15:900–4. doi: 10.5713/ajas.2002.900
8. Laca EA. Spatial memory and food searching mechanisms of cattle. *J Range Manag.* (1998) 370–8. doi: 10.2307/4003320
9. Tommasi L, Vallortigara G. Searching for the center: spatial cognition in the domestic chick (*Gallus gallus*). *J Exp Psychol Anim Behav Process*. (2000) 26:477–86. doi: 10.1037//0097-7403.26.4.477
10. Della Chiesa A, Speranza M, Tommasi L, Vallortigara G. Spatial cognition based on geometry and landmarks in the domestic chick (*Gallus gallus*). *Behav Brain Res.* (2006) 175:119–27. doi: 10.1016/j.bbr.2006.08.012
11. Tommasi L, Vallortigara G, Zanforlin M. Young chickens learn to localize the centre of a spatial environment. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol.* (1997) 180:567–72.
12. Braithwaite VA, Armstrong JD, McAdam HM, Huntingford FA. Can juvenile Atlantic salmon use multiple cue systems in spatial learning? *Anim Behav.* (1996) 51:1409–15. doi: 10.1006/anbe.1996.0144
13. Bailey DW, Rittenhouse LR, Hart RH, Richards RW. Characteristics of spatial memory in cattle. *Appl Anim Behav Sci.* (1989) 23:331–40. doi: 10.1016/0168-1591(89)90101-9
14. Salvanes AGV, Moberg O, Ebbesson LO, Nilsen TO, Jensen KH, Braithwaite VA. Environmental enrichment promotes neural plasticity and cognitive ability in fish. *Proc R Soc B* (2013) 280:20131331. doi: 10.1098/rspb.2013.1331
15. Laughlin K, Huck M, Mendl M. Disturbance effects of environmental stimuli on pig spatial memory. *Appl Anim Behav Sci.* (1999) 64:169–80.
16. Laughlin K, Mendl M. Costs of acquiring and forgetting information affect spatial memory and its susceptibility to interference. *Anim Behav.* (2004) 68:97–103. doi: 10.1016/j.anbehav.2003.10.019
17. Gupta SK, Behera K, Pradhan CR, Mandal AK, Sethy K, Behera D, et al. Cognitive performance for 2 strains of broiler birds in homogenous and mixed grouping system. *J Vet Behav*. (2017) 20:109–15. doi: 10.1016/j.jveb.2017.03.008
18. Wichman A, Heikkilä M, Valros A, Forkman B, Keeling LJ. Perching behaviour in chickens and its relation to spatial ability. *Appl Anim Behav Sci.* (2006) 105:165–79. doi: 10.1016/j.applanim.2006.05.009
19. Doyle RE, Freire R, Cowling A, Knott SA, Lee C. Performance of sheep in a spatial maze is impeded by negative stimuli. *Appl Anim Behav Sci.* (2014) 151:36–42. doi: 10.1016/j.applanim.2013.11.009
20. Hunter DS, Hazel SJ, Kind KL, Liu H, Marini D, Owens JA, et al. Do I turn left or right? Effects of sex, age, experience and exit route on maze test performance in sheep. *Physiol Behav.* (2015) 139:244–53. doi: 10.1016/j.physbeh.2014.11.037
21. Coulon M, Nowak R, Andanson S, Petit B, Lévy F, Boissy A. Effects of prenatal stress and emotional reactivity of the mother on emotional and cognitive abilities in lambs. *Dev Psychobiol.* (2015) 57:626–36. doi: 10.1002/dev.21320
22. Jansen J, Bolhuis JE, Schouten WG, Spruijt BM, Wiegant VM. Spatial learning in pigs: effects of environmental enrichment and individual characteristics on behaviour and performance. *Anim Cogn.* (2009) 12:303–15. doi: 10.1007/s10071-008-0191-y
23. Hirata M, Takeno N. Do cattle (Bos taurus) retain an association of a visual cue with a food reward for a year? *Anim Sci J.* (2014) 85:729–34. doi: 10.1111/asj.12210
24. Hirata M, Tomita C, Yamada K. Use of a maze test to assess spatial learning and memory in cattle: can cattle traverse a complex maze? *Appl Anim Behav Sci.* (2016) 180:18–25. doi: 10.1016/j.applanim.2016.04.004
25. Kovalčik K, Kovalčik M. Learning ability and memory testing in cattle of different ages. *Appl Anim Behav Sci.* (1986) 15:27–9.
26. Brockmark S, Adriaenssens B, Johnsson JI. Less is more: density influences the development of behavioural life skills in trout. *Proc R Soc B* (2010) 277:3035–43. doi: 10.1098/rspb.2010.0561
27. White SL, Wagner T, Gowan C, Braithwaite VA. Can personality predict individual differences in brook trout spatial learning ability? *Behav Process.* (2017) 141:220–8. doi: 10.1016/j.beproc.2016.08.009
28. Horvath K, Fernandez M, Miller-Cushon EK. The effect of feeding enrichment in the milk-feeding stage on the cognition of dairy calves in a T-maze. *Appl Anim Behav Sci.* (2017) 187:8–14. doi: 10.1016/j.applanim.2016.11.016

29. Nordquist RE, Heerkens JL, Rodenburg TB, Boks S, Ellen ED, van der Staay FJ. Laying hens selected for low mortality: behaviour in tests of fearfulness, anxiety and cognition. *Appl Anim Behav Sci.* (2011) 131:110–22. doi: 10.1016/j.applanim.2011.02.008
30. Benhaim D, Begout ML, Chatain B. Unfamiliar congener used as a visual attractor in wild caught and domesticated sea bass (*Dicentrarchus labrax*) placed in a T-maze. *J Aquac Res Dev.* (2013) 4:169. doi: 10.4172/2155-9546.1000169
31. Benhaïm D, Bégout ML, Lucas G, Chatain B. First insight into exploration and cognition in wild caught and domesticated sea bass (*Dicentrarchus labrax*) in a maze. *PLoS ONE* (2013) 8:e65872. doi: 10.1371/journal.pone.0065872
32. Sugnaseelan S, Prescott NB, Broom DM, Wathes CM, Phillips CJ. Visual discrimination learning and spatial acuity in sheep. *Appl Anim Behav Sci.* (2013) 147:104–11. doi: 10.1016/j.applanim.2013.04.012
33. Hosoi E, Rittenhouse LR, Swift DM, Richards RW. Foraging strategies of cattle in a Y-maze: influence of food availability. *Appl Anim Behav Sci.* (1995) 43:189–96. doi: 10.1016/0168-1591(95)00565-A
34. Tommasi L, Polli C. Representation of two geometric features of the environment in the domestic chick (*Gallus gallus*). *Anim Cogn.* (2004) 7:53–59. doi: 10.1007/s10071-003-0182-y
35. Freire R, Rogers LJ. Experience-induced modulation of the use of spatial information in the domestic chick. *Anim Behav.* (2005) 69:1093–100. doi: 10.1016/j.anbehav.2004.09.009
36. Bolhuis JE, Oostindjer M, Hoeks CW, de Haas EN, Bartels AC, Ooms M, et al. Working and reference memory of pigs (*Sus scrofa* domesticus) in a holeboard spatial discrimination task: the influence of environmental enrichment. *Anim Cogn.* (2013) 16:845–50. doi: 10.1007/s10071-013-0646-7
37. Grimberg-Henrici CG, Vermaak P, Bolhuis JE, Nordquist RE, van der Staay FJ. Effects of environmental enrichment on cognitive performance of pigs in a spatial holeboard discrimination task. *Anim Cogn.* (2016) 19:271–83. doi: 10.1007/s10071-015-0932-7
38. Roelofs S, Nordquist RE, van der Staay FJ. Female and male pigs' performance in a spatial holeboard and judgment bias task. *Appl Anim Behav Sci.* (2017) 191:5–16. doi: 10.1016/j.applanim.2017.01.016
39. Antonides A, Schoonderwoerd AC, Nordquist RE, van der Staay FJ. Very low birth weight piglets show improved cognitive performance in the spatial cognitive holeboard task. *Front Behav Neurosci.* (2015) 9:43. doi: 10.3389/fnbeh.2015.00043
40. Gifford AK, Cloutier S, Newberry RC. Objects as enrichment: effects of object exposure time and delay interval on object recognition memory of the domestic pig. *Appl Anim Behav Sci.* (2007) 107:206–17. doi: 10.1016/j.applanim.2006.10.019
41. Foster TM, Temple W, Mackenzie C, DeMello LR, Poling A. Delayed matching to sample performance of hens: effects of sample duration and response requirements during the sample. *J Exp Anal Behav.* (1995) 64:9–31. doi: 10.1901/jeab.1995.64-19
42. Forkman B. Domestic hens have declarative representations. *Anim Cogn.* (2000) 3:135–7. doi: 10.1007/s100710000074
43. Cozzutti C, Vallortigara G. Hemispheric memories for the content and position of food caches in the domestic chick. *Behav Neurosci.* (2001) 115:305. doi: 10.1037/0735-7044.115.2.305
44. Vallortigara G, Regolin L, Rigoni M, Zanforlin M. Delayed search for a concealed imprinted object in the domestic chick. *Anim Cogn.* (1998) 1:17–24. doi: 10.1007/s100710050003
45. Regolin L, Rugani R, Pagni P, Vallortigara G. Delayed search for social and nonsocial goals by young domestic chicks, *Gallus gallus* domesticus. *Anim Behav.* (2005) 70:855–64. doi: 10.1016/j.anbehav.2005.01.014
46. Briefer EF, Haque S, Baciadonna L, McElligott AG. Goats excel at learning and remembering a highly novel cognitive task. *Front Zool.* (2014) 11:20. doi: 10.1186/1742-9994-11-20
47. Held S, Mendl M, Devereux C, Byrne RW. Foraging pigs alter their behaviour in response to exploitation. *Anim Behav.* (2002) 64:157–65. doi: 10.1006/anbe.2002.3044
48. Held SD, Byrne RW, Jones S, Murphy E, Friel M, Mendl MT. Domestic pigs, *Sus scrofa*, adjust their foraging behaviour to whom they are foraging with. *Anim Behav.* (2010) 79:857–62. doi: 10.1016/j.anbehav.2009.12.035
49. Held S, Mendl M, Devereux C, Byrne RW. Behaviour of domestic pigs in a visual perspective taking task. *Behaviour* (2001) 138:1337–54. doi: 10.1163/156853901317367627
50. Broom DM, Sena H, Moynihan KL. Pigs learn what a mirror image represents and use it to obtain information. *Anim Behav.* (2009) 78:1037–41. doi: 10.1016/j.anbehav.2009.07.027
51. Gieling ET, Mijdam E, van der Staay FJ, Nordquist RE. Lack of mirror use by pigs to locate food. *Appl Anim Behav Sci.* (2014) 154:22–9. doi: 10.1016/j.applanim.2014.02.016
52. McBride SD, Perentos N, Morton AJ. Understanding the concept of a reflective surface: can sheep improve navigational ability through the use of a mirror? *Anim Cogn.* (2015) 18:361–71. doi: 10.1007/s10071-014-0807-3
53. Kristensen HH, Jones RB, Schofield CP, White RP, Wathes CM. The use of olfactory and other cues for social recognition by juvenile pigs. *Appl Anim Behav Sci.* (2001) 72:321–33. doi: 10.1016/S0168-1591(00)00209-4
54. McLeman MA, Mendl M, Jones RB, White R, Wathes CM. Discrimination of conspecifics by juvenile domestic pigs, *Sus scrofa*. *Anim Behav.* (2005) 70:451–61. doi: 10.1016/j.anbehav.2004.11.013
55. Gieling ET, Musschenga MA, Nordquist RE, van der Staay FJ. Juvenile pigs use simple geometric 2D shapes but not portrait photographs of conspecifics as visual discriminative stimuli. *Appl Anim Behav Sci.* (2012) 142:142–53. doi: 10.1016/j.applanim.2012.10.018
56. Peirce JW, Leigh AE, Kendrick KM. Configurational coding, familiarity and the right hemisphere advantage for face recognition in sheep. *Neuropsychologia* (2000) 38:475–83. doi: 10.1016/S0028-3932(99)00088-3
57. Kendrick KM, Atkins K, Hinton MR, Broad KD, Fabre-Nys C, Keverne B. Facial and vocal discrimination in sheep. *Anim Behav.* (1995) 49:1665–76. doi: 10.1016/0003-3472(95)90088-8
58. Bradshaw RH. Discrimination of group members by laying hens Gallus domesticus. *Behav. Process.* (1991) 24:143–51. doi: 10.1016/0376-6357(91)90006-L
59. Abeyesinghe SM, McLeman MA, Owen RC, McMahon CE, Wathes CM. Investigating social discrimination of group members by laying hens. *Behav. Processes* (2009) 81:1–13. doi: 10.1016/j.beproc.2008.11.017
60. Hagen K, Broom DM. Cattle discriminate between individual familiar herd members in a learning experiment. *Appl Anim Behav Sci.* (2003) 82:13–28. doi: 10.1016/S0168-1591(03)00053-4
61. Souza AS, Jansen J, Tempelman RJ, Mendl M, Zanella AJ. A novel method for testing social recognition in young pigs and the modulating effects of relocation. *Appl Anim Behav Sci.* (2006) 99:77–87. doi: 10.1016/j.applanim.2005.09.008
62. Ryan CM, Lea SE. Images of conspecifics as categories to be discriminated by pigeons and chickens: slides, video tapes, stuffed birds and live birds. *Behav. Process.* (1994) 33:155–75. doi: 10.1016/0376-6357(94)90064-7
63. Coulon M, Baudoin C, Heyman Y, Deputte BL. Cattle discriminate between familiar and unfamiliar conspecifics by using only head visual cues. *Anim Cogn.* (2011) 14:279–90. doi: 10.1007/s10071-010-0361-6
64. Coulon M, Deputte BL, Heyman Y, Baudoin C. Individual recognition in domestic cattle (Bos taurus): evidence from 2D-images of heads from different breeds. *PLoS ONE* (2009) 4:e4441. doi: 10.1371/journal.pone.0004441
65. Baldwin BA, Meese GB. The ability of sheep to distinguish between conspecifics by means of olfaction. *Physiol Behav.* (1977) 18:803–8. doi: 10.1016/0031-9384(77)90187-1
66. Brown GE, Brown JA. Do rainbow trout and Atlantic salmon discriminate kin? *Can. J. Zool.* (1992) 70:1636–40. doi: 10.1139/z92-227
67. Munksgaard L, DePassille AM, Rushen J, Herskin MS, Kristensen AM. Dairy cows' fear of people: social learning, milk yield and behaviour at milking. *Appl Anim Behav Sci.* (2001) 73:15–26. doi: 10.1016/S0168-1591(01)00119-8
68. Veissier I. Observational learning in cattle. *Appl Anim Behav Sci.* (1993) 35:235–43. doi: 10.1016/0168-1591(93)90139-G
69. Figueroa J, Solà-Oriol D, Manteca X, Pérez JF. Social learning of feeding behaviour in pigs: effects of neophobia and familiarity with the demonstrator conspecific. *Appl Anim Behav Sci.* (2013) 148:120–7. doi: 10.1016/j.applanim.2013.06.002

70. Sherwin CM, Heyes CM, Nicol CJ. Social learning influences the preferences of domestic hens for novel food. *Anim Behav.* (2002) 63:933–42. doi: 10.1006/anbe.2002.2000
71. Kaminski J, Riedel J, Call J, Tomasello M. Domestic goats, Capra hircus, follow gaze direction and use social cues in an object choice task. *Anim Behav.* (2005) 69:11–8. doi: 10.1016/j.anbehav.2004.05.008
72. Baciadonna L, McElligott AG, Briefer EF. Goats favour personal over social information in an experimental foraging task. *PeerJ* (2013) 1:e172. doi: 10.7717/peerj.172
73. Nawroth C, Baciadonna L, McElligott AG. Goats learn socially from humans in a spatial problem-solving task. *Anim Behav.* (2016) 121:123–9. doi: 10.1016/j.anbehav.2016.09.004
74. Pitcher BJ, Briefer EF, Baciadonna L, McElligott AG. Cross-modal recognition of familiar conspecifics in goats. ?*R Soc Open Sci.* (2017) 4:160346. doi: 10.1098/rsos.160346
75. Nawroth C, Ebersbach M, von Borell E. Juvenile domestic pigs (*Sus scrofa* domestica) use human-given cues in an object choice task. *Anim Cogn.* (2014) 17:701–13. doi: 10.1007/s10071-013-0702-3
76. Nawroth C, von Borell E, Langbein J. Exclusion performance in dwarf goats (Capra aegagrus hircus) and sheep (Ovis orientalis aries). *PLoS ONE* (2014) 9:e93534. doi: 10.1371/journal.pone.0093534
77. Albiach-Serrano A, Bräuer J, Cacchione T, Zickert N, Amici F. The effect of domestication and ontogeny in swine cognition (*Sus scrofa* scrofa and S. s. domestica). *Appl Anim Behav Sci.* (2012) 141:25–35. doi: 10.1016/j.applanim.2012.07.005
78. Langbein J, Siebert K, Nuernberg G. Concurrent recall of serially learned visual discrimination problems in dwarf goats (Capra hircus). *Behav. Processes* (2008) 79:156–64. doi: 10.1016/j.beproc.2008.07.004
79. Nawroth C, Prentice PM, McElligott AG. Individual personality differences in goats predict their performance in visual learning and non-associative cognitive tasks. *Behav. Processes* (2017) 134:43–53. doi: 10.1016/j.beproc.2016.08.001
80. Zebunke M, Langbein J, Manteuffel G, Puppe B. Autonomic reactions indicating positive affect during acoustic reward learning in domestic pigs. *Anim Behav.* (2011) 81:481–9. doi: 10.1016/j.anbehav.2010.11.023
81. Ligout S, Porter RH, Bon R. Social discrimination in lambs: persistence and scope. *Appl Anim Behav Sci.* (2002) 76:239–48. doi: 10.1016/S0168-1591(02)00015-1
82. Nawroth C, Ebersbach M, von Borell E. A note on pigs' knowledge of hidden objects. *Arch Anim Breed.* (2013) 56:861–72. doi: 10.7482/0003-9438-56-086
83. Nawroth C, von Borell E, Langbein J. Object permanence in the dwarf goat (Capra aegagrus hircus): perseveration errors and the tracking of complex movements of hidden objects. *Appl Anim Behav Sci.* (2015) 167:20–6. doi: 10.1016/j.applanim.2015.03.010
84. Whistance LK, Sinclair LA, Arney DR, Phillips CJC. Trainability of eliminative behaviour in dairy heifers using a secondary reinforcer. *Appl Anim Behav Sci.* (2009) 117:128–36. doi: 10.1016/j.applanim.2009.01.004
85. Nation K, Birge A, Lunde E, Cudd T, Goodlett C, Washburn S. Video-based data acquisition system for use in eye blink classical conditioning procedures in sheep. *Behav Res Methods* (2017) 49:1838–51. doi: 10.3758/s13428-016-0826-x
86. Johnson TB, Stanton ME, Goodlett CR, Cudd TA. Eyeblink classical conditioning in the preweanling lamb. *Behav Neurosci.* (2008) 122:722. doi: 10.1037/0735-7044.122.3.722
87. Wredle E, Rushen J, De Passillé AM, Munksgaard L. Training cattle to approach a feed source in response to auditory signals. *Can J Anim Sci.* (2004) 84:567–72. doi: 10.4141/A03-081
88. Moe RO, Nordgreen J, Janczak AM, Spruijt BM, Zanella AJ, Bakken M. Trace classical conditioning as an approach to the study of reward-related behaviour in laying hens: a methodological study. *Appl Anim Behav Sci.* (2009) 121:171–8. doi: 10.1016/j.applanim.2009.10.002
89. Folkedal O, Fernö A, Nederlof MA, Fosseidengen JE, Cerqueira M, Olsen RE, et al. Habituation and conditioning in gilthead sea bream (*Sparus aurata*): effects of aversive stimuli, reward and social hierarchies. *Aquac Res.* (2018) 49:335–40. doi: 10.1111/are.13463
90. Vindas MA, Folkedal O, Kristiansen TS, Stien LH, Braastad BO, Mayer I, et al. Omission of expected reward agitates Atlantic salmon (Salmo salar). *Anim Cogni.* (2012) 15:903–11. doi: 10.1007/s10071-012-0517-7
91. Champion RA, Matthews LR. An operant-conditioning technique for the automatic measurement of feeding motivation in cows. *Comput Electron Agric.* (2007) 57:115–22. doi: 10.1016/j.compag.2007.02.008
92. Holm L, Jensen MB, Jeppesen LL. Calves' motivation for access to two different types of social contact measured by operant conditioning. *Appl Anim Behav Sci.* (2002) 79:175–94. doi: 10.1016/S0168-1591(02)00137-5
93. van Eck LM, Antonides A, Nordquist RE, van der Staay FJ. Testing post-weaning food motivation in low and normal birth weight pigs in a runway and operant conditioning task. *Appl Anim Behav Sci.* (2016) 181:83–90. doi: 10.1016/j.applanim.2016.05.010
94. Vaughan A, de Passillé AM, Stookey J, Rushen J. Operant conditioning of urination by calves. *Appl Anim Behav Sci.* (2014) 158:8–15. doi: 10.1016/j.applanim.2014.06.009
95. Rugani R, Regolin L, Vallortigara G. Discrimination of small numerosities in young chicks. *J Exp Psychol Anim Behav Process.* (2008) 34:388. doi: 10.1037/0097-7403.34.3.388
96. Rugani R, Regolin L, Vallortigara G. Rudimental numerical competence in 5-day-old domestic chicks (*Gallus gallus*): identification of ordinal position. *J Exp Psychol Anim Behav Process.* (2007) 33:21. doi: 10.1037/0097-7403.33.1.21
97. Griffin AS, Guillette LM, Healy SD. Cognition and personality: an analysis of an emerging field. *Trends Ecol Evol.* (2015) 30:207–14. doi: 10.1016/j.tree.2015.01.012
98. Sih A, Bell AM, Johnson JC, Ziemba RE. Behavioral syndromes: an integrative overview. *Q Rev Biol.* (2004) 79:241–77. doi: 10.1086/422893
99. Colditz IG, Hine BC. Resilience in farm animals: biology, management, breeding and implications for animal welfare. *Anim Prod Sci.* (2016) 56:1961–83. doi: 10.1071/AN15297
100. Pearl J. *Causality: Models, Reasoning and Inference*. Cambridge: Cambridge University Press (2000).
101. Rowe C, Healy SD. (2014) Measuring variation in cognition. *Behav Ecol.* 25:1287–92. doi: 10.1093/beheco/aru090
102. Barrett L. Why brains are not computers, why behaviorism is not satanism, and why dolphins are not aquatic apes. *Behav Anal.* (2016) 39:9–23. doi: 10.1007/s40614-015-0047-0
103. Erhard HW, Boissy A, Rae MT, Rhind SM. Effects of prenatal undernutrition on emotional reactivity and cognitive flexibility in adult sheep. *Behav Brain Res.* (2004) 151:25–35. doi: 10.1016/j.bbr.2003.08.003
104. Andrade C, Alwarshetty M, Sudha S, Chandra JS. Effect of innate direction bias on T-maze learning in rats: implications for research. *J Neurosci Methods* (2001) 110:31–5. doi: 10.1016/S0165-0270(01)00415-0
105. da Costa MJP, Broom DM. Consistency of side choice in the milking parlour by Holstein–Friesian cows and its relationship with their reactivity and milk yield. *Appl Anim Behav Sci.* (2001) 70:177–86. doi: 10.1016/S0168-1591(00)00158-1
106. Meehl PE. Why summaries of research on psychological theories are often uninterpretable. *Psychol Rep.* (1990) 66:195–244.
107. Rohrer JM. Thinking clearly about correlations and causation: graphical causal models for observational data. *Adv Methods Pract Psychol Sci.* (2018) 1:27–42. doi: 10.1177/2515245917745629
108. Freund J, Brandmaier AM, Lewejohann L, Kirste I, Kritzler M, Krüger A, et al. (2013) Emergence of individuality in genetically identical mice. *Science* 340:756–9. doi: 10.1126/science.1235294
109. Rice D, Barone Jr S. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect.* (2000) 108:511. doi: 10.1289/ehp.00108s3511
110. Conrad MS, Johnson RW. The domestic piglet: an important model for investigating the neurodevelopmental consequences of early life insults. *Annu Rev Anim Biosci.* (2015) 3:245–64. doi: 10.1146/annurev-animal-022114-111049
111. Glover V, O'connor TG, O'Donnell K. Prenatal stress and the programming of the HPA axis. *Neurosci Biobehav Rev.* (2010) 35:17–22. doi: 10.1016/j.neubiorev.2009.11.008
112. Brunton PJ. Effects of maternal exposure to social stress during pregnancy: consequences for mother and offspring. *Reproduction* (2013) 146:R175–89. doi: 10.1530/REP-13-0258.

113. Weinstock M. The long-term behavioural consequences of prenatal stress. *Neurosci Biobehav Rev*. (2008) 32:1073–86. doi: 10.1016/j.neubiorev.2008.03.002
114. Jarvis S, Moinard C, Robson SK, Baxter E, Ormandy E, Douglas AJ, et al. Programming the offspring of the pig by prenatal social stress: neuroendocrine activity and behaviour. *Horm Behav*. (2006) 49:68–80. doi: 10.1016/j.yhbeh.2005.05.004
115. Rodricks CL, Gibbs ME, Jenkin G, Miller SL. The effect of hypoxia at different embryonic ages on impairment of memory ability in chicks. *Int J Dev Neurosci*. (2008) 26:113–8. doi: 10.1016/j.ijdevneu.2007.08.006
116. Hernandez CE, Harding JE, Oliver MH, Bloomfield FH, Held SD, Matthews LR. Effects of litter size, sex and periconceptional ewe nutrition on side preference and cognitive flexibility in the offspring. *Behav Brain Res.* (2009) 204:82–7. doi: 10.1016/j.bbr.2009.05.019
117. Gaillard C, Meagher RK, von Keyserlingk MA, Weary DM. Social housing improves dairy calves' performance in two cognitive tests. *PLoS ONE* (2014) 9:e90205. doi: 10.1371/journal.pone.0090205
118. Vieira ADP, de Passillé AM, Weary DM. Effects of the early social environment on behavioral responses of dairy calves to novel events. *J Dairy Sci.* (2012) 95:5149–55. doi: 10.3168/jds.2011-5073
119. Van der Meulen J, Koopmans SJ, Dekker RA, Hoogendoorn A. Increasing weaning age of piglets from 4 to 7 weeks reduces stress, increases post-weaning feed intake but does not improve intestinal functionality. *Animal* (2010) 4:1653–61. doi: 10.1017/S1751731110001011
120. Krachun C, Rushen J, de Passillé AM. Play behaviour in dairy calves is reduced by weaning and by a low energy intake. *Appl Anim Behav Sci.* (2010) 122:71–6. doi: 10.1016/j.applanim.2009.12.002
121. Sumner BE, D'Eath RB, Farnworth MJ, Robson S, Russell JA, Lawrence AB, et al. Early weaning results in less active behaviour, accompanied by lower 5-HT1A and higher 5-HT2A receptor mRNA expression in specific brain regions of female pigs. *Psychoneuroendocrinology* (2008) 33:1077–92. doi: 10.1016/j.psyneuen.2008.05.004
122. Yuan Y, Jansen J, Charles D, Zanella AJ. The influence of weaning age on post-mixing agonistic interactions in growing pigs. *Appl Anim Behav Sci.* (2004) 88:39–46. doi: 10.1016/j.applanim.2004.01.012
123. Napolitano F, Braghieri A, Cifuni GF, Pacelli C, Girolami A. Behaviour and meat production of organically farmed unweaned lambs. *Small Rumin Res*. (2002) 43:179–84. doi: 10.1016/S0921-4488(02)00015-9
124. Poletto R, Steibel JP, Siegford JM, Zanella AJ. Effects of early weaning and social isolation on the expression of glucocorticoid and mineralocorticoid receptor and 11β-hydroxysteroid dehydrogenase 1 and 2 mRNAs in the frontal cortex and hippocampus of piglets. *Brain Res*. (2006) 1067:36–42. doi: 10.1016/j.brainres.2005.10.001
125. Zhang LX, Levine S, Dent G, Zhan Y, Xing G, Okimoto D, et al. Maternal deprivation increases cell death in the infant rat brain. *Dev Brain Res.* (2002) 133:1–11. doi: 10.1016/S0926-6410(01)00118-5
126. Kikusui T, Ichikawa S, Mori Y. Maternal deprivation by early weaning increases corticosterone and decreases hippocampal BDNF and neurogenesis in mice. *Psychoneuroendocrinology* (2009) 34:762–72. doi: 10.1016/j.psyneuen.2008.12.009
127. Klein SL, Schiebinger L, Stefanick ML, Cahill L, Danska J, De Vries GJ, et al. Opinion: sex inclusion in basic research drives discovery. *Proc Natl Acad Sci USA*. (2015) 112:5257–8. doi: 10.1073/pnas.1502843112
128. Holden C. Sex and the suffering brain: researchers are seeking biological reasons for the widespread gender differences in the prevalence and symptomatology of mental disorders. *Science* (2005) 308:1574–8. doi: 10.1126/science.308.5728.1574
129. Palanza P. Animal models of anxiety and depression: how are females different? *Neurosci Biobehav Rev.* (2001) 25:219–33. doi: 10.1016/S0149-7634(01)00010-0
130. Dalla C, Pitychoutis PM, Kokras N, Papadopoulou-daifoti Z. Sex differences in animal models of depression and antidepressant response. *Basic Clin Pharmacol Toxicol.* (2010) 106:226–33. doi: 10.1111/j.1742-7843.2009.00516.x
131. Dalla C, Pitychoutis PM, Kokras N, Papadopoulou-Daifoti Z. Sex differences in response to stress and expression of depressive-like behaviours in the rat. In: Neill JC, Kulkarni J, editors. *Biological Basis of Sex Differences in Psychopharmacology. Current Topics in Behavioral Neurosciences*, Vol 8. Berlin; Heidelberg: Springer (2010). p. 97–118. doi: 10.1007/7854_2010_94
132. Shors TJ. Stressful experience and learning across the lifespan. *Annu Rev Psychol.* (2006) 57:55–85. doi: 10.1146/annurev.psych.57.102904.190205
133. Shors TJ, Lewczyk C, Pacynski M, Mathew PR, Pickett J. Stages of estrous mediate the stress-induced impairment of associative learning in the female rat. *Neuroreports* (1998) 9:419–23. doi: 10.1097/00001756-199802160-00012
134. Conrad CD, Jackson JL, Wieczorek L, Baran SE, Harman JS, Wright RL, et al. Acute stress impairs spatial memory in male bit not female rats: influence of estrous cycle. *Pharmacol Biochem Behav*. (2004) 78:569–79. doi: 10.1016/j.pbb.2004.04.025
135. Bowman RE, Beck KD, Luine VN. Chronic stress effects on memory: sex differences in performance and monoaminergic activity. *Horm Behav.* (2003) 43:48–59. doi: 10.1016/S0018-506X(02)00022-3
136. Kitraki E, Kremmyda O, Youlatos D, Alexis MN, Kittas C. Gender-dependent alterations in corticosteroid receptor status and spatial performance following 21 days of restraint stress. *Neuroscience* (2004) 125:47–55. doi: 10.1016/j.neuroscience.2003.12.024
137. Jonasson Z. Meta-analysis of sex differences in rodent models of learning and memory: a review of behavioural and biological data. *Neurosci Biobehav Rev*. (2005) 28:811–25. doi: 10.1016/j.neubiorev.2004.10.006
138. Lighthall NR, Mather M, Gorlick MA. Acute stress increases sex differences in risk seeking in the balloon analogue risk task. *PLoS ONE* (2009) 4:e6002. doi: 10.1371/journal.pone.0006002
139. Maney DL. Perils and pitfalls of reporting sex differences. *Philos Trans R Soc B* (2016) 371:20150119. doi: 10.1098/rstb.2015.0119
140. Murphy E, Nordquist RE, van der Staay FJ. Responses of conventional pigs and Göttingen miniature pigs in an active choice judgement bias task. *Appl Anim Behav Sci.* (2013) 148:64–76. doi: 10.1016/j.applanim.2013.07.011
141. Dall SRX, Houston AI, McNamara JM. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecol Lett*. (2004) 7:734–9. doi: 10.1111/j.1461-0248.2004.00618.x
142. Reale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. Integrating animal temperament within ecology and evolution. *Biol Rev Camb Philos Soc.* (2007) 82:291–318. doi: 10.1111/j.1469-185X.2007.00010.x
143. Westneat DF, Wright J, Dingemanse NJ. The biology hidden inside residual within-individual phenotypic variation. *Biol Rev Camb Philos Soc*. (2015) 90:729–43. doi: 10.1111/brv.12131
144. Koolhaas JM, Korte SM, De Boer SF, Van Der Vegt BJ, Van Reenen CG, Hopster H, et al. Coping styles in animals: current status in behavior and stress-physiology. *Neurosci Biobehav Rev*. (1999) 23:925–35. doi: 10.1016/S0149-7634(99)00026-3
145. Sih A, Del Giudice M. Linking behavioural syndromes and cognition: a behavioural ecology perspective. *Philos Trans R Soc Lond B Biol Sci.* (2012) 367:2762–72. doi: 10.1098/rstb.2012.0216
146. de Haas EN, Lee C, Hernandez CE, Naguib M, Rodenburg TB. Individual differences in personality in laying hens are related to learning a colour cue association. *Behav Process.* (2017) 134:37–42. doi: 10.1016/j.beproc.2016.11.001
147. Bensky MK, Paitz R, Pereira L, Bell AM. Testing the predictions of copings styles theory in threespined sticklebacks. *Behav Process.* (2017) 136:1–10. doi: 10.1016/j.beproc.2016.12.011
148. Campbell DT, Fiske DW. Convergent and discriminant validation by the multitrait-multimethod matrix. *Psychol Bull.* (1959) 56:81–105. doi: 10.1037/h0046016
149. Moors A, Ellsworth PC, Scherer KR, Frijda NH. Appraisal theories of emotion: state of the art and future development. *Emot Rev*. (2013) 5:119–24. doi: 10.1177/1754073912468165
150. Mendl M, Burman OH, Paul ES. An integrative and functional framework for the study of animal emotion and mood. *Proc R Soc B* (2010) 277:2895–904. doi: 10.1098/rspb.2010.0303
151. Raoult C, Moser J, Gygax L. Mood as cumulative expectation mismatch: a test of theory based on data from non-verbal cognitive bias tests. *Front Psychol.* (2017) 8:2197. doi: 10.3389/fpsyg.2017.02197
152. Nettle D, Bateson M. The evolutionary origins of mood and its disorders. *Curr Biol.* (2012) 22:R712–21. doi: 10.1016/j.cub.2012.06.020

153. Bateson M. Optimistic and pessimistic biases: a primer for behavioural ecologists. *Curr Opin Behav Sci*. (2016) 12:115–21. doi: 10.1016/j.cobeha.2016.09.013
154. Asher L, Friel M, Griffin K, Collins LM. Mood and personality interact to determine cognitive biases in pigs. *Biol Lett*. (2016) 12:20160402. doi: 10.1098/rsbl.2016.0402
155. Baciadonna L, McElligott AG. The use of judgement bias to assess welfare in farm livestock. *Anim Welf.* (2015) 24:81–91. doi: 10.7120/09627286.24.1.081
156. Martin LL, Clore GL. *Theories of Mood and Cognition: A User's Guidebook*. Psychology Press (2013).
157. Wathan J, Proops L, Grounds K, Mccomb K. Horses discriminate between facial expressions of conspecifics. *Sci Rep*. (2016) 6:38322. doi: 10.1038/srep38322
158. Lee C, Verbeek E, Doyle R, Bateson M. Attention bias to threat indicates anxiety differences in sheep. *Biol Lett.* (2016) 12:20150977. doi: 10.1098/rsbl.2015.0977
159. Eldar E, Rutledge RB, Dolan RJ, Niv Y. Mood as representation of momentum. *Trends Cogn Sci.* (2016) 20:15–24. doi: 10.1016/j.tics.2015.07.010
160. Watanabe M, Cromwell HC, Tremblay L, Hollerman JR, Hikosaka K, Schultz W. Behavioral reactions reflecting differential reward expectations in monkeys. *Exp Brain Res.* (2001) 140:511–8. doi: 10.1007/s002210100856
161. Fisher WW, Thompson RH, Piazza CC, Crosland K, Gotjen D. On the relative reinforcing effects of choice and differential consequences. *J Appl Behav Anal.* (1997) 30:423–38. doi: 10.1901/jaba.1997.30-423
162. DeLeon IG, Fisher WW, Rodriguez-Catter V, Maglieri K, Herman K, Marhefka JM. Examination of relative reinforcement effects of stimuli identified through pretreatment and daily brief preference assessments. *J Appl Behav Anal.* (2001) 34:463–73. doi: 10.1901/jaba.2001.34-463
163. Clay AW, Bloomsmith MA, Marr MJ, Maple TL. Systematic investigation of the stability of food preferences in captive orangutans: implications for positive reinforcement training. *J Appl Anim Welf Sci*. (2009) 12:306–313. doi: 10.1080/10888700903163492
164. Deaner RO, Khera AV, Platt ML. Monkeys pay per view: adaptive valuation of social images by rhesus macaques. *Curr Biol.* (2005) 15:543–8. doi: 10.1016/j.cub.2005.01.044
165. Færevik G, Jensen MB, Bøe KE. Dairy calves social preferences and the significance of a companion animal during separation from the group. *Appl Anim Behav Sci*. (2006) 99:205–21. doi: 10.1016/j.applanim.2005.10.012
166. Boissy A, Dumont B. Interactions between social and feeding motivations on the grazing behaviour of herbivores: sheep more easily split into subgroups with familiar peers. *Appl Anim Behav Sci.* (2002) 79:233–45. doi: 10.1016/S0168-1591(02)00152-1
167. Dumont B, Boissy A. Grazing behaviour of sheep in a situation of conflict between feeding and social motivations. *Behav Process.* (2000) 49:131–8. doi: 10.1016/S0376-6357(00)00082-6
168. Pedersen LJ, Jensen MB, Hansen SW, Munksgaard L, Ladewig J, Matthews L. Social isolation affects the motivation to work for food and straw in pigs as measured by operant conditioning techniques. *Appl Anim Behav Sci.* (2002) 77:295–309. doi: 10.1016/S0168-1591(02)00066-7
169. De Jonge FH, Tilly SL, Baars AM, Spruijt BM. On the rewarding nature of appetitive feeding behaviour in pigs (*Sus scrofa*): do domesticated pigs contrafreeload? *Appl Anim Behav Sci.* (2008) 114:359–72. doi: 10.1016/j.applanim.2008.03.006
170. Fu Y, Chen Y, Li L, Wang Y, Kong X, Wang J. Food restriction affects Y-maze spatial recognition memory in developing mice. *Int J Dev Neurosci.* (2017) 60:8–15. doi: 10.1016/j.ijdevneu.2017.03.010
171. Ferreira FR, Spini VBMG, Lopes EJ, Lopes RFF, Moreira EA, Amaral MAF, et al. Effect of feed restriction on learning, memory and stress of rodents. *Biosci J.* (2006) 22:91–7.
172. Bukhari SHF, Clark OE, Williamson LL. Maternal high fructose diet and neonatal immune challenge alter offspring anxiety-like behavior and inflammation across the lifespan. *Life Sci.* (2018) 197:114–21. doi: 10.1016/j.lfs.2018.02.010
173. Rytych JL, Elmore MR, Burton MD, Conrad MS, Donovan SM, Dilger RN, et al. Early life iron deficiency impairs spatial cognition in neonatal piglets. 1:2. *J Nutr.* (2012) 142:2050–6. doi: 10.3945/jn.112.165522
174. Antonides A, Schoonderwoerd AC, Scholz G, Berg BM, Nordquist RE, van der Staay FJ. Pre-weaning dietary iron deficiency impairs spatial learning and memory in the cognitive holeboard task in piglets. *Front Behav Neurosci.* (2015) 9:291. doi: 10.3389/fnbeh.2015.00291
175. Val-Laillet D, Besson M, Guérin S, Coquery N, Randuineau G, Kanzari A, et al. A maternal Western diet during gestation and lactation modifies offspring's microbiota activity, blood lipid levels, cognitive responses, and hippocampal neurogenesis in Yucatan pigs. *FASEB J*. 31:2037–49. doi: 10.1096/fj.201601015R
176. Clouard C, Kemp B, Val-Laillet D, Gerrits WJ, Bartels AC, Bolhuis JE. Prenatal, but not early postnatal, exposure to a Western diet improves spatial memory of pigs later in life and is paired with changes in maternal prepartum blood lipid levels. *FASEB J.* (2016) 30:2466–475. doi: 10.1096/fj.201500208R
177. Wang B. Molecular mechanism underlying sialic acid as an essential nutrient for brain development and cognition−3. *Adv Nutr.* (2012) 3:465S−72S. doi: 10.3945/an.112.001875
178. Wang B, Yu B, Karim M, Hu H, Sun Y, McGreevy P, et al. Dietary sialic acid supplementation improves learning and memory in piglets. *Am J Clin Nutr.* (2007) 85:561–9. doi: 10.1093/ajcn/85.2.561
179. Mayer EA. Gut feelings: the emerging biology of gut–brain communication. *Nat Rev Neurosci.* (2011) 12:453. doi: 10.1038/nrn3071
180. Galland L. The gut microbiome and the brain. *J Med Food* (2014) 17:1261–72. doi: 10.1089/jmf.2014.7000
181. Hoffman JD, Parikh I, Green SJ, Chlipala G, Mohney RP, Keaton M, et al. Age drives distortion of brain metabolic, vascular and cognitive functions, and the gut microbiome. *Front Aging Neurosci.* (2017) 9:298. doi: 10.3389/fnagi.2017.00298
182. Xu Y, Zhou H, Zhu Q. The impact of microbiota-gut-brain axis on diabetic cognition impairment. *Front Aging Neurosci.* (2017) 9:106. doi: 10.3389/fnagi.2017.00106
183. MacFabe DF, Cain NE, Boon F, Ossenkopp KP, Cain DP. Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: relevance to autism spectrum disorder. *Behav Brain Res.* (2011) 217:47–54. doi: 10.1016/j.bbr.2010.10.005
184. Bailey MT, Coe CL. Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. *Dev Psychobiol.* (1999) 35:146–55.
185. O'Mahony SM, Marchesi JR, Scully P, Codling C, Ceolho AM, Quigley EM, et al. Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biol Psychiatry* (2009) 65:263–7. doi: 10.1016/j.biopsych.2008.06.026
186. Jašarević E, Howerton CL, Howard CD, Bale TL. Alterations in the vaginal microbiome by maternal stress are associated with metabolic reprogramming of the offspring gut and brain. *Endocrinology* (2015) 156:3265–76. doi: 10.1210/en.2015-1177
187. Hedges DW, Woon FL. Early-life stress and cognitive outcome. *Psychopharmacology* (2011) 214:121–30. doi: 10.1007/s00213-010-2090-6
188. Weinstock M. Sex-dependent changes induced by prenatal stress in cortical and hippocampal morphology and behaviour in rats: an update. *Stress* (2011) 14:604–13. doi: 10.3109/10253890.2011.588294
189. Jašarević E, Howard CD, Misic AM, Beiting DP, Bale TL. Stress during pregnancy alters temporal and spatial dynamics of the maternal and offspring microbiome in a sex-specific manner. *Sci Rep*. (2017) 7:44182. doi: 10.1038/srep44182
190. Tun HM, Konya T, Takaro TK, Brook JR, Chari R, Field CJ, et al. Exposure to household furry pets influences the gut microbiota of infants at 3–4 months following various birth scenarios. *Microbiome* (2017) 5:40. doi: 10.1186/s40168-017-0254-x
191. Magnusson KR, Hauck L, Jeffrey BM, Elias V, Humphrey A, Nath R, et al. Relationships between diet-related changes in the gut microbiome and cognitive flexibility. *Neuroscience* (2015) 300:128–40. doi: 10.1016/j.neuroscience.2015.05.016
192. Kinross JM, Darzi AW, Nicholson JK. Gut microbiome-host interactions in health and disease. *Genome Med.* (2011) 3:14. doi: 10.1186/gm228
193. Robinson WS. Ecological correlations and the behavior of individuals. *Am Sociol Rev.* (1950) 15:351–7. doi: 10.2307/2087176

194. Nicol CJ, Caplen G, Edgar J, Richards G, Browne WJ. Relationships between multiple welfare indicators measured in individual chickens across different time periods and environments. *Anim Welf.* (2011) 20:133–43.
195. Thornton A, Lukas D. Individual variation in cognitive performance: developmental and evolutionary perspectives. *Philos Trans R Soc Lond B Biol Sci.* (2012) 367:2773–83. doi: 10.1098/rstb.2012.0214
196. Gelman A, Carlin J. Beyond power calculations: assessing Type S (sign) and Type M (magnitude) errors. *Perspect Psychol Sci.* (2014) 9:641–51. doi: 10.1177/1745691614551642
197. Simmons JP, Nelson LD, Simonsohn U. False positive psychology: undisclosed flexibility in data collection and analysis allows presenting anything as significant. *Psychol Sci.* (2011) 22:1359–66. doi: 10.1177/0956797611417632
198. Errington TM, Iorns E, Gunn W, Tan FE, Lomax J, Nosek BA. An open investigation of the reproducibility of cancer biology research. *eLife* (2014) 3:e04333. doi: 10.7554/eLife.04333.001
199. Chang AC, Li P. *Is Economics Research Replicable? Sixty Published Papers From Thirteen Journals Say "Usually not"*. Finance and Economics Discussion Series. Washington, DC: Board of Governors of the Federal Reserve System (2015).
200. Huston M. Artifical intelligence faces reproducibility crisis. *Science* (2018) 359:725–6. doi: 10.1126/science.359.6377.725
201. Smaldino PE, McElreath R. The natural selection of bad science. *R Soc Open Sci.* (2016) 3:160384. doi: 10.1098/rsos.160384
202. Voelkl B, Würbel H. Reproducibility crisis: are we ignoring reaction norms? *Trends Pharmacol Sci.* (2016) 37:509–10. doi: 10.1016/j.tips.2016.05.003
203. Gelman A, Hill J. *Data Analysis Using Regression and Multilevel/Hierarchical Models*. Cambridge: Cambridge University Press (2007).
204. Stevens KN, Asher L, Griffin K, Friel M, O'Connell N, Collins LM. A comparison of inferential analysis methods for multilevel studies: implications for drawing conclusions in animal welfare science. *Appl Anim Behav Sci.* (2017) 197:101–11. doi: 10.1016/j.applanim.2017.08.002
205. Harrison XA, Donaldson L, Correa-Cano ME, Evans J, Fisher DN, Goodwin CED, et al. A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ* (2018) 6:e4794. doi: 10.7717/peerj.4794
206. McElreath R, Koster J. Using multilevel models to estimate variation in foraging returns. *Hum Nat.* (2014) 25:100–200. doi: 10.1007/s12110-014-9193-4
207. Kruschke JK, Vanpaemel W. Bayesian estimation in hierarchical models. In: Busemeyer J, Townsend J, Wang ZJ, Eidels A, editors. *The Oxford Handbook of Computational and Mathematical Psychology*. Oxford: Oxford University Press (2015). p. 279–99.
208. Duncan C, Jones K, Moon G. Context, composition and heterogeneity: using multilevel models in health research. *Soc Sci Med.* (1998) 46:97–117. doi: 10.1016/S0277-9536(97)00148-2
209. Dingemanse NJ, Dochtermann NA. Quantifying individual variation in behaviour: mixed-effect modelling approaches. *J Anim Ecol.* (2013) 82:39–54. doi: 10.1111/1365-2656.12013
210. Lee MD. How cognitive modeling can benefit from hierarchical Bayesian models. *J Math Psychol.* (2011) 55:1–7. doi: 10.1016/j.jmp.2010.08.013
211. Dingemanse NJ, Kazem AJ, Reale D, Wright J. Behavioural reaction norms: animal personality meets individual plasticity. *Trends Ecol Evol.* (2010) 25:81–9. doi: 10.1016/j.tree.2009.07.013
212. Cleasby IR, Nakagawa S, Schielzeth H. Quantifying the predictability of behaviour: statistical approaches for the study of between-individual variation in the within-individual variance. *Methods Ecol Evol.* (2015) 6:27–37. doi: 10.1111/2041-210X.12281
213. Nakagawa S, Schielzeth H. Repeatability for Gaussian and non Gaussian data: a practical guide for biologists. *Biol Rev Camb Philos Soc.* (2010) 85:935–56. doi: 10.1111/j.1469-185X.2010.00141.x
214. van Horik JO, Langley EJG, Whiteside MA, Madden JR. Differential participation in cognitive tests is driven by personality, sex, body condition and experience. *Behav Processes* (2017) 134:22–230. doi: 10.1016/j.beproc.2016.07.001
215. Bringmann LF, Vissers N, Wichers M, Geschwind N, Kuppens P, Peeters F, et al. A network approach to psychopathology: new insights into clinical longitudinal data. *PLoS ONE* (2013) 8:e60188. doi: 10.1371/journal.pone.0060188
216. Maas CJ, Hox JJ. Sufficient sample sizes for multilevel modeling. *Methodology* (2005) 1:86–92. doi: 10.1027/1614-2241.1.3.85
217. Klein RA, Ratcliff KA, Vianello M, Adams RB Jr, Bahnik S, Bernstein MJ, et al. Investigating variation in replicability: a "many labs" replication project. *Soc Psychol.* (2014) 45:142–52. doi: 10.1027/1864-9335/a000178

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Low Birth Weight Impairs Acquisition of Spatial Memory Task in Pigs

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In commercial pig farming, an increasing number of low birth weight (LBW) piglets are born, due to selection for large litter sizes. While LBW piglets have a higher risk of pre-weaning mortality, a considerable number of these piglets survive to slaughter age. In humans, LBW is a risk factor for long-term cognitive impairments. In pigs, studies examining the post-weaning effects of LBW on cognition have reported contradictory results. Therefore, the current study aimed to assess the effects of LBW on cognitive development in pigs using an improved study design, by (1) testing a larger sample size than previous studies, (2) assessing acute and chronic stress responses to account for a potential altered stress response in LBW pigs, and (3) testing both female and male pigs to account for potential confounding effects of sex. Learning and memory of 20 LBW pigs and 20 normal birth weight (NBW) pigs, both groups consisting of 10 females and 10 males, were compared using a spatial holeboard task. In this task, pigs had to learn and remember the locations of hidden food rewards. After a pig had successfully acquired the task, it was presented with two successive reversal phases during which it was presented with a new configuration of reward locations. The holeboard allows for simultaneous assessment of working and reference memory, as well as measures of motivation, exploration, and behavioral flexibility. Mixed model ANOVAs revealed a transiently impaired reference memory performance of LBW pigs, implying they had more difficulty learning their reward configuration in the holeboard. Also, LBW piglets showed increased pre-weaning hair cortisol concentrations compared to their NBW siblings. No other effects of LBW were found. Sex had no direct or interaction effects on any measures of holeboard performance or stress. It is possible that the enriched housing conditions applied during our study had an ameliorating effect on our pigs' cognitive development. Overall, our results suggest LBW has a negative effect on post-weaning cognitive performance in pigs. This could have welfare consequences as cognitive skills are required for pigs to learn how to correctly respond to their environment.

Keywords: pigs, cognition, birth weight, sex differences, spatial learning, spatial holeboard task, cortisol

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INTRODUCTION

Piglets born with low birth weight (LBW) are an increasingly common occurrence on commercial pig farms. This is a result of selection for increased sow fecundity, leading to larger litters. With increasing litter size, sows may be unable to provide sufficient nutrients and oxygen for the optimal development of all fetuses (1, 2). This explains the more frequent occurrence of LBW piglets in larger litters (3). Besides a sub-optimal prenatal development, LBW piglets also have a higher risk of pre-weaning mortality (4). While this results in a relatively higher number of LBW piglets dying during the farrowing stage compared to piglets with normal birth weight (NBW), there is still a considerable number of LBW piglets surviving to slaughter age at ~6 months old (5).

The sub-optimal development of LBW offspring has been associated with postnatal cognitive impairments in a variety of species. In humans, LBW has been linked to learning difficulties throughout adolescence (6, 7). Impaired cognitive development associated with LBW has also been studied in a variety of animal models, most frequently in rats and sheep [although contrary to pigs and humans, LBW has to be experimentally induced in these models—(8)]. For example, LBW has been linked to spatial memory deficits in rats (9). Together, these findings suggest that LBW could have a long-term impact on cognitive functioning. Pigs' prenatal brain development has similarities to humans', including a period of rapid brain growth *in utero* (10). It is possible that the cognitive impairments resulting from a sub-optimal intra-uterine environment are also comparable.

Understanding whether LBW also causes a long-term impairment of cognitive functioning in pigs is crucial, as such an impairment may influence their abilities to cope with housing and rearing conditions. Pigs are presented with multiple challenges to their learning and memory abilities in the common conditions of a commercial farm (11). For example, piglets have to learn how to acquire food from a feeder after being weaned (12), be able to recognize conspecifics and remember the organization of the dominance hierarchy to avoid unnecessary aggression (13, 14) and if available, be able to successfully interact with cognitive enrichment (15). Pigs need to be able to learn and remember how to interact with their environment, creating predictability, and controllability, which have been shown to reduce stress (16).

Studies examining the effects of LBW on cognitive development in pigs have produced contradictory results. A study comparing pre-weaning spatial learning abilities of LBW and NBW piglets found LBW to be associated with the expected impaired performance (17). After weaning, one study found LBW to be associated with the predicted cognitive impairments, with LBW pigs showing impaired reversal learning in a spatial learning task compared to NBW pigs (18). Other studies reported a comparable performance of LBW and NBW pigs, finding no effects of LBW on spatial learning ability (19) or associative learning (20, 21). There has also been a report of improved cognitive performance in LBW pigs, with LBW being associated with improved spatial learning (22). Together, these studies do not provide a consensus on the long-term impact of LBW on the cognitive development of pigs. Rather, they show the need for further replication of cognitive studies with LBW pigs, applying methodological improvements to increase the quality of results.

None of the studies assessing the long-term effects of LBW on cognitive development took a potential effect of their study subjects' sex into account. Although it is unlikely that sex in itself has an influence on baseline cognitive performance in pigs (23), it is possible that females and males perform differently under the influence of stress. For example, spatial learning and memory is typically impaired as a consequence of chronic stress (24). Such negative effects of chronic stress appear to be more prominent for males than females (25). The opposite has been found for acute stress, which causes a more detrimental effect on females' cognitive performance (26). Possible sex-dependent effects of stress are relevant when assessing cognition in LBW animals, as LBW may lead to altered functioning of the HPA-axis. For example, LBW piglets show increased plasma cortisol concentrations throughout the first week after birth (27, 28), and show an exaggerated cortisol response to a physiological stressor (administration of insulin or ACTH) at 3 months of age (29). Together, these results suggest that stressors may have a more detrimental effect on pigs with LBW. Considering that females and males may be differentially affected by such stressors, taking both sex of the study subjects and measures of stress into account is of importance when assessing the effects of LBW on cognition.

A suitable task to assess the effects of LBW on learning and memory in pigs is the spatial holeboard task. The holeboard is a free-choice maze task consisting of an open arena in which pigs have to learn and remember the locations of hidden food rewards (30). Such a task is highly ecologically relevant for pigs, as it is based on their natural foraging behavior (31). Furthermore, the spatial holeboard allows for simultaneous assessment of multiple behavioral variables. The most important cognitive measures provided by the spatial holeboard are reference and working memory. Reference memory is required for information that remains relevant over a longer time period, such as how well a pig remembers the locations of rewards and how many locations contain a reward (32). Reference memory can be quantified as the ratio between visits to rewarded and unrewarded locations (30). Working memory is required for information that is relevant for a shorter time span, such as which locations have already been visited within a single training trial (33). This information is irrelevant in subsequent trials and consequently, working memory must be reset between trials. Working memory can be quantified as the ratio between first visits and all visits (including revisits) to a location (30). Besides measures of spatial learning and memory, the holeboard can also be used to assess motivation (by measuring latency to first visit or the time interval between visits), exploration (by measuring which locations are visited) and behavioral flexibility [by applying a reversal of the task—(30)]. The spatial holeboard task has already successfully been applied to assess spatial cognition in pigs, showing it is sensitive enough to detect even mild cognitive impairments [e.g., (34)].

The current study aimed to assess the long-term effects of LBW on learning and memory in pigs, as assessed by the spatial holeboard task. Several improvements to previous studies were applied. First, a larger sample size was included, with 20 LBW and 20 NBW pigs being tested. This doubles the sample size used in previous studies to assess baseline effects of birth weight on post-weaning cognition (18, 19, 22). Second, as LBW pigs may suffer from an altered stress response, hair and salivary cortisol concentrations were included as measures of chronic and acute stress, respectively. Finally, female and male pigs were tested to account for a potential confounding effect of sex. Based on studies assessing cognitive effects of LBW in humans (6, 7) and earlier studies with pigs at various ages (17, 18) it was expected that LBW would cause an impaired cognitive development in pigs. This would result in decreased performance in the spatial holeboard, compared to NBW pigs. Furthermore, LBW pigs were expected to show an altered stress response, resulting in higher basal hair cortisol concentrations compared to NBW pigs and an exaggerated salivary cortisol increase after a stressor.

MATERIALS AND METHODS

Ethical Note

All methods that demanded the handling of live animals were reviewed and approved by the local animal welfare body (Animal Welfare Body Utrecht) and were conducted in accordance with the recommendations of the EU directive 2010/63/EU.

Animals

Twenty pairs of piglets [(Yorkshire × Dutch Landrace) × Duroc] from 15 different litters were selected from the commercial pig breeding farm of Utrecht University, resulting in 20 LBW pigs and 20 NBW pigs (10 pairs of females and 10 pairs of males). Selection occurred in two separate rounds of 20 piglets (ten LBW-NBW pairs) to ensure availability of LBW piglets. During each selection round, all piglets born over a period of 1 week were weighed within 24 h after birth. LBW piglets were selected based on three criteria: (1) a minimum of 1 SD below the average birth weight of the litter, (2) a minimum of 1 SD below the average birth weight of the study population, yielding a maximum birth weight of 1,050 grams, and (3) from a minimum litter size of 10 piglets. For each LBW piglet, a NBW sibling was selected based on two criteria: (1) of the same sex as the selected LBW piglet, and (2) a birth weight closest to the litter average. To increase food intake and thereby survival rates of LBW piglets, cross-fostering of non-selected siblings was applied when litter size exceeded the sow's number of functional teats. Additionally, all litters were provided with milk replacer at 2–3 days of age. One female LBW piglet and one male NBW piglet died of natural causes during the early stages of training in the holeboard. Their data was excluded from analysis, resulting in a final sample size of 38 pigs. Of these, one female LBW piglet could not participate in the second reversal phase due to lameness.

Housing

The selected pigs were weaned and moved to the research facility (located next to the commercial farm), at ∼4 weeks of age. They were housed in four adjacent pens (∼4 × 5 m) in a naturally ventilated building. For each selection round, LBW and NBW pigs were housed separately. Pens had concrete floors and contained a covered piglet nest. Each day, the pens were cleaned and supplied with fresh straw bedding. To protect the piglets from the cold, the nest was equipped with rubber mats on the floor and transparent polyvinyl chloride (PVC) slats hanging in front of the entrance. Additionally, piglet nests contained heat lamps until the pigs were ∼8 weeks old. Minimum and maximum temperatures were recorded daily outside the piglet nest and ranged from 0 to 27°C. To avoid effects of heat stress, pigs were only tested if they voluntarily entered the holeboard apparatus. Pigs received $^1/_3$ of their daily food ration in the morning (prior to testing) and the remaining $^2/_3$ in the afternoon (after testing). Water was provided *ad libitum*. Each pig had a number sprayed on its back to facilitate individual recognition of the pigs.

Spatial Holeboard Task

Apparatus

The holeboard apparatus (manufactured by Ossendrijver B.V., Achterveld, The Netherlands) consisted of a square arena (5.3 × 5.3 m) with a synthetic slatted floor, surrounded by synthetic walls (80 cm high). The holes in the arena consisted of 16 food bowls placed in a 4 × 4 matrix (**Figure 1**), in which food rewards could be hidden. Pigs could enter the arena to search for these rewards via a surrounding corridor (40 cm wide), which gave access to one of four guillotine doors (operated by a rope and pulley system) placed in the walls surrounding the arena. By using four different starting positions, pigs cannot rely on a fixed search pattern to solve the task (30). Instead, pigs had to rely on extra-maze cues (such as the position of the experimenter outside the arena) to orient themselves inside the holeboard. A baited hole would contain two chocolate candies (M&M's® Milk Chocolate) as a reward. Each food bowl was fixed with a false bottom, beneath which four candies were placed to avoid providing the pigs with scent cues about the locations of the baited holes (**Figure 1**). Additionally, each bowl was covered with a synthetic red ball (JollyBall Dog Toy, ø 24 cm, 1,400 g, Jolly Pets, Ohio, USA) to avoid visual discrimination between baited and non-baited holes. The pigs were trained to lift the ball off a food bowl in order to obtain the food reward. If a pig soiled the holeboard during testing, it was rinsed immediately to avoid the development of scent cues. Additionally, the entire holeboard was rinsed daily. During testing, visits to holes were automatically recorded using custom made software (SeaState5, Delft, The Netherlands). When a ball was lifted off a food bowl, the connection between a magnet in the ball and a sensor in the bowl was interrupted. This signal was registered by an interface (LabJack) and sent to a laptop. A revisit to a hole was only recorded if a pig visited another hole in between or if 10 s passed in between successive visits to the same hole.

Training and Testing

After the pigs were moved to the research facility, training started by habituating the pigs to the presence of and being handled by the researchers. The pigs were then gradually habituated to being inside the holeboard apparatus. Initially, pigs were allowed to explore the holeboard in groups of ten. Group size was then gradually decreased until the pigs explored the holeboard individually.

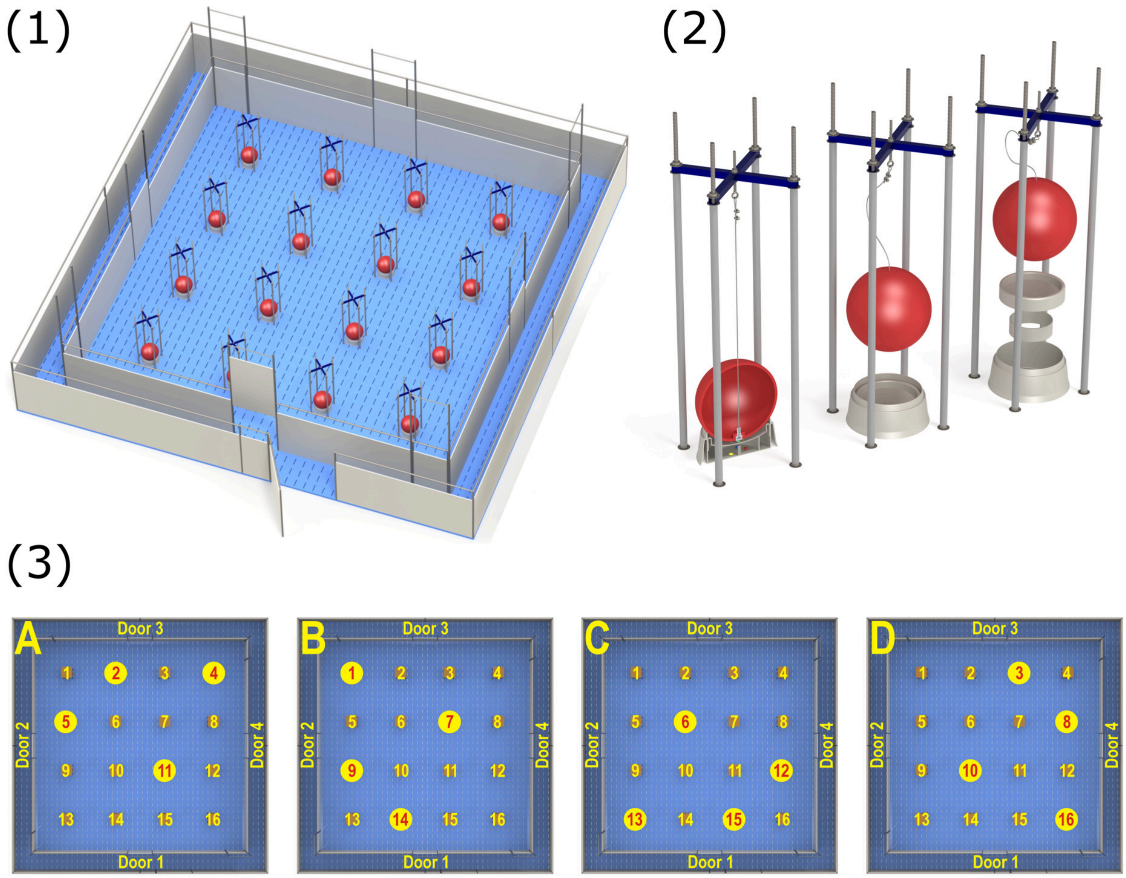

FIGURE 1 | **(1)** Overview of the spatial holeboard apparatus. **(2)** Details of the food bowls (holes). Each bowl is covered with a red ball to hide visual cues. The bowls are equipped with a false bottom covering four candies to mask odor cues. **(3)** The different reward configurations (A–D) used during holeboard training. Baited holes are highlighted (illustrations: Yorrit van der Staay).

Testing trials started when all pigs were able to lift the balls off the food bowls (at this point, pigs were ∼8 weeks old). Each pig performed two consecutive trials daily. At the start of a trial, a pig was let into the corridor surrounding the holeboard arena. When it reached an open entrance into the arena (one of four entrances was randomly chosen prior to each trial), it could freely search for food rewards by visiting holes (i.e., lifting the ball covering a food bowl). A trial ended when a pig managed to find all rewards or when a maximum trial duration of 7.5 min had passed, whichever occurred first.

The holeboard experiment consisted of four consecutive phases: habituation (four trials), acquisition (44–76 trials), first reversal (24–44 trials) and second reversal (20 trials). During habituation trials, all 16 holes contained a reward. This encouraged the pigs to visit as many holes as possible during each habituation trial. After the habituation trials, the acquisition phase started. Each pig was assigned one of four possible reward configurations (**Figure 1**). A reward configuration consisted of a subset of four baited holes (the remaining 12 holes did not contain a food reward). Each pig continued training on its assigned reward configuration for the duration of the acquisition phase. Reward configurations were randomly assigned but balanced for birth weight category and sex.

There were two criteria for a pig to complete the acquisition phase, based on previous holeboard studies with pigs (18, 23): a pig had to (1) complete a minimum of 44 acquisition trials, and (2) reach a reference memory score of at least 0.7 (see section Ameliorating Effects of Enrichment, Variables for calculation of reference memory) for two consecutive training days (consisting of four consecutive trials). This criterion performance indicated a pig had successfully learned the locations of the four baited holes. After completing the acquisition phase, a pig was assigned a new reward configuration for the first reversal phase (**Table 1**). The pigs now had to learn to retrieve their rewards in a new set of holes (e.g., a pig that was trained on configuration A during acquisition was now switched to configuration C). Pigs could complete the first reversal phase after a minimum of 24 reversal trials and reaching the same criterion level for reference memory performance that was set during the acquisition phase. After completing the first reversal phase, a second reversal was applied where pigs were again trained on a new reward configuration (**Table 1**).

TABLE 1 | Combinations of reward configurations used for the holeboard experiment.

Combination	Phase		
	Acquisition	1st reversal	2nd reversal
1	A	C	B
2	B	D	C
3	C	A	D
4	D	B	A

Behavioral Variables

For each trial in the holeboard, the following variables were analyzed:

- **Working memory**, calculated as the number of visits that yielded a reward divided by the total number of visits (including revisits) to baited holes.
- **Reference memory**, calculated as the total number of visits to baited holes divided by the total number of visits to all holes. Reference memory was further divided into components reflecting spatial orientation and spatial pattern learning:
    - **Rotational reference memory** reflects rotational errors made while a pig was orienting himself after entering the holeboard, i.e. reference memory mistakes made prior to finding the first reward (35). This measure was calculated as 1 divided by the total number of visits to all holes up to and including the first rewarded visit.
    - **Spatial pattern reference memory** reflects a pig's ability to successfully complete the spatial pattern formed by the reward configuration, i.e., reference memory mistakes made after finding the first reward (36). This measure was calculated as total number of visits to baited holes divided by the total number of visits to all holes excluding visits made before finding the first reward.
- **Trial duration**, **latency to first visit** and **latency to first reward**, calculated as average time in seconds elapsed between entering the holeboard and performing the required action. When a pig failed to perform the required action, a maximum value of 450 s was assigned.
- **Inter-visit interval**, calculated as the average time in seconds between two successive visits.
- **Total number of visits, number of different locations (holes) visited** and **number of rewards found**, calculated as absolute counts.

Additionally, **trials to criterion** was calculated as the number of trials needed to reach criterion performance for the acquisition and first reversal phase.

Cortisol Analysis

Hair Cortisol

Hair samples were collected at weaning and at the end of the experiment, when the pigs were ~5 months old. Hair was taken from the left flank of each pig with a razor (single edged disposable prep razor, Kai Medical, Solingen, Germany; a new razor was used for each sample). Hair cortisol concentration was determined based on the protocol by Davenport et al. (37). In short, samples were washed and dried, after which ~35 mg of hair was ground with a bead beater (TissueLyser II, QIAGEN Benelux B.V., Antwerp, Belgium) for a minimum of 2 × 15 min at 30 Hz, in 2 mL tubes containing three 2.3 mm steal beads (BioSpec, Lab Services B.V., Breda, the Netherlands). After grinding, 1 mL methanol was added and samples were incubated for 24 h with slow rotation to extract corticosteroids. Of the extract, 0.6 mL was dried using a vacuum centrifuge. Dried extracts were dissolved in 0.3 mL phosphate buffer. Hair cortisol concentrations were then determined in duplo using a Salimetrics Salivary Cortisol ELISA kit. Intra-assay and inter-assay coefficients of variation (CV) were 7.1 and 23.1%, respectively. The higher inter-assay CV implies plate-to-plate variation (i.e., different plates produced different cortisol concentrations for the same sample). To avoid an influence of inter-assay CV on group comparisons, samples were balanced across plates for birth weight and sex.

Salivary Cortisol

Saliva samples were collected from each pig prior to and after their first individual habituation trial in the holeboard. Pre-stressor samples were collected at ~14:00 in the afternoon in their home pens. Post-stressor samples were taken ~20 min after a pig's trial in the holeboard, to allow for the peak in cortisol response to develop (38). Saliva was collected by allowing each pig to chew on two cotton swabs (Cotton Swabs 150 mm × 4 mm WA 2PL; Heinz Herenz, Hamburg, Germany) until they were sufficiently moistened. Then, the swabs were centrifuged using saliva collection tubes (Salivette, Sarstedt, Germany) at around 3,524 g for 10 min at 10°C. Saliva samples were stored at −20°C until salivary cortisol concentration was determined in duplo using a Coat-a-Count radioimmunoassay kit (Siemens Healthcare Diagnostics BV, The Hague, the Netherlands). Intra-assay and inter-assay CVs were 4.8 and 1.6%, respectively.

Statistical Analysis

All statistical analyses were performed using R statistical software, version 3.4.2 (39). For linear mixed models, package nlme (40) was used. For each mixed model the random effect structure was assessed using Restricted Maximum Likelihood (REML) estimation. Final selection of random effect structure was based on Akaike's information criterion (AIC). Round (first or second round of selected animals) did not improve fit of mixed models and was therefore dropped from further analysis. Statistical significance was set at $P < 0.05$. Effect size was calculated as Pearson's r based on contrasts. Unless indicated otherwise, results are presented as mean ± SEM.

Birth Weight and Growth

Average birth weight of LBW and NBW pigs was compared using Welch's *t*-test. The effect of birth weight on pigs' weekly weight gain from weaning until 5 months of age was analyzed using a linear mixed model with Birth weight, Week and Birth weight × Week interaction as fixed effects. Random effect structure consisted of random slopes and intercepts for Subject nested within Litter.

Holeboard Data

For all variables scored during the acquisition and reversal phases, means of four successive trials (trial blocks) were calculated. Furthermore, to assess the effect of transitioning to a reversal phase, the last trial block of the acquisition phase was compared to the first trial block of the first reversal phase. The same was done for the transition from first to second reversal phase. The effect of birth weight on pigs' learning curves during acquisition, transition and reversal phases for all holeboard variables were analyzed using a linear mixed model with Birth Weight, Sex, Trial Block, and their two-way interactions as fixed effects. Random effect structure consisted of random intercepts for Subject nested within Litter and a first-order autoregressive correlation structure for residuals to account for repeated measures within subjects. The habituation phase was analyzed similarly, but with Trial as a fixed effect instead of Trial Block. Durations and latencies were $\log_{10}$ transformed to improve the distribution of residuals. Finally, trials to criterion for the acquisition and first reversal phase were compared using a linear mixed model with Birth Weight, Sex, and Birth Weight × Sex interaction as fixed effects and random intercepts for Litter.

Cortisol Concentrations

The effects of birth weight on pigs' hair cortisol concentrations at weaning and 5 months of age were analyzed using a linear mixed model with Birth weight, Sex, and Birth weight × Sex interaction as fixed effects and random intercepts for Litter. Hair samples collected at weaning from three pigs (1 LBW male, 1 NBW male, and 1 NBW female) were insufficient for cortisol analysis. Therefore, hair cortisol analysis on samples at weaning was performed on the remaining 35 samples.

The effects of birth weight on salivary cortisol concentrations before and after a pig's first individual trial in the holeboard were analyzed using a linear mixed model with Birth Weight, Sex, Sample, and all two-way interactions as fixed effects and random slopes and intercepts for Subject. Salivary cortisol concentrations were $\log_{10}$ transformed to improve distribution of residuals. Saliva collected from one LBW male was insufficient for cortisol analysis. Therefore, salivary cortisol analysis was performed on samples collected from the remaining 37 animals.

RESULTS

Birth Weight and Growth

LBW piglets had on average a lower birth weight than NBW piglets [LBW: 0.81 kg ± 0.02, NBW: 1.45 kg ± 0.05; $t_{(27.31)} = -11.63$, $P < 0.001$; $r = 0.91$]. LBW piglets continued to have lower body weight throughout the duration of the experiment [Birth weight: $F_{(1,22)} = 34.30$, $P < 0.001$; $r = 0.79$; **Figure 2**] and had a slower growth rate than the NBW piglets [Birth weight × Week: $F_{(15,482)} = 10.39$, $P < 0.001$; **Figure 2**].

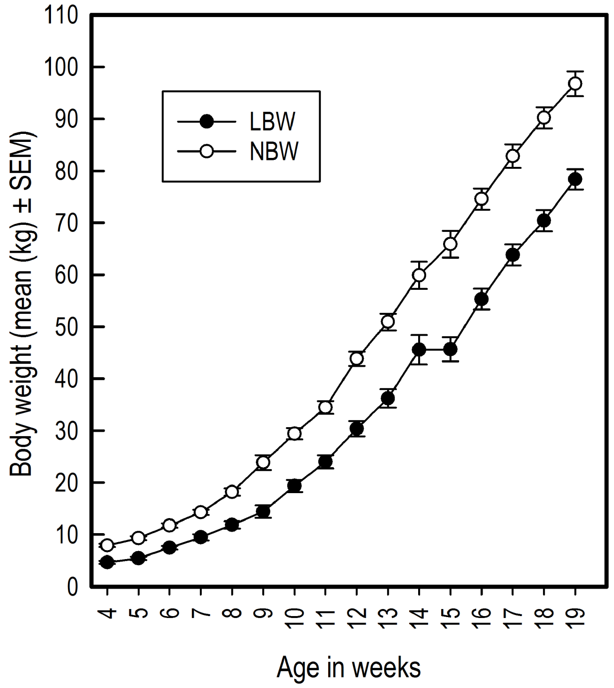

FIGURE 2 | Average body weight in kilograms of LBW and NBW pigs from weaning until the end of the experiment. For week 15, data from only ten LBW pigs was available, causing an appearance of lack of growth from week 14 to 15. This is an artifact due to missing data.

Spatial Holeboard Task

Habituation

Birth weight and sex did not influence pigs' performance during the habituation trials (Supplementary Table 1). During habituation, all pigs showed a comparable exploration of the holeboard based on total visits and locations (holes) visited.

Spatial Learning and Memory

Working Memory

Neither birth weight nor sex had an effect on working memory (WM) scores during any phase of the experiment (**Figure 3**; **Table 2**). All pigs improved their WM scores as training progressed during the acquisition phase, first reversal and second reversal [Trial blocks: Acquisition, $F_{(10,350)} = 16.13$, $P < 0.001$; First reversal, $F_{(5,174)} = 55.39$, $P < 0.001$; Second reversal, $F_{(4,136)} = 89.16$, $P < 0.001$]. After a transition to a new configuration of baited holes, all pigs showed an initial decrease in WM scores [Trial blocks: First transition, $F_{(1,34)} = 139.19$, $P < 0.001$; Second transition, $F_{(1,34)} = 175.11$, $P < 0.001$].

Reference Memory

Birth weight affected reference memory (RM) scores during the acquisition phase [Birth weight: $F_{(1,20)} = 5.76$, $P = 0.026$, $r = 0.12$; **Figure 3**; **Table 2**], with LBW piglets scoring lower than NBW piglets. This difference was transient, with no effect of birth weight on RM scores during either the first or second reversal phase [Birth weight: First reversal, $F_{(1,20)} = 0.01$, $P = 0.926$;

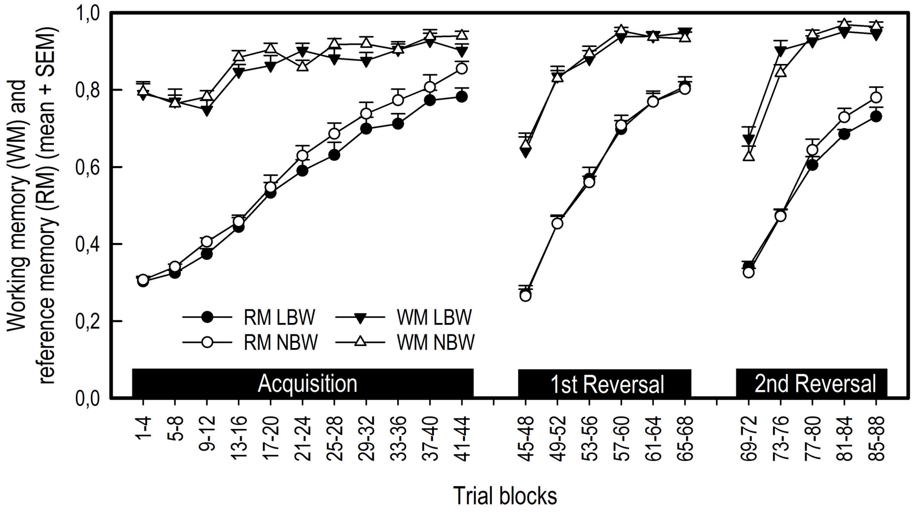

FIGURE 3 | Average working memory (WM) and reference memory (RM) scores of LBW and NBW pigs in the spatial holeboard task per trial block. There was no significant effect of sex on holeboard performance, either as a main effect or interaction with birth weight. Therefore, the data for males and females have been combined.

Second reversal, $F_{(1, 19)} = 1.65$, $P = 0.214$]. Similarly, there was a trend for LBW piglets to require a higher number of trials to complete the acquisition phase compared to NBW piglets [LBW: 53.26 ± 2.27, NBW: 48.00 ± 1.70; $F_{(1, 20)} = 4.19$, $P = 0.054$]. No effect of birth weight was found on the number of trials required to complete the first reversal phase [LBW: 29.26 ± 1.38, NBW: 29.79 ± 1.08; $F_{(1, 20)} = 0.08$, $P = 0.776$]. Sex had no effect on trials to criterion during the acquisition phase [Sex: $F_{(1, 20)} = 2.46$, $P = 0.132$; Sex × Birth weight: $F_{(1, 20)} = 0.46$, $P = 0.504$] or the first reversal phase [Sex: $F_{(1, 20)} = 0.08$, $P = 0.775$; Sex × Birth weight: $F_{(1, 20)} = 1.41$, $P = 0.249$], nor did it influence RM scores during any phase of the experiment (**Table 2**). All pigs improved their RM scores during the acquisition, first reversal and second reversal phases [Trial blocks: Acquisition, $F_{(10, 350)} = 104.71$, $P < 0.001$; First reversal, $F_{(5, 175)} = 173.34$, $P < 0.001$; Second reversal, $F_{(4, 136)} = 54.74$, $P < 0.001$]. After each transition to a reversal phase, all pigs showed an initial decrease in RM scores [Trial blocks: First transition, $F_{(1, 35)} = 1787.92$, $P < 0.001$; Second transition, $F_{(1, 34)} = 1882.18$, $P < 0.001$].

RM scores can be separated into a rotational and a spatial pattern component. Birth weight influenced rotational RM (rRM) scores for certain specific trial blocks during the acquisition phase and transition to the first reversal phase, as indicated by Birth weight × Trial blocks interactions. For the acquisition phase, LBW pigs had lower rRM scores for trials 21–24 and 25–28 [Birth weight × Trial blocks: $F_{(10, 350)} = 2.04$, $P = 0.029$]. For the first transition phase, LBW pigs had higher rRM scores for the final trial block of the acquisition phase and lower rRM scores for the first trial block of the first reversal phase [Birth weight × Trial blocks: $F_{(1, 35)} = 5.27$, $P = 0.028$]. Together, these findings do not represent a systematic difference between LBW and NBW pigs for rRM scores (**Table 2**). However, a general effect of birth weight was found for spatial pattern RM scores during the acquisition phase [Birth weight: $F_{(1, 20)} = 5.51$, $P = 0.029$, $r = 0.12$], suggesting the difference found between LBW and NBW pigs in RM performance reflects a difference in spatial pattern learning. No effect of birth weight on subsequent phases was found [Birth weight: First reversal, $F_{(1,20)} = 0.22$, $P = 0.647$; Second reversal, $F_{(1, 19)} = 1.30$, $P = 0.269$]. A difference between female and male pigs was only found for certain trial blocks during the transition from acquisition to first reversal phase and during the second reversal phase. Male pigs had higher rRM scores for the final trial block of the acquisition phase [Sex × Trial blocks: $F_{(1, 35)} = 4.15$, $P = 0.049$] and higher sRM scores for the final trial block of the second reversal phase [Sex × Trial blocks: $F_{(4, 136)} = 2.97$, $P = 0.022$]. Similar to the general RM scores, both rotational and spatial pattern RM scores improved within training phases, but initially decreased when pigs were transitioned to a reversal phase (**Table 2**).

Duration Measures

Birth weight had an effect on the latency to first reward during the acquisition phase, with LBW pigs taking longer than NBW pigs to find their first reward [Birth weight: $F_{(1, 20)} = 6.40$, $P = 0.012$, $r = 0.35$]. This finding was due to a difference between groups for the first trial blocks and thus does not reflect a systematic difference in performance between LBW and NBW pigs. Similarly, female pigs had higher inter-visit intervals compared to male pigs during the first trial block of the second reversal phase [Sex × Trial blocks: $F_{(4,136)} = 4.07$, $P = 0.004$]. No other effects of birth weight or sex on duration measures were found (Trial duration, Latency to first visit, Latency to first reward and Inter-visit interval; Supplementary Table 1). Most duration measures decreased as training progressed during the acquisition, first reversal and second reversal phase, with pigs needing less time to finish a trial. Latency to first visit increased during the acquisition phase, likely due to pigs learning

TABLE 2 | Spatial memory performance of low birth weight and normal birth weight pigs in the spatial holeboard task, during an acquisition (Acq), first transition (Trans I), first reversal (Rev I), second transition (Trans II), and second reversal (Rev II) phase.

	Birth weight (BW)			Sex			BW × Sex			Trial blocks			BW × Trial blocks			Sex × Trial blocks		
Measure Phase	***F***	***df***	***P≤***	***F***	***df***	***P≤***	***F***	***df***	***P≤***	***F***	***df***	***P≤***	***F***	***df***	***P≤***	***F***	***df***	***P≤***
WM																		
Acq	2.22	1,20	0.152	0.18	1,20	0.674	1.63	1,20	0.217	**16.13**	**10,350**	**<0.001**	1.03	10,350	0.422	1.25	10,350	0.258
Trans I	0.08	1,20	0.780	0.47	1,20	0.450	1.45	1,20	0.242	**139.19**	**1,34**	**<0.001**	0.56	1,34	0.459	0.06	1,34	0.803
Rev I	0.02	1,20	0.894	0.00	1,20	<0.999	0.37	1,20	0.549	**55.39**	**5,174**	**<0.001**	0.20	5,174	0.964	0.39	5,174	0.855
Trans II	1.45	1,19	0.243	0.03	1,19	0.858	*3.32*	*1,19*	*0.084*	**175.11**	**1,34**	**<0.001**	1.22	1,34	0.277	0.01	1,34	0.934
Rev II	0.73	1,19	0.402	0.16	1,19	0.691	0.46	1,19	0.505	**89.16**	**4,136**	**<0.001**	*2.01*	*4,136*	*0.096*	0.34	4,136	0.854
RM																		
Acq	**5.76**	**1,20**	**0.026**	1.03	1,20	0.322	0.93	1,20	0.344	**104.71**	**10,350**	**<0.001**	0.58	10,350	0.832	1.04	10,350	0.407
Trans I	0.21	1,20	0.649	0.00	1,20	0.978	0.01	1,20	0.931	**1787.92**	**1,35**	**<0.001**	0.08	1,35	0.783	0.37	1,35	0.549
Rev I	0.01	1,20	0.926	0.66	1,20	0.427	0.01	1,20	0.920	**173.34**	**5,175**	**<0.001**	0.07	5,175	0.996	0.86	5,175	0.508
Trans II	0.00	1,19	0.976	0.54	1,19	0.470	0.00	1,19	0.979	**1882.18**	**1,34**	**<0.001**	1.63	1,34	0.210	0.30	1,34	0.589
Rev II	1.65	1,19	0.214	1.75	1,19	0.201	0.24	1,19	0.633	**54.74**	**4,136**	**<0.001**	1.10	4,136	0.362	*2.35*	*4,136*	*0.058*
rRM																		
Acq	0.45	1,20	0.509	0.23	1,20	0.640	0.10	1,20	0.758	**49.90**	**10,350**	**<0.001**	**2.04**	**10,350**	**0.029**	0.73	10,350	0.695
Trans I	0.10	1,34	0.749	1.23	1,34	0.276	0.08	1,34	0.778	**567.30**	**1,35**	**<0.001**	**5.27**	**1,35**	**0.028**	**4.15**	**1,35**	**0.049**
Rev I	2.54	1,20	0.126	0.86	1,20	0.366	0.83	1,20	0.374	**82.78**	**5,175**	**<0.001**	1.34	5,175	0.249	1.57	5,175	0.172
Trans II	0.48	1,19	0.497	*3.26*	*1,19*	*0.087*	1.43	1,19	0.246	**164.70**	**1,34**	**<0.001**	0.05	1,34	0.829	0.00	1,34	0.945
Rev II	1.63	1,19	0.217	0.01	1,19	0.914	1.42	1,19	0.248	**43.10**	**4,136**	**<0.001**	0.03	4,136	0.999	0.41	4,136	0.804
sRM																		
Acq	**5.51**	**1,20**	**0.029**	1.22	1,20	0.283	1.24	1,20	0.279	**108.61**	**10,350**	**<0.001**	0.60	10,350	0.811	0.97	10,350	0.472
Trans I	0.08	1,20	0.782	1.38	1,20	0.254	1.72	1,20	0.204	**1198.26**	**1,35**	**<0.001**	0.33	1,35	0.569	0.00	1,35	0.988
Rev I	0.22	1,20	0.647	0.10	1,20	0.763	0.04	1,20	0.850	**140.71**	**5,175**	**<0.001**	0.23	5,175	0.948	1.06	5,175	0.385
Trans II	0.01	1,19	0.906	0.40	1,19	0.533	0.75	1,19	0.396	**977.78**	**1,34**	**<0.001**	1.35	1,34	0.254	0.67	1,34	0.419
Rev II	1.30	1,19	0.269	1.59	1,19	0.222	0.16	1,19	0.697	**152.19**	**4,136**	**<0.001**	1.21	4,136	0.310	**2.97**	**4,136**	**0.022**

Effects printed in bold have associated probabilities of < 0.05*. Effects printed in italics have associated probabilities of* $0.10 > P \geq 0.05$*. WM, working memory; RM, reference memory; rRM, rotational reference memory; sRM, spatial pattern reference memory.*

to approach a rewarded location for their first visit, instead of simply visiting the nearest hole upon entering the holeboard [Trial block: Acquisition, $F_{(10,\ 350)} = 13.35$, $P < 0.001$]. Most duration measure scores initially increased after a transition to a reversal. The only exception was latency to first visit, which remained stable after the start of both reversal phases [Trial block: First transition, $F_{(1,35)} = 3.09$, $P = 0.087$; Second transition, $F_{(1,\ 34)} = 0.26$, $P = 0.615$]. As the second reversal progressed, only a trend for an increase in latency to first visit was found [Trial block: Second reversal, $F_{(4,\ 136)} = 2.33$, $P = 0.059$].

Exploration Measures

No systematic effects of birth weight or sex were found for any of the exploration measures assessed (Total number of visits, Number of locations visited and Number of rewards found). LBW pigs found less rewards than NBW pigs during the first trial blocks of the acquisition phase [Birth weight × Trial blocks: $F_{(10,350)} = 2.13$, $P = 0.022$]. Female pigs visited more locations than male pigs during the final trial blocks of the second reversal phase [Sex × Trial blocks: $F_{(4,136)} = 2.84$, $P = 0.027$]. No other effects of birth weight or sex were found. Scores for all exploration measures improved as pigs progressed during the acquisition, first reversal and second reversal phases (Supplementary Table 1). As training progressed, pigs required fewer total visits, visited fewer locations, and found a higher number of rewards. The opposite was true when pigs were subjected to the reversal phases.

Cortisol Concentrations

Hair Cortisol

At weaning, cortisol concentration in flank hair of LBW piglets was higher than that of NBW piglets [LBW: 33.20 ± 1.68, NBW: 29.26 ± 1.41; $F_{(1,18)} = 5.38$, $P = 0.032$, $r = 0.34$]. Sex did not influence hair cortisol concentration at weaning [Sex: $F_{(1,18)} = 0.00$, $P = 0.975$; Sex × Birth weight: $F_{(1,18)} = 0.04$, $P = 0.847$]. The difference between birth weight categories was no longer present in hair samples collected at 5 months of age, at the end of the experiment [LBW: 20.61 ± 1.14, NBW: 21.84 ± 1.88; $F_{(1,20)} = 0.33$, $P = 0.575$]. Again, sex did not influence hair cortisol concentration [Sex: $F_{(1,20)} = 2.96$, $P = 0.101$; Sex × Birth weight: $F_{(1,20)} = 0.52$, $P = 0.480$].

Salivary Cortisol

Performing the first individual trial in the spatial holeboard task caused an increase in salivary cortisol concentration for all piglets [$F_{(1,34)} = 31.53$, $P < 0.001$; **Figure 4**]. No effects of birth weight [Birth weight: $F_{(1,33)} = 2.55$, $P = 0.120$; Birth weight × Sample: $F_{(1,34)} = 0.01$, $P = 0.924$; **Figure 4**] or sex [Sex: $F_{(1,33)} = 1.15$, $P = 0.292$; Sex x Sample: $F_{(1,34)} = 0.18$, $P = 0.677$; Sex x Birth weight: $F_{(1,33)} = 1.21$, $P = 0.279$] were found on salivary cortisol concentrations.

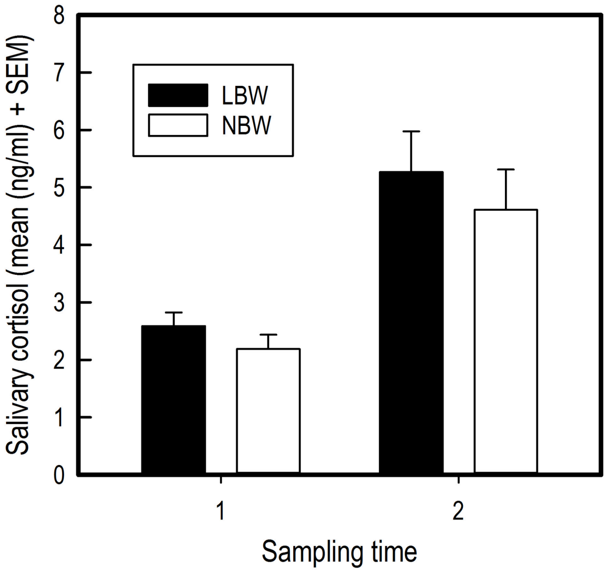

FIGURE 4 | Average salivary cortisol concentrations of LBW and NBW pigs before and after a stressor. There was no significant effect of sex on cortisol concentrations, either as a main effect or interaction with birth weight. Therefore, the data for males and females have been combined.

DISCUSSION

The present study assessed the effects of LBW on post-weaning cognitive performance in pigs. To this end, it is important that the LBW piglets selected for our study actually represented a different population than the selected piglets with NBW. Indeed, LBW piglets had significantly lower birth weights than NBW piglets. Furthermore, the LBW piglets selected for our study had birth weights comparable to or smaller than those of piglets assessed in other LBW studies [e.g., (18, 19, 22)]. In pigs, birth weight is the main measure used to determine whether intra-uterine growth restriction has occurred (41, 42). Head morphology has been suggested as an additional measure, where a relatively large head is considered a sign of the so-called "brain sparing effect", i.e., placental insufficiency resulting in prioritized brain development (22, 43). However, head morphology has been shown to correlate with birth weight (43) and can also be confounded by differences in head shape between different pig breeds (own, non-systematic observations). Therefore, birth weight remains the best indicator that the LBW pigs used in our study suffered from limited nutrients and oxygen *in utero*. The found difference in body weight of LBW and NBW pigs persisted throughout the duration of the experiment. This shows LBW pigs continued to experience impaired weight gain well beyond weaning. Long-term effects on growth have also been shown in previous studies with LBW pigs [e.g., (22, 44)]. Such a lack of catch-up growth shown by LBW offspring is considered an additional risk factor for cognitive impairment in humans (6).

Based on previous studies assessing the effects of LBW in both humans and pigs [e.g., (6, 17, 18)], it was expected that LBW pigs would have an impaired cognitive development as shown by lower memory scores in the spatial holeboard task compared to NBW pigs. Both groups of pigs were able to acquire the task, producing similar learning curves to previous holeboard studies with pigs [e.g. (23, 45)]. Pigs improved their performance as training progressed, as shown by increasing memory scores and decreasing latencies and exploration. In line with our expectation, LBW pigs had lower reference memory (RM) scores during the acquisition phase of the experiment. Additionally, LBW pigs had higher average hair cortisol concentrations (HCC) than NBW pigs in samples taken at weaning. This implies LBW pigs experienced more chronic stress during pre-weaning development. Both the cognitive impairment and the increased HCC found for LBW pigs were transient, likely due to the enriched housing conditions applied during this study.

Effects of LBW on Spatial Learning and Memory

Birth weight was found to cause a mild cognitive impairment, based on spatial learning and memory in the holeboard task. Compared to NBW pigs, LBW pigs showed lower RM scores as the acquisition phase of the experiment progressed. This finding indicates that LBW pigs had more difficulty learning and remembering the locations of food rewards in the holeboard. It is unlikely this effect of LBW was caused by a difference in motivation between LBW and NBW pigs to perform the task. Both groups showed comparable scores on measures of motivation, such as the latency to first visit and the inter-visit interval. This is corroborated by an earlier study comparing food motivation of LBW and NBW pigs (46).

Our finding of impaired cognitive development is supported by earlier studies showing decreased learning and memory associated with LBW in pigs (17, 18). Similarly, LBW in humans has been shown to cause learning difficulties throughout adolescence (6, 7), as well as impaired spatial learning (47). However, there have also been studies which have shown LBW pigs to have a comparable, or even superior, cognitive performance compared to NBW pigs (19–22). Several factors could have contributed to this discrepancy in results.

First, it is difficult to compare the results found by Antonides *et al.* (who reported improved cognitive performance of LBW pigs) to those of other studies assessing post-weaning cognition in LBW pigs, including the current study. This is due to large differences in housing conditions. Their pigs were removed from the sow at 4–6 days of age, whereas other studies applied weaning at 4 weeks of age, comparable to standard commercial practice. Abrupt changes in neonatal environment have been shown to impact piglet development, resulting in increased behavioral and physiological signs of stress (48, 49). Additionally, there was a considerable difference in stocking density. Antonides *et al.* provided 0.625–1.25 m^2 space per pig, whereas the other studies provided a minimum of $\sim$2 m^2 per pig (18–21). A higher stocking density affects pig welfare mainly through increased aggression (50). This could have impacted NBW pigs more, as they remain larger than LBW pigs. Taken together, these differences in housing conditions may have influenced the pigs' early development, hindering direct comparison of results.

Second, the findings of previous studies examining LBW pigs have all been based on smaller sample sizes than applied in our study. Smaller sample sizes increase the probability of chance findings (51), potentially leading to contradictory results in replication studies. Other factors influencing cognitive abilities, e.g., personality (52), could then lead to a significant difference between groups that does not reflect the effects of birth weight. In particular, several studies reporting comparable cognitive performance of NBW and LBW pigs have based their results on relatively small sample sizes. For example, Murphy and colleagues (20) compared six NBW to five LBW pigs in a conditional discrimination task, where both groups were equally capable of learning the task. Similarly, Gieling and colleagues (19) found comparable spatial holeboard performance of LBW and NBW pigs by using litter as the experimental unit in data analysis (i.e., average performance of LBW or NBW litter mates was analyzed instead of individual performance of each pig). This resulted in a loss of statistical power by reducing the effective sample size. Interestingly, visual inspection of the RM scores of their pigs show a similar pattern to the current study. Control LBW pigs (half of the animals were prenatally treated with an anti-oxidative drug) have lower average RM scores toward the end of the acquisition phase.

Finally, it is possible that LBW has not consistently been found to impair cognition in pigs due to the use of different cognitive tasks in different studies. Cognitive development of LBW pigs has been assessed using measures of spatial learning (17–19, 22) and associative learning (20, 21). Spatial learning in a holeboard task and associative learning in a conditional discrimination task have previously been found to be independent measures of cognition in pigs (53). Perhaps no effects of LBW were found in associative learning studies with pigs because they assessed a cognitive domain that is less vulnerable to impairment as a result of LBW. Such specific effects of LBW, with varying effects on different cognitive tasks, have previously been reported for humans (54) and rats (55). That LBW does not have a general negative effect on cognitive development in pigs is also supported by our finding of decreased RM, but not working memory (WM) scores. This is corroborated by earlier holeboard studies (18, 19, 22), where LBW and NBW pigs show very comparable WM learning curves during the acquisition of the holeboard task [although one study found an effect of LBW on WM scores during reversal learning, implying impaired behavioral flexibility—(18)]. Furthermore, when separating the general RM scores into rotational RM scores based on the ability of orientation within the environment (35) and spatial pattern RM scores based on spatial pattern learning (36), our results show that birth weight only affected spatial pattern RM scores. After entering the holeboard, LBW and NBW pigs were equally capable of orienting themselves and locating a baited hole. However, completing the spatial pattern of baited holes after finding this first reward was impaired in LBW pigs. This provides further evidence that LBW could have specific effects on different cognitive domains. It would be relevant for future studies to assess the effects of LBW on additional cognitive domains in pigs, mainly those that are relevant for their welfare. For example, as social behaviors are of such importance to pig welfare (13), testing the effects of LBW on social cognition is recommended.

Effects of LBW on Pre-weaning Chronic Stress

Previous studies with pigs have shown that LBW causes an altered functioning of the HPA axis. LBW pigs show increased baseline cortisol levels, both pre- and post-weaning (27, 56). Furthermore, LBW pigs show an exaggerated acute stress response (29). It was therefore expected that the LBW pigs in our study would suffer from a similar increase in HPA axis activity, namely an increase in hair cortisol concentration compared to NBW pigs, indicative of chronic stress and an exaggerated increase in salivary cortisol compared to NBW pigs after exposure to an acute stressor. These expectations were only partially confirmed.

HCC was used as a non-invasive measure of chronic stress (57). As cortisol is incorporated into the growing hair shaft, HCC allows for assessment of HPA axis activity over a longer time period than other biomarkers of stress. HCC as a measure of chronic stress has previously been assessed in pigs [e.g., (22, 58)]. For example, barren housing leads to a higher HCC in pigs (58). These results are comparable to chronic stress assessment using HCC in other species, with long-term stress leading to increased HCC (37).

At weaning, LBW pigs showed an increased HCC compared to NBW pigs. This suggests that LBW pigs experienced more chronic stress while in the farrowing pens. It is known that LBW piglets experience more physiological stressors after farrowing. For example, LBW piglets suffer from impaired thermoregulation (59) and are less likely to acquire a (desirable) teat when suckling (60) compared to their NBW siblings. However, in our study the effects of such stressors were mitigated by providing extra heating in the farrowing pens (both heat lamps and floor heating), as well as applying cross-fostering to ensure the number of piglets in a litter did not exceed the sow's number of teats. It is possible that in addition to the increased physiological stressors, LBW piglets display an exaggerated response to chronic environmental stressors. Housing piglets in farrowing pens, in which the sow is constrained in a farrowing crate, can lead to behavioral signs of decreased welfare, such as decreased play behavior (61). This could be due to the lack of space and limited opportunities for sow-piglet interactions (62). Based on our results, LBW piglets are more chronically stressed in the farrowing environment than their NBW siblings. Future studies are required to establish which specific physiological or environmental stressors are responsible for LBW piglets' chronic stress.

Ameliorating Effects of Enrichment

Only a mild impairment of spatial cognition was found, along with a transient increase in chronic stress. It is likely that the effects of LBW found were ameliorated by the enriched housing conditions applied during this study.

Enriched housing has been shown to improve cognitive performance in pigs (34, 63). Furthermore, it has been suggested that training animals—in particular if training extends over a longer time period—may act as cognitive enrichment (64). Together, the environmental and cognitive enrichment applied in the current study may have alleviated the effects of adverse conditions, such as LBW. Therefore, it is possible that the LBW pigs in our study performed better than they would have done if they had been housed in the barren conditions that are standard practice on most commercial farms. Future studies exploring the post-weaning cognitive abilities of LBW pigs in different housing conditions are encouraged.

Several indications were found that enrichment also had an ameliorating effect on LBW pigs' stress response. First, in contrast to pre-weaning HCC, post-weaning HCC was not influenced by birth weight. This suggests both groups of pigs were experiencing similar levels of chronic stress once they were moved to the research facilities. Previous studies have shown enriched housing conditions decrease stress as measured by HCC and serum cortisol concentration in pigs (58, 65). Therefore, it is likely that in our study, neither LBW nor NBW pigs experienced chronic stress after weaning. Similar findings have been reported by Murphy *et al.*, who compared mean salivary cortisol concentration (SCC) and found no difference between LBW and NBW pigs housed in enriched conditions [(21), cf. (22)].

Second, we found no exaggerated acute stress response in LBW pigs. In our study, SCC was used as a non-invasive measure of acute stress (66). Both LBW and NBW pigs showed increased SCC after performing the first individual trial in the holeboard, indicating the applied stressor was successful (38). However, this increase in SCC was similar for LBW and NBW pigs. This provides further suggestion that the enrichment applied during our study had an ameliorating effect on LBW pigs' stress response, as a previous study has found LBW pigs to show an exaggerated response to acute stress (29). Future research aimed at the comparison of HPA axis functioning between LBW and NBW pigs housed in standard commercial conditions is encouraged. A longitudinal study of hair cortisol on commercial pig farms should be feasible, as collection of samples is non-invasive. The acute stress response of LBW and NBW pigs could be compared by collecting saliva samples prior to and after common stressors on commercial farms, such as ear tagging and tail docking at a few days old or mixing animals after weaning.

No Sex Effects on Cognition or Cortisol

Our study is the first to control for an effect of sex on the cognitive development of LBW pigs. This was done to account for a possible sex-dependent effect of stress on learning and memory, as has been found in other species (25, 26). Such effects were expected to be exaggerated in LBW pigs, due to their altered HPA-axis functioning (28–30, 58). However, sex did not systematically influence any of the measures for spatial learning and memory in the holeboard, possibly because we failed to find an exaggerated stress response in our LBW pigs (see section Ameliorating Effects of Enrichment).

In a previous study examining the effects of sex on spatial holeboard performance, male pigs showed impaired behavioral flexibility when faced with a reversal of the task (23). This result was not corroborated in the current study. Independent of birth weight, female and male pigs showed a similar exploration of the holeboard and were equally able to find the rewarded holes during the reversal phase. These contrasting results could be due to a difference in housing conditions between the two studies, with the previous study housing females and males separately. In our study, pigs were grouped according to birth weight category, resulting in mixed-sex groups. The effects of mixed- vs. single-sex housing in pigs in relation to their behavior and stress response has not yet received extensive scientific attention. Whether being housed in a mixed-sex group is more or less stressful may differ for males and females, as male pigs have been shown to engage in more aggressive behaviors than females (67, 68). It has also been shown that male aggression is provoked more in mixed-sex groups than when males are housed separately from females (68). As group composition appears to influence aggressive interactions and thereby social stress (69), it is possible that it also impacts pigs' behavioral flexibility.

CONCLUSION

Our results show that LBW causes a transient cognitive impairment in weaned pigs, as measured by a spatial holeboard task. An impaired development of spatial cognition could have adverse effects on the welfare of LBW pigs, as they require spatial learning and memory to correctly respond to their environment. For example, remembering specific locations, such as food and water sites, preferred areas for resting or the preferred areas of dominant conspecifics, is relevant to pig welfare (70, 71). LBW pigs also showed a transient increase in HCC, implying increased chronic stress in the farrowing environment. It is likely the effects of LBW found were mitigated by the enriched housing conditions applied during this study. Therefore, future studies assessing the cognitive development and stress responses of LBW pigs in commercial housing conditions are encouraged.

AUTHOR CONTRIBUTIONS

FvdS, RN, and SR contributed to conception and design of the study. IvB, SM, and SR contributed to data acquisition. SR performed statistical analysis and wrote the first draft of the manuscript. All authors contributed to manuscript revision and have read 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.2018.00142/full#supplementary-material

REFERENCES

1. Père M-C, Etienne M. Uterine blood flow in sows: effects of pregnancy stage and litter size. *Reprod Nutr Dev.* (2000) 40:369–82. doi: 10.1051/rnd:2000105
2. Wähner M, Fischer K. Current physiological aspects of fetal growth and parturition in the pig. *Biotechnol Anim Husb*. (2005) 21:135–48. Available Online at: http://istocar.bg.ac.rs/sr/home-bah/
3. Rutherford K, Baxter E, D'Eath R, Turner S, Arnott G, Roehe R, et al. The welfare implications of large litter size in the domestic pig I: biological factors. *Anim Welf.* (2013) 22:199–218. doi: 10.7120/09627286.22.2.199
4. Galiot L, Lachance I, Laforest J-P, Guay F. Modelling piglet growth and mortality on commercial hog farms using variables describing individual animals, litters, sows and management factors. *Anim Reprod Sci.* (2018) 188:57–65. doi: 10.1016/j.anireprosci.2017.11.009
5. Calderón Díaz JA, Boyle LA, Diana A, Leonard FC, Moriarty JP, McElroy MC, et al. Early life indicators predict mortality, illness, reduced welfare and carcass characteristics in finisher pigs. *Prev Vet Med.* (2017) 146:94–102. doi: 10.1016/j.prevetmed.2017.07.018
6. Lindström L, Wikström A-K, Bergman E, Lundgren M. Born small for gestational age and poor school performance – how small is too small? *Horm Res Paediatr*. (2017) 88:215–23. doi: 10.1159/000477905
7. Yu B, Garcy AM. A longitudinal study of cognitive and educational outcomes of those born small for gestational age. *Acta Paediatr*. (2018) 107:86–94. doi: 10.1111/apa.13993
8. Hunter DS, Hazel SJ, Kind KL, Owens JA, Pitcher JB, Gatford KL. Programming the brain: common outcomes and gaps in knowledge from animal studies of IUGR. *Physiol Behav*. (2016) 164:233–48. doi: 10.1016/j.physbeh.2016.06.005
9. Camprubí Camprubí M, Balada Caballé R, Ortega Cano JA, Ortega de la Torre M de los A, Duran Fernández-Feijoo C, Girabent-Farrés M, et al. Learning and memory disabilities in IUGR babies: functional and molecular analysis in a rat model. *Brain Behav*. (2017) 7:e00631. doi: 10.1002/brb3.631
10. Dobbing J, Sands J. Comparative aspects of the brain growth spurt. *Early Hum Dev*. (1979) 3:79–83. doi: 10.1016/0378-3782(79)90022-7
11. Wechsler B, Lea SEG. Adaptation by learning: its significance for farm animal husbandry. *Appl Anim Behav Sci*. (2007) 108:197–214. doi: 10.1016/j.applanim.2007.03.012
12. Magowan E, McCann MEE, O'Connell NE. The effect of feeder type and change of feeder type on growing and finishing pig performance and behaviour. *Anim Feed Sci Technol*. (2008) 142:133–43. doi: 10.1016/j.anifeedsci.2007.06.035
13. Coutellier L, Arnould C, Boissy A, Orgeur P, Prunier A, Veissier I, et al. Pig's responses to repeated social regrouping and relocation during the growing-finishing period. *Appl Anim Behav Sci*. (2007) 105:102–14. doi: 10.1016/j.applanim.2006.05.007
14. Li Y, Wang L. Effects of previous housing system on agonistic behaviors of growing pigs at mixing. *Appl Anim Behav Sci*. (2011) 132:20–6. doi: 10.1016/j.applanim.2011.03.009
15. Zebunke M, Puppe B, Langbein J. Effects of cognitive enrichment on behavioural and physiological reactions of pigs. *Physiol Behav*. (2013) 118:70–9. doi: 10.1016/j.physbeh.2013.05.005
16. Wiepkema PR, Koolhaas JM. Stress and animal welfare. *Anim Welf*. (1993) 2:195–218.
17. Radlowski EC, Conrad MS, Lezmi S, Dilger RN, Sutton B, Larsen R, et al. A neonatal piglet model for investigating brain and cognitive development in small for gestational age human infants. *PLoS ONE* (2014) 9:e91951. doi: 10.1371/journal.pone.0091951
18. Gieling ET, Park SY, Nordquist RE, van der Staay FJ. Cognitive performance of low- and normal-birth-weight piglets in a spatial hole-board discrimination task. *Pediatr Res*. (2012) 71:71–6. doi: 10.1038/pr.2011.5
19. Gieling ET, Antonides A, Fink-Gremmels J, ter Haar K, Kuller WI, Meijer E, et al. Chronic allopurinol treatment during the last trimester of pregnancy in sows: effects on low and normal birth weight offspring. *PLoS ONE* (2014) 9:e86396. doi: 10.1371/journal.pone.0086396
20. Murphy E, Kraak L, Nordquist RE, van der Staay FJ. Successive and conditional discrimination learning in pigs. *Anim Cogn*. (2013) 16:883–93. doi: 10.1007/s10071-013-0621-3
21. Murphy E, Kraak L, van den Broek J, Nordquist RE, van der Staay FJ. Decision-making under risk and ambiguity in low-birth-weight pigs. *Anim Cogn*. (2015) 18:561–72. doi: 10.1007/s10071-014-0825-1

22. Antonides A, Schoonderwoerd AC, Nordquist RE, van der Staay FJ. Very low birth weight piglets show improved cognitive performance in the spatial cognitive holeboard task. *Front Behav Neurosci*. (2015) 9:43. doi: 10.3389/fnbeh.2015.00043
23. Roelofs S, Nordquist RE, van der Staay FJ. Female and male pigs' performance in a spatial holeboard and judgment bias task. *Appl Anim Behav Sci*. (2017) 191:5–16. doi: 10.1016/j.applanim.2017.01.016
24. Conrad CD. A critical review of chronic stress effects on spatial learning and memory. *Prog Neuropsychopharmacol Biol Psychiatry* (2010) 34:742–55. doi: 10.1016/j.pnpbp.2009.11.003
25. Luine VN, Beck KD, Bowman RE, Frankfurt M, MacLusky NJ. Chronic stress and neural function: accounting for sex and age. *J Neuroendocrinol*. (2007) 19:743–51. doi: 10.1111/j.1365-2826.2007.01594.x
26. Healy SD, Bacon IE, Haggis O, Harris AP, Kelley LA. Explanations for variation in cognitive ability: behavioural ecology meets comparative cognition. *Behav Processes* (2009) 80:288–94. doi: 10.1016/j.beproc.2008.10.002
27. Klemcke HG, Lunstra DD, Brown-Borg HM, Borg KE, Christenson RK. Association between low birth weight and increased adrenocortical function in neonatal pigs. *J Anim Sci*. (1993) 71:1010–8. doi: 10.2527/1993.7141010x
28. Wise T, Stone RT, Vernon MW. Relationships of serum estriol, cortisol and albumin concentrations with pig weight at 110 days of gestation and at birth. *Biol Neonate* (1991) 59:114–9.
29. Poore KR, Fowden AL. The effect of birth weight on hypothalamo-pituitary-adrenal axis function in juvenile and adult pigs. *J Physiol*. (2003) 547:107–16. doi: 10.1113/jphysiol.2002.024349
30. van der Staay FJ, Gieling ET, Pinzón NE, Nordquist RE, Ohl F. The appetitively motivated "cognitive" holeboard: a family of complex spatial discrimination tasks for assessing learning and memory. *Neurosci Biobehav Rev*. (2012) 36:379–403. doi: 10.1016/j.neubiorev.2011.07.008
31. Graves HB. Behavior and ecology of wild and feral swine (Sus scrofa). *J Anim Sci*. (1984) 58:482–92.
32. Olton DS, Papas BC. Spatial memory and hippocampal function. *Neuropsychologia* (1979) 17:669–82. doi: 10.1016/0028-3932(79)90042-3
33. Dudchenko PA. An overview of the tasks used to test working memory in rodents. *Neurosci Biobehav Rev*. (2004) 28:699–709. doi: 10.1016/j.neubiorev.2004.09.002
34. Grimberg-Henrici CGE, Vermaak P, Elizabeth Bolhuis J, Nordquist RE, van der Staay FJ. Effects of environmental enrichment on cognitive performance of pigs in a spatial holeboard discrimination task. *Anim Cogn*. (2016) 19:271–83. doi: 10.1007/s10071-015-0932-7
35. Cheng K, Newcombe NS. Is there a geometric module for spatial orientation? squaring theory and evidence. *Psychon Bull Rev*. (2005) 12:1–23. doi: 10.3758/BF03196346
36. Brown MF, Wintersteen J. Spatial patterns and memory for locations. *Anim Learn Behav*. (2004) 32:391–400. doi: 10.3758/BF03196036
37. Davenport MD, Tiefenbacher S, Lutz CK, Novak MA, Meyer JS. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. *Gen Comp Endocrinol*. (2006) 147:255–61. doi: 10.1016/j.ygcen.2006.01.005
38. Merlot E, Mounier AM, Prunier A. Endocrine response of gilts to various common stressors: a comparison of indicators and methods of analysis. *Physiol Behav*. (2011) 102:259–65. doi: 10.1016/j.physbeh.2010.11.009
39. R Core Team. *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. (2017). Available online at: https://www.R-project.org/
40. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. *nlme: Linear and Nonlinear Mixed Effects Models*. (2018). Available online at: https://CRAN.R-project.org/package=nlme
41. Gieling ET. *Pigs as Animal Model for Low-Birth-Weight Babies*. PhD thesis Utrecht University (2013).
42. Wu G, Bazer FW, Wallace JM, Spencer TE. Board-Invited Review: intrauterine growth retardation: implications for the animal sciences. *J Anim Sci*. (2006) 84:2316–37. doi: 10.2527/jas.2006-156
43. Amdi C, Krogh U, Flummer C, Oksbjerg N, Hansen CF, Theil PK. Intrauterine growth restricted piglets defined by their head shape ingest insufficient amounts of colostrum. *J Anim Sci*. (2013) 91:5605–13. doi: 10.2527/jas.2013-6824
44. Rehfeldt C, Kuhn G. Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis1. *J Anim Sci*. (2006) 84:E113–23. doi: 10.2527/2006.8413_supplE113x
45. Arts JWM, van der Staay FJ, Ekkel ED. Working and reference memory of pigs in the spatial holeboard discrimination task. *Behav Brain Res*. (2009) 205:303–6. doi: 10.1016/j.bbr.2009.06.014
46. van Eck LM, Antonides A, Nordquist RE, van der Staay FJ. Testing post-weaning food motivation in low and normal birth weight pigs in a runway and operant conditioning task. *Appl Anim Behav Sci*. (2016) 181:83–90. doi: 10.1016/j.applanim.2016.05.010
47. Leitner Y, Heldman D, Harel S, Pick CG. Deficits in spatial orientation of children with intrauterine growth retardation. *Brain Res Bull*. (2005) 67:13–8. doi: 10.1016/j.brainresbull.2005.04.017
48. van Nieuwamerongen SE, Soede NM, van der Peet-Schwering CMC, Kemp B, Bolhuis JE. Gradual weaning during an extended lactation period improves performance and behavior of pigs raised in a multi-suckling system. *Appl Anim Behav Sci*. (2017) 194:24–35. doi: 10.1016/j.applanim.2017.05.005
49. de Ruyter EM, van Wetter WHEJ, Lines DS, Plush KJ. Gradually reducing sow contact in lactation is beneficial for piglet welfare around weaning. *Appl Anim Behav Sci*. (2017) 193:43–50. doi: 10.1016/j.applanim.2017.03.011
50. Fu L, Li H, Liang T, Zhou B, Chu Q, Schinckel AP, et al. Stocking density affects welfare indicators of growing pigs of different group sizes after regrouping. *Appl Anim Behav Sci*. (2016) 174:42–50. doi: 10.1016/j.applanim.2015.10.002
51. Button KS, Ioannidis JPA, Mokrysz C, Nosek BA, Flint J, Robinson ESJ, et al. Power failure: why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci*. (2013) 14:365–76. doi: 10.1038/nrn3475
52. Mazza V, Eccard JA, Zaccaroni M, Jacob J, Dammhahn M. The fast and the flexible: cognitive style drives individual variation in cognition in a small mammal. *Anim Behav*. (2018) 137:119–32. doi: 10.1016/j.anbehav.2018.01.011
53. Roelofs S, Murphy E, Ni H, Gieling E, Nordquist RE, van der Staay FJ. Judgement bias in pigs is independent of performance in a spatial holeboard task and conditional discrimination learning. *Anim Cogn*. (2017) 20:739–53. doi: 10.1007/s10071-017-1095-5
54. Ford RM, Griffiths S, Neulinger K, Andrews G, Shum DHK, Gray PH. Impaired prospective memory but intact episodic memory in intellectually average 7- to 9-year-olds born very preterm and/or very low birth weight. *Child Neuropsychol*. (2017) 23:954–79. doi: 10.1080/09297049.2016.1216091
55. Caprau D, Schober ME, Bass K, O'Grady S, Ke X, Block B, et al. Altered expression and chromatin structure of the hippocampal IGF1r gene is associated with impaired hippocampal function in the adult IUGR male rat. *J Dev Orig Health Dis*. (2012) 3:83–91. doi: 10.1017/S2040174411000791
56. Poore KR, Forhead AJ, Gardner DS, Giussani DA, Fowden AL. The effects of birth weight on basal cardiovascular function in pigs at 3 months of age. *J Physiol*. (2002) 539:969–78. doi: 10.1013/jphysiol.2001.012926
57. Meyer JS, Novak MA. Minireview: hair cortisol: a novel biomarker of hypothalamic-pituitary-adrenocortical activity. *Endocrinology* (2012) 153:4120–7. doi: 10.1210/en.2012-1226
58. van der Staay FJ, van Zutphen JA, de Ridder MM, Nordquist RE. Effects of environmental enrichment on decision-making behavior in pigs. *Appl Anim Behav Sci*. (2017) 194:14–23. doi: 10.1016/j.applanim.2017.05.006
59. Herpin P, Damon M, Le Dividich J. Development of thermoregulation and neonatal survival in pigs. *Livest Prod Sci*. (2002) 78:25–45. doi: 10.1016/S0301-6226(02)00183-5
60. Le Dividich J, Charneca R, Thomas F. Relationship between birth order, birth weight, colostrum intake, acquisition of passive immunity and pre-weaning mortality of piglets. *Span J Agric Res*. (2017) 15:e0603. doi: 10.5424/sjar/2017152-9921
61. Held SDE, Špinka M. Animal play and animal welfare. *Anim Behav*. (2011) 81:891–9. doi: 10.1016/j.anbehav.2011.01.007
62. Singh C, Verdon M, Cronin GM, Hemsworth PH. The behaviour and welfare of sows and piglets in farrowing crates or lactation pens. *Animal* (2017) 11:1210–21. doi: 10.1017/S1751731116002573
63. Sneddon IA, Beattie VE, Dunne L, Neil W. The effect of environmental enrichment on learning in pigs. *Anim Welf*. (2000) 9: 373–83. Available Online at: https://www.ufaw.org.uk/the-ufaw-journal/animal-welfare

64. Manteuffel G, Langbein J, Puppe B. From operant learning to cognitive enrichment in farm animal housing: bases and applicability. *Anim Welf.* (2009) 18:87–95. Available Online at: https://www.ufaw.org.uk/the-ufaw-journal/animal-welfare
65. Carreras R, Mainau E, Arroyo L, Moles X, González J, Bassols A, et al. Housing conditions do not alter cognitive bias but affect serum cortisol, qualitative behaviour assessment and wounds on the carcass in pigs. *Appl Anim Behav Sci.* (2016) 185:39–44. doi: 10.1016/j.applanim.2016.09.006
66. Hellhammer DH, Wüst S, Kudielka BM. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology* (2009) 34:163–71. doi: 10.1016/j.psyneuen.2008.10.026
67. Clark CCA, D'Eath RB. Age over experience: consistency of aggression and mounting behaviour in male and female pigs. *Appl Anim Behav Sci.* (2013) 147:81–93. doi: 10.1016/j.applanim.2013.04.014
68. Colson V, Orgeur P, Courboulay V, Dantec S, Foury A, Mormède P. Grouping piglets by sex at weaning reduces aggressive behaviour. *Appl Anim Behav Sci.* (2006) 97:152–71. doi: 10.1016/j.applanim.2005.07.006
69. Camerlink I, Peijnenburg M, Wemelsfelder F, Turner SP. Emotions after victory or defeat assessed through qualitative behavioural assessment, skin lesions and blood parameters in pigs. *Appl Anim Behav Sci.* (2016) 183:28–34. doi: 10.1016/j.applanim.2016.07.007
70. Held S, Mendl M, Laughlin K, Byrne RW. Cognition studies with pigs: livestock cognition and its implication for production. *J Anim Sci.* (2002) 80:E10–7. doi: 10.2134/animalsci2002.0021881200800ES10003x
71. Mendl M, Laughlin K, Hitchcock D. Pigs in space: spatial memory and its susceptibility to interference. *Anim Behav.* (1997) 54:1491–508. doi: 10.1006/anbe.1997.0564

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Search Behavior in Goat (*Capra hircus*) Kids From Mothers Kept at Different Animal Densities Throughout Pregnancy

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Individual differences in cognitive performance are often reported but factors related to variation within species are rarely addressed. Goats *(Capra hircus)* have been subjects of many cognitive studies recently but without focus on individual variation. Among others, factors such as prenatal stress and sex of the individual have been proposed as possible explanations for individual variation in cognitive skills. We aimed to study whether prenatal environment, prenatal stress, litter size, sex, and birth weight influences search behavior skills of goat kids. Pregnant Norwegian dairy goats were exposed to different spatial allowance (namely 1.0, 2.0, or 3.0 m^2 per animal) within the commercially applied range during pregnancy and their serum cortisol levels were measured six times within this period. Twenty-six of the kids born entered a three-stage searching task with increasing difficulty when they were 6 weeks old. The tasks included finding a bucket of milk: while moving (stage 1), after moving and disappearing behind a curtain (stage 2), and moving behind a displacement device and the device moving behind a curtain while hiding the bucket (stage 3). We found that prenatal animal density had no effect on the search skills of the offspring, while kids with higher prenatal maternal cortisol levels performed better at the highest stage tested: finding an object after single invisible displacement. At this stage, singleton kids and males performed better than twins and females. Birth weight had no effect at this stage. The findings suggest that maternal cortisol in the observed range had a facilitating effect on cognitive development of goat kids.

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INTRODUCTION

Animals often follow the trajectories of prey, predators, and conspecifics; however, should the object become hidden, an animal which has the ability to mentally reconstruct the object would have a distinct advantage (1, 2). Searching behavior, observed when animals make attempts to find objects, may be a manifestation of object permanence skills (3). Object permanence is the cognitive capacity to understand that objects continue to exist even when they have disappeared from view and the ability to represent their unseen displacement trajectory. Standardized tests based on Piaget's theory of object permanence (3) are widely used in developmental and comparative research [reviewed by Jaakkola (1)]. They rely on simple non-verbal behaviors and the tasks can be adapted to suit the sensory and motor characteristics of different species (4, 5), thus making them ecologically valid. So far, most of the studies focusing on object permanence skills or search behavior in the different species place the emphasis on the highest level of cognitive performance in the species or the stage of cognitive performance achieved by the subjects. They normally discuss the results in comparison with other species [e.g., (4–10)], or in relation to the effects of differences in the testing procedure applied within species [e.g., (5, 6)]. This kind of goal often leads to involving test subjects which are fully mature, have ample experience with experimental settings, often with other types of cognitive tasks, and may be kept in an enriched environment compared to most of their conspecifics (e.g., experimental animals and human-raised individuals). Previous studies have aimed to trace the development of object permanence skills with longitudinal studies [for a review see (6)]. Although these studies indicate the potential cognitive skills of the species, they rarely focus on the striking individual variations shown in the different tasks (for example, variation in the level of skill at maturity or rate of skill development) and do not shed light on the causes for individual variation. According to a review by Thornton and Lukas (11), causes of individual variation in cognitive performance are, in general, understudied. Few studies of object permanence to date have taken into account the effects of prenatal stress and environmental enrichment during development (12, 13), yet prenatal, perinatal, and early postnatal environments were found to affect the cognitive development of animals (14). The direction of these effects depends on timing, length, and intensity of the stimulus, as well as the measurement applied [for review (15)]. Prenatal stress can have different effects in males than females [such as, sex tested, for a review see (16)]. For instance, male rat offspring showed impaired learning and memory skills after exposing pregnant mothers to restraint stress, while these cognitive skills were unchanged in the female offspring (17).

Recently, goat cognition has become a topic of interest as there is increasing evidence that goats can perform well in different learning and memory tasks. Goats are group-living, browsing animals and their behavior is greatly influenced by the way they perceive, process, and memorize information from their environment (18). They are able to use direct and indirect information to locate a food reward (19, 20), are capable of solving complex learning and memory tasks (21), can learn socially from humans in spatial tasks (22), and, given the opportunity, they will actually seek cognitive challenges (23). Previous work on goat cognition also shows that goats have excellent vision, responding not only to spatial and temporal variations of visual stimuli such as different shapes (24–27) but are also able to concurrently recall between five and seven different discrimination problems that they had previously learned and retained over several weeks (18). Good visual perception and learning skills can also be expected since they are prerequisites for the social recognition skills/abilities present in these animals [e.g., (18, 28); but see (29)]. These skills are crucial for goats which are a highly social species living in stable, individualized social groups (30, 31). Specific aspects of personality, namely sociability and exploration, were found to have an effect on cognitive performance in discrimination learning and non-associative food searching task in this species (32). In one study, adult dwarf goats, as a group, showed remarkable skills when a food item was hidden in one of two non-identical cups and the position of the cups were visible changed, crossing in the view of the animals (33). As goats are sensitive to aspects of their social environments, variations to their social environment such as group size, group stability, and space allocation (29, 31, 34–38) can have an effect on their cognition. For example, Langbein and colleagues found that a simple relocation (a normal husbandry routine) resulted in impaired (albeit minor) visual memory retrieval abilities in goats (39). This relationship between stress and cognitive abilities has already been well-described in human psychology and has been applied more recently (though to a lesser extent) to non-human animals (40, 41).

Adult Norwegian dairy goats are housed at relatively high animal densities during pregnancy and experience higher levels of social stress in terms of more agonistic interactions than goats kept at lower densities (38). In a parallel study, we found that prenatal social stress inflicted via high stocking densities negatively affected the behavioral development of goat kids (29). Prenatal stress is also known to affect cognition in animals during development [for reviews see (15, 42–44)]. Brain neurogenesis, structure, and function can be dramatically affected by the environmental conditions that an animal experiences during prenatal development (45–47). Specifically, the hippocampus has been comprehensively shown to be deleteriously affected by prenatal stress (48–53). Since the hippocampus processes learning, memory, and spatial and contextual information, it is probably the most crucial brain region in object permanence comprehension (54). Direct links between object permanence performance and prenatal stress (12, 13), frontal lobe activity (55, 56), and hair cortisol levels [an indicator of chronic stress; (57)] have been found. Interestingly, a parallel study conducted on sheep found a significantly higher total spine density in apical dendrites of the CA1 pyramidal neurons in the hippocampus of lambs born to mothers held at a treatment density of 1.0 m^2 throughout gestation than lambs from 3.0 m^2 (58). Therefore, it is likely that prenatal stress due to reduced space allowance will affect the cognitive processes of 6-week-old goat kids.

The goals of the present study were: (a) to assess the individual variation in cognitive capabilities of 6-week-old goat kids using tasks based on methods from early stages of Piaget's object permanence tasks; and (b) to examine whether prenatal stress via increased animal densities, sex of the subjects, or litter size impacted these abilities. We predicted that a high prenatal density would have negative effects on the cognitive skills of the kids and, as a result, kids born from the high prenatal density treatment would be less capable of comprehending searching tasks than kids born from the lower densities at this age. No effects of sex of the kids were predicted based on earlier studies comparing cognitive skills in goat (21).

MATERIALS AND METHODS

Animals and Treatment During Gestation

Healthy, pregnant, dehorned Norwegian dairy goats from the experimental goat herd of the Norwegian University of Life

Sciences, Ås, Norway were used in the experiment. Ethical rules stated by Forsøksdyrutvalget (the Norwegian Committee for Research Animals, www.fdu.no) which satisfy the European Union (EU) animal testing directive (86/609/EEC), the Council of Europe Convention on Laboratory Animals (ETS 123; http://conventions.coe.int/Treaty/en/Treaties/Html/123.htm) and the legislations for keeping farm animals and small ruminants in Norway (www.mattilsynet.no) were followed. In addition, all study practices were reviewed and approved by the Norwegian University of Life Sciences' Institutional Animal Care and Use Committee, The Animal Production Experimental Center.

The herd is kept on pasture in the mountains during the summer period. In September (2011), the goats were transported from pasture to the farm in Ås and were housed individually due to measurement of feed consumption in a nutritional experiment, with visual, olfactory, and limited physical access to each other, causing minimal stress in relation to isolation. Beginning in mid-October, the goats were placed into groups of 15–35. During this time, the hay and concentrate provided was reduced in order to terminate lactation. Approximately 2 weeks later, in early November, the prenatal density treatment began. The goats were not synchronized and were inseminated or mated between the end of October and mid-November. One buck was used for mating and semen from three other bucks was used for insemination. Fifty-four multiparous female goats, aged 2.8 ± 0.1 years and weighing 50.2 ± 1.0 kg were selected based on confirmation of pregnancy (by not returning to estrus and/or ultrasound investigation 3–7 weeks after mating or insemination) and expected time of parturition. These goats were evenly distributed in herds of six animals (a total of 18 animals per treatment) in densities of 1.0, 2.0, or 3.0 m^2 per animal (low-density: pens 276 × 650 cm each; medium-density: pens 189 × 632 cm, 224 × 540 cm, 276 × 435 cm; high-density: pens 189 × 317 cm, 224 × 270 cm, 224 × 270 cm, see (38) for specifics on goat allocation and the pen densities chosen). The goats were kept in stable groups and not mixed with new individuals throughout their entire pregnancy until their kids were 5 weeks old.

The treatment pens were indoors, in one of two insulated, mechanically ventilated rooms in the same building with a constant room temperature of approximately 10°C. Artificial lighting provided a 7:17 h light: dark regime with lights on at 8 a.m. in addition to natural lighting through windows along either side of the building. The pens were made of 1.5 m high solid walls (15 mm plywood) which prevented physical contact between groups. Flooring consisted of expanded metal flooring with a 60 cm solid wood area at the rear end of the pen where sawdust was laid for bedding. The pens were cleaned in the morning and afternoon after feeding. During this time, fresh bedding was added to the solid floor area. Free access to fresh water, grass silage, and salt blocks with copper were provided. The front of each pen had six eating places (one for each goat) which provided access to a common feeding trough. Silage was supplied every morning and afternoon. The goats were also fed 0.2 kg of concentrate feed every morning throughout most of the experimental period. The concentrate was gradually increased to 0.5 kg in the last part of pregnancy (from mid-January until kidding) when the feed was complemented with hay in the afternoon to stimulate the goats' digestion. At the time of expected birth, each goat was isolated from the herd until 24 h after parturition to allow for maternal care and bonding. After the 24-h post-parturition period, the goats and their kids were returned to their treatment herd. The feed openings (eating places) in the pens allowed kids to move freely between their home pen and separate kid areas which had solid wooden floors and free access to hay. The birth of the kids was staggered over a 5-week period from the beginning of February to the beginning of March.

One goat from the medium-density treatment aborted 16 days before the expected date of parturition. This goat was removed from the experimental pen for 8 days for observation, medicated, and returned to the same experimental pen until the end of the treatment. A stillborn kid was born in the medium-density treatment (most likely due to complications at birth) and the mother could not be saved. One goat from the low-density treatment gave birth to two live and two stillborn kids (the latter two were immature). Finally, a live-born singleton kid from the high-density treatment had to be removed for a parallel study. Only data from the remaining 51 litters (low-density: $n = 18$; medium-density: $n = 16$; and high-density: $n = 17$) are presented.

Goat Kids

Beginning when the kids turned 3 weeks of age, in addition to having free access to their mothers, the kids were introduced to free access to warm goat milk from a milk bucket with four artificial teats affixed to the wall in each kid area. Each kid was also handled and hand fed via a bottle affixed with an artificial teat at least once a day. This was done to teach the kids to suckle milk from a source other than their mothers and to ensure that the kids had learned that the milk bucket was a positive stimulus. By 4.5 weeks of age, the kids' access to their mothers was blocked as per standard procedure in order to begin the weaning process and following behavioral tests at 5 weeks of age for another study investigating anxiety in a novel environment and sociality (29), the testing arena became the kids' home pen (375 * 660 cm; **Figure 1**). This change was carried out prior to testing to ensure that fear or stress of a novel area did not have an effect on the cognitive performance of animals (59). At this time, the kids from all treatments were housed together in this pen and the experimenters did not have access to information about their treatments. The kids had free access to the milk buckets throughout this period until the end of testing at 7 weeks of age. Milk was not provided after 17:00 the days preceding test days but free access to water and solid food (hay, silage, and concentrate) was. Prior to testing, the test kids were herded out of the test arena to a pen in a room adjacent to the experimental room to minimize pre-test handling.

Data Collection

Birth Weight and Cortisol Levels

Individual kids were sexed and ear-tagged within 12–36 h after birth. At the same time, their weights were measured on an electric scale.

Blood was taken and processed as described in Vas et al. (38). Blood samples were collected from the mothers of the

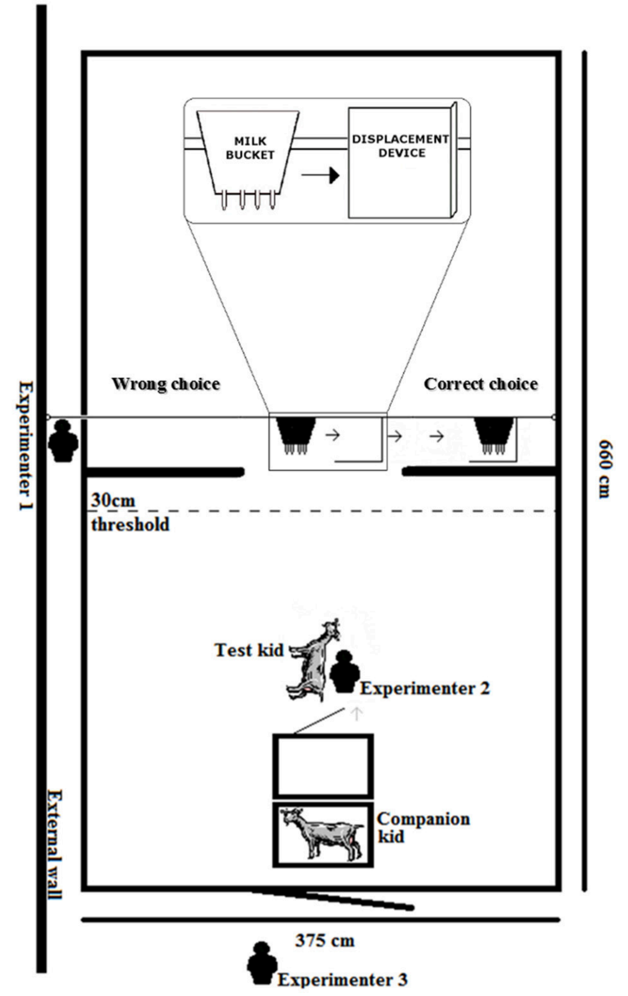

FIGURE 1 | The experimental set up of the test arena. The test during the Invisible displacement task with three experimenters, the test kid, a companion kid remaining in its cage and the milk bucket sliding first into the L-shaped box then both moving behind the right screen. A choice to the left would be considered a fail while a choice to the right would be considered correct.

kids via venipuncture in the jugular vein three times during pregnancy (in the first, second, and last third of pregnancy), on two consecutive days in each period before the morning feeding (between 7:00 and 8:30). Blood samples from the kids were collected when the kids turned 3 weeks of age, on two consecutive days. All the sample collections were performed with minimal disturbance of the goats, gentle handling, and by trained and experienced assistants. Samples were collected into heparinized tubes (Vacutainer, Becton and Dickinson, Leuven, Belgium). After storage at −4°C for 2 days, samples were centrifuged at 3,000 × g for 15 min. After removing the plasma, samples were stored again at −20°C. The samples were analyzed for cortisol by the Hormon Laboratory of the Oslo University Hospital, using electrochemiluminescence immunoassay (ECLIA, Roche Cobas Cortisol assay) by using Roche Elecsys E immunoanalyzer system (Roche Diagnostics, Mannheim, Germany). For more details see Vas et al. (38). The cortisol values of the six samples from the mother goats and the two values from the kids were averaged resulting in one single cortisol value for each goat.

The "Search Test"

The week the kids turned 6 weeks of age, they were presented with a series of "object permanence tests" which were a modification of the tests used by Gagnon and Doré (60). The testing period was staggered over a 5-week period as there were 5 weeks separating the first birth from the last. The kids were separated into five groups accordingly.

Apparatus

The design of the apparatus was adapted to the morphological characteristics of goat kids (60, 61). An artificial milk bucket (27 * 30 * 20 cm), identical in appearance with the one the kids were familiar with, was used as the target object. Two 125 * 114 cm opaque curtains (hereafter referred to as screens) were hung across the test arena (332 cm from the entrance door and 328 cm from the back wall; **Figure 1**). These provided hiding locations on either side for the milk bucket but with a 125 cm opening so that the kids had full view and access to the bucket when the bucket was positioned in the center. Forty-five centimeters behind the screens, metal wiring was strung in a loop (65 cm above the floor) with pulleys on either side allowing the milk bucket to be hung and drawn to either side behind the screens (**Figure 1**). The metal wire holding the milk bucket went through a hole in the wall of the test arena allowing a researcher to pull the bucket to the left or right, manipulating the movement of the milk bucket, while remaining hidden. This controlled for any inadvertent experimenter cues given through the manipulation of the bucket (such as choose the last or first box touched by either the experimenter or displacement device; see (5) for a review). The bottom of the milk bucket was approximately 30 cm from the floor, roughly the height of a goat udder. For the invisible displacement task, a 36 * 38 * 24 cm L-shaped wooden frame, which was completely covered with the same opaque fabric as the curtains, was attached on a third line so that the bucket would move behind the frame, catch the "L" and drag the frame to either side (**Figures 1**, **2**). From the kids' perspectives, it appeared as though the milk bucket completely disappeared behind or into the displacement device. The L-shaped wooden frame (the displacer) could be attached from the top or flipped and attached by the bottom, effectively switching which side it would be dragged. As the apparatus was stored in the same building as the goats and both screens had milk splashed on them, olfactory cues were not a concern (1, 5, 60, 62–64).

"Habituation" trials

Before the first day of testing, habituation trials were conducted to allow the kids to become familiarized with the apparatus (10, 63). The habituation trials were done in pairs as goats are highly sensitive to separation (65–67) and a companion allowed the kids to feel more secure while becoming accustomed to the experimental set up. Competition between the two kids to gain access to the milk should not have been an issue as the milk bucket had four artificial teats and four kids were often seen sucking simultaneously before testing. Kids were also

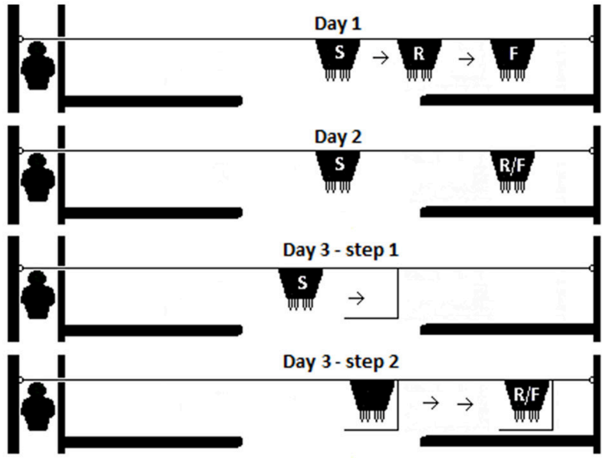

FIGURE 2 | The test procedure for each day. Visible displacement with early search was tested on Day 1. On Day 2, kids were tested for their performance at the Visible displacement task. On the final day of testing (Day 3), a simple Invisible displacement task was conducted in two steps (Day 3—step 1 and Day 3—step 2). The start of each trial (S), the release of the test kid (R), and in the final position (F) of the bucket are indicated for each stage tested.

never observed “forfeiting” a teat to another kid; therefore, the most dominant kids did not monopolize the bucket during the habituation trials. The milk bucket was placed in the center of the room, equal distance from either screen with the artificial teats facing toward the cages so that the test kid had full view of both the bucket and the teats facing the kid. During the habituation trials, the cages were placed side by side parallel to the apparatus and the kids were placed in the cages. Both kids were released simultaneously and allowed 2 min from release to suck from the bucket. They were then collected, returned to the cages for 30 s and re-released. Three habituation trials were conducted in a row. If a kid latched onto the artificial teat and attempted to suck milk, they were marked as sucking. As Pepperberg (5) stated, in order for Piagetian tasks to accomplish the goal of determining levels of cognitive processing, the animal must be motivated to engage in the task at hand. Cognitive performances can be highly influenced by motivations such as the subjective value of the reward (68), being distracted by other events, and current stress level of the animal. The habituation trials were used to allow the most motivated kid to be chosen from twin litters as the test kid and exclude the non-motivated kids from further testing. Twenty-five kids were exposed to habituation trials from the low-density treatment, of which, 14 sucked. In the medium-density, 11 out of the 28 kids sucked, and in the high-density, 14 of the 22 kids sucked. If both kids from a twin litter were marked as sucking for the same amount of trial, then one kid was chosen at random to be the test kid of that litter. Twenty-six kids from the 51 litters were used as test kids (for details about number of males and females, singleton and twin kids see **Table 2**).

Procedure

In the test arena, two cages were placed side by side perpendicularly to the apparatus to house the test kid (in the cage closer to the apparatus) and a familiar “companion” (not a test kid; **Figure 1**). A “companion kid,” that was housed together with the test kid, was used to avoid the test kid performing behaviors as a result of separation from group mates (59). The “companion kid” was changed after approximately three kids were tested.

Three experimenters were used to administer the search tests (**Figure 1**). Experimenter 1 sat outside the test arena. Experimenter 1 was able to pull the metal wire holding the milk bucket to the left or right while remaining hidden. Half of the trials were conducted pulling the bucket behind the left screen and half behind the right in a semi-randomized order to control for side preference (for example, R-L-L-R-L-R-R-L-R-L; see **Table 1** for the number of trials conducted at each stage). The pattern of the trials was alternated with the first trial beginning to the right or to the left for every other kid for all stages. Experimenter 1 sat in this spot prior to the test kid entering the room with a list stating the predetermined order of which side the bucket was to be pulled. This allowed Experimenter 1 to remain hidden until the last trial was completed and the test kid was taken out of the test room. Therefore, the test kid should not have been influenced by the presence of Experimenter 1 and Experimenter 1 had no knowledge of which kid was being tested. Experimenter 2 was responsible for handling the kid. She placed the kid in the cage, removed, and restrained the kid by gently holding its body with the head oriented toward the apparatus but preventing it from moving toward the apparatus before the predetermined release time. After the kid’s release, Experimenter 2 stood motionless with her eyes averted from the test kid and the apparatus until a choice was made, she then retreated to the corner so that the kid was neither attracted to nor received any inadvertent behavioral cues from her. Upon release, the test kid was allowed 30 s to make a choice. Once the kid passed the 30 cm threshold before the screens (**Figure 1**), a choice was considered to have been made. The test kid had to walk behind the correct screen to access the bucket. If the kid sucked on the milk bucket within the 30 s of the trial, it was allowed to suck for 10 s regardless of whether the choice was considered a successful choice or not. This allowed for reinforcement of the stimulus, prevented any behaviors of frustration which may have arisen upon not receiving an expected milk reward, and, most importantly, prevented any potential negative feedback of the procedure where each failure to find the object behind a selected screen could result in an extinction trial for the association. The kid was collected and placed in the cage after it had sucked 10 s or until 30 s had past if the kid did not suck, whichever occurred first. The test kid spent 30 s in its cage in between each trial. Experimenter 2 manipulated a stopwatch timing the trials, collected the kid, and placed it in the cage between trials. Experimenter 3 stood outside the test arena and recorded whether a kid was successful on each trial (see “Scoring” for passing criterion).

Four test scenarios were administered over three consecutive days within the same week with the stages increasing in difficulty each day [**Figure 2**; (4, 61, 63)]. Three tests were visible displacement problems (Days 1 and 2) and one was invisible displacement (Day 3). On all days, the following pre-test procedure was conducted: The cages were placed adjacent

TABLE 1 | Passing criterion for each stage tested.

Stage tested (test day)	Number of trials conducted	Passing criterion (number of correct choices)	Probability[a]	Probability of reaching stage[b]
VDE[c] (Day 1)	10	8	0.05	0.05
VD[d] (Day 2)	11	8	0.11	<0.001
IVD[e] (Day 3)	10	8	0.05	<0.0001

[a] Probability of reaching success criterion at current stage by chance.
[b] Probability of a single kid advancing through the previous stages and reaching success criterion at current stage by chance.
[c] The Visible displacement with early search task.
[d] The Visible displacement task.
[e] The Invisible displacement task.

TABLE 2 | Sample sizes, means and interquartile range (IQR) of success rates at different stages.

		Sum	Prenatal maternal density[a]			Sex		Litter size	
			High	Medium	Low	Females	Males	Singleton	Twin
Visible early	N[b]	26	10	7	9	9	17	8	18
	Mean[c]	0.915	0.940	0.914	0.887	0.864	0.941	0.988	0.882
	Lower quartile	0.900	0.925	0.850	0.900	0.800	0.900	0.900	0.825
	Higher quartile	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Visible	N[b]	22	8	7	8	7	16	8	15
	Mean[c]	0.800	0.854	0.721	0.815	0.846	0.780	0.831	0.783
	Lower quartile	0.730	0.798	0.640	0.745	0.820	0.708	0.730	0.685
	Higher quartile	0.910	0.910	0.820	0.933	0.910	0.910	0.910	0.910
Invisible	N[b]	16	6	4	6	6	10	5	11
	Mean[c]	0.829	0.800	0.875	0.828	0.783	0.857	0.900	0.797
	Lower quartile	0.800	0.800	0.825	0.800	0.800	0.800	0.800	0.700
	Higher quartile	1000.000	0.875	1.000	0.950	0.800	1.000	1.000	1.000

[a] Space allowance of 1.0 (High), 2.0 (Medium), or 3.0 (Low) m^2 per animal provided to pregnant goat mothers.
[b] Number of subjects participating.
[c] Mean of the success rate, which is successful choices divided by number of trials when a choice was made.

to the apparatus at the entrance of the experimental pen. The milk bucket was placed in the center of the room filled with milk equidistant from either screen. The "companion kid" was brought into the test room, allowed to roam freely in the test arena, and suck from the milk bucket for 2 min and then placed in the cage. This was done in order to facilitate calm behavior of the "companion kid." Afterwards, the test kid was brought into the test room and placed into the adjacent cage. Each test kid (in the cage closest to the apparatus (**Figure 1**) was tested individually. The test kid was gently removed from the cage, held facing the apparatus directly in front of the cage, and released without moving the bucket to allow the kid to suck on the bucket as a warm-up trial. After 30 s, the test kid was collected and put into the cage. This pre-test warm-up trial was conducted to reinforce the milk bucket as a stimulus.

Single visible displacement with initiation of the search movement ("Visible displacement with early search") When 60 s had passed after the kid was placed in the cage after the initial warm-up trial, Experimenter 2 removed the kid from the cage and held it as during the warm-up trial. The bucket was slid horizontally and the test kid was released *as* the bucket began to go behind the screen (**Figure 2**: Day 1).

Single visible displacement ("Visible displacement") If the Visible displacement with early search stage permanence criteria was achieved (see later criteria), the kids were tested in Single visible displacement tasks. The testing procedure was identical to Day 1 except that the bucket was slid horizontally until fully concealed *before* releasing the test kid. The kid was released after all movement of the bucket was completed (**Figure 2**: Day 2). The pattern was changed and an 11th trial was added which allowed for three consecutive trials to be conducted behind the same screen (for example, R-L-L-R-L-R-R-L-L-L-R). Care was taken that the number of consecutive trials to one side was less than five to control for trial and error/place learning (69, 70). Again, the pattern of the trials was alternated for every other kid.

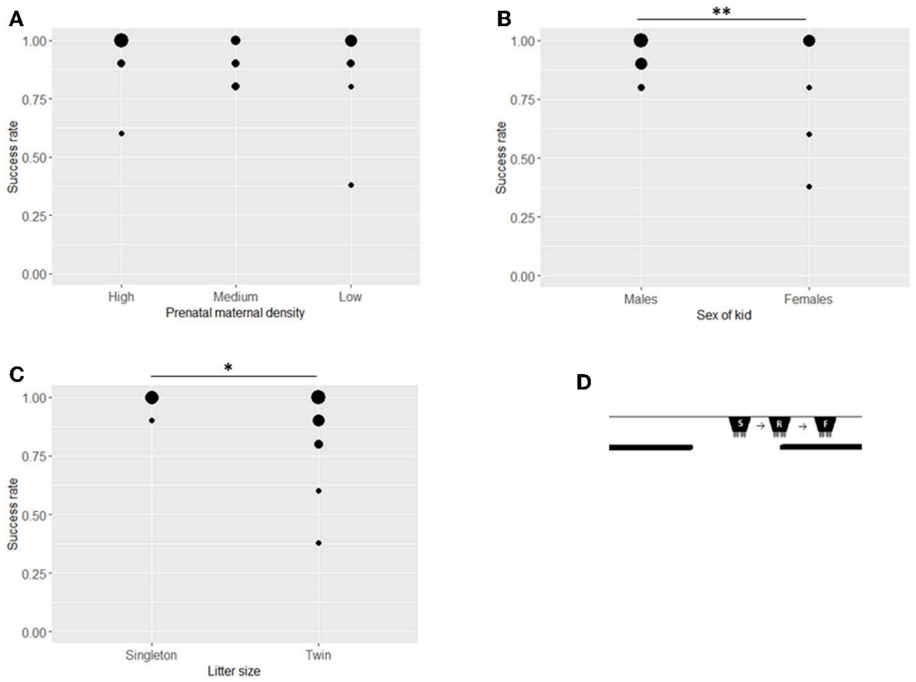

FIGURE 3 | Success rates of kids in the Visible early task. Success rates (number of successful choices divided by number of trials when a choice was made) in the Visible early task by treatment **(A)**, sex **(B)**, and litter size **(C)**. The position of the bucket at the start of each trial (S), the release of the test kid (R) and in the final position (F) in the Visible early task **(D)**. Asterisks indicate significant differences between groups (at $^{*}P < 0.05$ and $^{**}P < 0.005$ level). Size of dots refer to number of overlapping data points.

Simple invisible displacement task ("Invisible displacement") Based on traditional invisible displacement tasks and invisible transposition tasks administered to dogs (*Canis familiaris:* (7, 71), cats (*Felis catus:* (71), and jackdaws (*Corvus monedula:* (72) the milk bucket was slid horizontally into a displacement device (the L-shaped box) so that it was completely hidden in full view of the test kid (**Figure 2**: Day 3—step 1). The bucket remained in the displacement device while it (with bucket behind) was slid, in full view of the kid, behind either test screen (**Figure 2**: Day 3—Step 2). The kid was released when both the bucket and the displacement device had come to a complete stop.

Scoring Kids were deemed successful on a trial if the orientation of their head was toward the side the bucket was positioned when it was at the threshold 30 cm before the screen (**Figure 1**) and sucked from the milk bucket. A threshold of 30 cm before the screen was chosen as that was the last point where the test kid could not see the bucket behind either screen if they were positioned in the center as they approached the screen. If a kid suddenly looked the opposite way of its trajectory (as if to check behind the other screen) and/or suddenly changed trajectory from the wrong side to the correct side after the 30 cm threshold, it was recorded as an incorrect choice as it was assumed the kid simply saw the bucket. Similar to other studies, a response was scored as incorrect when the kid chose the wrong screen. A trial was scored as "no choice" when the kid did not make a response within 30 s of release and the trial was not included in the total number of trials given for analysis. Although it may be that "no choice" was made due to lack of motivation or because of uncertainty about the location of the reward, these two causes could not be distinguished behaviorally. In total, there were only 8 trials where kids did not make a choice. Two portable cameras (SONY HDR-SR12) were set up at either side of the test arena to record behaviors. In addition to recording behaviors through direct observations, choices were confirmed via video analyses by one experimenter. In the case of discrepancies recorded through direct observations and video analyses (0.4% of trials fell under this category), a minimum of two experimenters reanalyzed the footage. All experimenters were blind to the treatment condition of each kid.

The number of trials conducted at any stage were kept to a minimum to control for the possibility of training or learning [see e.g., (5, 73)] and to avoid saturation. Ten trials were conducted each day with the exception of the extra trial on Day 2, when eleven trials were given. In all conditions when a choice was made the probability of chance success was 0.5. Therefore, according to the exact binomial tests (57, 60, 74), passing criterion was as in **Table 1**. Consequently, subjects were failed and testing was discontinued if three trials were scored as "incorrect" in total at a single stage. Since the search tasks were administered with the stages increasing in difficulty (4, 61, 63) it was unlikely that if a kid failed a stage it would advance to succeed at the next, more difficult stage (60, 70); therefore, if a kid failed at a stage then the test was terminated for the kid.

Two kids which had successfully performed at the "Visible displacement task" were not tested on the "Invisible displacement.” Direct observations had marked them as failing at the visible displacement; however, upon later analyses of the videos, it was determined that they had, in fact, successfully completed that stage. Overall, 664 trials were included in analyses (not including warm-up trials), 567 of which were scored as “successful.”

Statistical Method

R statistics software (Version 3.3.3) was employed to run all statistical models. Birth weight, maternal and kid cortisol values were standardized by twice of the standard deviation to deal with skewed distribution (the mean was subtracted from the value and then, divided by twice of the standard deviation of the sample).

First, effect of sex and litter size on birth weight were tested with Kruskal-Wallis tests. Generalized linear models were applied (with binomial distribution, log link) to evaluate the effect of sex (female or male kid) and litter size (singleton or twin) on the number of successful choices compared to the total number of trials when the kid made a choice. In addition, the treatment (with three levels: low, medium, or high prenatal density), standardized values of the following, continuous variables: blood cortisol level of the mothers, that of the kids, and the birth weight of the kids were added as covariates to the models.

RESULTS

Males had higher birth weights compared to females ($Chi^2 = 7.172$, df = 1, $P = 0.007$; mean ± SE for males: 3.69 ± 0.14 kg, females: 3.00 ± 0.20 kg). Kids born as singletons or twins had comparable weights at birth ($Chi^2 = 1.123$, $df = 1$, $P = 0.289$, singletons: 3.59 ± 0.24 kg. twins: 3.39 ± 0.15 kg).

As an overview **Table 2** presents the success rate (number of successful choices divided by number of trials when a choice was made) of the kids in the different treatment groups, sexes, and litter sizes at the three stages together with sample sizes.

Visible Displacement With Early Search

In this first stage (**Figure 3D**), 26 goat kids participated, with an average success rate of 91.5% (**Table 2**). Prenatal maternal density had no significant effect on the success rate of early search of visible displacement of the kids ($ß = 0.016$, $SE = 0.328$, $z = 0.049$, $P = 0.960$, **Figure 3A**) but males performed better than females at this stage ($ß = -1.985$, $SE = 0.665$, z = -2.985, $P = 0.002$, **Table 2**, **Figure 3B**) and singleton kids had a higher success rate than kids from twin litters ($ß = -2.429$, SE = 1.082, $z = -2.245$, $P = 0.025$, **Table 2**, **Figure 3C**). Neither the cortisol level of the mother (26.06 ± 1.81 nmol/l, $ß = 0.198$, $SE = 0.289$, $z = 0.685$, $P = 0.494$) nor that of the kid (29.52 ± 7.15 nmol/l, $ß = 0.016$, $SE = 0.247$, $z = 0.064$, $P = 0.949$) affected the success rate significantly but kids with higher birth weights had lower success rates ($ß = -1.218$, SE = 0.388, $z = -3.140$, $P = 0.002$).

Visible Displacement

In sum, twenty-three kids were tested in the visible displacement task (**Figure 4D**). Prenatal maternal density had no significant effect on success rate in the visible displacement task ($ß = -0.146$, $SE = 0.216$, $z = -0.675$, $P = 0.450$, **Figure 4A**). The two sexes and kids from singleton vs. twin litters showed a similar performance (sex: $ß = -0.058$, $SE = 0.546$, $z = -0.106$, $P = 0.915$, litter size: $ß = -0.508$, $SE = 0.372$, $z = -1.365$, $P = 0.172$; **Figures 4B,C**). Maternal and kid cortisol levels and birth weight did not affect the performance (maternal cortisol: $ß = 0.319$, $SE = 0.209$, $z = 1.526$, $P = 0.127$, kid cortisol: $ß = -0.043$, $SE = 0.176$, $z = -0.244$, $P = 0.808$, weight: $ß = -0.263$, $SE = 0.223$, $z = -1.178$, $P = 0.239$).

Invisible Displacement

Sixteen kids participated in Invisible displacement tasks (**Figure 5D**). There was no effect of prenatal maternal density on performance ($ß = -0.514$, $SE = 0.378$, $z = -1.359$, $P = 0.174$, **Table 2**, **Figure 5A**). Males had a higher success rate compared to females ($ß = -1.736$, $SE = 0.766$, $z = -2.265$, $P = 0.024$, **Table 2**, **Figure 5B**) and singleton kids performed better than kids from twin litters ($ß = -1.110$, $SE = 0.566$, $z = -1.961$, $P = 0.050$, **Table 2**, **Figure 5C**). A higher level of maternal cortisol level was associated with a higher success rate at this stage ($ß = 0.677$, $SE = 0.325$, $z = 2.082$, $P = 0.037$). Kid cortisol level or birth weight did not have any significant effect on success rate in the invisible displacement task (kid cortisol: $ß = 0.321$, $SE = 0.235$, $z = 1.363$, $P = 0.173$, birth weight: $ß = -0.202$, $SE = 0.338$, $z = -0.597$, $P = 0.550$).

DISCUSSION

The adult goats (mothers of the test kids in the present study) kept in the high density showed more agonistic (offensive and defensive) and slightly less socio-positive interactions compared to goats kept in the low density in a previous study (38). Interestingly, blood cortisol levels of the mothers were found to be comparable in the different density treatments in our earlier study (38), possibly indicating individual variation in line with individual responses to the varying space allowance or other factors. Kids of these mothers were the subjects of the present study. However, contrary to what was predicted, the applied prenatal density treatment as an expected cause of prenatal stress did not affect the performance of the kids at any level of the cognitive tasks.

Although maternal space allowance, *per se*, had no effect, kids whose mothers had higher cortisol values during pregnancy performed better in the Invisible displacement search task. Goat kids were subjected to three different tasks. In the Visible displacement task with early search, kids could initiate searching behavior when the target object was only partly hidden and partly seen. At this stage, simply following the trajectory of the object is enough to be successful and no memory is required. At the next stage, during the Visible displacement task, kids were restrained for a longer time and could begin to approach only when the target object was no longer visible. In this task, although some level of working memory is required, subjects approaching the place where the target disappeared could easily find the target. In the Invisible displacement task, however, the target disappeared at first behind an occluding barrier and moved further in occlusion to its final destination. To be successful

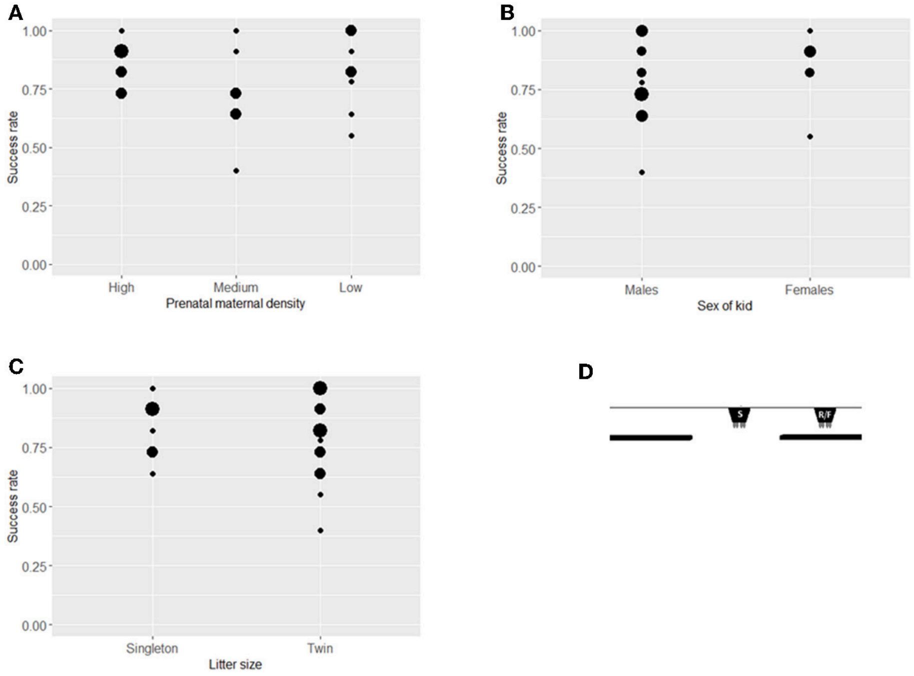

FIGURE 4 | Success rates of kids in the Visible displacement task. Success rates (number of successful choices divided by number of trials when a choice was made) in the Visible displacement task (mean ± SE) by treatment **(A)**, sex **(B)**, and litter size **(C)**. The position of the bucket at the start of each trial (S), the release of the test kid (R) and in the final position (F) in the Visible displacement task **(D)**. There was no difference between groups at $P < 0.05$ level. Size of dots refer to number of overlapping data points.

in this task, kids had to follow the trajectory of the movement further compared to where the target disappeared from view. This stage is regarded as the most cognitively demanding in our setting because of the longer time gap between the last point at which the target is seen and the initiation of movement as well as the longer distance between the last point at which the target is visible and its final location.

There are several possible factors which can, to some degree, explain individual variations in cognitive skills. Prenatal stress, stress experienced by pregnant mother during pregnancy, and its effect on offspring are understudied and the results, mainly in rats, are complex. Different kinds of prenatal stress were found to increase [e.g., (74)] or, in the majority of studies, decrease cognitive performance in the offspring [e.g., (49, 51, 75–78)]. The significance of the effect also depends on the type of cognitive skill and the specific methods applied to evaluate it (79). An intense, long-lasting prenatal stress in the period of pregnancy when the HPA axis is developing (timing depending on species) is hypothesized to impair cognitive development and skills in the offspring [for reviews in humans see (80–84), reviews in animals e.g., (14–16, 85)]. At the same time, prenatal maternal stress was found to facilitate development of cognitive skills in some studies. For instance, language skills were improved in prenatally stressed 11 years old girls compared to non-stressed girls (86). Improved cognitive skills were also found in children exposed to stress prenatally in another human study (87). The explanation for these contradictory effects of prenatal stress most likely lies in the timing of the stressor as mid-gestational stress was associated with improved learning in two studies on male rat offspring (88, 89).

The findings of no effect of density but a link to maternal cortisol suggest that prenatally elevated maternal cortisol levels could lead to enhanced cognitive skills in goat kids. The interpretation of blood serum cortisol level as indicator of stress has to be done with caution due to multiple factors affecting corticosteroide levels (90). Still, it is often used as indicator of acute or chronic stress in animals under experimental conditions, where experimental setup (e.g., multiple sampling) and animal management (e.g., strict feeding regimes) can control for some of the possible environmental (e.g., feeding time) and animal-related (e.g., species, breed, lactation status, age of animals) factors leading to variation in corticosteroide levels. Producing offspring with better cognitive abilities in challenging, moderately stressful, and unpredictable environments may be adaptive from an evolutionary point of view as the offspring may be more capable of handling cognitive challenges. Furthermore, the inconsistency in our results regarding treatment and maternal cortisol suggests that individual stress in pregnant goats may be indicated more directly by maternal cortisol levels than space allowance measured at group level. The applied animal densities in this study were in range of the common practice in Norway and complied with space allowance regulations of goats kept indoors within the European Union. The experimental densities were aimed to model commercial conditions and

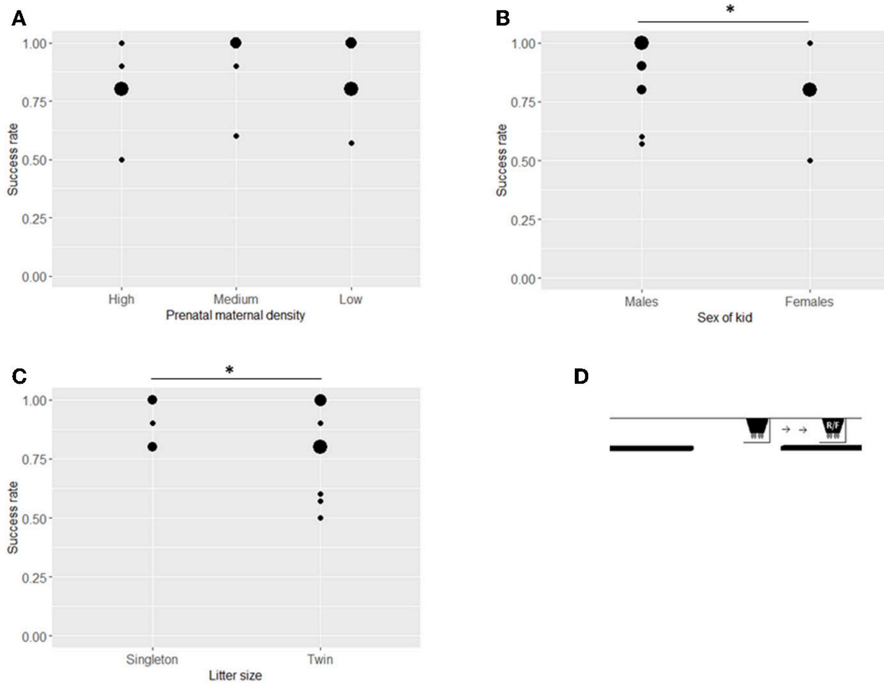

FIGURE 5 | Success rates of kids in the Invisible displacement task. Success rates (number of successful choices divided by number of trials when a choice was made) in the Invisible displacement task (mean ± SE) by treatment **(A)**, sex **(B)**, and litter size **(C)**. The position of the bucket at the start of each trial (S), the release of the test kid (R) and in the final position (F) in the Invisible displacement task **(D)**. Asterisks indicate significant differences between groups (at $P < 0.05$ level). Size of dots refer to number of overlapping data points.

suggested that even the highest animal density represented a manageable, moderate stress level for goats. Similar managed environments may impact individuals differently based on, for instance, the group composition including behavioral profile of group members, individual relationships, the animals' rank position, and coping style at least when the environment is not too restrictive. Therefore, our findings emphasize the importance of individual animal-based welfare indicators compared to resource-based measures.

An additional cause of observable individual variations in cognitive skills may be the sex of the animals. In our study, males had higher success rates both at the low-level Visible displacement with early search and at the highest level tested in the Invisible displacement tasks compared to females, while the performance of the two sexes was comparable in the Visible displacement task. Males weighed more at birth compared to females but animals with lighter birth weight performed better in the Visible displacement with early search task. Therefore, weight cannot be a simple explanation for the found difference between the two sexes. We can speculate that the difference at least in the Visible displacement with early search task, where there was no need for high cognitive abilities, may be caused by a stronger motivation to feed in males or higher stress levels in females. Considering the better performance of males in the Invisible displacement task, several studies indicate that there are sex differences in specific aspects of cognitive skills. The presence, absence, or direction of differences are highly dependent not only on the skill but on the methods used to evaluate (77, 79). Regarding cognition in goats specifically, no sex differences were reported in adult goats learning and recalling a new object manipulation task (21) and no differences were found between males and females in visual discrimination and a non-associative cognition task in another study (32). Furthermore, female and male goat kids were equally unable to discriminate between a familiar and an unfamiliar test kid in a social discrimination task (29). This study may be the first reporting sex differences in cognitive skills in goats. Here, we raise several hypotheses for this observed sex difference, particularly in the Invisible displacement task. First, that the observed superior performance of males over females is a true difference in cognitive skills in these tasks. Most of the published goat cognition studies involve subjects from a single sex [e.g., only males (91, 92), only females (18, 19, 23, 25, 28, 93–95)] or both sexes but their performance is not compared (20, 21, 26, 96). In earlier object permanence studies, no sex differences were reported to our knowledge possibly partly because this comparison was not the focus in the majority of studies. Second, cognitive performance may be influenced by motivation (68). All subjects were exposed to habituation trials to pick the highest motivated kid from twin litters and to involve only motivated kids before the test trials. At the same time, motivation could change from day to day according to actual needs, for instance, depending on hunger, thirst, tiredness, or stimuli immediately before testing. Third, while a mild level of stress (arousal) may enhance cognitive performance, high levels of stress can impair attention span and other relevant skills (40, 41). In an earlier study, female and male goat kids of comparable age were found to have similar basal cortisol values and responded similarly in a social test (35). A social isolation and a social test performed on the subjects in the present study were reported in a separate paper (29). There, males showed lower levels of sociality (measured as approaching stimuli kids in an unfamiliar arena) but there was no difference between males and females in the number of vocalizations or escape attempts made in a social isolation situation. Fourth, cognitive impairment caused by prenatal stress can be reversed by early postnatal environment, e.g., by secure attachment between infant and mother in humans (76), better mothering skills in rodents (97), or environmental enrichment in rats (98, 99). It may be that even though males and females were exposed to a similar amount of prenatal stress and similar postnatal environments, the prenatal stress had different effect on cognitive skills in females than in males. This was shown, for instance, in passive avoidance learning of rats of mothers exposed to restraint stress during pregnancy (77).

The blood serum cortisol level of kids at 3 weeks age collected by venipuncture did not predict later performance of these kids in the search tasks. A relationship was predicted, as both blood sampling and testing procedures included handling of the test kids and some restraint. Although blood was collected by experienced assistants and the measurement was aimed to indicate basal cortisol levels, we cannot exclude that the young kids, less used to human handling reacted to the procedure and cortisol levels were raised by the actual time of sampling. In theory, it is possible that individuals more reactive to an environmental stimuli (kids with higher blood cortisol values) would be more aroused in moderately stressful situations and this increased arousal would lead to better cognitive performance or, if the situation causes a high stress reaction, cognitive performance could be impaired. By the time of testing, kids were regularly handled by the experimenters and habituation and warm-up trials were planned to eliminate unnecessary stress. Therefore, we assume that the stress reactivity of the goat kids played a minimal role in successfulness in the search tasks and this can be an explanation to the lack of relationship between blood serum cortisol values at 3 weeks of age and cognitive performance at 6 weeks of age.

Higher performance was shown in the Visible displacement with early search task by kids with lower birth weight. This task probably did not require a high level of cognitive performance as the search behavior was initiated when the target object was still visible for the kids. Therefore, the difference in the success rate might be a consequence of difference in motivation or stress level in the kids. Kids born as singleton were often found to be heavier at birth [e.g., (88, 100–102)] and at later ages, up to until 90 days old, than kids born in twin litters [e.g., (89)] but no difference was found here in birth weight between singletons and kids born as twins similarly to an earlier study (35). Furthermore, singleton kids gain more weight on a daily average compared to kids from twin litters (89, 103), but to our knowledge, there is no information about whether higher weight gain of singletons is paralleled with higher motivation to feed. Baxter et al. (88) report differences between singletons and twins in frequency of sucking attempts made, singleton kids having more contacts at the udder and more unsuccessful sucking attempts compared to twin kids. In the present study, singleton kids performed better both in the Visible displacement with early search task and in the Invisible displacement task. Presumably, singleton kids may receive more nutrients [singleton lambs are usually heavier at birth and have lower mortality rate compared to twin lambs (104)] and maternal care from their mothers, and these nutritional and social benefits can lead to enhanced cognitive development. Although ewes with twin litters show higher total maternal investment indicated by more high-pitched vocalization (indicating anxious behavior) and more grooming behavior (indicating better caretaking) early postnatally (104, 105), which may be comparable to goat mothers with singleton or twin litters, the maternal effort is less then doubled leading to lower maternal care per offspring in twin litters compared to kids born as singleton offspring. Early postnatal environment, and especially maternal care and maternal style has an important effect on behavioral development in many mammalian species and higher maternal care can facilitate stress-resilience and cognitive skills (16, 97, 104–108).

In this study, we aimed to place emphasis on individual differences in cognitive skills, namely search behavior in goat kids, and to evaluate the effects of factors which can contribute to the variation in this skill. We demonstrated variations in search behavior at different levels in young goats, a new candidate species for cognitive research. Goat kids were tested at a specific age of development: 6 weeks old. In commercial herds in Norway, kids are separated from their mothers and solid food is introduced in an increasing amount at this age. Therefore, motivation for exploration, neophilia, and memory and learning skills are crucial in coping in farm conditions.

In summary, prenatal maternal animal densities did not affect performance of 6-week-old goat kids in a search task, but elevated maternal cortisol levels during pregnancy contributed to better cognitive skills in the offspring. Males and kids from singleton litters outperformed females and kids from twin litters at higher levels of searching tasks and searched more successfully at earlier stages.

AUTHOR CONTRIBUTIONS

JV contributed to the planning and design of the experiment, data collection, statistical analysis, and manuscript writing. RMC contributed to planning and design of the experiment, main responsibilities lay in data collection, and manuscript writing. ILA contributed to the planning of the experiment and manuscript writing as the leader of the project. All authors have read and approved the last version of the manuscript.

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REFERENCES

1. Jaakkola K. Do animals understand invisible displacement? A critical review. *J Comp Psychol.* (2014) 128:225–39. doi: 10.1037/a0035675
2. Dumas C. Figurative and spatial information and search behaviour in dogs (*Canis familiaris*). *Behav Processes* (1998) 42:101–6.
3. Piaget J. *The Construction of Reality in the Child*. New York, NY: International Universities Press (1954).
4. Collier-Baker E, Davis JM, Suddendorf T. Do dogs (*Canis familiaris*) understand invisible displacement? *J Comp Psychol.* (2004) 118:421–33. doi: 10.1037/0735-7036.118.4.421
5. Pepperberg IM. The value of the Piagetian framework for comparative cognitive studies. *Anim Cogn.* (2002) 5:177–82. doi: 10.1007/s10071-002-0148-5
6. Gómez JC. Species comparative studies and cognitive development. *Trends Cogn Sci.* (2005) 9:118–25. doi: 10.1016/j.tics.2005.01.004
7. Fiset S, Plourde V. Object permanence in domestic dogs (*Canis lupus familiaris*) and gray wolves (*Canis lupus*). *J Comp Psychol.* (2013) 127:115–27. doi: 10.1037/a0030595
8. Güntürkün O, Ströckens F, Scarf D, Colombo M. Apes, feathered apes, and pigeons: differences and similarities. *Curr Opin Behav Sci.* (2017) 16:35–40. doi: 10.1016/j.cobeha.2017.03.003
9. Rooijakkers EF, Kaminski J, Call J. Comparing dogs and great apes in their ability to visually track object transpositions. *Anim Cogn.* (2009) 12:789–96. doi: 10.1007/s10071-009-0238-8
10. de Blois ST, Novak MA, Bond M. Object permanence in orangutans (*Pongo pygmaeus*) and squirrel monkeys (*Saimiri sciureus*). *J Comp Psychol.* (1998) 112:137–52. doi: 10.1037//0735-7036.112.2.137
11. Thornton A, Lukas D. Individual variation in cognitive performance: developmental and evolutionary perspectives. *Philos Trans R Soc B Biol Sci.* (2012) 367:2773–83. doi: 10.1098/rstb.2012.0214
12. Gamer E, Gallant D, Grunebaum H. An evaluation of 1-year-olds on a test of object permanence. *Arch Gen Psychiatry* (1976) 33:311–7. doi: 10.1001/archpsyc.1976.01770030029004
13. Schneider ML. Prenatal stress exposure alters postnatal behavioral expression under conditions of novelty challenge in rhesus monkey infants. *Dev Psychobiol.* (1992) 25:529–40.
14. Chapillon P, Patin V, Roy V, Vincent A, Caston J. Effects of pre- and postnatal stimulation on developmental, emotional, and cognitive aspects in rodents: a review. *Dev Psychobiol.* (2002) 41:373–87. doi: 10.1002/dev.10066
15. Weinstock M. The long-term behavioural consequences of prenatal stress. *Neurosci Biobehav Rev.* (2008) 32:1073–86. doi: 10.1016/j.neubiorev.2008.03.002
16. Weinstock M. Gender differences in the effects of prenatal stress on brain development and behaviour. *Neurochem Res.* (2007) 32:1730–40. doi: 10.1007/s11064-007-9339-4
17. Zagron G, Weinstock M. Maternal adrenal hormone secretion mediates behavioural alterations induced by prenatal stress in male and female rats. *Behav Brain Res.* (2006) 175:323–8. doi: 10.1016/j.bbr.2006.09.003
18. Langbein J, Siebert K, Nuernberg G. Concurrent recall of serially learned visual discrimination problems in dwarf goats (*Capra hircus*). *Behav Process.* (2008) 79:156–64. doi: 10.1016/j.beproc.2008.07.004
19. Nawroth C, von Borell E, Langbein J. Exclusion performance in dwarf goats (*Capra aegagrus hircus*) and sheep (*Ovis orientalis aries*). *PLoS ONE* (2014) 9:e93534. doi: 10.1371/journal.pone.0093534
20. Kaminski J, Riedel J, Call J, Tomasello M. Domestic goats, *Capra hircus*, follow gaze direction and use social cues in an object choice task. *Anim Behav.* (2005) 69:11–8. doi: 10.1016/j.anbehav.2004.05.008
21. Briefer EF, Haque S, Baciadonna L, McElligott AG. Goats excel at learning and remembering a highly novel cognitive task. *Front Zool.* (2014) 11:20. doi: 10.1186/1742-9994-11-20
22. Nawroth C, Baciadonna L, McElligott AG. Goats learn socially from humans in a spatial problem-solving task. *Anim Behav.* (2016) 121:123–9. doi: 10.1016/j.anbehav.2016.09.004
23. Langbein J, Siebert K, Nürnberg G. On the use of an automated learning device by group-housed dwarf goats: do goats seek cognitive challenges? *Appl Anim Behav Sci.* (2009) 120:150–8. doi: 10.1016/j.applanim.2009.07.006
24. Provenza FD, Lynch JJ, Burritt EA, Scott CB. How goats learn to distinguish between novel foods that differ in postingestive consequences. *J Chem Ecol.* (1994) 20:609–24.
25. Roitberg E, Franz H. Oddity learning by African dwarf goats (*Capra hircus*). *Anim Cogn.* (2004) 7:61–7. doi: 10.1007/s10071-003-0190-y
26. Blakeman NE, Friend TH. Visual discrimination at varying distances in Spanish goats. *Appl Anim Behav Sci.* (1986) 16:279–83. doi: 10.1016/0168-1591(86)90120-6
27. Baldwin BA. Operant studies on shape discrimination in goats. *Physiol Behav.* (1979) 23:455–9.
28. Keil NM, Imfeld-Mueller S, Aschwanden J, Wechsler B. Are head cues necessary for goats (*Capra hircus*) in recognising group members? *Anim Cogn.* (2012) 15:913–21. doi: 10.1007/s10071-012-0518-6
29. Chojnacki RM, Vas J, Andersen IL. The effects of prenatal stocking densities on the fear responses and sociality of goat (*Capra hircus*) kids. *PLoS ONE* (2014) 9:e94253. doi: 10.1371/journal.pone.0094253
30. O'Brien PH. Feral goat social organization: a review and comparative analysis. *Appl Anim Behav Sci.* (1988) 21:209–21. doi: 10.1016/0168-1591(88)90110-4
31. Patt A, Gygax L, Wechsler B, Hillmann E, Palme R, Keil NM. The introduction of individual goats into small established groups has serious negative effects on the introduced goat but not on resident goats. *Appl Anim Behav Sci.* (2012) 138:47–59. doi: 10.1016/j.applanim.2012.02.012
32. Nawroth C, Prentice PM, McElligott AG. Individual personality differences in goats affect their performance in visual learning and non-associative cognitive tasks. *Behav Processes* (2017) 134:43–53. doi: 10.1016/j.beproc.2016.08.001
33. Nawroth C, von Borell E, Langbein J. Object permanence in the dwarf goat (*Capra aegagrus hircus*): perseveration errors and the tracking of complex movements of hidden objects. *Appl Anim Behav Sci.* (2015) 167:20–6. doi: 10.1016/j.applanim.2015.03.010
34. Andersen IL, Bøe KE. Resting pattern and social interactions in goats—The impact of size and organisation of lying space. *Appl Anim Behav Sci.* (2007) 108:89–103. doi: 10.1016/j.applanim.2006.10.015
35. Andersen IL, Roussel S, Ropstad E, Braastad BO, Steinheim G, Janczak AM, Jørgensen GM, Bøe KE. Social instability increases aggression in groups of dairy goats, but with minor consequences for the goats' growth, kid production and development. *Appl Anim Behav Sci.* (2008) 114:132–48. doi: 10.1016/j.applanim.2008.01.007
36. Andersen IL, Tønnesen H, Estevez I, Cronin GM, Bøe KE. The relevance of group size on goats' social dynamics in a production environment. *Appl Anim Behav Sci.* (2011) 134:136–43. doi: 10.1016/j.applanim.2011.08.003
37. Barroso FG, Alados CL, Boza J. Social hierarchy in the domestic goat: effect on food habits and production. *Appl Anim Behav Sci.* (2000) 69:35–53. doi: 10.1016/s0168-1591(00)00113-1
38. Vas J, Chojnacki R, Kjøren MF, Lyngwa C, Andersen IL. Social interactions, cortisol and reproductive success of domestic goats (*Capra hircus*) subjected to different animal densities during pregnancy. *Appl Anim Behav Sci.* (2013) 147:117–26. doi: 10.1016/j.applanim.2013.04.009
39. Langbein J, Nürnberg G, Puppe B, Manteuffel G. Self-controlled visual discrimination learning of group-housed dwarf goats (*Capra hircus*):

behavioral strategies and effects of relocation on learning and memory. *J Comp Psychol.* (2006) 120:58–66. doi: 10.1037/0735-7036.120.1.58

40. Dantzer R. Can farm animal welfare be understood without taking into account the issues of emotion and cognition? *J Anim Sci.* (2002) 80:E1–9. doi: 10.2134/ANIMALSCI2002.0021881200800ES10002X
41. Held S, Mendl M, Laughlin K, Byrne RW. Cognition studies with pigs: livestock cognition and its implication for production. *J Anim Sci.* (2002) 80:E10–7. doi: 10.2527/ANIMALSCI2002.0021881200800ES10003X
42. Braastad BO, Osadchuk L V, Lund G, Bakken M. Effects of prenatal handling stress on adrenal weight and function and behaviour in novel situations in blue fox cubs (*Alopex lagopus*). *Appl Anim Behav Sci.* (1998) 57:157–69.
43. Charil A, Laplante DP, Vaillancourt C, King S. Prenatal stress and brain development. *Brain Res Rev.* (2010) 65:56–79. doi: 10.1016/j.brainresrev.2010.06.002
44. Kaiser S, Sachser N. The effects of prenatal social stress on behaviour: mechanisms and function. *Neurosci Biobehav Rev.* (2005) 29:283–94. doi: 10.1016/j.neubiorev.2004.09.015
45. van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. *Nat Rev Neurosci.* (2000) 1:191–8. doi: 10.1038/35044558
46. Lema SC, Hodges MJ, Marchetti MP, Nevitt GA. Proliferation zones in the salmon telencephalon and evidence for environmental influence on proliferation rate. *Comp Biochem Physiol Part A Mol Integr Physiol.* (2005) 141:327–35. doi: 10.1016/j.cbpb.2005.06.003
47. Ickes BR, Pham TM, Sanders LA, Albeck DS, Mohammed AH, Granholm AC. Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain. *Exp Neurol.* (2000) 164:45–52. doi: 10.1006/exnr.2000.7415
48. Clayton NS. Memory and the hippocampus in food-storing birds: a comparative approach. *Neuropharmacology* (1998) 37:441–52.
49. Coulon M, Wellman CL, Marjara IS, Janczak AM, Zanella AJ. Early adverse experience alters dendritic spine density and gene expression in prefrontal cortex and hippocampus in lambs. *Psychoneuroendocrinology* (2013) 38:1112–21. doi: 10.1016/j.psyneuen.2012.10.018
50. Lemaire V, Koehl M, Le Moal M, Abrous DN. Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. *Proc Natl Acad Sci USA.* (2000) 97:11032–7. doi: 10.1073/pnas.97.20.11032
51. Jarrard LE. What does the hippocampus really do? *Behav Brain Res.* (1995) 71:1–10. doi: 10.1016/0166-4328(95)00034-8
52. Wu J, Song TB, Li YJ, He KS, Ge L, Wang LR. Prenatal restraint stress impairs learning and memory and hippocampal PKCbeta1 expression and translocation in offspring rats. *Brain Res.* (2007) 1141:205–13. doi: 10.1016/j.brainres.2007.01.024
53. Yang J, Han H, Cao J, Li L, Xu L. Prenatal stress modifies hippocampal synaptic plasticity and spatial learning in young rat offspring. *Hippocampus* (2006) 16:431–6. doi: 10.1002/hipo
54. Pollok B, Prior H, Güntürkün O. Development of object permanence in food-storing magpies (*Pica pica*). *J Comp Psychol.* (2000) 114:148–57. doi: 10.1037//0735-7036.114.2.148
55. Baird AA, Kagan J, Gaudette T, Walz KA, Hershlag N, Boas DA. Frontal lobe activation during object permanence: data from near-infrared spectroscopy. *Neuroimage* (2002) 16:1120–6. doi: 10.1006/nimg.2002.1170
56. Diamond A, Zola-Morgan S, Squire LR. Successful performance by monkeys with lesions of the hippocampal formation on AB and object retrieval, two tasks that mark developmental changes in human infants. *Behav Neurosci.* (1989) 103:526–37. doi: 10.1037/0735-7044.103.3.526
57. Dettmer AM, Novak MFSX, Novak MA, Meyer JS, Suomi SJ. Hair cortisol predicts object permanence performance in infant rhesus macaques (*Macaca mulatta*). *Dev Psychobiol.* (2009) 51:706–13. doi: 10.1002/dev.20405.Hair
58. Averós X. Space allowance in dairy sheep: effects on lamb brain morphology and behaviour. In: *Animal Welfare Indicators 3rd Annual Conference*. Prague.
59. Gieling ET, Nordquist RE, van der Staay FJ. Assessing learning and memory in pigs. *Anim Cogn.* (2011) 14:151–73. doi: 10.1007/s10071-010-0364-3
60. Gagnon S, Doré FY. Cross-sectional study of object permanence in domestic puppies (*Canis familiaris*). *J Comp Psychol.* (1994) 108:220–32.
61. Dumas C, Doré FY. Cognitive development in kittens (*Felis catus*): a cross-sectional study of object permanence. *J Comp Psychol.* (1989) 103:191–200. doi: 10.1037/0735-7036.103.2.191
62. Fedor A, Skollár G, Szerencsy N, Ujhelyi M. Object permanence tests on gibbons (*Hylobatidae*). *J Comp Psychol.* (2008) 122:403–17. doi: 10.1037/0735-7036.122.4.403
63. Deppe AM, Wright PC, Szelistowski WA. Object permanence in lemurs. *Anim Cogn.* (2009) 12:381–8. doi: 10.1007/s10071-008-0197-5
64. Gagnon S, Doré FY. Search behavior in various breeds of adult dogs (*Canis familiaris*): object permanence and olfactory cues. *J Comp Psychol.* (1992) 106:58–68.
65. Tomlinson KA, Price EO. The establishment and reversibility of species affinities in domestic sheep and goats. *Anim Behav.* (1980) 28:325–30. doi: 10.1016/S0003-3472(80)80040-6
66. Anderson US, Maple TL, Bloomsmith MA. A close keeper-nonhuman animal distance does not reduce undesirable behavior in contact yard goats and sheep. *J Appl Anim Welf Sci.* (2004) 7:59–69. doi: 10.1207/s15327604jaws0701_4
67. Price EO, Thos J. Behavioral responses to short-term social isolation in sheep and goats. *Appl Anim Ethol.* (1980) 6:331–9.
68. Miller HC, Bender C. The breakfast effect: dogs (*Canis familiaris*) search more accurately when they are less hungry. *Behav Processes* (2012) 91:313–7. doi: 10.1016/j.beproc.2012.09.012
69. Salwiczek LH, Emery NJ, Schlinger B, Clayton NS. The development of caching and object permanence in Western Scrub-Jays (*Aphelocoma californica*): which emerges first? *J Comp Psychol.* (2009) 123:295–303. doi: 10.1037/a0016303
70. Doré FY, Dumas C. Psychology of animal cognition: Piagetian studies. *Psychol Bull.* (1987) 102:219–33. doi: 10.1037/0033-2909.102.2.219
71. Doré FY, Fiset S, Goulet S, Dumas M-C, Gagnon S. Search behavior in cats and dogs: interspecific differences in working memory and spatial cognition. *Anim Learn Behav.* (1996) 24:142–9.
72. Ujfalussy DJ, Miklósi Á, Bugnyar T. Ontogeny of object permanence in a non-storing corvid species, the jackdaw (*Corvus monedula*). *Anim Cogn.* (2013) 16:405–16. doi: 10.1007/s10071-012-0581-z
73. Jaakkola K, Guarino E, Rodriguez M, Erb L, Trone M. What do dolphins (*Tursiops truncatus*) understand about hidden objects? *Anim Cogn.* (2010) 13:103–20. doi: 10.1007/s10071-009-0250-z
74. Hoffmann A, Rüttler V, Nieder A. Ontogeny of object permanence and object tracking in the carrion crow, Corvus corone. *Anim Behav.* (2011) 82:359–67. doi: 10.1016/j.anbehav.2011.05.012
75. Leroy H, Depiereux E, Giffroy J-M, Diederich C. Effect of prenatal environment on learning abilities in puppies and in adult dogs. *J Vet Behav.* (2009) 4:253. doi: 10.1016/j.jveb.2009.06.008
76. Bergman K, Sarkar P, Glover V, O'Connor TG. Maternal prenatal cortisol and infant cognitive development: moderation by infant-mother attachment. *Biol Psychiatry* (2010) 67:1026–32. doi: 10.1016/j.biopsych.2010.01.002
77. Gué M, Bravard A, Meunier J, Veyrier R, Gaillet S, Recasens M, et al. Sex differences in learning deficits induced by prenatal stress in juvenile rats. *Behav Brain Res.* (2004) 150:149–57. doi: 10.1016/S0166-4328(03)00250-X
78. Modir F, Elahdadi Salmani M, Goudarzi I, Lashkarboluki T, Abrari K. Prenatal stress decreases spatial learning and memory retrieval of the adult male offspring of rats. *Physiol Behav.* (2014) 129:104–9. doi: 10.1016/j.physbeh.2014.02.040
79. Szuran TF, Pliska V, Pokorny J, Welzl H. Prenatal stress in rats: effects on plasma corticosterone, hippocampal glucocorticoid receptors, and maze performance. *Physiol Behav.* (2000) 71:353–62. doi: 10.1016/s0031-9384(00)00351-6
80. Kingston D, McDonald S, Austin M-P, Tough S. Association between prenatal and postnatal psychological distress and toddler cognitive development: a systematic review. *PLoS ONE* (2015) 10:e0126929. doi: 10.1371/journal.pone.0126929
81. Tarabulsy GM, Pearson J, Vaillancourt-Morel M-P, Bussières E-L, Madigan S, Lemelin J-P, et al. Meta-analytic findings of the relation between maternal prenatal stress and anxiety and child cognitive outcome. *J Dev Behav Pediatr.* (2014) 35:38–43. doi: 10.1097/DBP.0000000000000003

82. Van den Bergh BRH, van den Heuvel MI, Lahti M, Braeken M, de Rooij SR, Entringer S, et al. Prenatal developmental origins of behavior and mental health: the influence of maternal stress in pregnancy. *Neurosci Biobehav Rev.* (2017). doi: 10.1016/j.neubiorev.2017.07.003. [Epub ahead of print].
83. Talge NM, Neal C, Glover V. Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? *J Child Psychol Psychiatry Allied Discip.* (2007) 48:245–61. doi: 10.1111/j.1469-7610.2006.01714.x
84. Dunkel Schetter C, Tanner L. Anxiety, depression and stress in pregnancy: implications for mothers, children, research, and practice. *Curr Opin Psychiatry* (2012) 25:141–8. doi: 10.1097/YCO.0b013e3283503680.Anxiety
85. Weinstock M. The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain Behav Immun.* (2005) 19:296–308. doi: 10.1016/j.bbi.2004.09.006
86. King S, Dancause K, Turcotte-Tremblay A-M, Veru F, Laplante DP. Using natural disasters to study the effects of prenatal maternal stress on child health and development. *Birth Defects Res Part C* (2012) 96:273–88. doi: 10.1002/bdrc.21026
87. Li J, Robinson M, Malacova E, Jacoby P, Foster J, van Eekelen A. Maternal life stress events in pregnancy link to children's school achievement at age 10 years. *J Pediatr.* (2013) 162:483–9. doi: 10.1016/j.jpeds.2012.09.007
88. Baxter EM, Mulligan J, Hall SA, Donbavand JE, Palme R, Aldujaili E, et al. Positive and negative gestational handling influences placental traits and mother-offspring behavior in dairy goats. *Physiol Behav.* (2016) 157:129–38. doi: 10.1016/j.physbeh.2016.02.001
89. Zhang C-Y, Zhang Y, Xu D-Q, Li X, Su J, Yang L-G. Genetic and phenotypic parameter estimates for growth traits in Boer goat. *Livest Sci.* (2009) 124:66–71. doi: 10.1016/j.livsci.2008.12.010
90. Mormède P, Andanson S, Aupérin B, Beerda B, Guémené D, Malmkvist J, et al. Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. *Physiol Behav.* (2007) 92:317–39. doi: 10.1016/j.physbeh.2006.12.003
91. Langbein J, Nürnberg G, Manteuffel G. Visual discrimination learning in dwarf goats and associated changes in heart rate and heart rate variability. *Physiol Behav.* (2004) 82:601–9. doi: 10.1016/j.physbeh.2004.05.007
92. Langbein J, Siebert K, Nuernberg G, Manteuffel G. The impact of acoustical secondary reinforcement during shape discrimination learning of dwarf goats (*Capra hircus*). *Appl Anim Behav Sci.* (2007) 103:35–44. doi: 10.1016/j.applanim.2006.04.019
93. Langbein J. Investigations on training, recall and reversal learning of a Y-maze by dwarf goats (*Capra hircus*): the impact of lateralisation. *Behav Processes* (2012) 89:304–10. doi: 10.1016/j.beproc.2011.12.013
94. Kaminski J, Call J, Tomasello M. Goats' behaviour in a competitive food paradigm: evidence for perspective taking? *Behaviour* (2006) 143:1341–56. doi: 10.1163/156853906778987542
95. Pascual-Alonso M, María GA, Sepúlveda WS, Villarroel M, Aguayo-Ulloa L, Galindo F, et al. Identity profiles based on social strategies, morphology, physiology, and cognitive abilities in goats. *J Vet Behav.* (2013) 8:458–65. doi: 10.1016/j.jveb.2013.05.003
96. Duvaux-Ponter C, Rigalma K, Roussel-Huchette S, Schawlb Y, Ponter AA. Effect of a supplement rich in linolenic acid, added to the diet of gestating and lactating goats, on the sensitivity to stress and learning ability of their offspring. *Appl Anim Behav Sci.* (2008) 114:373–94. doi: 10.1016/j.applanim.2008.01.021
97. Maccari S, Piazza P V, Kabbaj M, Barbazanges A, Simon H, Le Moal M. Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. *J Neurosci.* (1995) 15:110–6.
98. Yang J, Hou C, Ma N, Liu J, Zhang Y, Zhou J, et al. Enriched environment treatment restores impaired hippocampal synaptic plasticity and cognitive deficits induced by prenatal chronic stress. *Neurobiol Learn Mem.* (2007) 87:257–63. doi: 10.1016/j.nlm.2006.09.001
99. Xie T, Wang W, Jia L, Mao Z, Qu Z, Luan S, et al. Environmental enrichment restores cognitive deficits induced by prenatal maternal seizure. *Brain Res.* (2012) 1470:80–8. doi: 10.1016/j.brainres.2012.06.034
100. Acín C, Martín-Burriel I, Monleón E, Lyahyai J, Pitarch JL, Serrano C, et al. Prion protein gene variability in Spanish goats. Inference through susceptibility to classical scrapie strains and pathogenic distribution of peripheral PrP(sc.). *PLoS ONE* (2013) 8:e61118. doi: 10.1371/journal.pone.0061118
101. Adams GJ, Johnson KG. Sleep-wake cycles and other night-time behaviours of the domestic dog *Canis familiaris*. *Appl Anim Behav Sci.* (1993) 36:233–48. doi: 10.1016/0168-1591(93)90013-F
102. Adams GJ, Johnson KG. Sleep and nocturnal behaviour in domestic dogs. *Aust Vet Pract.* (1991) 21:144–7.
103. Liu W, Zhang Y, Zhou Z. Adjustment for non-genetic effects on body weight and size in Angora goats. *Small Rumin Res.* (2005) 59:25–31. doi: 10.1016/j.smallrumres.2004.11.006
104. Dwyer CM, Lawrence AB. Variability in the expression of maternal behaviour in primiparous sheep: effects of genotype and litter size. *Appl Anim Behav Sci.* (1998) 58:311–30. doi: 10.1016/S0168-1591(97)00148-2
105. Dwyer CM, McLean KA, Deans LA, Chirnside J, Calvert SK, Lawrence AB. Vocalisations between mother and young in sheep: effects of breed and maternal experience. *Appl Anim Behav Sci.* (1998) 58:105–19. doi: 10.1016/S0168-1591(97)00113-5
106. Richetto J, Calabrese F, Meyer U, Riva MA. Prenatal versus postnatal maternal factors in the development of infection-induced working memory impairments in mice. *Brain Behav Immun.* (2013) 33:190–200. doi: 10.1016/j.bbi.2013.07.006
107. (FEDIAF) TEPFIF. *Nutritional Guidelines for Complete and Complementary Pet Food for Cats and Dogs* (2016). p. 1–100.
108. O'Connell NE, Beattie VE, Sneddon IA, Breuer K, Mercer JT, Rance KA, et al. Influence of individual predisposition, maternal experience and lactation environment on the responses of pigs to weaning at two different ages. *Appl Anim Behav Sci.* (2005) 90:219–32. doi: 10.1016/j.applanim.2004.08.012

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Better, Not Just More—Contrast in Qualitative Aspects of Reward Facilitates Impulse Control in Pigs

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Delay-of-gratification paradigms, such as the famous "Marshmallow Test," are designed to investigate the complex cognitive concepts of self-control and impulse control in humans and animals. Such tests determine whether a subject will demonstrate impulse control by choosing a large, delayed reward over an immediate, but smaller reward. Documented relationships between impulsive behavior and aggression in humans and animals suggest important implications for farm animal husbandry and welfare, especially in terms of inadequate social behavior, tail biting and maternal behavior. In a preliminary study, we investigated whether the extent of impulse control would differ between quantitatively and qualitatively different aspects of reward in pigs. Twenty female piglets were randomly divided into two groups, with 10 piglets each. After a preference test to determine individual reward preference among six different food items, a discrimination test was conducted to train for successful discrimination between different amounts of reward (one piece vs. four pieces) and different qualitative aspects of reward (highly preferred vs. least preferred food item). Then, an increasing delay (2, 4, 8, 16, 24, 32 s) was introduced for the larger/highly preferred reward. Each piglet could choose to get the smaller/least preferred reward immediately or to wait for the larger/highly preferred reward. Piglets showed clear differences in their preference for food items. Moreover, the "quality group" displayed faster learning in the discrimination test (number of sessions until 90% of the animals completed the discrimination test: "quality group"—3 days vs. "quantity group"—5 days) and reached a higher level of impulse control in the delay-of-gratification test compared to the "quantity group" (maximum delay that was mastered: "quality group"—24 s vs. "quantity group"—8 s). These results demonstrate that impulse control is present in piglets but that the opportunity to get a highly preferred reward is more valued than the opportunity to get more of a given reward. This outcome also underlines the crucial role of motivation in cognitive test paradigms. Further investigations will examine whether impulse control is related to traits that are relevant to animal husbandry and welfare.

Keywords: impulsivity, delay of gratification, delay choice task, discrimination learning, reward learning, preference test, motivation, pigs

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INTRODUCTION

During their daily life, animals face many decisions, including social conflict, predator avoidance, feeding and mating. Therefore, time is a critical factor in each choice situation, resulting in an intertemporal choice problem, e.g., fight now and risk injury or withdraw and fight later, or, leave a food patch now after eating a small amount of food, or stay longer to obtain more (Stevens and Stephens, 2010, p. 361 ff.). Studies on intertemporal choice are closely related to studies on self-control or impulse control aimed at investigating the trade-off between far-sighted decisions and short-term temptations (Logue, 1988; Berns et al., 2007). Impulse control as a cognitive function is part of the neural network of inhibitory control, which is in turn a core element of executive functions (Diamond, 2013). Impulse control enables behavior control as well as withstanding internal predispositions and external temptations in order to adopt appropriate behaviors for different situations (Bari and Robbins, 2013). To investigate impulse control, diverse paradigms have been used to address two different forms of impulsivity, impulsive action (motor impulsivity) and impulsive choice/decision-making (cognitive impulsivity; Monterosso and Ainslie, 1999; Winstanley et al., 2006).

The main approach to studying impulsive choice uses tests involving delay of gratification/reward, wherein subjects have the choice between a more immediate but smaller reward and a delayed, larger reward (Leonardi et al., 2012; Beran, 2015). Two different types of tasks, namely, delay choice and delay maintenance, can be used to investigate different components of delayed gratification (Addessi et al., 2013; Paglieri et al., 2013). Delay choice tasks are directly linked to intertemporal choice (see above), giving the subjects the choice between a smaller, sooner reward and a larger, delayed reward with no possibility to change their choice (Stevens and Mühlhoff, 2012). Such intertemporal choice or self-control tasks have been successfully used in a wide range of species, such as insects (e.g., Cheng et al., 2002), fish (e.g., Mühlhoff et al., 2011), birds (e.g., Ainslie, 1974; Vick et al., 2010), rodents (e.g., Tobin and Logue, 1994; Brunner and Hen, 1997), and primates (e.g., Stevens et al., 2005; Rosati et al., 2007). These studies show that in addition to humans, animals can also choose to wait for a larger/better outcome, at least up to a certain delay. With increasing delay, the choice of the larger/better reward usually decreases in the form of a hyperbolic function, which is suggested to be due to devaluation or discounting of the reward (Reynolds et al., 2002; Madden and Bickel, 2010). Thus, delay discounting results in mainly impulsive choices across a wide range of species (Stevens and Stephens, 2010). The same authors point to a critical aspect: “In an evolutionary approach, a preference for immediate rewards appears not impulsive but adaptive in naturally occurring behavioral situations. [...] Decision mechanisms adapted to a common foraging problem may not work as well in an artificial laboratory situation.” (Stevens and Stephens, 2010, p. 383). In contrast, in delay maintenance tasks, such as the accumulation task or the exchange task, subjects are able to take a smaller reward at any time or can choose to wait until the delivery of the larger/better reward (Beran, 2002; Evans and Beran, 2007; Beran et al., 2016). Studies on sustaining the choice of a delay to reward in animals, mainly on birds and primates, show that they are able to withstand temptations for up to several minutes in favor of a larger or more preferred reward (Stevens et al., 2011; Evans et al., 2012; Addessi et al., 2013; Auersperg et al., 2013; Hillemann et al., 2014; Koepke et al., 2015).

Many studies have investigated the features and mechanisms of intertemporal choice and delayed gratification in terms of species ecology and the evolution and economy of decision making in humans and animals (e.g., Loewenstein et al., 2003; Stevens, 2014; Beran, 2015). Several decades ago, Walter Mischel performed studies on delay-of-gratification in children, which later became known as the “Marshmallow Test” (Mischel et al., 1989). The children could choose to either take one marshmallow immediately (small reward) or to wait for the return of the experimenter (delay) to get two marshmallows (large reward). Subsequent studies revealed a surprising relationship between the degree of impulse control as a child and academic and social competence as well as stress coping and attention abilities later in life (Mischel et al., 2011). Several studies in humans and animals have also suggested that individual variation in impulsivity is a behavioral or even a personality trait (Kirkpatrick et al., 2014; Velázquez-Sánchez et al., 2014; Ciardelli et al., 2017) that is partly related to aggressive behavior (Brunner and Hen, 1997; Cervantes and Delville, 2007; Coppens et al., 2014) and affected by stressors (Metcalfe and Mischel, 1999). This line of research is valuable to be extended to a group of species, not much investigated so far, for which cognitive research on self-control and possible links to aggression and stress coping behavior would be beneficial in terms of health, welfare and animal protection. In farm animals, many problems still exist on-farm in terms of maternal behavior, injurious behavior (e.g., aggression, tail biting, feather pecking) and stereotypes (Keeling and Jensen, 2017). It can be noted that, despite all the animals in a group have nearly the same environmental conditions, not all animals show these behavioral problems. Thus, individual variation in impulse control seems to be a promising approach to understanding individual variation in stress coping behavior and mechanisms that finally lead to behavioral problems in farm animals, such as pigs.

Pigs, as omnivores, exhibit a flexible foraging ecology and occupy a wide variety of habitats (Leaper et al., 1999). Moreover, their brains show a developed prefrontal cortex comparable to that of humans and non-human primates (Kornum and Knudsen, 2011), providing them with pronounced cognitive abilities (Zebunke et al., 2011; Marino and Colvin, 2015; Düpjan et al., 2017). The prefrontal cortex is also linked to a capacity for impulse control (Fuster, 2015). Thus, pigs have a certain capacity for impulse control, which has been previously explored in only one study (Melotti et al., 2013). In this study, the authors investigated the behavior of pigs in a delay choice paradigm. They found that the pigs were willing to wait for 12 to 50 s for a larger reward. The aim of the present experiment was to expand knowledge and to examine the effect of different contrasts in reward (different amount [quantitative aspect] vs. differentially preferred items [qualitative aspects]) on the level of impulsivity/impulse control in a delay maintenance task in pigs. This is a pilot study for a larger research project investigating the phenomenon of impulse control in pigs and its relationships with personality, social behavior, emotional coping and other cognitive capacities, as well as its possible impact on animal husbandry and welfare.

ANIMALS, MATERIALS, AND METHODS

Ethical Statement

All animal care and experimental procedures were performed in accordance with the German welfare requirements for farm animals and the ASAB/ABS Guidelines for the Use of Animals in Research (Anonymous, 2016). All experimental procedures 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. no. 7221.3-2-016/16).

Subjects and Housing

The experiment was conducted between May and July of 2016. We used 20 female German Landrace piglets, born in April of 2016 and raised in scan farrowing pens in the experimental pig unit of the Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany (Stabenow and Manteuffel, 2002). The piglets were weaned at 28 days of age and transported to an experimental room in the same unit. The piglets were randomly divided into two groups of 10 piglets each and were moved into adjacent pens measuring 3 × 5 m. The pens contained several nipple drinkers with water *ad libitum* and a trough with an animal to feeding space ratio of 2:1. The piglets received 90% of their recommended feeding amount divided into two portions of 45%: once around midday, after the experiment, and the rest at ∼3:30 p.m. (Lindermayer et al., 1994). This procedure was chosen to ensure motivation for participation in the experiment due to empty troughs in the morning. The piglets could receive the other 10% of the recommended feeding amount by consuming the available rewards during the experiment. The partially slatted floor was cleaned daily and covered with a mixture of chopped straw, wood shavings and hemp pellets. The pens each contained four or five balls of hard rubber, fixed with metal chains and used as environmental enrichment. At the beginning, directly after weaning, the pens were equipped with heat lamps to help the piglets maintain their body temperature. A few weeks later, with rising outdoor temperatures, the heat lamps were removed. In addition to natural daylight, the experimental room was artificially illuminated from 7:30 a.m. to 4:00 p.m. During the experiment, one piglet in one group was treated due to lameness, and one piglet in the other group was treated due to reddish urine.

Experimental Setup

The experimental pen was located near the holding pens of the piglets so that it was not necessary to transport the piglets a long way and isolate them socially during the individual experiments. The experimental pen was a combination of two single pens and measured 2 × 2 m (**Figure 1**). Opposite to the entrance, the pen wall was replaced by a metal grid with two openings of ∼20 cm each (made by removing one bar in each case). The openings were wide enough that a piglet could put its head and ears through. The width of the openings could be adjusted for the growing piglets by metal bars, inserted separately, that could be fixed at the top to prevent their removal by the piglets. The distance between the openings was ∼1 m. A wooden sliding board (1.20 × 0.25 m) with two metal puppy feeding dishes (diameter: 20 cm) inserted at the level of the openings was installed behind the grid to present the rewards [see **Figure 1**, see also (Nawroth et al., 2015)]. The experimenter was positioned behind the sliding board to manually operate it. Two wooden sliding doors were attached between the grid and the sliding board so that access to the rewards could be regulated. With open sliding doors and a pulled-back sliding board, the piglets could not reach the rewards. Six different food items, differing in qualitative aspects (i.e, visual and olfactory cues), were used during the experiment as potential rewards: standard food pellets, uncooked pasta (Penne), chocolate M&M's®, pieces of fresh apple (Jonagold), pieces of cheese (young Gouda) and chicken sausage.

Experimental Procedure

One experimenter managed the experimental protocol weekdays between 8 and 11:30 a.m. All the piglets were individually marked. The animals of one group ("quantity") started all the tests first, followed by the animals in the other group ("quality"). Within each group, the order of the piglets was pseudorandomized for each session.

Habituation

The experimental procedure started 4 days after weaning with a stepwise habituation of the piglets to the experimental pen, the experimental setup and the food rewards. For this purpose, the experimenter was present, the sliding doors opened, the sliding board was fixed toward the grid and the dishes were filled with standard food pellets (day 1–3) or a mixture of food rewards (day 4–7). As a first step, in two sessions (day 1), five piglets per group could explore the experimental pen for 10 min. This process was followed by two sessions per day (day 2–5), with two piglets exploring the experimental pen for 5 min. The first session of day 6 followed the same procedure (two piglets for 5 min). In the second session, each piglet could individually explore the experimental pen for 2 min, as was the procedure for the two sessions on day 7. After each of the two sessions per day and before regular feeding, a mixture of all the food rewards was placed in the standard feeding troughs of both groups to habituate the piglets to the unknown food and to prevent potential neophobia (Roura et al., 2008). The habituation was followed by a preference test, lasting 3 days. Thereafter, another habituation session followed to refamiliarize the animals with the experimental setup. Then, the function of the sliding doors (initially closed) and the sliding board (initially pulled back) were introduced. Each piglet completed 12 trials with one piece of its most preferred food item as a reward in one dish. In each trial, one of the sliding doors was opened and, when the piglet put its head and ears through the opening (i.e., it made a choice), the sliding board with the reward dish was pushed toward the piglet. After the reward was consumed or after 30 s with no choice made (timeout), the sliding door was closed, and

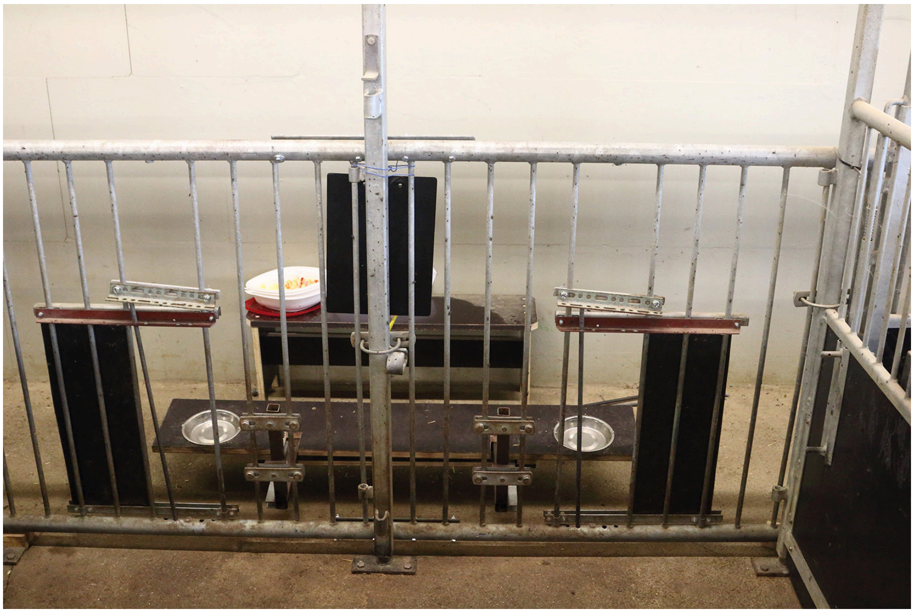

FIGURE 1 | Experimental pen with the experimental setup: grating with closable openings to the reward dishes fixed in a sliding board. The experimenter was positioned behind the grating and managed the openings and the sliding board, as well as baiting the reward dishes.

a new trial began. The order of the reward side opened to each piglet was pseudorandomized (6 × left, 6 × right).

Preference Test

The preference test was adapted from Hillemann et al. (2014) and was also performed in the experimental pen. We used two spoons that were placed close to each other through the metal grid to present two different food items to the piglets (one piece of each). We used the six different food items mentioned above (pellets, penne, M&M's®, apple, cheese, sausage), which resulted in 15 test combinations of two items. Each combination was repeated three times, for a total of 45 trials per animal (a total of 900 trials across all animals). One session, consisting of 15 consecutive trials, was completed per day and per piglet so that all 15 possible food combinations were presented in pseudorandomized order. The food item that was ingested first was considered preferred. In cases where no choice was made by the piglet within 30 s (timeout), the trial outcome was considered an "omission" or refusal of both food items. By analyzing the choices and omissions for each piglet, the most and the least preferred food items were determined. In cases where there was an equal preference for two or more food items (this was the case for three piglets), we decided upon cheese, as this was among the most highly preferred foods and seemed to be a widely desired item. The least preferred reward was pellets and we did not continue to use this reward in further trials, as it was difficult to dose compared to pieces of penne or M&M's®.

Discrimination Test

During the discrimination test, the piglets learned to discern differences in the reward presented on two specified sides of the sliding board. One group received different amounts of a reward (i.e., contrast in the quantitative aspect, one piece vs. four pieces of the subject's most preferred food item), and the other group received differentially preferred rewards, referring to qualitative aspects of reward (one piece of the most vs. one piece of the least preferred food item). For simplicity, the groups were further referred to as "quantity group" and "quality group." The rewarded side (left vs. right, larger/highly preferred vs. smaller/least preferred) was always the same for individual piglets and was pseudorandomized across the piglets, with an equal distribution of side-reward property combinations (i.e., 5 × left larger, 5 × left smaller, 5 × left highly preferred, 5 × left least preferred). Each piglet completed one session per day, with 12 trials each, and a 30 s timeout; if the piglet refused to make a choice within 30 s, the trial was rated as "omission." Each trial started with the sliding board pulled back out of reach for the piglets, and the doors closed. After equipping both reward dishes with the according rewards, the door(s) were opened and the piglet was able to put its head through one of the openings. Each session started with four forced choice trials wherein only one of the two sliding doors was opened to ensure that the piglets experienced the reward opportunities on both sides. First, in two trials, the side with the larger/highly preferred reward was opened, followed by two trials with the side with the smaller/least preferred reward. In the following eight free choice trials, both sliding doors were opened, giving the piglet the opportunity to make a choice. A choice was made when the piglet put its head and ears through the opening. In this case, the table was immediately pushed to the piglet so that it was able to consume the chosen reward. Thereafter, the table was pulled back, both doors closed and a new trial began. The piglets were not able to consume the other, un-chosen reward, as the sliding door on that side was closed immediately after the piglet had made its choice. The discrimination test was performed until each piglet passed the learning criterion, meaning it chose the larger/highly preferred reward in at least seven of the eight free choice trials, which corresponds to a significant preference according to the binomial test (Melotti et al., 2013). When a piglet passed the learning criterion, it started directly in the next session with the delay maintenance test for impulse control.

Delay Maintenance Test

The test procedure in the delay maintenance test was nearly the same as in the discrimination test (see above). Here, the number of forced choice trials was decreased, whereby the number of free choice trials was increased and an increasing delay was introduced to the free choice trials [see (Melotti et al., 2013)]. Thus, in the case of choosing the smaller/least preferred reward, the table with the reward was pushed immediately to the piglet, whereas in the case of choosing the larger/highly preferred reward, the table was pushed to the piglet only after a certain delay, keeping both reward options in view during the delay. Therefore, the piglet always had the opportunity to revise its decision during the delay and to switch to the other side that provided an immediate reward [as examples see **Supplementary Videos** for waiting (**Supplementary Video 1**), immediate reward (**Supplementary Video 2**), and switching (**Supplementary Video 3**)]. Thus, the current task is seen as a delay maintenance task, despite the fact that the experimental setup is similar to classical intertemporal choice tasks testing for delay choice (Addessi et al., 2014). Again, a 30-s timeout was used: if the piglet refused to make a choice within 30 s, the trial was rated as "omission."

Each session started with two forced choice trials, first opening the side with the larger/highly preferred reward and after the piglet's choice (i.e., putting head and ears through the opening), giving access to the reward only after a delay, as in the following free choice trials. This process was followed by a one-time opening of the side with the smaller/least preferred reward, giving access without delay. Thereafter, there were ten free choice trials, giving the piglet the opportunity to wait for the larger/highly preferred reward or to choose the immediate but smaller/least preferred reward. The delay increased in the following steps: 2 s, 4 s, 8 s, 16 s, 24 s, 32 s (40, 50 s–these steps were planned for but not tested). Similar to the discrimination test, we used a significant binomial test to decide whether an animal significantly chose to wait for the larger/highly preferred reward and thus successfully passed a delay step, meaning the piglet waited for the larger/highly preferred reward in at least nine out of 10 free choice trials. Consequently, the animal received the next higher delay in the following session. We also used the binomial test to decide whether an animal significantly failed a delay step due to choosing the immediate reward, switching from waiting to the immediate reward during the delay or omitting a choice until timeout (for at least nine out of the 10 free choice trials). Consequently, for these animals the experiment was finished. Animals that showed indifferent behavior (i.e., no clear waiting and no clear failing) received the same delay again in the next session, i.e. the animals repeated the same delay step as long as they did not reach the criteria for successful waiting (next delay step in the following session) or significant failing (experiment finished), respectively. The experiment was finished when all the animals left the delay maintenance test due to significantly failing a delay step.

Statistical Analysis

In each trial of the preference test, the animal could make a choice by choosing one food item over another for ingestion, or, could omit a choice by refusing both food items. We first calculated the total proportion of choices and omissions with regard to the number of total trials. The proportions of choices and omissions of the single food items are presented for both groups as well as for each individual.

For the discrimination test the maximum number of sessions needed to pass the learning criterion was compared between the groups using the Mann-Whitney test (due to the non-normal distribution of the data).

In the delay maintenance test, we excluded subjects from the experiment as soon as they significantly failed to wait. Therefore, with increasing delay, we had to deal with a decreasing number of animals. Nevertheless, to statistically compare both groups, we first examined the total number of sessions completed by the two groups using an unpaired t-test (after confirming normal distribution of the data). Then, we analyzed the maximum delay step that was successfully mastered by each individual in both groups. Therefore, we transformed the categorical delay steps (2, 4, 8, 16, 24, 32 s) into a continuous variable (delay step: 1, 2, 3, 4, 5, 6) and used the Mann-Whitney Test (due to non-normal distribution of the data) to test the median scores of the two samples for significant differences. We also categorized the piglets' strategies as wait (for the larger/highly preferred reward), immediate (choosing the smaller/least preferred reward), switch (revision of the choice from waiting to immediate reward during the delay) and omit (making no choice within 30 s) and calculated the proportion of trials for each strategy. The single strategies were compared between the groups across all the trials using an unpaired t-test (after confirming normal distribution of the data) and, when necessary, a Welch correction due to unequal standard deviations (this was the case for the switching strategy). For one of the piglets from the quality group, the delay step of 4 s was accidently skipped, so that those data are missing (see **Supplementary Table 4**).

All the statistical analyses were performed in GraphPad InStat (Version 3.06, GraphPad Software, La Jolla California USA, www.graphpad.com).

RESULTS

Preference Test

Out of 900 total trials, the piglets made 804 choices (89.3%) and 96 omissions (10.7%, see **Supplementary Table 1**). The most preferred items were apple, cheese and sausage. These items accounted for more than 75% of the preferences (see **Figure 2**; **Supplementary Table 2**). In total, 17 animals preferred one item more than all the others, while three animals preferred two or

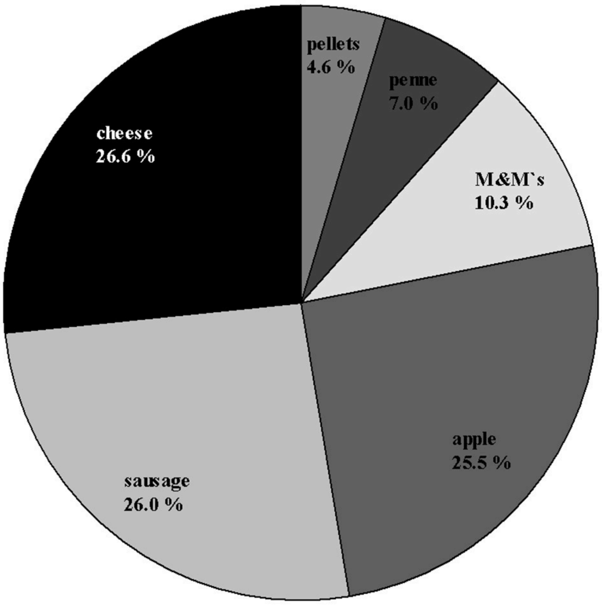

FIGURE 2 | Percentage distribution of choices ($N = 804$) across the rewards used in the preference test for all animals in both groups.

three items equally. This outcome resulted in 24 highly preferred items (rather than 20–the number of animals): 7 × sausage, 7 × cheese, 6 × apple, 3 × M&M's®, 1 × penne. Roughly summarized, the animals preferred 14 × savory tastes, 9 × sweet taste, and 1 × neutral taste. The most refused items were pellets, penne and M&M's®, especially in the three combinations of these items.

Discrimination Test

The individual number of sessions needed to pass the learning criterion varied: it ranged from 2 to 10 sessions. In both groups, one piglet needed 10 sessions. Apart from that, the other nine piglets of the "quantity group" needed a maximum of five sessions to pass the learning criterion, while the other nine piglets of the "quality group" needed a maximum of just three sessions. On average, there was no significant difference between the groups ("quantity group": 3.9 ± 2.4 sessions, "quality group" 3.2 ± 2.4 sessions; $U = 33.0$, $P = 0.2$, n1 = n2 = 10; **Figure 3**). The proportion of omissions was quite low. During a total of 568 free choice trials, only 22 omissions occurred (3.9%) in just two animals ("quantity group": animal 5, 4 omissions; "quality group": animal 8, 18 omissions). An overview of the individual choices (larger/highly preferred, smaller/least preferred, omission) is given in **Supplementary Table 3**.

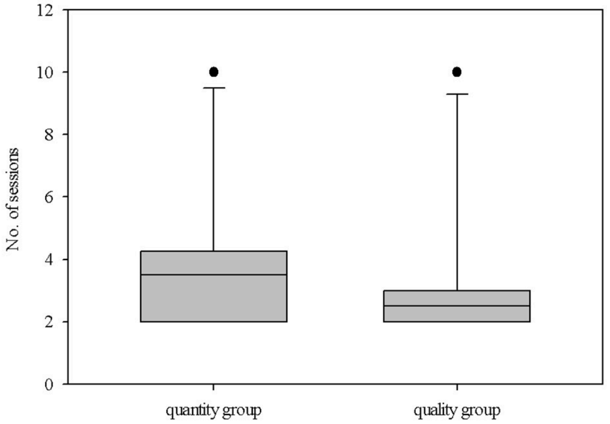

FIGURE 3 | Performance of the animals in both groups (quantitative difference in reward [amount: 1:4] vs. qualitative difference in reward [differentially preferred items: low: high]) during the discrimination test, i.e., the number of sessions needed to reach the learning criterion (significantly choosing the larger/highly preferred reward). The boxplot shows the distribution of the data from both groups with the 25, 50th (median), and 75th percentiles as gray boxes, the 90th percentile as a whisker and black circles as outliers.

Delay Maintenance Test

The total number of sessions completed by the individual piglets ranged from three to 13, and the total number of trials across all the piglets was 1650. In comparing the total number of sessions between the groups, it emerged that the "quantity group" completed on average more sessions than the "quality group," but the difference was not significant ("quantity group": 9.2 ± 3.1 sessions, "quality group": 7.3 ± 2.8 sessions; $t_{18} = 1.435$, $P = 0.168$). The delay steps that were successfully passed by the piglets ranged from 0 s (that is, the piglets did not even master the first delay step of waiting 2 s for the reward) to 24 s. The maximum delay step, mastered by two animals from the "quantity group," was 8 s, while two animals from the "quality group" mastered the maximum of 24 s. Comparing the maximum delay steps mastered between both groups showed that the "quantity group" successfully mastered, on average, the second delay step of 4 s (2.0 ± 0.67) and the "quality group" showed a tendency to successfully master the next higher delay step of 8 s (3.0 ± 1.83, $U = 27.0$, $P = 0.088$, n1 = n2 = 10). **Table 1** illustrates the decreasing number of animals and the decreasing proportion of trials with waiting with each increasing delay. The proportions of trials with choosing the immediate reward, switching and omissions did not show a clear pattern across the increasing delay steps. The proportions of the different strategies shown by the piglets in relation to all the trials are shown in **Figure 4**. The "quantity group" chose the immediate reward in a significantly larger proportion of trials than did the "quality group" ("quantity group": 22.8 ± 8.3%, "quality group": 15.0 ± 6.1%; $t_{18} = 2.392$, $P = 0.028$). The proportion of trials with successful waiting ("quantity group": 58.2 ± 6.3%, "quality group"; 64.5 ± 11.3%; $t_{18} = 1.525$, $P = 0.145$), switching ("quantity group": 13.2 ± 12.3%, "quality group": 7.2 ± 5.8%; $t_{18} = 1.41$, $P = 0.176$) and omissions ("quantity group": 5.8 ± 8.3%, "quality group": 13.4 ± 12.4%; $t_{18} = 1.622$, $P = 0.122$) was not significantly different between groups. Nevertheless, numerical differences indicate that animals of the "quantity group" were prone to show the switching strategy, whereas animals of the "quality group" were prone to omit any choice. However, the high standard deviations indicate a high individual variation. An overview of the individual choices (successful waiting, switching, immediate reward, omission) is given in **Supplementary Table 4**.

TABLE 1 | Overall performance of the animals in both groups (quantitative difference in reward [amount] vs. qualitative difference in reward [differentially preferred items]) during the delay maintenance test.

Performance/group	Delay step											
	2 s		4 s		8 s		16 s		24 s		32 s	
	Quantity	Quality	Quantity	Quality	Quantity	Quality	Quantity	Quality	Quantity	Quality	Quantity	Quality
Successful animals [*n*]	10	8	8	7	2	7	0	5	0	2	0	0
Waiting [% of trials]	73.21	74.21	67.74	87.78	40.00	77.78	22.86	53.85	0	48.57	0	25.71
Immediate [% of trials]	17.86	12.63	19.35	10.00	36.54	10.56	24.29	20.77	0	15.71	0	17.14
Switching [% of trials]	4.64	0	2.26	0	18.46	1.67	42.86	23.08	0	15.71	0	20.00
Omission [% of trials]	4.29	13.16	10.65	2.22	5.00	10.00	10.00	2.31	0	20.00	0	37.14

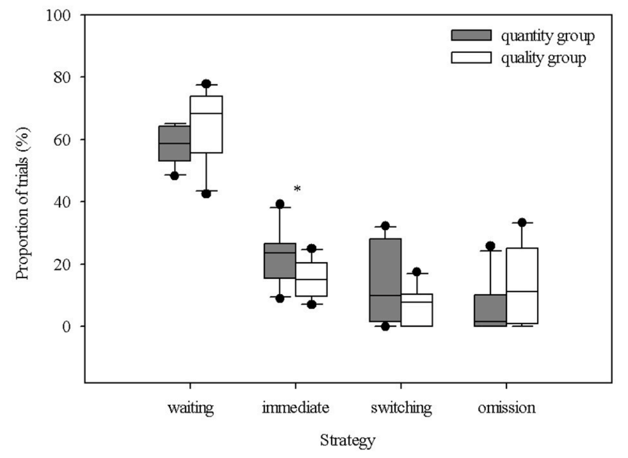

FIGURE 4 | Percentage distribution of the different behavioral strategies during decision-making across all trials of the animals in both groups (quantitative difference in reward [amount: 1:4] vs. qualitative difference in reward [differentially preferred items: low: high]) during the delay maintenance test. The boxplot shows the distribution of the data within the single strategies with the 25, 50th (median), and 75th percentiles as gray boxes, the 90th percentile as a whisker and black circles as outliers. The asterisk indicates a significant difference between both groups revealed with an unpaired *t*-test (* = $P < 0.05$).

DISCUSSION

The piglets in the current study showed individual food preferences. While we found no influence of the type of contrast between rewards (amount vs. differentially preferred items) on learning in a discrimination test, the level of impulse control varied depending on the contrast between rewards.

Preference Test

The individual preferences for potential food reward items and the distribution of choices made across the food items were quite heterogeneous and diverse (**Figure 2**). Preferences ranged from a near equal distribution of choices across all the food items to a sharply skewed distribution for individuals who refused half of the food items completely. It is obvious that in cognitive experimental setups, where motivation is crucial, the reward type itself plays an important role. In many experiments, the same food reward for all animals is used to standardize the experiment. In the case of pigs, either standard food was used after food deprivation (Dantzer et al., 1980; de Jong et al., 2000; Elizabeth Bolhuis et al., 2004), or mostly sweet food rewards were used, such as M&M's® (de Jonge et al., 2008; Gieling et al., 2013), apple/applesauce (Douglas et al., 2012; Melotti et al., 2013; Düpjan et al., 2017), raisins (McLeman et al., 2005; de Jonge et al., 2008) and chocolate raisins (Bolhuis et al., 2013). In the current study, in more than half of the choices made, a food item with a savory taste was preferred (cheese, sausage), and in a another third, a food item with a sweet taste was preferred (apple, M&M's®). The results clearly demonstrate that in pigs, similar to humans, tastes are quite different; consequently, the motivation to do something to get a standardized food reward differs among individuals. Therefore, in experiments using a food reward, it is more appropriate to perform a preference test in advance and to use individually preferred food items in order to attain high motivation. However, this approach must be tested in further experiments, to determine whether a preference for a special food item, once developed, is stable across time in pigs.

Discrimination Test

The piglets needed 2–10 sessions (16 to 80 free choice trials) to reach the learning criterion (significantly choose the larger/highly preferred of two possible rewards). In the study by Melotti et al. (2013), pigs needed on average 3.6 sessions (28.8 free choice trials) to significantly choose the lever providing four pieces of apple instead of one. The current study used the same learning criterion (at least seven out of eight free choice trials = success rate of 87.5%), and our results were in the same range ("quality group"−3.2 sessions, 25.6 ± 19.5 free choice trials; "quantity group"−3.9 sessions, 31.2 ± 19.0 free choice trials). In other studies, animals needed a somewhat higher number of trials for successful discrimination, e.g., domestic chickens needed 32 ± 9 to 64 ± 55 trials to discriminate between a rewarded and a non-rewarded object (Croney et al., 2007), and domestic horses took 50.9 ± 10.3 to 74.6 ± 20.4 trials to choose the reward from three patterned cards (Mader and Price, 1980). In those studies, animals had a choice between a reward and no reward, which should have facilitated discrimination learning; however the rewards were not visible and were coded with abstract objects, which complicated their ability to learn the relationship between the object and the reward. In the current study, the piglets from the group with differentially preferred rewards were somewhat (numerically, but not statistically significantly) faster at completing the task (**Figure 3**). A study in capuchin monkeys with a reversed-reward contingency task showed that learning the task with qualitative differences in reward seemed to be intrinsically easier compared to quantitative differences (Anderson et al., 2008). An additional study in capuchin monkeys showed that contrast in reward quality affected the animals more than contrast in quantity (Talbot et al., 2018). Probably, one of the reasons that affect learning speed and decision making is that qualitative aspects of reward comprise more cues (visual, olfactory) compared to quantitative aspects of reward (just visual cues), which can facilitate discrimination and learning. It should be noted that the piglets in this study were not really hungry due to regular feeding after the experiment, and they did not have completely empty feeding troughs in the morning before testing. Therefore, the reward during the experiment was more a bonus than an essential resource. Furthermore, the contrast in qualitative aspects of reward might result in an increased incentive and motivation compared to the contrast in quantitative aspects (Berridge, 2009).

Delay Maintenance Test

In the delay maintenance test, the "quantity group" chose the smaller/least preferred but immediate reward significantly more often, showing less impulse control. This result is supported by the lower maximum delay step of 8 s mastered by two animals of this group compared to the maximum of 24 s achieved by two animals of the "quality group" as well as by a higher number of piglets choosing to wait in the "quality group" (**Table 1**). Moreover, the "quantity group" completed more sessions than the "quality group," despite the fact that they did not reach higher delays. This means that they completed more sessions in the single delay steps without reaching the success criterion for the next step or the failure criterion to finish the experiment, i.e., they showed ambivalent behavior. This result indicates that impulse control is partially present but that the opportunity to get a highly preferred reward is more valued by the piglets than the opportunity to get more of a given reward (Hillemann et al., 2014). Additionally, in other species, the importance or incentive value of more preferred rewards has been shown to be higher compared to a higher amount of reward; therefore, the incentive/motivation to wait is higher [corvids: Dufour et al. (2012); Wascher et al. (2012), cockatoos: Auersperg et al. (2013), parrots: Koepke et al. (2015), capuchins: Drapier et al. (2005), dogs: Brucks et al. (2017)] or the discounting of the reward value with increasing delay is lower (Monterosso and Ainslie, 1999). Hillemann et al. (2014) also stated that corvids waited more often when the difference in the preference of the reward was more pronounced (e.g., low-mid vs. low-high). In the current study, rewards which were least preferred were used as contrast to the highly preferred reward, and the results show that the animals were prone to omit any choice with increasing delay, probably due to the low hedonic value of the immediate reward (Berridge, 2009). It has to be taken into account that the piglets (similar to the majority of animals in such tests) were not food-deprived and that they did not incur any nutritional disadvantage by refusing rewards.

With increasing delay, the animals of the "quantity group" tended to switch to the immediate reward while waiting for the larger reward. Melotti et al. (2013) used a delay choice setup with a continuous increase in delay in their study, with no possibility for the pigs to switch once a choice was made and with no visibility of the different amounts of the rewards (i.e., during choice the rewards were represented by the left or right lever, respectively). The results showed that the pigs were able to wait for 12–50 s, tolerating considerably higher delays than were achieved in our study (0–24 s). In contrast to Melotti et al. (2013), our study used a delay maintenance paradigm, giving the pigs the opportunity to switch to the immediate reward at any time, with visible rewards that differed in quantitative aspects (amount) as well as qualitative aspects (preference). With regard to the quantitative aspect of the reward, from reversed reward contingency tasks in primates, it is known that the prepotent impulse to choose a higher over a lower quantity leads to a widespread failure of these animals in this task (Vlamings et al., 2006; Anderson et al., 2008). The same "go for more" effect could be demonstrated in a hybrid delay task showing that monkeys, after initially choosing the larger/later option (delay choice task), were not able to wait until the delivery of the complete reward (delay maintenance/accumulation task; Paglieri et al., 2013). This demonstrates that delay choice and delay maintenance tasks are not equivalent (Addessi et al., 2013). With regard to the visibility aspect of the reward, the performance of primates in the reversed reward contingency task is enhanced when the rewards were covered with colored lids (Vlamings et al., 2006) or were represented by symbols (Addessi and Rossi, 2011). In delay choice tasks, the monkeys more often chose the smaller/sooner option when rewards were represented by symbols or were covered (Genty et al., 2012; Addessi et al., 2014). These results demonstrate that non-visibility of the rewards can override the "go for more" impulse. With regard to the qualitative aspect of the reward, in reversed reward contingency tasks, performance increased when rewards were used that differed in preference (Anderson et al., 2008). In two separate self-control exchange tasks in which animals needed to exchange a less-preferred reward to obtain a more-preferred reward, both chimpanzees (Beran et al., 2016) and capuchin monkeys (Parrish et al., 2018) were more successful on trials with differences in qualitative aspects vs. differences in quantitative aspects of the reward. Finally, in delay-of-gratification tests in pigeons and children, the capacity for impulse control was higher when the rewards were not visible and differed in qualitative aspects (Mischel, 1974; Grosch and Neuringer, 1981). Thus, in the current study, choosing a visible, quantitatively larger (but delayed) reward first (before switching to the smaller immediate reward) rather reflects the initial impulsive choice of "go for more" and not necessarily the intentional choice to wait and therefore may not demonstrate real impulse control in view of a temptation (Addessi et al., 2013; Hillemann et al., 2014). Furthermore, we used a discontinuous increase in delay, i.e., the delay increased only when the individual animal significantly chose to wait, which resulted in a repeated presentation of the same delay. Maybe these effects, i.e., the "go for more" choice with no opportunity of modification and the continuous increase in delay until a stop criterion was reached, led to increased delays for the pigs in the study by Melotti et al. (2013).

According to optimal foraging theory (Charnov, 1976; Stephens and Krebs, 1986; Herrnstein et al., 2000), gain rate (i.e., amount of food/energy intake per unit of time) plays an important role in decision making in inter-temporal choice tasks. Thus, in addition to the quantity of the reward and the delay until delivery of the reward, handling/manipulation time and the duration of inter-trial intervals also matter in choice tasks (Izawa et al., 2003; Aoki et al., 2006; Matsushima et al., 2008). However, we did not control for or manipulate the handling time of the different food amounts, e.g., by making the smaller reward harder accessible (Held et al., 2005). Handling time, regarded as the time the pigs needed to consume the whole amount of food, was difficult to determine exactly. The current setup did not allow us to see when all pieces were consumed, while pigs also tended to further interact with an empty dish. This led to a large inter- as well as intra-individual variability in handling time, independent of the amount of reward. The best strategy for the pigs to maximize profitability, i.e., gain per time (delay + handling), would have been to keep the handling time as short as possible. But the large variability in handling time shows that gain rate maximization seemed not to be the main motivation. Possible factors that contributed to this variability could be intra-individual differences to obtain food, e.g., due to our lack of control on the food intake that each subject had prior to testing, as well as inter-individual differences in foraging behavior (Bolnick et al., 2003). Additionally, our current study design did not allow varying the inter-trial interval, e.g., by trial-specifically adjusting the total trial length. In a recent review, Sjoberg and Johansen (2018) argue that in several studies on delay discounting the delay plays a pivotal role, while the inter-trial interval had little or no effect on choice. Nevertheless, we cannot completely exclude that our results are confounded by other variables like inter-trial interval and handling time. This has to be investigated in further studies specially designed to answer the question on which factors contribute to pigs' choices in delay of gratification tasks.

Compared to several other species, which are able to wait several minutes [primates: Beran et al. (1999), corvids: Hillemann et al. (2014), dogs: Leonardi et al. (2012)] or up to weeks and months [humans: Frederick et al. (2002)], piglets achieved a relatively low level of impulse control, which is also common in several other species [see Figure 13.6. in Stevens and Stephens (2010, p. 380)]. The reasons for these species differences in impulse control, as discussed in Stevens and Stephens (2010), are the structure of the natural habitat (i.e., rich vs. poor) and the domain of selection (i.e., the natural foraging environment including feeding ecology and social competition; Stevens, 2014). Unfortunately, no studies exist that directly investigate the impulse control capacity of wild boar or other wild pig species, especially in terms of economic decision making. Only one study describes a coincidental but locally restricted observation of wild boar washing soiled food in a zoo (Sommer et al., 2016). The authors argued that the capacity of delaying gratification could have facilitated this behavior. However, Korte et al. (2009) presented a framework discussing the effects of artificial selection of production traits in farm animals with regards to their metabolism, hormone and brain physiology and, consequently, their behavior. The comparably low impulse control of piglets could reflect the "hawk behavioral strategy," which, accompanied by active, aggressive and bold behavior, increases vulnerability to injurious behavior and stereotypes, such as tail biting. Despite this, and similar to other non-domesticated species, pigs showed considerable individual differences in their impulse control capacity. Two factors that could contribute to this high inter-individual variation are diverging social behavior and personality traits. For example, within the scope of "social foraging theory," individuals can adapt different foraging strategies (e.g., producer, scrounger) and therefore could show differences in impulse control depending on their position within the social network (Giraldeau and Dubois, 2008). Moreover, individuals possess different personalities that are shaped by evolution and ecology (Dingemanse and Wolf, 2010; Réale et al., 2010). For example, within the scope of "coping theory" individuals can adapt to be more active or passive in the face of challenges and thus may also show differences in impulse control (Lazarus, 1993; Zebunke et al., 2015, 2017). This is a starting point for further studies investigating the relationships between impulse control and personality as well as social behavior traits. Moreover, especially in light of field applications, individual differences in impulse control could also play a role in the problematic behaviors that emerge in intensive housing conditions due to stressful conditions, such as injurious behavior (e.g., aggression, tail biting) and stereotypes. Further knowledge about the features, mechanisms and relationships of impulse control to other traits could help to find approaches that increase farm animal welfare.

CONCLUSION

The results of the current study show that in studies that rely on the motivation of the participating subjects, care must be taken in reward selection, which makes a preliminary preference test useful. Similar to other species, pigs showed higher impulse control when the reward differed in qualitative aspects rather than quantitative aspects. The inter-individual variance in impulse control measured in this study could be seen as a starting point for future studies aimed at the understanding of the relevance of impulse control in farmed species in the context of animal welfare. Broader knowledge regarding impulse control could help to assess the reaction of the animals to differentially available resources and to adapt husbandry practices to species/breed specific demands and individual differences in this behavioral aspect.

AUTHOR CONTRIBUTIONS

MZ conceived and planned the experiments, performed the analytic calculations and wrote the manuscript. MK carried out the experiments and collected the data. NM helped shape the analysis and manuscript. JL and BP contributed to the idea, the planning and supervised the project.

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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.2018.02099/full#supplementary-material

Supplementary Table 1 | Overview of the omissions during the preference test: percentage of omissions with regard to the number of total trials (N = 45) per animal, percentage of items refused by the individual animals (group = "quantity" [amount: 1:4], "quality" [differentially preferred items: low:high]; animal = 1-10) from both groups (group = "quantity" [amount: 1:4], "quality" [differentially preferred items: low : high]; animal = all) as well as in total (group = all, animal = all). Averaged values within the groups and across both groups are highlighted in bold.

Supplementary Table 2 | Overview of the choices during the preference test: percentage of choices made with regard to the number of total trials (N = 45) per animal, percentage of items chosen by individual animals (group = "quantity" [amount: 1:4], "quality" [differentially preferred items: low:high]; animal = 1-10) from both groups (group = "quantity" [amount: 1:4], "quality" [differentially preferred items: low:high]; animal = all) as well as in total (group = all, animal = all). Averaged values within the groups and across both groups are highlighted in bold. Individual, highly preferred items are highlighted in bold and italicized. The food items chosen as individual rewards for the following tests are underlined. For the group with qualitative differences in reward ("group quality," differentially preferred items: low:high), the individual food items used as the least preferred reward are marked as crossed-out characters.

Supplementary Table 3 | Overview of the performance of each pig during the discrimination test over the course of the sessions for both groups ("quantity": with a quantitative difference in reward, i.e., 1 vs. 4 pieces of the most preferred reward; "quality": with a qualitative difference in reward, i.e., 1 piece of the most preferred vs. 1 piece of the least preferred reward). Each symbol indicates a decision during a trial: black circle = choice of the larger/highly preferred reward; white circle = choice of the smaller/least preferred reward; - = omission.

Supplementary Table 4 | Overview of the performance of single pigs in the two groups ("quantity": with a quantitative difference in reward, i.e., 1 vs. 4 pieces of the most preferred reward; "quality": with a qualitative difference in reward, i.e., 1 piece of the most preferred vs. 1 piece of the least preferred reward) during the course of the delay maintenance test with increasing delays (2 s, 4 s, 8 s, 16 s, 24 s, 32 s) to the larger/highly preferred reward. Each symbol indicates a decision during a trial: black circle = successful waiting for the larger/highly preferred reward; ⋂ = switching, i.e., first choosing to wait, but switching to the immediate reward before the end of the delay; white circle = choosing the immediate smaller/least preferred reward; - = omission. The rows within each individual indicate repeated sessions within one delay step due to not reaching the learning criterion (significant binomial testing of successful against unsuccessful waiting, i.e., switching, immediate, omission).

Supplementary Video 1 | Video sample of a pig of the "quantity group" (amount: left = 1 piece vs. right = 4 pieces) choosing to wait a delay of 8 s for the larger reward (right side).

Supplementary Video 2 | Video sample of a pig of the "quantity group" (amount: left = 1 piece vs. right = 4 pieces) choosing the small, immediate reward (left side).

Supplementary Video 3 | Video sample of a pig of the "quantity group" (amount: left = 1 piece vs. right = 4 pieces) switching from the larger (right side) to the smaller reward (left side) during the delay of 8 s.

REFERENCES

Addessi, E., Bellagamba, F., Delfino, A., De Petrillo, F., Focaroli, V., Macchitella, L., et al. (2014). Waiting by mistake: symbolic representation of rewards modulates intertemporal choice in capuchin monkeys, preschool children and adult humans. *Cognition* 130, 428–441. doi: 10.1016/j.cognition.2013.11.019

Addessi, E., Paglieri, F., Beran, M. J., Evans, T. A., Macchitella, L., De Petrillo, F., et al. (2013). Delay choice versus delay maintenance: different measures of delayed gratification in capuchin monkeys (*Cebus apella*). *J. Comp. Psychol.* 127, 392–398. doi: 10.1037/a0031869

Addessi, E., and Rossi, S. (2011). Tokens improve capuchin performance in the reverse-reward contingency task. *Proc. R. Soc. B Biol. Sci.* 278, 849–854. doi: 10.1098/rspb.2010.1602

Ainslie, G. W. (1974). Impulse control in Pigeons. *J. Exp. Anal. Behav.* 21, 485–489. doi: 10.1901/jeab.1974.21-485

Anderson, J. R., Hattori, Y., and Fujita, K. (2008). Quality before quantity: rapid learning of reverse-reward contingency by capuchin monkeys (*Cebus apella*). *J. Comp. Psychol.* 122, 445–448. doi: 10.1037/a0012624

Anonymous (2016). Guidelines for the treatment of animals in behavioural research and teaching. *Anim. Behav.* 111, 1–9. doi: 10.1016/S0003-3472(15)00461-3

Aoki, N., Csillag, A., and Matsushima, T. (2006). Localized lesions of arcopallium intermedium of the lateral forebrain caused a handling-cost aversion in the domestic chick performing a binary choice task. *Er. J. Neurosci.* 24, 2314–2326. doi: 10.1111/j.1460-9568.2006.05090.x

Auersperg, A. M., Laumer, I. B., and Bugnyar, T. (2013). Goffin cockatoos wait for qualitative and quantitative gains but prefer 'better' to 'more'. *Biol. Lett.* 9:20121092. doi: 10.1098/rsbl.2012.1092

Bari, A., and Robbins, T. W. (2013). Inhibition and impulsivity: behavioral and neural basis of response control. *Prog. Neurobiol.* 108, 44–79. doi: 10.1016/j.pneurobio.2013.06.005

Beran, M. J. (2002). Maintenance of self-imposed delay of gratification by four chimpanzees (Pan troglodytes) and an orangutan (*Pongo pygmaeus*). *J. Gen. Psychol.* 129, 49–66. doi: 10.1080/00221300209602032

Beran, M. J. (2015). The comparative science of "self-control": what are we talking about? *Front. Psychol.* 6:51. doi: 10.3389/fpsyg.2015.00051

Beran, M. J., Rossettie, M. S., and Parrish, A. E. (2016). Trading up: chimpanzees (Pan troglodytes) show self-control through their exchange behavior. *Anim. Cogn.* 19, 109–121. doi: 10.1007/s10071-015-0916-7

Beran, M. J., Savage-Rumbaugh, E. S., Pate, J. L., and Rumbaugh, D. M. (1999). Delay of gratification in chimpanzees (*Pan troglodytes*). *Dev. Psychobiol.* 34, 119–127.

Berns, G. S., Laibson, D., and Loewenstein, G. (2007). Intertemporal choice–toward an integrative framework. *Trends Cogn. Sci.* 11, 482–488. doi: 10.1016/j.tics.2007.08.011

Berridge, K. C. (2009). 'Liking' and 'wanting' food rewards: brain substrates and roles in eating disorders. *Physiol. Behav.* 97, 537–550. doi: 10.1016/j.physbeh.2009.02.044

Bolhuis, J. E., Oostindjer, M., Hoeks, C. W. F., de Haas, E. N., Bartels, A. C., Ooms, M., et al. (2013). Working and reference memory of pigs (*Sus scrofa domesticus*) in a holeboard spatial discrimination task: the influence of environmental enrichment. *Anim. Cogn.* 16, 845–850. doi: 10.1007/s10071-013-0646-7

Bolnick, D. I., Svanback, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., et al. (2003). The ecology of individuals: incidence and implications of individual specialization. *Am. Nat.* 161, 1–28. doi: 10.1086/343878

Brucks, D., Soliani, M., Range, F., and Marshall-Pescini, S. (2017). Reward type and behavioural patterns predict dogs' success in a delay of gratification paradigm. *Sci. Rep.* 7:42459. doi: 10.1038/srep42459

Brunner, D., and Hen, R. (1997). Insights into the neurobiology of impulsive behavior from serotonin receptor knockout mice. *Ann. N. Y. Acad. Sci.* 836, 81–105.

Cervantes, M. C., and Delville, Y. (2007). Individual differences in offensive aggression in golden hamsters: a model of reactive and impulsive aggression? *Neuroscience* 150, 511–521. doi: 10.1016/j.neuroscience.2007.09.034

Charnov, E. L. (1976). Optimal foraging, the marginal value theorem. *Theoret. Popul. Biol.* 9, 129–136. doi: 10.1016/0040-5809(76)90040-X

Cheng, K., Peña, J., Porter, M. A., and Irwin, J. D. (2002). Self-control in honeybees. *Psychon. Bull. Rev.* 9, 259–263. doi: 10.3758/bf03196280

Ciardelli, L. E., Weiss, A., Powell, D. M., and Reiss, D. (2017). Personality dimensions of the captive California sea lion (*Zalophus californianus*). *J. Comp. Psychol.* 131, 50–58. doi: 10.1037/com0000054

Coppens, C. M., de Boer, S. F., Buwalda, B., and Koolhaas, J. M. (2014). Aggression and aspects of impulsivity in wild-type rats. *Aggress. Behav.* 40, 300–308. doi: 10.1002/ab.21527

Croney, C. C., Prince-Kelly, N., and Meller, C. L. (2007). A note on social dominance and learning ability in the domestic chicken (*Gallus gallus*). *Appl. Anim. Behav. Sci.* 105, 254–258. doi: 10.1016/j.applanim.2006.05.001

Dantzer, R., Arnone, M., and Mormede, P. (1980). Effects of frustration on behavior and plasma corticosteroid levels in pigs. *Physiol. Behav.* 24, 1–4. doi: 10.1016/0031-9384(80)90005-0

de Jong, I. C., Prelle, I. T., van de Burgwal, J. A., Lambooij, E., Korte, S. M., Blokhuis, H. J., et al. (2000). Effects of environmental enrichment on behavioral responses to novelty, learning, and memory, and the circadian rhythm in cortisol in growing pigs. *Physiol. Behav.* 68, 571–578. doi: 10.1016/S0031-9384(99)00212-7

de Jonge, F. H., Ooms, M., Kuurman, W. W., Maes, J. H. R., and Spruijt, B. M. (2008). Are pigs sensitive to variability in food rewards? *Appl. Anim. Behav. Sci.* 114, 93–104. doi: 10.1016/j.applanim.2008.01.004

Diamond, A. (2013). Executive functions. *Ann. Rev. Psychol.* 64, 135–168. doi: 10.1146/annurev-psych-113011-143750

Dingemanse, N. J., and Wolf, M. (2010). Recent models for adaptive personality differences: a review. *Philo. Trans. R Soc. B Biol. Sci.* 365, 3947–3958. doi: 10.1098/rstb.2010.0221

Douglas, C., Bateson, M., Walsh, C., Bédué, A., and Edwards, S. A. (2012). Environmental enrichment induces optimistic cognitive biases in pigs. *Appl. Anim. Behav. Sci.* 139, 65–73. doi: 10.1016/j.applanim.2012.02.018

Drapier, M., Chauvin, C., Dufour, V., Uhlrich, P., and Thierry, B. (2005). Food-exchange with humans in brown capuchin monkeys. *Primates* 46, 241–248. doi: 10.1007/s10329-005-0132-1

Dufour, V., Wascher, C. A. F., Braun, A., Miller, R., and Bugnyar, T. (2012). Corvids can decide if a future exchange is worth waiting for. *Biol. Lett.* 8, 201–204. doi: 10.1098/rsbl.2011.0726

Düpjan, S., Stracke, J., Tuchscherer, A., and Puppe, B. (2017). An improved design for the spatial judgement task in domestic pigs. *Appl. Anim. Behav. Sci.* 187, 23–30. doi: 10.1016/j.applanim.2016.11.012

Elizabeth Bolhuis, J., Schouten, W. G. P., de Leeuw, J. A., Schrama, J. W., and Wiegant, V. A. (2004). Individual coping characteristics, rearing conditions and behavioural flexibility in pigs. *Behav. Brain Res.* 152, 351–360. doi: 10.1016/j.bbr.2003.10.024

Evans, T. A., and Beran, M. J. (2007). Delay of gratification and delay maintenance by rhesus macaques (*Macaca mulatta*). *J. Gen. Psychol.* 134, 199–216. doi: 10.3200/genp.134.2.199-216

Evans, T. A., Beran, M. J., Paglieri, F., and Addessi, E. (2012). Delaying gratification for food and tokens in capuchin monkeys (*Cebus apella*) and chimpanzees (Pan troglodytes): when quantity is salient, symbolic stimuli do not improve performance. *Anim. Cogn.* 15, 539–548. doi: 10.1007/s10071-012-0482-1

Frederick, S., Loewenstein, G., and O'Donoghue, T. (2002). Time discounting and time preference: a critical review. *J. Econ. Lit.* 40, 351–401. doi: 10.1257/002205102320161311

Fuster, J. M. (2015). *The Prefrontal Cortex.* London: Academic Press.

Genty, E., Karpel, H., and Silberberg, A. (2012). Time preferences in long-tailed macaques (*Macaca fascicularis*) and humans (Homo sapiens). *Anim. Cogn.* 15, 1161–1172. doi: 10.1007/s10071-012-0540-8

Gieling, E., Wehkamp, W., Willigenburg, R., Nordquist, R., Ganderup, N., and van der Staay, F. (2013). Performance of conventional pigs and Gottingen miniature pigs in a spatial holeboard task: effects of the putative muscarinic cognition impairer Biperiden. *Behav. Brain Func.* 9:4. doi: 10.1186/1744-9081-9-4

Giraldeau, L. A., and Dubois, F. (2008). Social foraging and the study of exploitative behavior. *Adv. Study Behav.* 38, 59–104. doi: 10.1016/S0065-3454(08)00002-8

Grosch, J., and Neuringer, A. (1981). Self-control in pigeons under the Mischel paradigm. *J. Exp. Anal. Behav.* 35, 3–21. doi: 10.1901/jeab.1981.35-3

Held, S., Baumgartner, J., KilBride, A., Byrne, R. W., and Mendl, M. (2005). Foraging behaviour in domestic pigs (Sus scrofa): remembering and prioritizing food sites of different value. *Anim. Cogn.* 8, 114–121. doi: 10.1007/s10071-004-0242-y

Herrnstein, R. J., Rachlin, H., and Laibson, D. I. (2000). *The Matching Law: Papers in Psychology and Economics*. Cambridge, MA: Harvard University Press.

Hillemann, F., Bugnyar, T., Kotrschal, K., and Wascher, C. A. F. (2014). Waiting for better, not for more: corvids respond to quality in two delay maintenance tasks. *Anim. Behav.* 90, 1–10. doi: 10.1016/j.anbehav.2014.01.007

Izawa, E.-I., Zachar, G., Yanagihara, S., and Matsushima, T. (2003). Localized lesion of caudal part of lobus parolfactorius caused impulsive choice in the domestic chick: evolutionarily conserved function of ventral striatum. *J. Neurosci.* 23, 1894–1902. doi: 10.1523/jneurosci.23-05-01894.2003

Keeling, L., and Jensen, P. (2017). "Abnormal behaviour, stress and welfare," in *The Ethology of Domestic Animals: An Introductory Text, 3rd Edn*. ed P. Jensen (Wallingford; Boston, MA: CABI), 119–134.

Kirkpatrick, K., Marshall, A. T., Smith, A. P., Koci, J., and Park, Y. (2014). Individual differences in impulsive and risky choice: effects of environmental rearing conditions. *Behav. Brain Res.* 269, 115–127. doi: 10.1016/j.bbr.2014.04.024

Koepke, A. E., Gray, S. L., and Pepperberg, I. M. (2015). Delayed gratification: a grey parrot (*Psittacus erithacus*) will wait for a better reward. *J. Comp. Psychol.* 129, 339–346. doi: 10.1037/a0039553

Kornum, B. R., and Knudsen, G. M. (2011). Cognitive testing of pigs (Sus scrofa) in translational biobehavioral research. *Neurosci. Biobehav. Rev.* 35, 437–451. doi: 10.1016/j.neubiorev.2010.05.004

Korte, S. M., Prins, J., Vinkers, C. H., and Olivier, B. (2009). On the origin of allostasis and stress-induced pathology in farm animals: celebrating darwin's legacy. *Vet. J.* 182, 378–383. doi: 10.1016/j.tvjl.2009.08.023

Lazarus, R. S. (1993). Coping theory and research–past, present, and future. *Psychosom Med.* 55, 234–247. doi: 10.1097/00006842-199305000-00002

Leaper, R., Massei, G., Gorman, M. L., and Aspinall, R. (1999). The feasibility of reintroducing Wild Boar (*Sus scrofa*) to Scotland. *Mammal Rev.* 29, 239–259. doi: 10.1046/j.1365-2907.1999.2940239.x

Leonardi, R. J., Vick, S. J., and Dufour, V. (2012). Waiting for more: the performance of domestic dogs (Canis familiaris) on exchange tasks. *Anim. Cogn.* 15, 107–120. doi: 10.1007/s10071-011-0437-y

Lindermayer, H., Propstmeier, G., and Straub, K. (1994). *Fütterungsberater Schwein–Ferkel, Zuchtschweine, Mastschweine*. Munich: Ulmer.

Loewenstein, G., Read, D., and Baumeister, R. F. (2003). *Time and Decision: Economic and Psychological Perspectives of Intertemporal Choice*. New York, NY: Russell Sage Foundation.

Logue, A. W. (1988). Research on self-control: an integrating framework. *Behav. Brain Sci.* 11, 665–709. doi: 10.1017/S0140525X00053978

Madden, G. J. E., and Bickel, W. K. E. (2010). *Impulsivity: The Behavioral and Neurological Science of Discounting.* Washington, DC: American Psychological Association.

Mader, D. R., and Price, E. O. (1980). Discrimination learning in horses: effects of breed, age and social dominance. *J. Anim. Sci.* 50, 962–965.

Marino, L., and Colvin, C. M. (2015). Thinking pigs: a comparative review of cognition, emotion, and personality in *Sus domesticus*. *Int. J. Comp. Psychol.* 28:23859. Available online at: https://escholarship.org/uc/item/8sx4s79c

Matsushima, T., Kawamori, A., and Bem-Sojka, T. (2008). Neuro-economics in chicks: foraging choices based on amount, delay and cost. *Brain Res. Bull.* 76, 245–252. doi: 10.1016/j.brainresbull.2008.02.007

McLeman, M. A., Mendl, M., Jones, R. B., White, R., and Wathes, C. M. (2005). Discrimination of conspecifics by juvenile domestic pigs, *Sus scrofa*. *Anim. Behav.* 70, 451–461. doi: 10.1016/j.anbehav.2004.11.013

Melotti, L., Thomsen, L. R., Toscano, M. J., Mendl, M., and Held, S. (2013). Delay discounting task in pigs reveals response strategies related to dopamine metabolite. *Physiol. Behav.* 120, 182–192. doi: 10.1016/j.physbeh.2013.08.009

Metcalfe, J., and Mischel, W. (1999). A hot/cool-system analysis of delay of gratification: dynamics of willpower. *Psychol. Rev.* 106, 3–19. doi: 10.1037/0033-295X.106.1.3

Mischel, W. (1974). "Processes in delay of gratification," in *Advances in Experimental Social Psychology*, ed L. Berkowitz (New York, NY: Academic Press), 249–292.

Mischel, W., Ayduk, O., Berman, M. G., Casey, B. J., Gotlib, I. H., Jonides, J., et al. (2011). 'Willpower' over the life span: decomposing self-regulation. *Soc. Cogn. Affect. Neurosci.* 6, 252–256. doi: 10.1093/scan/nsq081

Mischel, W., Shoda, Y., and Rodriguez, M. L. (1989). Delay of gratification in children. *Science* 244, 933–938. doi: 10.1126/science.2658056

Monterosso, J., and Ainslie, G. (1999). Beyond discounting: possible experimental models of impulse control. *Psychopharmacology* 146, 339–347. doi: 10.1007/pl00005480

Mühlhoff, N., Stevens, J. R., and Reader, S. M. (2011). Spatial discounting of food and social rewards in guppies (*Poecilia reticulata*). *Front. Psychol.* 2:68. doi: 10.3389/fpsyg.2011.00068

Nawroth, C., von Borell, E., and Langbein, J. (2015). Object permanence in the dwarf goat (*Capra aegagrus hircus*): perseveration errors and the tracking of complex movements of hidden objects. *Appl. Anim. Behav. Sci.* 167, 20–26. doi: 10.1016/j.applanim.2015.03.010

Paglieri, F., Focaroli, V., Bramlett, J., Tierno, V., McIntyre, J. M., Addessi, E., et al. (2013). The hybrid delay task: can capuchin monkeys (*Cebus apella*) sustain a delay after an initial choice to do so? *Behav. Process.* 94, 45–54. doi: 10.1016/j.beproc.2012.12.002

Parrish, A. E., James, B. T., Rossettie, M. S., Smith, T., Otalora-Garcia, A., and Beran, M. J. (2018). Investigating the depletion effect: self-control does not waiver in capuchin monkeys. *Anim. Behav. Cogn.* 5, 118–138. doi: 10.26451/abc.05.01.09.2018

Réale, D., Dingemanse, N. J., Kazem, A. J. N., and Wright, J. (2010). Evolutionary and ecological approaches to the study of personality. *Philo. Trans. R. Soc. B-Biol. Sci.* 365, 3937–3946. doi: 10.1098/rstb.2010.0222

Reynolds, B., de Wit, H., and Richards, J. (2002). Delay of gratification and delay discounting in rats. *Behav. Proces.* 59, 157. doi: 10.1016/S0376-6357(02)00088-8

Rosati, A. G., Stevens, J. R., Hare, B., and Hauser, M. D. (2007). The evolutionary origins of human patience: temporal preferences in chimpanzees, bonobos, and human adults. *Curr. Biol.* 17, 1663–1668. doi: 10.1016/j.cub.2007.08.033

Roura, E., Humphrey, B., Tedó, G., and Ipharraguerre, I. (2008). Unfolding the codes of short-term feed appetence in farm and companion animals. A comparative oronasal nutrient sensing biology review. *Can. J. Anim. Sci.* 88, 535–558. doi: 10.4141/CJAS08014

Sjoberg, E. A., and Johansen, E. B. (2018). Impulsivity or sub-optimal reward maximization in delay discounting? a critical discussion. *Hum. Ethol. Bull.* 33, 22–36. doi: 10.22330/heb/332/022-036.

Sommer, V., Lowe, A., and Dietrich, T. (2016). Not eating like a pig: European wild boar wash their food. *Anim. Cogn.* 19, 245–249. doi: 10.1007/s10071-015-0903-z

Stabenow, B., and Manteuffel, G. (2002). A better welfare for nursing sows without increased piglet loss applying peri-parturition short term crating. *Archiv Für Tierzucht-Arch. Anim. Breed.* 45, 53–60. doi: 10.5194/aab-45-53-2002

Stephens, D. W., and Krebs, J. R. (1986). *Foraging Theory.* Princeton, NJ: Princeton University Press.

Stevens, J. R. (2014). Evolutionary pressures on primate intertemporal choice. *Proc. R. Soc. B Biol. Sci.* 281:20140499. doi: 10.1098/rspb.2014.0499

Stevens, J. R., Hallinan, E. V., and Hauser, M. D. (2005). The ecology and evolution of patience in two New World monkeys. *Biol. Lett.* 1, 223–226. doi: 10.1098/rsbl.2004.0285

Stevens, J. R., and Mühlhoff, N. (2012). Intertemporal choice in lemurs. *Behav. Process.* 89, 121–127. doi: 10.1016/j.beproc.2011.10.002

Stevens, J. R., Rosati, A. G., Heilbronner, S. R., and Mühlhoff, N. (2011). Waiting for grapes: expectancy and delayed gratification in bonobos. *Int. J. Comp. Psychol.* 24, 99–111. Available online at: https://escholarship.org/uc/item/4km2r37j

Stevens, J. R., and Stephens, D. W. (2010). "The adaptive nature of impulsivity," in *Impulsivity–The Behavioral and Neurological Science of Discounting*, eds G. J. Madden, and W. K. Bickel (Washington, DC: American Psychological Association), 361–387.

Talbot, C. F., Parrish, A. E., Watzek, J., Essler, J. L., Leverett, K. L., Paukner, A., et al. (2018). The influence of reward quality and quantity and spatial proximity on the responses to inequity and contrast in Capuchin Monkeys (*Cebus [Sapajus] apella*). *J. Comp. Psychol.* 132, 75–87. doi: 10.1037/com0000088

Tobin, H., and Logue, A. W. (1994). Self-control across species (*Columba livia*, *Homo sapiens*, and *Rattus norvegicus*). *J. Comp. Psychol.* 108, 126–133.

Velázquez-Sánchez, C., Ferragud, A., Moore, C. F., Everitt, B. J., Sabino, V., and Cottone, P. (2014). High trait impulsivity predicts food addiction-like behavior in the rat. *Neuropsychopharmacology* 39, 2463–2472. doi: 10.1038/npp.2014.98

Vick, S. J., Bovet, D., and Anderson, J. R. (2010). How do African grey parrots (*Psittacus erithacus*) perform on a delay of gratification task? *Anim. Cogn.* 13, 351–358. doi: 10.1007/s10071-009-0284-2

Vlamings, P. H. J. M., Uher, J., and Call, J. (2006). How the great apes (Pan troglodytes, Pongo pygmaeus, Pan paniscus, and Gorilla gorilla) perform on the reversed contingency task: The effects of food quantity and food visibility. *J. Exp. Psychol. Anim. Behav. Proces.* 32, 60–70. doi: 10.1037/0097-7403.32.1.60

Wascher, C. A., Dufour, V., and Bugnyar, T. (2012). Carrion crows cannot overcome impulsive choice in a quantitative exchange task. *Front. Psychol.* 3:118. doi: 10.3389/fpsyg.2012.00118

Winstanley, C. A., Eagle, D. M., and Robbins, T. W. (2006). Behavioral models of impulsivity in relation to ADHD: translation between clinical and preclinical studies. *Clin. Psychol. Rev.* 26, 379–395. doi: 10.1016/j.cpr.2006.01.001

Zebunke, M., Langbein, J., Manteuffel, G., and Puppe, B. (2011). Autonomic reactions indicating positive affect during acoustic reward learning in domestic pigs. *Anim. Behav.* 81, 481–489. doi: 10.1016/j.anbehav.2010.11.023

Zebunke, M., Nürnberg, G., Melzer, N., and Puppe, B. (2017). The backtest in pigs revisited-inter-situational behaviour and animal classification. *Appl. Anim. Behav. Sci.* 194, 7–13. doi: 10.1016/j.applanim.2017.05.011

Zebunke, M., Repsilber, D., Nürnberg, G., Wittenburg, D., and Puppe, B. (2015). The backtest in pigs revisited–an analysis of intra-situational behaviour. *Appl. Anim. Behav. Sci.* 169, 17–25. doi: 10.1016/j.applanim.2015.05.002

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More Than Eggs – Relationship Between Productivity and Learning in Laying Hens

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The intense selection of chickens for production traits, such as egg laying, is thought to cause undesirable side effects and changes in behavior. Trade-offs resulting from energy expenditure in productivity may influence other traits: in order to sustain energetic costs for high egg production, energy expenditure may be redirected away from specific behavioral traits. For example, such energetic trade-offs may change the hens' cognitive abilities. Therefore, we hypothesized highly productive laying hens to show reduced learning performance in comparison to moderate productive lines. We examined the learning ability of four chicken lines that differed in laying performance (200 versus 300 eggs/year) and phylogenetic origin (brown/white layer; respectively, within performance). In total 61 hens were tested in semi-automated Skinner boxes in a three-phase learning paradigm (initial learning, reversal learning, extinction). To measure the hens' learning performance within each phase, we compared the number of active decisions needed to fulfill a learning criteria (80% correct choices for learning, 70% no responses at extinction) using linear models. Differences between the proportions of hens per line that reached criterion on each phase of the learning tasks were analyzed by using a Kaplan–Meier (KM) survival analysis. A greater proportion of high productive hens achieved the learning criteria on each phase compared to less productive hens ($\text{Chi}^2{}_3 = 8.25$, $p = 0.041$). Furthermore, high productive hens accomplished the learning criteria after fewer active decisions in the initial phase ($p = 0.012$) and in extinction ($p = 0.004$) compared to the less selected lines. Phylogenetic origin was associated with differences in learning in extinction. Our results contradict our hypothesis and indicate that the selection for productivity traits has led to changes in learning behavior and the high productive laying hens possessed a better learning strategy compared to moderate productive hens in a feeding-rewarding context. This better performance may be a response to constraints resulting from high selection as it may enable these hens to efficiently acquire additional energy resources. Underlying mechanisms for this may be directly related to differences in neuronal structure or indirectly to foraging strategies and changes in personality traits such as fearfulness and sociality.

Keywords: domestication, selection, cognition, discrimination learning, poultry, laying hens, layer, extinction

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INTRODUCTION

Domestication, a process whereby an animal is shaped genetically and phenotypically while living under human supervision (Price, 1999; Jensen, 2006), led to immense but similarly directed phenotypical and behavioral changes of animals, the so-called domesticated phenotype (Jensen, 2014). More recently, intense selection for productivity traits of domesticated livestock animals has led to additional phenotypic and behavioral changes, which are considered to have potential negative effects on animal welfare (Rauw et al., 1998).

The modern chicken, *Gallus gallus domesticus*, is a good example for this process, as its behavior has been influenced and shaped by domestication and later on by selection for productivity traits (Hale, 1962; Price, 1998; Schütz and Jensen, 2001). Their domestication was not a linear process and occurred several times at different places in the world (Wood-Gush, 1959; Lyimo et al., 2014; Woldekiros and D'Andrea, 2017), leading to phylogenetic variation in layer chickens with strain-specific features (de Haas et al., 2013; Lyimo et al., 2014). Still, all domesticated chickens share, to a certain degree, similar phenotypical and behavioral modifications in comparison to the ancestral red jungle fowl (Jensen, 2006).

Modern layer breeds still do exhibit mostly the same behavior repertoire as the red jungle fowl, but with changed frequencies and/or intensities (Hale, 1962; Price, 1998; Schütz and Jensen, 2001; Jensen, 2006). Further, selection of domestic hens for increased productivity traits, such as egg laying, may have led to additional modification of specific behavioral traits, like reduced aggression or sociality (Schütz and Jensen, 2001; Schütz et al., 2001; Väisänen and Jensen, 2003; Lindqvist and Jensen, 2009).

Chickens are known to possess a complex behavioral repertoire, including sophisticated cognitive abilities (Krause et al., 2006; Nicol, 2015; Marino, 2017; Garnham and Løvlie, 2018). Like other animals, they use learning as a key mechanism to adapt to their physical and social environment (Jensen, 2006). This appears first directly after hatching with remarkable filial imprinting (Bolhuis, 1991) and continues throughout life, for example, in foraging contexts, where chickens learn to orientate, (re)locate specific food resources, or can be trained in artificial situations to use operant feeders (Nicol, 2015). Understanding the cognitive abilities of chickens and other livestock animals can have crucial impact on their husbandry and production and thus on their welfare (Nicol, 1996; Abeyesinghe et al., 2005; Smith and Johnson, 2012). As humans tend to expect animals with greater cognitive similarities to humans to be more likely to suffer (Serpell, 2004; Smith and Johnson, 2012), which is, from a scientific perspective, not reasonable (Dawkins, 2001). However, as domestication and selection have influenced particular behavioral traits, the contingent question arises, whether the cognitive abilities of domesticated and selected animals have been influenced and altered as well. In chickens, Lindqvist and Jensen (2009) have shown that the domesticated, high selected White Leghorn chickens (males and females) perform worse in a spatial learning task, compared to the ancestral red jungle fowl. However, it is difficult to say, whether the observed differences were related to the phylogenetic origin of the chickens, effects of domestication or effects of selection for productivity traits (see also Schütz et al., 2001).

An underlying mechanism, which could cause behavioral changes as reduced learning abilities in highly selected animals could be productivity-induced trade-offs, which are also predicted by the resource allocation theory (Beilharz et al., 1993; Mignon-Grasteau et al., 2005). Evolutionary adaptation should have resulted in an optimal energy allocation between self-preservation and reproductive processes in order to maximize evolutionary fitness in wild animals, like the red jungle fowl. Schütz and Jensen (2001) have hypothesized that modern layers, in contrast, may have shifted more of their energy resources toward reproduction, e.g., increased egg yield, which could lead to trade-offs represented in the behavior of the chickens.

It seems to be reasonable to assume that trade-offs might have occurred, as egg productivity increased over the last decades in layer hybrids by about 1% per year, which equates to about two additional eggs per year (Flock and Heil, 2002; McKay, 2008). Annual egg production of laying hens was at about 150 eggs in the 1940s (Klauder, 1948), whereas today, annual egg production is about 300. Layers of both white and brown lines produce more than 300 eggs/year with a very high feed conversion efficiency (Lieboldt et al., 2015a). Neuronal processes are metabolically particular costly (Brady et al., 2011; Bullmore and Sporns, 2012; Kuzawa et al., 2014), which led us to the assumption that trade-offs in cognitive skills are likely to have taken place in response to the increased egg yield (Van der Waaij, 2004; Mirkena et al., 2010).

Thus, our aim in this study was to determine whether intensive selection of domesticated layer lines for high egg yields has altered their cognitive skills. Hence, we focus, as a proxy for these skills, on the learning abilities and the flexibility in learning. We tested hens of four domesticated chicken lines in an operant conditioning task, consisting of (i) discrimination learning, (ii) a reversal learning, followed by (iii) an extinction procedure.

The four laying lines we tested, varied, using a crossed design, in their level of egg yield and their phylogenetic origin (white versus brown shell layers). Thus, two laying lines were high productive: WLA (originating from White Leghorn, 325 eggs/year) and BLA (originating from Rode Island, 310 eggs/year). The two others were moderate productive lines: R11 (originating from White Leghorn, 200 eggs/year) and L68 (originating from New Hampshire, 205 eggs/year). Growth rates, feed conversion rates, productivity, and other relevant physiological characteristics of this lines have been described in great detail in previous studies (Granevitze et al., 2009; Lieboldt et al., 2015a,b, 2016; Polasky et al., 2016; Höhne et al., 2017). For example, is the daily feed intake per mass of hens 68.73 g food/kg for WLA, 62.61 g/kg for BLA and lower for the moderate productive lines with 51.98 g/kg for L68 and 58.37 g/kg for R11 (Lieboldt et al., 2015a). According to those performance data, both high productive lines are similar to each other, as well as both moderate productive lines. A specific phylogenetic characteristic is that both brown layer lines, i.e., BLA and

L68, are heavier than both white layer lines (Lieboldt et al., 2015a).

We expected that if cognitive abilities of the hens have been altered in response to the selection for productivity, the two high productive lines should behave similarly regardless of their different phylogenetic background and that the same should be true for the two moderate productive lines. Furthermore, we assume resource trade-offs to appear and therefore that hens of high productive laying lines would possess reduced learning performances, indicated by reduced learning abilities and slower flexibility, compared to hens with a lower level of egg yield.

MATERIALS AND METHODS

Animals and Housing

We used hens of four purebred layer lines (*Gallus gallus domesticus*) differing in two dimensions in a crossed two by two design: (i) egg laying performance: high annual egg laying rate of approximately 310 eggs/year and moderate annual egg laying rate of approximately 200 eggs/year and (ii) phylogenetic origin: white layers that lay eggs with white shells and brown layers (Lieboldt et al., 2015b). The two lines with high annual egg performance are the WLA (high egg laying performance and white layer) and the BLA (high egg laying performance and brown layer). Both are originating from Lohmann Tierzucht GmbH. The two lines with moderate egg laying performance are the R11 (moderate egg laying performance and white layer) and L68 (moderate egg laying performance and brown layer). These lines originated from resource populations at the Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Mariensee, Germany. R11 has been kept at the FLI since the 1960s (Hartmann, 1987), and L68 was bred in the 1970s (VEG Vogelsang, Lieboldt et al., 2015b). The two white layer lines, WLA and R11, are of White Leghorn origin and cluster phylogenetically close together (Granevitze et al., 2009; Lyimo et al., 2014), but they are genetically distant from the two brown layers. Also both brown layer lines, BLA and L68, cluster genetically close together (Lyimo et al., 2014). BLA originates from Rhode Island, whereas L68 originates from New Hampshire (model described in Granevitze et al., 2009; Lieboldt et al., 2015a,b, 2016; Polasky et al., 2016; Höhne et al., 2017; Krause and Schrader, 2018). Chickens from all four lines were incubated and hatched simultaneously at the Institute of Animal Welfare and Animal Husbandry, FLI, Celle, Germany. At hatching, each chick was equipped with an individually numbered wing-tag for identification. All chicks were raised together and under identical conditions until the 16th week of age. Thereafter, the hens were kept in four adjacent compartments in a stable, separated by line, with 4 m^2 floor area each. Here, the hens had access to group nests, perches, litter, pecking blocks and an additional sand tank for dustbathing. In their home compartments, the hens had *ad libitum* access to water and standard commercial layer food. The light–dark cycle was set to 14 L:10 D. For this experiment, we used a total of 61 hens (BLA: $n = 17$, L68: $n = 18$, WLA: $n = 13$, and R11: $n = 13$), that were 45 weeks old at the beginning of the experiment, thus in the laying period.

Experimental Setup

The whole experiment, i.e., habituation, screen training, and the three learning phases, were carried out in four identical custom-built test-boxes (**Figure 1**), located in an adjacent room to the home-compartments of the hens. Each box consisted of plexiglass walls (width, depth, height: 55 cm × 46.5 cm × 66 cm) with a TFT monitor on one side (model DT-121-A from Distronic (Distronic, Hochheim/Main, Germany)). The display of this monitor (12.1 in (height × wide: 19 cm × 25 cm,) was a SVGA 600 × 800 pixel model (LB121S02-TD01 from Philips (Philips Deutschland GmbH, Hamburg, Germany)). Over the monitor laid a frame (IR Touch-kit 121.-A301, Citron GmbH, Augsburg, Germany) at a distance of 1.0 cm. This frame created a mesh of infrared light beams across the monitor in order to recognize pecks. When a beam was broken, e.g., when a chicken beak pecked against the monitor, the position was recorded by *x*- and *y*-coordinates. Underneath this set up of screen and frame, a foldable food trough (height × wide × depth: 1.5 cm × 4 cm × 8 cm) was placed (see **Figure 1**). To reward a hen, this trough could be filled with wheat grains (approximately 22 wheat grains, e.g., 2 g, per turn) by a computer controlled disperser (model craft, RB350-600-0A101R, Conrad Electronics, Hirschau, Germany). The time a hen was enabled to feed, in case of a reward, could be controlled and was 5 s in the screen training and the three learning levels. Thereafter, the trough was automatically cleared. The amount of the food reward was higher than the hens could eat during that time. The wheat grains were

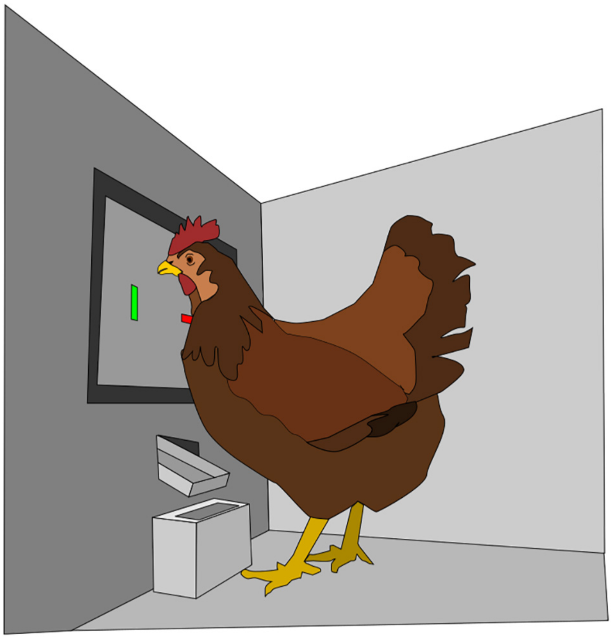

FIGURE 1 | Schematic illustration of the test box (width, depth, height: 55 cm × 46.5 cm × 66 cm). The black touchscreen (height × wide: 19 cm × 25 cm, diagonal: 31 cm) is on the back wall, displaying two different stimuli, the colored bars. Wheat grain rewards are delivered to a food trough (height × wide × depth: 1.5 cm × 4 cm × 8 cm) below the screen. The walls are made out of clear plexiglass.

stored in a container outside the box and not visible to the tested hens. When a food reward was delivered to the hen, a small white LED light close to the through turned on as well. Loudspeakers (Logitech PC stereo Z120 1.2W, Logitech Europe S.A., Lausanne, Switzerland) were placed above the monitor in the corners of the test-boxes, playing acoustic sound (*Windows Default*) when the screen was touched. A custom-made computer program, written in C++ (Microsoft Visual Studio, 2010) controlled the complete electronic set up of the box, e.g., sound, light, reward delivery, touchscreen, and monitor. For additional observation of the hens, a video camera was installed above each learning test-box. The hens were not able to see each other from inside the box.

Training, Testing, and Learning Criteria

In this experiment, the hens were observed in successive habituation, screen training, and learning phases, consisting of differential learning, reversal learning and extinction (see **Table 1**). While training and testing, the hens could obtain wheat grain rewards and had always *ad libitum* access to food and water in their home compartments. Thus, the hens were not food restricted. The protocol of training this operant conditioning task was adapted after Mar et al. (2013). For all experimental phases, the hens were individually taken from their home compartments and gently placed in to the test-boxes. Each day, 4 days a week, the hens stayed in the test box for one session to a maximum of 20 min. The time in the box, separated from the conspecifics, was slowly increased during the habituation phase (see **Table 1**). However, if a hen participated in the experiment and made quick decisions, it could decrease its time in the box, since each hen was asked to make 20 decisions per session (equal 20 trials, see **Supplementary Figure S3**). Alternatively, the time in the box ended after the 20 min.

If a hen did not succeed to finish one of the screen training levels or one of the three learning phases within 20 sessions, it was excluded from the further experiment. To successfully finish one of those phases, except the extinction, a hen needed 80% correct decisions out of at least 10 decisions. This learning criteria differs from 50% chance level and is in accordance to other learning studies (e.g., Garner et al., 2006; Nawroth et al., 2014; Brust and Guenther, 2015). To successfully finish the extinction, the hen needed to demonstrate no responses in 70% of at least 10 trials.

Stimuli

The chosen stimuli, a gray circle (diameter: 2 cm, color in RGB values: R = 224, G = 224, B = 224) for screen training and a green bar (high × length: 10 mm × 40 mm, color in RGB values: R = 20, G = 184, B = 29) and a red bar (high × length: 10 mm × 40 mm, color in RGB values: R = 237, G = 28, B = 36, see **Supplementary Figures S1, S2**) for the three different learning level, where presented on a black screen and were all detectable for the hens visual physiology (Osorio et al., 1999).

Habituation and Screen Training

The habituation phase was subdivided into five sessions (**Table 1**). Throughout this, the hens were slowly trained to stay calm and separated in the test box (**Figure 1**) and to find the food rewards in the trough. For this habituation phase, the test hen was placed in the box on the first day for 5 min with *ad libitum* access to wheat grains. During the 4 following days, the hen's time in the box increased while the access to the food reward decreased. On the fifth day of habituation, a hen stayed 15 min in the box and wheat grains were given though the delivery system with 5 s feeding time (**Table 1**).

After that, the hens continued with the screen training phase, which amount of sessions depended on the individual participation of the hens. While screen training, the hens learned to use the screen in combination with the food reward delivery system. As a stimulus for this, we used gray circle on black background (2 cm diameter, see **Supplementary Figure S1**) presented at a randomized position on the touchscreen. The screen training was again subdivided in tree level during which the hens were successively trained to peck five times on the circle in order to receive a reward (see **Table 1**). When a hen finished

TABLE 1 | Phases of the experiment and their specific characteristic.

	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

The amount of sessions a hen needed for screen training and the learning phases depended on the individual learning behavior.

the habituation sessions and screen training level successfully, it was allowed to continue with the learning tasks.

Learning Tasks

Phase 1: Discrimination Learning

For the discrimination learning, the hens needed to learn to differentiate between two simultaneously shown colored bars, red and green (see **Supplementary Material**), independent of the bars orientation. It was randomly selected, whether a hen learned that red or green was the rewarded color. Furthermore, the side of the screen on which the rewarded bar appeared was randomized, to avoid side preferences (de Haas et al., 2017a,b). Pecking on the black screen was neither rewarded nor counted as a wrong decision. If a hen made a correct decision, thus pecking on the correct bar, it was rewarded and allowed to feed on wheat grains that were provided in the trough for 5 s before the next trial appeared. Therefore, a black screen was shown for 20 s (inter-component time) and after that, the two colored bars appeared again with a randomized position (left or right) and orientation (horizontal or vertical). If a hen made a wrong decision, no reward was given, and a black screen appeared for 5 s, followed again by 20 s of inter-component time. After that, the previous shown bars appeared at the same position again (correction trial, see **Supplementary Figure S3**). Hens solved the differential learning when they made 80% correct decisions of at least 10 decisions.

Phase 2: Reversal Learning

When a hen entered the reversal learning phase, the initial unrewarded color was rewarded and the initial rewarded color was unrewarded. Everything else, e.g., inter-component time and feeding time of the reward remained the same. The reversal learning was successfully finished after 80% correct decisions of at least ten decisions. This form of learning provides two simultaneous learning tasks, and the previously learned association needs to be deleted while a new association needs to be learned (Coppens et al., 2010; Brust et al., 2014; Zidar et al., 2017).

Phase 3: Extinction

In the extinction phase, no food reward was provided and the extinction criteria was reached, when a hen did not respond to any of the symbols on the screen in 70% of a least 10 trials. If a hen did not peck, the symbols vanished after 20 s, followed by an inter-component time of 20 s. If a hen pecked on one of the symbols on the touchscreen, the black screen appeared for an inter-component time of 20 s.

Data Analysis

In order to compare the proportion of hens per line that achieved criterion on each phase of the screen training and learning task, we counted the number of hens per line, which were still participating in accordance to the above-mentioned criteria in the test at each phase. These numbers were analyzed them by using a Kaplan–Meier (KM) survival analysis (for criteria, see section "Materials and Methods").

To compare the learning performance of the hens, we analyzed the sum of their active decisions needed, to fulfill the learning criteria. The active decisions are the amount of correct and wrong decisions, whereby inactive trials with no decisions are not implicated. Throughout this, we aimed to correct for confounding motivation since a "no decision" does not reveal actual information about the learning process itself and might be likely influenced by other factors. The residuals of the average number of active decisions per phase were normal distributed and homogeneity of the variances was given; therefore, we analyzed the data with linear models (LMs). The two by two designed LMs consisted of the factors: phylogeny (two levels: white and brown layers) and productivity (two levels: high and moderate productive hens) and their interaction. Non-significant factors were not excluded from the model according to the recommendation of Forstmeier and Schielzeth (2011).

The data processing was performed using a custom – written Matlab (Matlab and Statistics Toolbox Release, 2017) script to summarize the data per training and learning level. The data per phase for the LMs were statistically analyzed using R 3.3.1, R Core Team (R Core Team, 2016) and Statistica 13 (Statistica, 2015) for the survival analysis.

Ethical Note

This study was approved by the German Lower Saxony State Office for Consumer Protection and Food Safety (LaVes) (# 33.19-42502-04-15/5054) and in accordance with German regulations on Animal Welfare.

RESULTS

Success in Participating Throughout the Test

The proportion of successful participation in the experiment differed between the four tested lines. A greater proportion of high productive hens, WLA and BLA, achieved the learning criteria on the screen training and learning levels, compared to moderate productive hens, R11 and L68 ($\mathrm{Chi}^2{}_3 = 8.25$, $p = 0.041$, see **Figure 2**).

Learning Performance

The differential learning performance was significantly affected by the different productivity levels of the hens, but not by differences in the phylogeny (LM: factor productivity: $F_{1,41} = 69.63$, $p = 0.011$; factor phylogeny: $F_{1,41} = 2.023$, $p = 0.163$; interaction productivity*phylogeny: $F_{1,41} = 2.792$, $p = 0.102$; **Figure 3**). The extinction was significantly affected by productivity and phylogeny (LM: factor productivity: $F_{1,33} = 9.543$, $p = 0.004$; factor phylogeny: $F_{1,33} = 8.588$, $p = 0.006$; interaction productivity*phylogeny: $F_{1,33} = 0.09$, $p = 0.766$; **Figure 3**). Thus, high productive hens, WLA and BLA, accomplished the learning criteria after fewer active decisions in the initial learning phase and the extinction, compared to the moderate productive lines, R11 and L68. Furthermore, the white hens, WLA and R11, needed less active decisions in the extinction compared to brown layers (**Figure 3**).

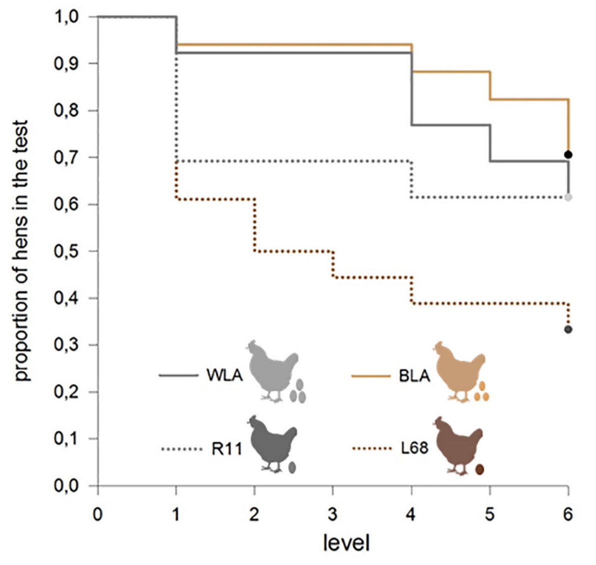

FIGURE 2 | Proportion of hens that successfully pass the learning tasks of each level. A greater proportion of high productive hens, WLA and BLA, achieved the learning criteria at the screen training and learning level, compared to moderate productive hens, R11 and L68 (Chi^2_3 = 8.25, $p = 0.041$).

In the reversal learning, all hens needed a similar amount of active decisions to fulfill the learning criteria (LM: factor productivity: $F_{1,36}$ = 1.924, p = 0.174; factor phylogeny: $F_{1,36}$ = 0.641, p = 0.423; interaction productivity*phylogeny: $F_{1,36}$ = 0.028, p = 0.867; **Figure 3**).

DISCUSSION

We found that a higher proportion of high productive hens achieved the learning criteria on each of the learning tasks compared to the moderate productive hens. Furthermore, high productive hens accomplished the learning criteria after fewer active decisions in the initial learning and in extinction compared to the hens of moderate productive lines. Reversal learning requires most likely higher levels of flexibility in learning and was solved similarly by hens from both lines and productivity levels. Phylogenetic background was only associated with differences in learning ability in extinction; white layer hens performed better than the brown lines. Both the higher success of the high productive lines in the different phases and their lower number of active decisions required to fulfill the learning criteria in the initial discrimination learning and extinction in this experiment indicate that there is no trade-off between their cognitive abilities in favor of egg laying. Rather, these hens seem to possess a more efficient learning strategy. The fewer active decisions to learn imply a faster association of the visual-acoustic cue with the food reward. This is particularly interesting in extinction, where no food is available and any activity results in additional energy expenditure. Thus, our initial hypothesis, that high productive laying hens would be worse in learning, caused by energy trade-offs, cannot be proven in this experiment. The opposite

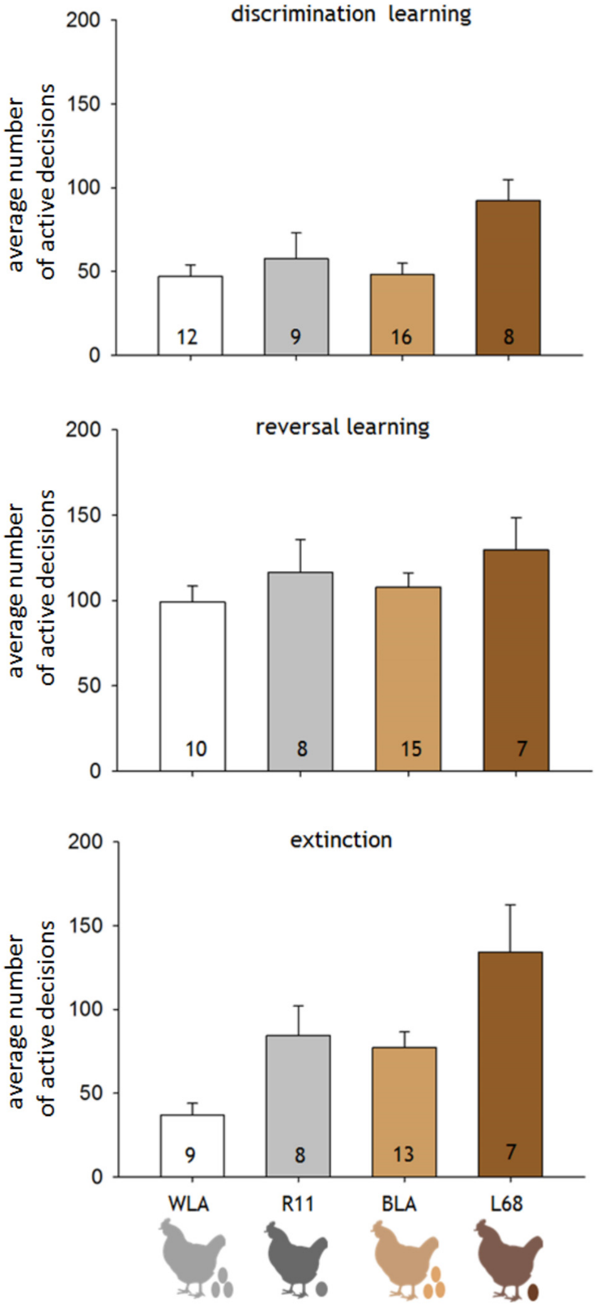

FIGURE 3 | Averaged number of active decisions (±SD) needed by hens of the different lines, WLA, R11, BLA, and L68, to fulfill the learning criteria of each phase. The number within each column represents the number of hens, which participated in that phase (*n*). Discrimination learning was significant affected by productivity (LM: factor productivity: $F_{1,41}$ = 69.63, p = 0.011; factor phylogeny: $F_{1,41}$ = 2.023, p = 1.63; interaction productivity*phylogeny: $F_{1,41}$ = 2.792, p = 0.102). Whereas reversal learning was more difficult for all hens, independent of their phylogenetic background or productivity level (LM: factor productivity: $F_{1,36}$ = 1.924, p = 0.174; factor phylogeny: $F_{1,36}$ = 0.641, p = 0.423; interaction productivity*phylogeny: $F_{1,36}$ = 0.028, p = 0.867). The average active decisions needed for the extinction level were significantly affected by phylogenetic origin and productivity (LM: factor productivity: $F_{1,33}$ = 9.543, p = 0.004; factor phylogeny: $F_{1,33}$ = 8.588, p = 0.006; interaction productivity*phylogeny: $F_{1,33}$ = 0.09, p = 0.766).

seems to be the case but is still highlighting that the effect of the recent intense selection for high egg yields has altered behavior and cognitive skills of laying hens. Speculatively, the observed more efficient learning in the high productive hens may be a strategy to optimize energy intake. However, direct effects of this in high selected hens may be related to changes in the brain structure and indirectly to changes in food motivation or changes in personality traits.

A factor ensuring the hens to participate in the learning tasks is the food reward, while the value of that reward may vary between hens from the different lines. The high productive laying hens have been shown to possess a higher energy and nutrition demand than the moderate productive layers, due to higher egg lay production (Lieboldt et al., 2015a). It is likely that this has resulted in an increased food motivation. Such an increase of food motivation has been observed in meat chickens intensively selected for intensive body growth (Bokkers and Koene, 2002). Thus, a higher motivation to obtain the reward may have contributed to the observed differences in learning ability between the productivity lines. However, it is worth noticing that the hens in our study had *ad libitum* access to food in their home pens and no food deprivation prior to testing so motivational differences may be relatively minimized. In addition, by analyzing the active decision, we further reduced a possible impact of motivation. Nevertheless, it would be interesting to rerun the experiments with another reward system than food, e.g., social reinforces or enabling comfort behavior like dust bathing. In order to get further information on whether the actual egg laying directly affects the learning performance, it would be also interesting to test the males of each line. This would reveal further information on, whether the selection for egg productivity selectively targets the female's genetical makeup.

Another reward-related influence on the results may be connected to differences in foraging strategies. Support for the idea that foraging strategies may have changed through selection is provided in the study by Schütz and Jensen (2001), who compared the behavior of high productive White leghorn layer hybrids with the red jungle fowl and the Swedish bantam chicken, a domesticated chicken line, which, however, is not selected for productivity. In their study, hens could either feed from an undiluted *ad libitum* food source or use a bowl where they needed to search and sort for food in wood-shavings (Schütz and Jensen, 2001). The high productive laying hens were more likely to eat from the undiluted food source, while the other two lines performed more foraging behavior and fed more often from the food source where searching and sorting was required. A similar underlying process might contribute to our findings, i.e., the higher number of active decisions needed to obtain a food reward shown by the moderate productive lines. These hens could use a more flexible foraging strategy, making them more likely to try the unrewarded stimulus, thereby requiring more active decisions to fulfill the learning criteria. Considering the moderate productive laying hen, as a slightly more native line, it can be argued that a flexible foraging strategy would be more effective in a natural environment, where resource patches are finite and variable. Higher flexibility provides the opportunity to learn about possible alternative food resources (Schütz and Jensen, 2001). High productive laying hens, arguably more adapted to domestic environments with secure food supplies can be more effective with a less flexible feeding strategy. Therefore, the high productive hens may have been more focused on exploiting one food source and may not even try other alternatives, once a successful strategy had been developed. To further investigate this idea, it would be also interesting to test other rewards, as mentioned above.

An important factor, which can influence the learning abilities of animals, is stress (Mendl, 1999). Stress can be caused by different factors, for example, by neophobia, general fearfulness or social stress, for e.g., caused by social isolation (Mendl et al., 1997; Mendl, 1999; Shaw and Schmelz, 2017), in which intensity an animal perceives or is affected by stress can also be related to its personality traits (de Haas et al., 2017a). Personality traits, in general, have been shown to influence learning in wild as well as in domesticated animals (Lansade and Simon, 2010; Guenther et al., 2014; de Haas et al., 2017a,b; Zidar et al., 2017). Furthermore, those traits have been described in fowl (Favati et al., 2015; Zidar et al., 2017; Dudde et al., 2018) and traits like sociality or fearfulness show some degree of heritability (Jensen, 2006; Garnham and Løvlie, 2018). Fearful hens, for example, may be poorer learners than less-fearful conspecifics (de Haas et al., 2017a,b). Further, Schütz et al. (2001) indicated that high productive laying hens may have a lower level of social motivation. Therefore, it is likely that in the here presented experiment, high productive lines may experience less social stress when separated from the flock for testing and therefore perform better in the given task. Such a result could be shown in goats, where individuals with a lower level of social motivation performed better in a visual discrimination task (Nawroth et al., 2017). In general, the here tested chicken lines could vary in their distribution of personality types (Dudde et al., 2018). Hence, in our learning task, phylogenetic background could have led to associated differences in the personality between the lines, which in turn, may have affected cognitive performance.

Nevertheless, it is possible that through the intensive selection process, the cognitive abilities in associative learning contexts of high productive hens have indeed improved. Underlying neurological mechanism that might have led to such an improved cognitive ability remains subject to speculation. Potentially, it could be related to adaptations of the neuronal structures, like neuronal density or lateralization, in high productive hens. Other studies have shown that brain structures can affect cognitive performance, e.g., the lateralization in chickens (Rogers et al., 2004; Daisley et al., 2010). The domestication process itself had a marked effect on the brain of domesticated animals, e.g., their brain size decreased (Kruska and Steffen, 2013). However, brain size itself does not correlate with cognitive function but more likely relates to changes in neuronal perception of acoustic or visual stimuli (Chittka and Niven, 2009). Therefore, it might be interesting to investigate from a behavioral perspective whether the sensitivity toward physical cues of high selected laying hens has altered. From a neurobiological perspective, it could be interesting to compare brain allometry, neuronal density, or lateralization of high selected laying hens in comparison to moderate productive lines.

CONCLUSION

Taken together, our results demonstrate that laying hens have good cognitive abilities, as they can learn reasonably complex tasks. In contrast to our initial hypotheses, these abilities do not seem to be limited by resource trade-offs, resulting from high selected levels of egg lay capacity. Instead, it seems that the selection for productivity traits has led to changes in learning behavior and the high productive laying hens showed a better learning performance compared to moderate productive hens in a feeding-rewarding context. These higher levels of performance may be in response to constraints imposed by high selection pressure on productivity, resulting in more efficient strategies to gain additional energy, which may ameliorate the trade-offs from selection on high egg yields. Underlying mechanisms for this may be directly related to differences in neuronal structure or indirectly to foraging strategies and changes in personality traits such as fearfulness and sociality.

AUTHOR CONTRIBUTIONS

AD, EK, LM, and LS conceived and designed the study and wrote the manuscript. AD collected the data. AD and EK analyzed the data.

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REFERENCES

Abeyesinghe, S. M., Nicol, C. J., Hartnell, S. J., and Wathes, C. M. (2005). Can domestic fowl, *Gallus gallus* domesticus, show self-control? *Anim. Behav.* 70, 1–11. doi: 10.1016/j.anbehav.2004.10.011

Beilharz, R., Luxford, B., and Wilkinson, J. (1993). Quantitative genetics and evolution: is our understanding of genetics sufficient to explain evolution? *J. Anim. Breed. Genet.* 110, 161–170. doi: 10.1111/j.1439-0388.1993.tb00728.x

Bokkers, E. A., and Koene, P. (2002). Sex and type of feed effects on motivation and ability to walk for a food reward in fast growing broilers. *Appl. Anim. Behav. Sci.* 79, 247–261. doi: 10.1016/S0168-1591(02)00151-X

Bolhuis, J. J. (1991). Mechanisms of avian imprinting: a review. *Biol. Rev.* 66, 303–345. doi: 10.1111/j.1469-185X.1991.tb01145.x

Brady, S., Siegel, G., Albers, R. W., and Price, D. (eds) (2011). *Basic Neurochemistry: Principles of Molecular, Cellular, and Medical Neurobiology*. Waltham, MA: Academic press.

Brust, V., and Guenther, A. (2015). Domestication effects on behavioural traits and learning performance: comparing wild cavies to guinea pigs. *Anim. Cogn.* 18, 99–109. doi: 10.1007/s10071-014-0781-9

Brust, V., Krüger, O., Naguib, M., and Krause, E. T. (2014). Lifelong consequences of early nutritional conditions on learning performance in zebra finches (*Taeniopygia guttata*). *Behav. Process.* 103, 320–326. doi: 10.1016/j.beproc.2014.01.019

Bullmore, E., and Sporns, O. (2012). The economy of brain network organization. *Nat. Rev. Neurosci.* 13, 336–349. doi: 10.1038/nrn3214

Chittka, L., and Niven, J. (2009). Are bigger brains better? *Curr. Biol.* 19, R995–R1008. doi: 10.1016/j.cub.2009.08.023

Coppens, C. M., de Boer, S. F., and Koolhaas, J. M. (2010). Coping styles and behavioural flexibility: towards underlying mechanisms. *Philos. Trans. R. Soc. B* 365, 4021–4028. doi: 10.1098/rstb.2010.0217

Daisley, J. N., Vallortigara, G., and Regolin, L. (2010). Logic in an asymmetrical (social) brain: transitive inference in the young domestic chick. *Soc. Neurosci.* 5, 309–319. doi: 10.1080/17470910903529795

Dawkins, M. I. S. (2001). Who needs consciousness? *Anim. Welf.* 10, S19–S29.

de Haas, E., Kemp, B., Bolhuis, J., Groothuis, T., and Rodenburg, T. (2013). Fear, stress, and feather pecking in commercial white and brown laying hen parent-stock flocks and their relationships with production parameters. *Poult. Sci.* 92, 2259–2269. doi: 10.3382/ps.2012-02996

de Haas, E. N., Lee, C., Hernandez, C. E., Naguib, M., and Rodenburg, T. B. (2017a). Individual differences in personality in laying hens are related to learning a colour cue association. *Behav. process.* 134, 37–42. doi: 10.1016/j.beproc.2016.11.001

de Haas, E. N., Lee, C., and Rodenburg, T. B. (2017b). learning and judgment can be affected by predisposed fearfulness in laying hens. *Front. Vet. Sci.* 4:113. doi: 10.3389/fvets.2017.00113

Dudde, A., Schrader, L., Weigend, S., Mathews, L. R., and Krause, E. T. (2018). More eggs but less social and more fearful? Differences in behavioral traits in relation to the phylogenetic background and productivity level in laying hens. *Appl. Anim. Behav. Sci.* (in press). doi: 10.1016/j.applanim.2018.08.017

Favati, A., Zidar, J., Thorpe, H., Jensen, P., and Løvlie, H. (2015). The ontogeny of personality traits in the red junglefowl, *Gallus gallus*. *Behav. Ecol.* 27, 484–493. doi: 10.1093/beheco/arv177

Flock, D., and Heil, G. (2002). Eine langzeitanalyse der leistungsentwicklung weißer und brauner legehybriden anhand von ergebnissen der amtlichen deutschen legeleistungsprüfungen von 1974/75 bis 1997/99. *Arch. Geflügelkunde* 66, 1–20.

Forstmeier, W., and Schielzeth, H. (2011). Cryptic multiple hypotheses testing in linear models: overestimated effect sizes and the winner's curse. *Behav. Ecol. Sociobiol.* 65, 47–55. doi: 10.1007/s00265-010-1038-5

Garner, J. P., Thogerson, C. M., Würbel, H., Murray, J. D., and Mench, J. A. (2006). Animal neuropsychology: validation of the intra-dimensional extra-dimensional set shifting task for mice. *Behav. Brain Res.* 173, 53–61. doi: 10.1016/j.bbr.2006.06.002

Garnham, L., and Løvlie, H. (2018). Sophisticated fowl: the complex behaviour and cognitive skills of chickens and red junglefowl. *Behav. Sci.* 8:13. doi: 10.3390/bs8010013

Granevitze, Z., Hillel, J., Feldman, M., Six, A., Eding, H., and Weigend, S. (2009). Genetic structure of a wide-spectrum chicken gene pool. *Anim. Genet.* 40, 686–693. doi: 10.1111/j.1365-2052.2009.01902.x

Guenther, A., Brust, V., Dersen, M., and Trillmich, F. (2014). Learning and personality types are related in cavies (*Cavia aperea*). *J. Comp. Psychol.* 128:74. doi: 10.1037/a0033678

Hale, E. (1962). "Domestication and the evolution of behavior," in *The Behaviour of Domestic Animals*, ed. E. S. E. Hafez (London: Baillieàre, Tindall & Cox Ltd.), 22–53.

Hartmann, W. (1987). "Genetic aspects of resistance to avian leukosis and Marek's disease," in *Proceedings of the 36th Annual National Breeder's Roundtable*, (Decatur, GA: Poultry Breeders of America), 34–72.

Höhne, A., Schrader, L., Weigend, S., and Petow, S. (2017). Ghrelin plasma concentration does not covary with energy demand in adult laying hens. *Domest. Anim. Endocrinol.* 61, 77–83. doi: 10.1016/j.domaniend.2017.06.006

Jensen, P. (2006). Domestication—from behaviour to genes and back again. *Appl. Anim. Behav. Sci.* 97, 3–15. doi: 10.1016/j.applanim.2005.11.015

Jensen, P. (2014). Behavior genetics and the domestication of animals. *Annu. Rev. Anim. Biosci.* 2, 85–104. doi: 10.1146/annurev-animal-022513-114135

Klauder, G. (1948). *Landwirtschaftliche Faustzahlen*. Berlin: Paul Parey.

Krause, E. T., Naguib, M., Trillmich, F., and Schrader, L. (2006). The effects of short term enrichment on learning in chickens from a laying strain (*Gallus gallus* domesticus). *Appl. Anim. Behav. Sci.* 101, 318–327. doi: 10.1016/j.applanim.2006.02.005

Krause, E. T., and Schrader, L. (2018). High, low, or familiar? Nest site preferences of experienced laying hens. *Br. Poult. Sci.* 59, 359–364. doi: 10.1080/00071668.2018.1470318

Kruska, D. C., and Steffen, K. (2013). Comparative allometric investigations on the skulls of wild cavies (*Cavia aperea*) versus domesticated guinea pigs (C. aperea f. porcellus) with comments on the domestication of this species. *Mammal. Biol.* 78, 178–186. doi: 10.1016/j.mambio.2012.07.002

Kuzawa, C. W., Chugani, H. T., Grossman, L. I., Lipovich, L., Muzik, O., Hof, P. R., et al. (2014). Metabolic costs and evolutionary implications of human brain development. *Proc. Natl. Acad. Sci. U.S.A.* 111, 13010–13015. doi: 10.1073/pnas.1323099111

Lansade, L., and Simon, F. (2010). Horses' learning performances are under the influence of several temperamental dimensions. *Appl. Anim. Behav. Sci.* 125, 30–37. doi: 10.1016/j.applanim.2010.02.010

Lieboldt, M., Frahm, J., Halle, I., Görs, S., Schrader, L., Weigend, S., et al. (2016). Metabolic and clinical response to *Escherichia coli* lipopolysaccharide in layer pullets of different genetic backgrounds supplied with graded dietary L-arginine. *Poult. Sci.* 95, 595–611. doi: 10.3382/ps/pev359

Lieboldt, M., Frahm, J., Schrader, L., Baulain, U., Henning, M., and Preisinger, R. (2015a). Phylogenic versus selection effects on growth development, egg laying and egg quality in purebred laying hens. *Eur. Poult. Sci.* 79:22.

Lieboldt, M., Halle, I., Frahm, J., Schrader, L., Weigend, S., Preisinger, R., et al. (2015b). Effects of long-term graded L-arginine supply on growth development, egg laying and egg quality in four genetically diverse purebred layer lines. *J. Poult. Sci.* 53, 8–21. doi: 10.2141/jpsa.0150067

Lindqvist, C., and Jensen, P. (2009). Domestication and stress effects on contrafreeloading and spatial learning performance in red jungle fowl (*Gallus gallus*) and White leghorn layers. *Behav. Process.* 81, 80–84. doi: 10.1016/j.beproc.2009.02.005

Lyimo, C., Weigend, A., Msoffe, P., Eding, H., Simianer, H., and Weigend, S. (2014). Global diversity and genetic contributions of chicken populations from African, Asian and European regions. *Anim. Genet.* 45, 836–848. doi: 10.1111/age.12230

Mar, A. C., Horner, A. E., Nilsson, S. R. O., Alsiö, J., Kent, B. A., Kim, C. H., et al. (2013). The touchscreen operant platform for assessing executive function in rats and mice. *Nat. Protoc.* 8, 1985–2005. doi: 10.1038/nprot.2013.123

Marino, L. (2017). Thinking chickens: a review of cognition, emotion, and behavior in the domestic chicken. *Anim. Cogn.* 20, 127–147. doi: 10.1007/s10071-016-1064-4

Matlab and Statistics Toolbox Release (2017). *Matlab and Statistics Toolbox Release*. Natick, MA: The MathWorks, Inc.

McKay, J. (2008). "The genetics of modern commercial poultry," in *Proceedings of the 23rd World's Poultry Congress*, Brisbane, QLD.

Mendl, M. (1999). Performing under pressure: stress and cognitive function. *Appl. Anim. Behav. Sci.* 65, 221–244. doi: 10.1016/S0168-1591(99)00088-X

Mendl, M., Laughlin, K., and Hitchcock, D. (1997). Pigs in space: spatial memory and its susceptibility to interference. *Anim. Behav.* 54, 1491–1508. doi: 10.1006/anbe.1997.0564

Mignon-Grasteau, S., Boissy, A., Bouix, J., Faure, J.-M., Fisher, A. D., Hinch, G. N., et al. (2005). Genetics of adaptation and domestication in livestock. *Livest. Sci.* 93, 3–14. doi: 10.1016/j.livprodsci.2004.11.001

Mirkena, T., Duguma, G., Haile, A., Tibbo, M., Okeyo, A., Wurzinger, M., et al. (2010). Genetics of adaptation in domestic farm animals: a review. *Livest. Sci.* 132, 1–12. doi: 10.1016/j.livsci.2010.05.003

Nawroth, C., Prentice, P. M., and McElligott, A. G. (2017). Individual personality differences in goats predict their performance in visual learning and non-associative cognitive tasks. *Behav. Process.* 134, 43–53. doi: 10.1016/j.beproc.2016.08.001

Nawroth, C., von Borell, E., and Langbein, J. (2014). Exclusion performance in dwarf goats (Capra aegagrus hircus) and sheep (*Ovis orientalis* aries). *PLoS One* 9:e93534. doi: 10.1371/journal.pone.0093534

Nicol, C. (1996). Farm animal cognition. *Anim. Sci.* 62, 375–391. doi: 10.1017/S1357729800014934

Nicol, C. J. (2015). *The Behavioural Biology of Chickens*. Wallingford: CABI. doi: 10.1079/9781780642499.0000

Osorio, D., Miklósi, A., and Gonda, Z. (1999). Visual ecology and perception of coloration patterns by domestic chicks. *Evol. Ecol.* 13, 673–689. doi: 10.1023/A:1011059715610

Polasky, C., Weigend, S., Schrader, L., and Berndt, A. (2016). Non-specific activation of CD8α-characterised γδ T cells in PBL cultures of different chicken lines. *Vet. Immunol. Immunopathol.* 179, 1–7. doi: 10.1016/j.vetimm.2016.07.008

Price, E. O. (1998). "Behavioral genetics and the process of animal domestication," in *Genetics and the Behavior of Domestic Animals*, ed. T. Grandin (San Diego, CA: Academic Press).

Price, E. O. (1999). Behavioral development in animals undergoing domestication. *Appl. Anim. Behav. Sci.* 65, 245–271. doi: 10.1016/S0168-1591(99)00087-8

R Core Team (2016). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Rauw, W., Kanis, E., Noordhuizen-Stassen, E., and Grommers, F. (1998). Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livest. Prod. Sci.* 56, 15–33. doi: 10.1016/S0301-6226(98)00147-X

Rogers, L. J., Zucca, P., and Vallortigara, G. (2004). Advantages of having a lateralized brain. *Proc. R. Soc. Lond. B* 271, 420–422. doi: 10.1098/rsbl.2004.0200

Schütz, K. E., Forkman, B., and Jensen, P. (2001). Domestication effects on foraging strategy, social behaviour and different fear responses: a comparison between the red junglefowl (*Gallus gallus*) and a modern layer strain. *Appl. Anim. Behav. Sci.* 74, 1–14. doi: 10.1016/S0168-1591(01)00156-3

Schütz, K. E., and Jensen, P. (2001). Effects of resource allocation on behavioural strategies: a comparison of red junglefowl (*Gallus gallus*) and two domesticated breeds of poultry. *Ethology* 107, 753–765. doi: 10.1046/j.1439-0310.2001.00703.x

Serpell, J. A. (2004). Factors influencing human attitudes to animals and their welfare. *Anim. Welf.* 13, 145–152.

Shaw, R. C., and Schmelz, M. (2017). Cognitive test batteries in animal cognition research: evaluating the past, present and future of comparative psychometrics. *Anim. Cogn.* 20, 1003–1018. doi: 10.1007/s10071-017-1135-1

Smith, C. L., and Johnson, J. (2012). The chicken challenge: what contemporary studies of fowl mean for science and ethics. *Between Species* 15, 75–102. doi: 10.15368/bts.2012v15n1.4

Statistica, D. (2015). *Dell Statistica Data Analysis Software System, Version 13*. Round Rock, TX: Dell Inc.

Väisänen, J., and Jensen, P. (2003). Social versus exploration and foraging motivation in young red junglefowl (*Gallus gallus*) and White Leghorn layers. *Appl. Anim. Behav. Sci.* 84, 139–158. doi: 10.1016/j.applanim.2003.07.001

Van der Waaij, E. (2004). A resource allocation model describing consequences of artificial selection under metabolic stress. *J. Anim. Sci.* 82, 973–981. doi: 10.2527/2004.824973x

Woldekiros, H., and D'Andrea, A. (2017). Early evidence for domestic chickens (*Gallus gallus* domesticus) in the Horn of Africa. *Int. J. Osteoarchaeol.* 27, 329–341. doi: 10.1002/oa.2540

Wood-Gush, D. (1959). A history of the domestic chicken from antiquity to the 19th century. *Poult. Sci.* 38, 321–326. doi: 10.3382/ps.0380321

Zidar, J., Sorato, E., Malmqvist, A.-M., Jansson, E., Rosher, C., Jensen, P., et al. (2017). Early experience affects adult personality in the red junglefowl: a role for cognitive stimulation? *Behav. Process.* 134, 78–86. doi: 10.1016/j.beproc.2016.06.003

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Valence of Facial Cues Influences Sheep Learning in a Visual Discrimination Task

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Sheep are one of the most studied farm species in terms of their ability to process information from faces, but little is known about their face-based emotion recognition abilities. We investigated (a) whether sheep could use images of sheep faces taken in situation of varying valence as cues in a simultaneous discrimination task and (b) whether the valence of the situation affects their learning performance. To accomplish this, we photographed faces of sheep in three situations inducing emotional states of neutral (ruminating in the home pen) or negative valence (social isolation or aggressive interaction). Sheep ($n = 35$) first had to learn a discrimination task with colored cards. Animals that reached the learning criterion ($n = 16$) were then presented with pairs of images of the face of a single individual taken in the neutral situation and in one of the negative situations. Finally, sheep had to generalize what they had learned to new pairs of images of faces taken in the same situation, but of a different conspecific. All sheep that learned the discrimination task with colored cards reached the learning criterion with images of faces. Sheep that had to associate a negative image with a food reward learned faster than sheep that had to associate a neutral image with a reward. With the exception of sheep from the aggression-rewarded group, sheep generalized this discrimination to images of faces of different individuals. Our results suggest that sheep can perceive the emotional valence displayed on faces of conspecifics and that this valence affects learning processes.

Keywords: sheep, faces, emotions, discrimination task, ovis aries, cognition

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INTRODUCTION

Faces are an essential source of information for social species ranging from primates to ungulates such as sheep. By looking at the face of another animal, individuals can obtain information about identity, emotional state, sexual attraction, or gaze direction (1). Sheep are one of the most studied livestock species in terms of face processing and are able to discriminate between faces of at least 50 conspecifics, and to remember these faces for up to 2 years (2). Sheep, like cattle, are also sensitive to social familiarity in faces and show preferences for familiar faces over unfamiliar ones (3, 4). Individual recognition based on faces is also stable over time in sheep; ewes trained to identify images of faces of 3-month-old lambs were able to discriminate the same lambs aged only 1 month (5).

In animals, emotional states can be expressed through vocalizations (6), odors (7), posture (8), or facial expressions (9). Outward expressions of emotions are a way of communicating social information to conspecifics as well as across species, as highlighted in recent studies of perception of human faces by dogs, horses, and even giant pandas (10–12). Despite their relative lack of facial mobility or of a facial musculature as complex as that of non-human primates, sheep display emotional expressions through their faces, and especially through ear postures (13, 14). The role of facial features such as eyes, mouth, and cheek muscles have also been identified in sheep facial expressions linked to pain (15). Moreover, conspecifics can distinguish between facial displays of emotions. Indeed, when presented with images of the face of the same familiar conspecific taken in a stressful (isolation) or in a calm situation, sheep showed a preference for the calm face (3).

Animals thus possess a wide variety of ways to express their emotions. For researchers in the field of animal welfare science, being able to assess animals' emotional states has been a major focus, moving from more traditional physiological and behavioral measures to the development of the cognitive bias tests (16). Cognitive bias refers to the influence that the valence of emotional states has on cognitive processes, leading to biases in judgment, memory, or attention (17). For instance, animals in negative emotional states make "pessimistic" judgments in judgment bias tests, while animals in positive emotional states show "optimistic" judgments. This method has been applied to several species, and has especially been used to assess the impact of husbandry practices on the welfare of farmed species [reviewed in Ref. (18)]. Most recently, judgment bias tests have even been extended to insects (19, 20).

The present study was part of a larger project investigating the potential use of images of faces of conspecifics as cues in judgment bias tests with small ruminants. Its first aim was to assess the ability of sheep to distinguish between facial displays of different emotional states. To that end, we first investigated whether sheep could learn to use images of faces of familiar conspecifics displaying different emotional states as cues in a simultaneous discrimination task. We took photos of sheep in three situations (social isolation, aggressive interaction, and ruminating in the home pen) that are considered to induce emotional states of different valence. In the first phase of training, we used simple colored cards, to ensure sheep could learn the discrimination task in the experimental setup. In the second phase, sheep were trained with pairs of images of faces taken from the same individual but in two different situations.

Since most cognitive bias studies use secondary reinforcers, an extensive training phase is required whereby animals learn to associate one cue with a positive consequence and another cue with a negative consequence. If the valence of the emotional state experienced by a sheep can be perceived by conspecifics in images of its face, then faces would be stimuli with an inherent value for the other animals observing them, and thus the training phase would not be necessary. Hence, the second aim of this study was to determine whether sheep perceived the valence of the emotional state displayed in an image of the face of a conspecific. Social familiarity has been shown to influence learning speed in discrimination tasks, with sheep learning to discriminate faster between faces of a familiar breed than between faces of an unfamiliar breed or between symbols (21). However, little is known about the influence of facial expressions of different emotional states on the learning process in a discrimination task. We hypothesized that learning speed is affected by the type of images rewarded, i.e., that the emotional valence displayed in the image of a face affects learning, but that during the first training phase with colored cards, the type of cards rewarded would not affect learning speed. Presenting images of faces has been shown to reduce stress in sheep (22) and images of conspecifics are primary reinforcers, i.e., they are naturally approached by sheep (23). We thus predicted that learning the association between an image of a neutral face and a reward but also generalizing this association to images of faces of new familiar individuals would be easier than the association between the image of a stressed face and a reward.

MATERIALS AND METHODS

Ethical Note

All experimental procedures were approved by the Scotland's Rural College (SRUC) Edinburgh Animal Ethics Committee (Protocol no. ED-AE-2-2014). Animals were closely monitored before, during, and after the study.

Animals and Housing

Testing took place between March and July 2014 at the SRUC Woodhouselee experimental farm at Easter Bush (UK). Forty non-pregnant female Scottish Mule sheep of 10–12 months of age (37.1 ± 4.8 kg) were used in this study. The sheep were born and reared on the experimental farm and were familiar with each other, having lived in the same flock for at least 6 months prior to the study. Four sheep (thereafter referred to as Photo Sheep) were pseudo-randomly selected based on body weight and had their faces filmed. This selection on body weight enabled us to select animals that were of average body weight (37.55 ± 3.6 kg). The positive correlation between live weight and hierarchy is well established in ungulates (24, 25) and choosing sheep of intermediate weight was done to avoid the selection of only dominant animals. The Photo Sheep did not take part in the discrimination task but were housed with the rest of the group until the end of the study.

All sheep were housed indoors in a straw-bedded pen (4 m × 12 m) for the duration of the experiment. Animals had *ad libitum* access to hay and water as their main diet. They were also fed a limited amount of concentrate pellets (0.5 kg per animal per day) after training every day and showed a high motivation to eat it. This allowed us to use concentrate pellets as reward in the tests without having to food-deprive the animals.

Habituation to Handling

Scottish Mules sheep are a hill breed and typically have limited contact with humans throughout the year. The experimental animals had little experience of human handling and living indoors, and therefore underwent a short phase of systematic desensitization to facilitate handling (26), and to limit the impact of handling stress on responses to tests. This habituation procedure involved four consecutive steps that allowed the animals to gradually adapt.

First, the animals were handled in three smaller groups (two groups of 13 and one group of 14 sheep). A group was only moved to the next step once all sheep went calmly through the previous step. For the first step, the group was moved into a small handling pen with the gate open. For the second step, the group was confined in a small handling pen with no human handler present. The third step consisted of confining the group in the same small handling pen, but with an experimenter standing just outside the pen. Finally, for the fourth step, the experimenter had to touch calmly every sheep within the group. Following the fourth step, each group of sheep was moved through raceways into 4 m × 4 m pen that served as the test arena. Once in the test arena, they remained there for 10 min and received a small amount of concentrate feed. This manipulation was repeated three times with the group size decreasing to five and then to two sheep.

Images of Faces

We filmed each of the four Photo Sheep in three situations. For each situation, short video clips of the Photo Sheep were taken with an HD camcorder (Legria HFM52, Canon, Tokyo, Japan) and frames with a full clear frontal view of the face were extracted from the video clips using Pinnacle Studio 17 (Pinnacle Systems, 2013). Then, using Adobe Photoshop CC (Adobe Systems, 2014), the faces were digitally cut from the frames and placed against a neutral beige background (RGB model: $R = 217$, $G = 202$, $B = 126$) and levels of brightness and contrast were adjusted (**Figure 1**).

Ruminating in the Home Pen

The Photo Sheep were filmed by a familiar experimenter while standing ruminating in their home pen. Rumination was considered to be a relaxed state of neutral valence and low arousal, similar to the one described in horses by Wathan et al. (27). Rumination has also been used as an indicator of habituation to a stressful situation in sheep (28). Animals had their ears in the frontal plane, showed no flared nostrils or wide eyes and were looking straight at the camera (**Figure 1**, a-1, b-1, c-1, d-1).

Social Isolation

Each of the Photo Sheep was isolated in a small pen (4.5 m × 4.5 m) with solid walls (approximately 140 cm high) for 90 s. The Photo Sheep were only isolated once. No visual contact with conspecifics was allowed, but the pen was located in the same building as the home pen, and so auditory and olfactory contact with other sheep was maintained. Short video clips were recorded by two hidden experimenters. All animals displayed stress-related behaviors such as increased locomotion, high pitched vocalizations (23, 29), and attempts to escape from the test pen (30). This situation was thus considered as inducing an emotional state of negative valence and high arousal (**Figure 1**, a-2, c-2).

Aggressive Interactions

A trough allowing access to concentrate feed to only one sheep at a time was placed in a test arena with solid walls (4.5 m × 4.5 m). Photo Sheep were paired for this situation and all possible pairs were filmed (six pairs). A given pair of Photo Sheep entered the test arena simultaneously and was given 2 min to interact while being filmed by two hidden experimenters. In each pair, both Photo Sheep showed agonistic behaviors such as head threats, head butts, or pushes (29, 31). Images of faces were created from faces of Photo Sheep filmed frontally and while initiating a bout of aggressive interaction (head threat) (**Figure 1**, b-2, d-2), and this situation was considered to have induced a negative emotional state of high arousal in both sheep.

Discrimination Task in a Two-Armed Maze

In the simultaneous discrimination task, sheep had to learn to associate one cue with a food reward and a second cue with a negative consequence. Positive reinforcement consisted of a food reward, namely a small amount of concentrate pellets (12.5 ± 1.5 g) placed in a bucket. Positive punishment consisted of

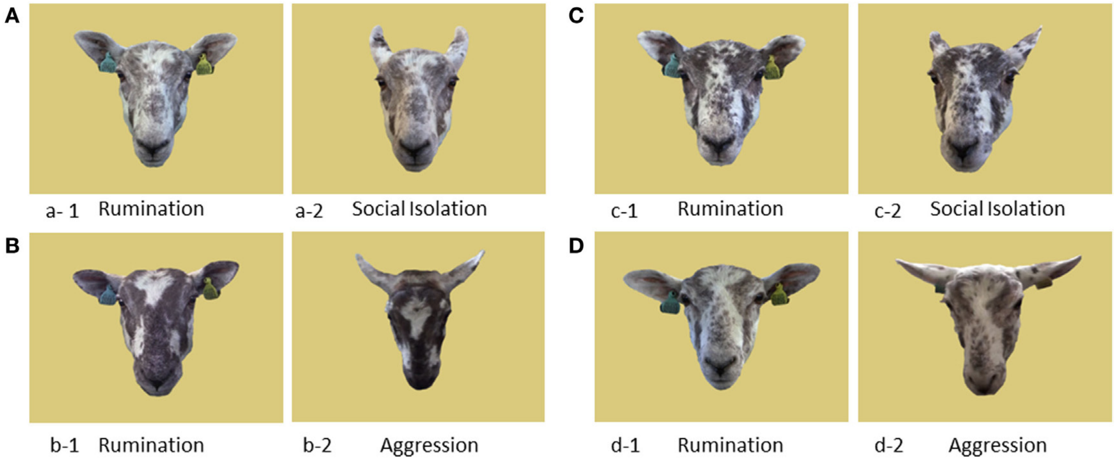

FIGURE 1 | Pairs of images obtained from four different Photo Sheep **(A–D)** and presented simultaneously in the maze during training and test sessions. **(A,B)** were used during training and **(C,D)** during tests.

keeping the sheep in social isolation for 60 s in the incorrect arm of the maze. A bucket containing pellets but closed with a mesh lid was also placed in the incorrect arm, so that the animal could see and smell but not eat the food (**Figure 2**). The type of cue used depended on the training phase. There was no previous evidence of sheep learning a discrimination task either in a similar setup or with images of faces. Consequently, if sheep had failed to learn the task with images of faces, it would have been impossible to distinguish whether this failure was due to the discrimination task being too complex, or because it was too difficult for the animals to process the new type of cues. Thus, before images of faces, simple colored cards were used as cues, to determine whether sheep were capable of learning the simultaneous discrimination task. Using cards in the first phase also allowed us to compare sheep's behavioral responses when presented with neutral stimuli and with images of faces. The experiment was divided into five consecutive phases: (1) habituation to the maze, (2) training with colored cards, (3) transition training, (4) training with images of faces, and (5) tests with new images of faces (**Figure 3**).

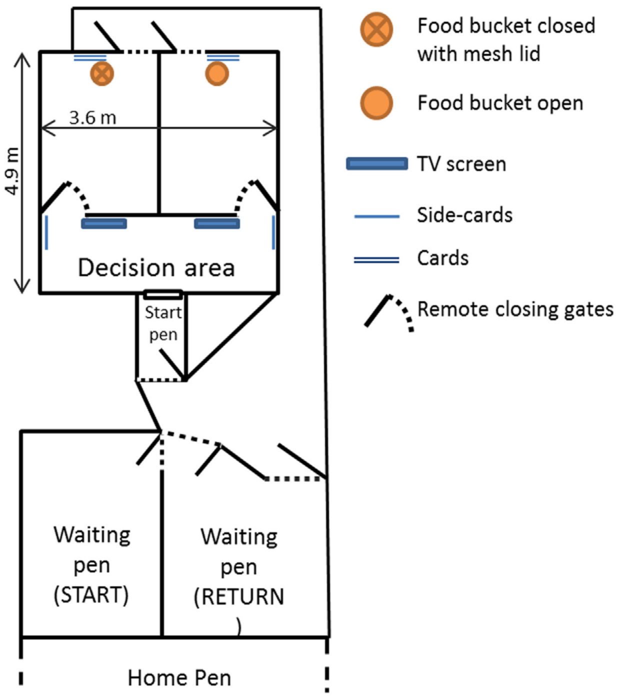

FIGURE 2 | Representation of the experimental setup. This includes the two-armed maze, the start pen and the start and return waiting pens, as well as the raceways connecting them. The position of the food buckets alternated between runs depending on which side the rewarded image was placed. "Side cards" and "cards" represent the cards where the cues displayed on the screens were repeated on laminated printed A3 sheets (approximately the size of the screen). The gates leading to the two arms of the maze could be closed remotely.

Experimental Setup

The discrimination task took place in a two-armed test maze (4.8 m × 3.6 m) with solid wooden walls (**Figure 2**). At the beginning of a trial, sheep were moved from the home pen into a waiting pen (labeled "START," **Figure 2**) which was connected to a start pen that gave access to the maze through a sliding door. A 2.5-m long wooden wall was placed at 1.9 m from the entry gate, with two open gates leading to the two arms of the maze. These gates could be closed remotely by an experimenter standing outside the maze once a sheep had entered one of the arms. This wall also supported two flat computer screens (48 cm diagonal), one at each end near the gates, on which the two cues were simultaneously displayed. The cue displayed on each screen was also shown twice in each corresponding arm: on a card hanging on the wall next to the remotely closing gate (hereafter referred to as side-card) and on another card placed on the rear wall of each arm. The area between the entry gate and the wall with the screens was referred to as the decision area, i.e., the area where the sheep had to choose between the two arms of the maze. Both arms of the maze had an exit door opening onto a raceway leading back to the "return" waiting pen that was adjacent to the home pen.

Habituation to the Experimental Setup

The habituation phase was divided into three steps over 2 days (**Figure 3**). Sheep were considered to be habituated once they no longer displayed a stress response while being handled or in the test-pen. Due to the preliminary phase of systematic

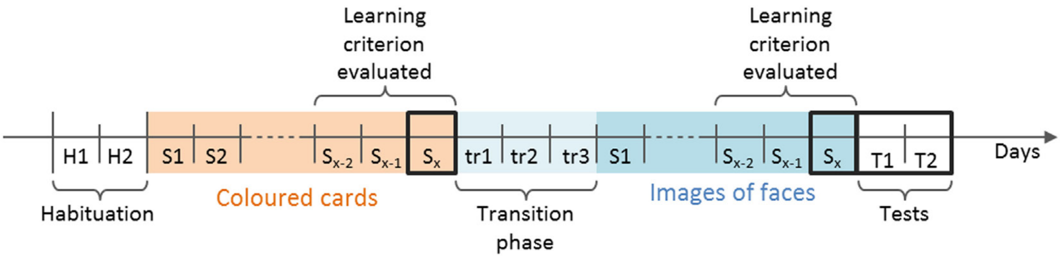

FIGURE 3 | Timeline of the five consecutive phases of the study. The total number of sessions needed to reach the learning criterion varied between animals (noted *x*), for Colored Cards, $x \leq 18$, for Images of faces $x \leq 15$. The learning criterion ($\geq$80% correct choices over two consecutive sessions, or $\geq$80% correct choices in two out of three consecutive sessions and $\geq$60% in the penultimate) was evaluated during the three last sessions of both the Colored Cards and the Image of faces phase, as indicated. Sessions framed by thick black lines were analyzed. During transition sessions, images of faces framed by the corresponding color (rewarded image = rewarded shade of green and *vice versa*) were presented. The final training sessions are noted S_x. H, habituation; S, training sessions; T, tests sessions; tr, transition sessions.

desensitization to human handling, habituation to the experimental setup was fairly short. On the first day, the sheep visited the maze in randomly allocated groups of three and were allowed to explore it for 3 min (all gates remained open). This was repeated three times consecutively per triad in total. The sheep were then randomly split into pairs and entered the maze three times consecutively for 2 min. On the second day of the habituation phase, sheep were brought into the maze individually for 1 min. Again, this was repeated three times consecutively for each individual. One sheep had to be removed from the study at that stage due to health issues, and thus 35 sheep were included in the next phase of the experiment.

Training Phases

First Training Phase: With Colored Cards

Two shades of green differing in tone and brightness were used as cues for the first training phase (light: Red = 240, Green = 241, Blue = 223; dark: Red = 122, Green = 188, Blue = 50). Sheep can easily distinguish shades of green that differ only in brightness (32). The chosen cues also differed slightly in tone and so ensured that a good contrast between the two colors would be maintained on the screens.

For half of the tested sheep, light green was the rewarded cue and dark green was the punished cue. The other half of the group received the opposite pairings. The rewarded side alternated following Gellerman series (33) to prevent the sheep from place learning. For the first eight runs in the maze, one of the remote-closing gates was closed prior to the sheep's entry. This preliminary conditioning session forced the animal to explore each possible side/reward combinations and their consequences (incorrect-left, correct-right, incorrect-right, and correct-left) twice. Side and type alternated, starting with incorrect-left, so that the final run was forced-rewarded. These preliminary conditioning runs in the maze were not taken into account in the analyses.

A training session then consisted of 10 consecutive runs through the maze. The order of the side/reward combination also followed Gellerman series (33) and was changed after each session to prevent the sheep from learning the order or developing a side bias. The outcome (side chosen and success or failure) was recorded for each run.

For the first 7 training sessions, since all 35 sheep could not be trained in 1 day, the group was split into two groups of 17 and 18 sheep. Each group went through a training session every other day. After the seventh training session, five animals that had shown consistent side biases (i.e., animals that consistently chose the same side for every run through the maze) were removed from the study. As a result, all animals then trained every day. A sheep reached the learning criterion once it had reached a minimum of 80% correct responses in two consecutive sessions, or 80% of correct choices in two out of three consecutive sessions and at least 60% correct choices in the penultimate session (i.e., ≥80%, ≥60%, ≥80%). This is equivalent to a minimum of 22 correct choices out of 30 consecutive runs, or a minimum of 73% of correct answers across 3 consecutive sessions. If after 18 training sessions the animals had still not reached the learning criterion, they were excluded from the next phase of the study.

Second Training Phase: With Images of Faces

In this phase of training, colors were replaced with images of the faces of the Photo Sheep (**Figures 1A,B**). A pair of cues consisted of two images of the same Photo Sheep: one taken in the neutral situation and one in one of the two negative situations (SI for social isolation or Aggr for aggressive interactions). To differentiate the neutral image paired with SI from the neutral image paired with Aggr, neutral images from SI-Neutral pairs are referred to as N_{SI} and neutral images from Aggr–Neutral pairs are referred to as N_{Aggr}. The type of rewarded image was attributed alternatively to each sheep that reached the learning criterion in the first phase (*n* sheep). The four types of images (SI, Aggr, N_{SI}, and N_{Aggr}) were allocated so that for half of the test sheep (*n*/2 sheep) the correct cue was an image from one of the negative situations (SI, *n*/4; Aggr, *n*/4), and for the other half, the correct cue was an image from the neutral situation (N_{SI}, *n*/4; N_{Aggr}, *n*/4). A given sheep was trained with images of the face of the same individual.

Each sheep went through three transition training sessions to facilitate the transfer of the colored card cues to the facial cues. For the first session (tr1, **Figure 3**), each face was framed by the color sharing the same attributes, i.e., the now rewarded face was framed by the color previously rewarded and *vice versa*. The color was also repeated on the side-card, and the card placed above the bucket in the arm was a repetition of the framed face. For the next two sessions (tr2 and tr3, **Figure 3**) the colored side-card was removed but the pictures were still framed in shades of green. These three transition training sessions were not included in the number of sessions needed to reach the learning criterion, as the aim of the experiment was to test the ability to learn to identify facial expressions, and not a combination of colored cards and expressions.

After the three transition sessions, the only cues available to the sheep to choose an arm of the maze were images of faces presented on the screens and repeated on the cards above the feed bucket (**Figure 2**). The learning criterion was the same as during the colored cards phase. As soon as a sheep had reached the learning criterion, it was moved to the test phase.

Test: Generalization to Images of New Familiar Individuals

The test phase consisted of two sessions of ten runs each, where the images presented to the test sheep were of the face of a different Photo Sheep. The Photo Sheep used in this test phase were also familiar with the test sheep, but images of their faces had never been presented in the maze (**Figures 1C,D**). The test sheep had to generalize the task they had learned to images of new familiar individuals to gain access to the food reward. The type of rewarded image did not change during this phase, e.g., sheep that had learned to associate SI images with a reward had to associate SI images of a new Photo Sheep with the reward.

Data Collection and Statistical Analysis

For both training phases (colored cards and images of faces), learning speed, i.e., the number of sessions needed to reach the learning criterion, was recorded for each sheep. For every run of the training and test phases, the outcome (success or error), the time from the sheep's entry into the maze (two front feet inside the maze) to its choice (gate arm closed behind the sheep) were recorded (LatChoice, seconds) from video files using The Observer 5.0 (Noldus Information Technology, Netherlands).

All analyses with non-parametric tests were conducted in Minitab 17 (Minitab Inc., PA, USA). Mixed models were run in GenStat 16th edition (VSN International Ltd., UK). Significance level was set at $P = 0.05$.

Due to the small number of animals (16 sheep were included in the second training phase, four for each type of image rewarded, see Section "Results" for more details), the two types of negative images (SI and Aggr) and the two types of neutral images (N_{SI}, when the second image was SI, N_{Aggr} when the second image of the pair was Aggr) were grouped under "negative images" and "neutral images," respectively, for analysis of learning speed. The four types of images were not grouped in other analyses. Data were tested for outliers using Grubb's test at a 5% level of significance and differences in learning speed between the categories of rewarded images were then analyzed by Mood's median tests, which are more robust than Kruskal–Wallis tests against outliers (34).

All sheep needed a different number of training sessions to reach the learning criterion, so for a given sheep the final training session of a phase did not necessarily have the same session number than for another sheep (e.g., the final training session could be Session 3 for one individual, and Session 14 for another). However in this final training session, all sheep were at a similar state of training and understanding of the task (at least 80% correct choices). Thus, the final training sessions of both training phases were analyzed (**Figure 3**, framed in black). The two test sessions, where sheep had to generalize the task, were also analyzed.

For the selected training and test sessions, the effect of the type of rewarded image (N_{Aggr}, N_{SI}, Aggr, or SI) on the success of a run (0 or 1) was analyzed by a generalized linear mixed model (GLMM) using a binomial distribution and logit link function. The type of rewarded image (N_{Aggr}, N_{SI}, Aggr, or SI) was included as a fixed effect in the final model, and Animal and Session were included as random effects, with Session nested within Animal.

If sheep chose at chance level during a test session, then the mean value of the success variable was 0.5. In that case, with the logit transformation used by the GLMM the mean would be as follows: $\text{logit}(0.5) = \ln(0.5/(1-0.5)) = 0$. To test whether sheep chose the correct image at above chance levels during the two test sessions, the confidence interval (CI) was calculated for the mean value of the success variable for each type of rewarded image (N_{Aggr}, N_{SI}, Aggr, or SI), based on the output from the GLMM analysis. If the CI included 0, it was not possible to tell whether the sheep had chosen above chance level.

LatChoice was transformed using a natural log function to conform to statistical assumptions. For the same selected training and test sessions (**Figure 3**), LatChoice was then analyzed by restricted maximum likelihood with repeated measurements, using a power model to account for correlations within subjects across time. Type of rewarded image (N_{Aggr}, N_{SI}, Aggr, or SI), outcome of the run (success or error), and their interactions were included as fixed effects. Random effects included Run and Animal. *Post hoc* pairwise comparisons were conducted using least significant difference tests. Normality of the residuals was checked graphically.

RESULTS

Training with Colored Cards

Sixteen sheep (46%) reached the learning criterion in 18 sessions or fewer. Only two sheep learned the task in fewer than 10 sessions (7% of total number of sheep or 12.5% of successful sheep) (**Figure 4**). There was no difference in learning speed between sheep that had to associate a light green card with the reward and sheep that had to associate a dark green card with the reward (Medians: 14 vs. 15 sessions, $\chi^2 = 0.25$, $df = 1$, $P = 0.614$).

There was no effect of the type of colored card rewarded (dark green or light green, $F_{1,37.7} = 0.28$, $P = 0.598$), Success (correct vs. incorrect choice, $F_{1,128.9} = 0.17$, $P = 0.683$), or the interaction between those two factors ($F_{1,131.2} = 0.80$, $P = 0.373$) on LatChoice.

Training with Images of Faces

All 16 sheep (100%) reached the learning criterion with images of faces within 15 training sessions. Fifteen of these animals (94%) reached the learning criterion after 11 sessions (**Figure 4**).

There was a significant difference in learning speed between sheep that had to associate a neutral image (N_{Aggr} or N_{SI}) with the reward and sheep that had to associate a negative image (SI or Aggr) with the reward. Sheep learned the task faster (i.e., needed fewer training sessions) when a negative image was rewarded (Medians: 4 vs. 7.5 sessions, $\chi^2 = 4.00$, $df = 1$, $P = 0.046$, **Figure 5**). Grubbs' test results showed that there were no outliers ($G = 1.17$, $P > 0.90$).

In the final training session, there was no effect of the type of image rewarded on the sheep's number of correct choices (Aggr: 9 ± 0.82, SI: 9.5 ± 1.0 N_{Aggr}: 8.25 ± 0.5, N_{SI}: 8.5 ± 0.58; $F_{3,153} = 0.83$, $P = 0.477$). There was no effect of the type of image rewarded (N_{Aggr}, N_{SI}, Aggr, or SI, $F_{3,34} = 0.23$, $P = 0.879$), Success ($F_{1,124.9} < 0.01$, $P = 0.949$), or the interaction between those factors ($F_{3,124.7} = 0.23$, $P = 0.879$) on LatChoice either.

Tests: Generalization to Images of New Familiar Individuals

The generalization of the task to pairs of images of faces of new familiar individuals was affected by the type of rewarded image (N_{Aggr}, N_{SI}, Aggr, or SI, $F_{3,26.8} = 3.43$, $P = 0.031$). Based on the CIs, sheep that had Aggr as their rewarded image did not choose the correct image at above chance levels, while sheep that had SI, N_{Aggr}, or N_{SI} as their rewarded image did (**Table 1**).

LatChoice was significantly higher when sheep made the correct choice than when they made a mistake (correct choice: 9.4 ± 7.8 s, wrong choice: 7.9 ± 10.8 s; $F_{1,292.9} = 13.26$, $P < 0.001$) but the type of image rewarded (N_{Aggr}, N_{SI}, Aggr, or SI, $F_{3,12.1} = 1.10$, $P = 0.385$) or its interaction with Success ($F_{3.297.9} = 0.40$, $P = 0.756$) had no effect on LatChoice.

DISCUSSION

We investigated whether sheep could discriminate between images of faces of familiar conspecifics taken in situations eliciting emotional states of neutral or negative valence, using a simultaneous discrimination task in a two-armed maze. We also

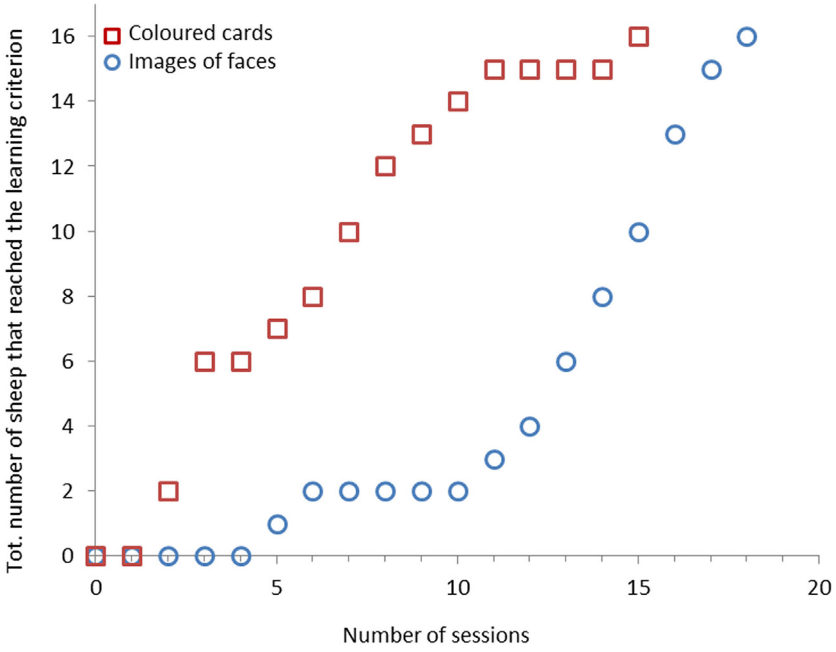

FIGURE 4 | Cumulative number of sheep ($n = 16$) that reached the learning criterion for each session. Training phase with colored cards is coded with blue circles and training phase with images of faces with red squares.

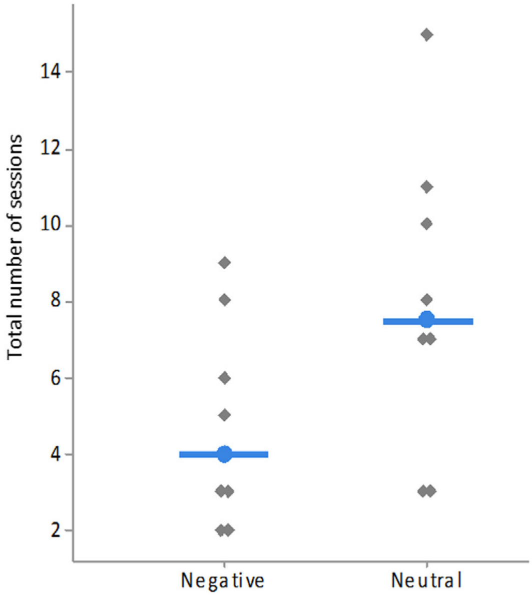

FIGURE 5 | Total number of sessions needed to reach the learning criterion by type of rewarded image (neutral = N_{Aggr} and N_{SI}, negative = Aggr, and SI). Each gray lozenge represents one individual sheep. Medians of each group are indicated by the blue bar and dot.

TABLE 1 | CI of the mean number of successes by type of image rewarded (N_{Aggr}, N_{SI}, Aggr, or SI).

	Mean	CI lower bound	CI higher bound	Backtransformed mean (mean % of success)
Aggr	−0.052	−0.654	0.551	49%
SI	0.754	0.32	1.376	68%
N_{Aggr}	1.206	0.538	1.875	77%
N_{SI}	1.137	0.475	1.798	76%

To test whether sheep chose the correct image at above chance levels during the two test sessions, the CI was calculated for the mean value of the success variable for each type of image rewarded, based on the output from the GLMM analysis. If sheep chose at chance level during a test session, then the mean value of the success variable was 0.5, so with the logit transformation used by the GLMM the mean was as follows: logit (0.5) = ln (0.5/(1−0.5)) = 0. So if the CI included 0, it was not possible to conclude that the sheep chose above chance level. Transformed CI and means are presented, and backtransformed means are included to help with interpretation. CI, confidence interval; GLMM, generalized linear mixed model.

assessed the influence of the valence of the rewarded image on learning and generalization processes. All sheep that learned the preliminary discrimination task with colored cards reached the learning criterion with images of faces. There was no difference in learning speed between the two shades of green of the colored cards; however, sheep learnt to associate the food reward with a negative image faster than with a neutral image.

Influence of the Type of Images of Faces on Learning Processes

As predicted, the type of image rewarded (neutral vs. negative) had an effect on the learning process, while the type of colored card rewarded (light or dark green) did not. However, we observed the opposite of our hypothesis regarding learning speed. Images of calm conspecifics are approached voluntarily by sheep (3) and can therefore be considered as primary reinforcers. We had originally proposed that sheep learning the association between images of ruminating Photo Sheep and food reward would reach the learning criterion faster than sheep learning the association with images taken in negative situations. However, sheep actually learned the task more quickly when their rewarded image was one of the two negatives images (SI or Aggr). It is of course possible that sheep learned to avoid the unrewarded image rather than to approach the rewarded one; however, this would not impact our findings regarding the ability of sheep to discriminate between images of faces.

In humans, negative stimuli (colored images of beetles, negatively valenced images from the International Affective Picture System database) induce stronger and faster responses (e.g., higher amplitude and shorter latency of electrophysiological markers, shorter response time in key pressing) than positive or neutral stimuli (colored images of buildings, neutral or positively valence images from the International Affective Picture System database (35, 36)). Similarly, in flash suppression studies of the perception of facial expressions, fearful expressions have been shown to gain access to awareness more quickly than neutral or happy expressions (37). In animals, a previous study showed that sheep were indeed more attentive (head turned to the screen for at least 2 s) toward videos showing agonistic interactions between conspecifics than toward videos showing ruminating sheep (38). Goats have also been shown to be more attentive toward images of faces of conspecifics photographed in a negative situation (ice pack applied to the udder) (39). From an evolutionary point of view, it is appropriate for animals to pay more attention to faces displaying negative emotions as they could signal the presence of potential threats. In our study, if the attention of sheep was increased toward negative images of faces, this may have aided them to learn to associate an image of a negative face and reward faster. The difference in learning speed could thus be initial evidence that sheep can not only distinguish between facial features, but that they also perceive the valence of the expression shown on the images. In this way, sheep would perceive images of faces taken in negative situations as at least as more interesting, but potentially as negative. This would represent a first step toward the use of images of faces in cognitive bias studies. Further studies are needed to determine if the differences in learning performances were due to sheep paying more attention to negative images, or if images taken in situations of high arousal but positive valence would have the same effect.

The generalization of the discrimination task to images of faces of new familiar individuals during tests was also affected by the type of image rewarded (N_{Aggr}, N_{SI}, Aggr, or SI). Only Aggr-rewarded sheep were not able to generalize the task to images of new familiar individuals. Since sheep that had N_{Aggr} as their rewarded image had no difficulties in generalizing the task, the poorer results from the Aggr-rewarded group cannot be explained by an increased difficulty in discriminating the neutral from the aggressive face in the new pair of cues. SI- and Aggr-rewarded sheep had reached the learning criterion faster. Consequently, these sheep had been exposed less often to images of faces than Neutral-rewarded sheep. Having a greater experience of the images might have helped the latter to be better at generalizing the task to new images. However, SI-rewarded sheep could generalize the task to new images and did not differ from Neutral-rewarded sheep in their ability to generalize. Therefore, the previous experience of images of faces cannot entirely explain the poorer performance from the Aggr-rewarded animals.

It is also possible that the identity of the Photo Sheep influenced the results from the Aggr-rewarded sheep. If the new Photo Sheep was a very dominant animal, seeing it presenting an aggressive expression might have prompted a strong avoidance response. However, we selected Photo Sheep so that they would be of average body weight since the positive correlation between live weight and hierarchy has been established in ungulates (24, 25). From that perspective, it is unlikely that all sheep from the Aggr-rewarded group were subordinate to the Photo Sheep, but that possibility cannot be excluded. Knowing the hierarchical relationships between the Photo Sheep and the tested animals, would have enabled us to clarify this point and to examine the influence of rank on learning speed.

Lastly, it is worth noting that during generalization sessions, sheep took longer to choose a branch when they made correct choices compared with incorrect choices. In juvenile pigs, similar longer response times for correct choices have been reported too (40). In this study, Nawroth et al. considered that these shorter response times were caused by impulsivity in the choice behavior of the piglets and suggested that subjects with non-impulsive approach behavior made more correct choice. They also encouraged to look at the latency to make a decision in a choice task at the individual level, rather than at the group level. Horses however have been observed to take longer to make an incorrect choice (41). This was interpreted as an uncertainty in decision making, due to awareness of the subject that it was potentially making the wrong choice. In our study, the more challenging task of transferring a rule to new cues might explain this variation, since no such difference in latency to choose was observed in the final training sessions which involved images of faces. This difference in latency to make a choice also indicates that sheep that made mistakes during the generalization sessions probably did not take time to process the two cues, but made a choice based on others factors.

Methodological Limitations

Only 16 out of 35 sheep succeeded in reaching the second training phase, with images of faces. We allowed sheep a maximum of 180 runs in the maze to learn the task during the Colored Cards phase. This criterion is within the range of learning performances of sheep in similar tasks that also involved pairs of cues presented alternatively on both sides (80–240) (42). Given a few more training sessions, more animals might have reached the learning criterion, and so we most likely only included the faster learners in the subsequent phases of the study. Despite the preliminary phase of systematic desensitization to human handling and the habituation phase, some individuals might not have habituated fully to the experimental setup, and still considered it a stressful environment. Since stress and negative emotional states impede cognitive abilities in sheep (43, 44), it is possible that faster learners were less fearful and found repeated handling and isolation less stressful. It is also possible that these animals had better cognitive abilities. Hill breeds of sheep might also not be ideal for cognitive studies due to their high emotional reactivity; lowland breeds such as Clun Forest sheep could be more indicated for such studies It should also be mentioned that our final sample size of 16 sheep is fairly small which affects the statistical power of our study. Therefore, our conclusions may not apply to the whole population of sheep. However we did establish that sheep can discriminate between images of faces taken in situations of varying valence, and that the valence of the situation influenced learning speed.

Finally, Bovet and Vauclair (45) raised a concern about using "pictorial stimuli" in animal studies without controlling for how images are perceived by the animals. In our study, we confirmed that reactions to images of faces differed from reactions to colored cards in sheep as differences in learning speed were identified with images of faces only, and were associated with the valence of the situation in which the faces were photographed. This suggests that images of faces were perceived as faces by the sheep, but further evidence is needed to draw strong conclusions on this matter.

CONCLUSION

Sheep discriminated between images of faces of conspecifics taken in an emotionally negative or neutral situation. Sheep were also able to generalize this discrimination to images of new faces, but this ability did not extend to images taken during aggressive interactions: sheep from the Aggr-rewarded group were unable to generalize the task. Learning was affected by the type of image displayed and differences in learning speed were associated with the valence of the situation in which the faces were photographed: sheep that had to associate a negative image with the reward learned faster than sheep that had to learn the neutral image-reward association. This suggests that sheep can perceive the valence of an emotional state displayed in an image of a face. This is an encouraging first step for the use of images of faces in cognitive bias studies.

ETHICS STATEMENT

All experimental procedures were approved by the Scotland's Rural College Edinburgh Animal Ethics Committee (Protocol no. ED-AE-2-2014). Animals were closely monitored before, during, and after the study.

AUTHOR CONTRIBUTIONS

LB conceived the study, collected and analyzed the data, and wrote the manuscript. HE conceived the study and contributed to the analysis of the data and writing of the manuscript. AW contributed to the conception of the study and the writing of the manuscript. AB contributed to the conception of the study and the writing of the manuscript. MH conceived the study, contributed to the data collection and analysis, and wrote the manuscript.

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REFERENCES

1. Leopold DA, Rhodes G. A comparative view of face perception. *J Comp Psychol* (2010) 124:233–51. doi:10.1037/a0019460
2. Kendrick KM, da Costa AP, Leigh AE, Hinton MR, Peirce JW. Sheep don't forget a face. *Nature* (2001) 414:165. doi:10.1038/35102669
3. Tate AJ, Fischer H, Leigh AE, Kendrick KM. Behavioural and neurophysiological evidence for face identity and face emotion processing in animals. *Philos Trans R Soc Lond B Biol Sci* (2006) 361:2155–72. doi:10.1098/rstb.2006.1937
4. Coulon M, Baudoin C, Heyman Y, Deputte B. Cattle discriminate between familiar and unfamiliar conspecifics by using only head visual cues. *Anim Cogn* (2011) 14:279–90. doi:10.1007/s10071-010-0361-6
5. Ferreira G, Keller M, Saint-Dizier H, Perrin G, Lévy F. Transfer between views of conspecific faces at different ages or in different orientations by sheep. *Behav Processes* (2004) 67:491–9. doi:10.1016/j.beproc.2004.08.005
6. Dupjan S, Schön PC, Puppe B, Tuchscherer A, Manteuffel G. Differential vocal responses to physical and mental stressors in domestic pigs (*Sus scrofa*). *Appl Anim Behav Sci* (2008) 114:105–15. doi:10.1016/j.applanim.2007.12.005
7. Terlouw EMC, Boissy A, Blinet P. Behavioural responses of cattle to the odours of blood and urine from conspecifics and to the odour of faeces from carnivores. *Appl Anim Behav Sci* (1998) 57:9–21. doi:10.1016/S0168-1591(97)00122-6
8. Siniscalchi M, Lusito R, Vallortigara G, Quaranta A. Seeing left- or right-asymmetric tail wagging produces different emotional responses in dogs. *Curr Biol* (2013) 23:2279–82. doi:10.1016/j.cub.2013.09.027
9. Waller BM, Micheletta J. Facial expression in non-human animals. *Emot Rev* (2013) 5:54–9. doi:10.1177/1754073912451503
10. Racca A, Guo K, Meints K, Mills DS. Reading faces: differential lateral gaze bias in processing canine and human facial expressions in dogs and 4-year-old children. *PLoS One* (2012) 7:e36076. doi:10.1371/journal.pone.0036076
11. Smith AV, Proops L, Grounds K, Wathan J, McComb K. Functionally relevant responses to human facial expressions of emotion in the domestic horse (*Equus caballus*). *Biol Lett* (2016) 12:20150907. doi:10.1098/rsbl.2015.0907
12. Li Y, Dai Q, Hou R, Zhang Z, Chen P, Xue R, et al. Giant pandas can discriminate the emotions of human facial pictures. *Sci Rep* (2017) 7:8437. doi:10.1038/s41598-017-08789-y
13. Reefmann N, Buetikofer Kàszas F, Wechsler B, Gygax L. Ear and tail postures as indicators of emotional valence in sheep. *Appl Anim Behav Sci* (2009) 118:199–207. doi:10.1016/j.applanim.2009.02.013
14. Boissy A, Aubert A, Désiré L, Greiveldinger L, Delval E, Veissier I. Cognitive sciences to relate ear postures to emotions in sheep. *Anim Welf* (2011) 20:47–56.
15. McLennan KM, Rebelo CJB, Corke MJ, Holmes MA, Leach MC, Constantino-Casas F. Development of a facial expression scale using footrot and mastitis as models of pain in sheep. *Appl Anim Behav Sci* (2016) 176:19–26. doi:10.1016/j.applanim.2016.01.007
16. Harding EJ, Paul ES, Mendl M. Animal behaviour: cognitive bias and affective state. *Nature* (2004) 427:312. doi:10.1038/427312a
17. Paul ES, Harding EJ, Mendl M. Measuring emotional processes in animals: the utility of a cognitive approach. *Neurosci Biobehav Rev* (2005) 29:469–91. doi:10.1016/j.neubiorev.2005.01.002
18. Baciadonna L, McElligott AG. The use of judgement bias to assess welfare in farm livestock. *Anim Welf* (2015) 24:81–91. doi:10.7120/09627286.24.1.081
19. Perry CJ, Baciadonna L, Chittka L. Unexpected rewards induce dopamine-dependent positive emotion-like state changes in bumblebees. *Science* (2016) 353:1529. doi:10.1126/science.aaf4454
20. d'Ettorre P, Carere C, Demora L, Le Quinquis P, Signorotti L, Bovet D. Individual differences in exploratory activity relate to cognitive judgement bias in carpenter ants. *Behav Processes* (2016) 134:63–9. doi:10.1016/j.beproc.2016.09.008

21. Kendrick KM, Atkins K, Hinton MR, Heavens P, Keverne B. Are faces special for sheep? Evidence from facial and object discrimination learning tests showing effects of inversion and social familiarity. *Behav Processes* (1996) 38:19–35. doi:10.1016/0376-6357(96)00006-X
22. Vandenheede M, Bouissou MF. Fear reactions of ewes to photographic images. *Behav Processes* (1994) 32:17–28. doi:10.1016/0376-6357(94)90024-8
23. da Costa AP, Leigh AE, Man MS, Kendrick KM. Face pictures reduce behavioural, autonomic, endocrine and neural indices of stress and fear in sheep. *Proc Biol Sci* (2004) 271:2077–84. doi:10.1098/rspb.2004.2831
24. Drickamer LC, Arthur RD, Rosenthal TL. Predictors of social dominance and aggression in gilts. *Appl Anim Behav Sci* (1999) 63:121–9. doi:10.1016/S0168-1591(99)00005-2
25. Landaeta-Hernàndez AJ, Rae DO, Kaske M, Archbald LF. Factors influencing social organization in postpartum Angus cows under confinement. Effect on cow-calf weight change. *Livest Sci* (2013) 152:47–52. doi:10.1016/j.livsci.2012.11.019
26. Hargreaves AL, Hutson GD. The effect of gentling on heart rate, flight distance and aversion of sheep to a handling procedure. *Appl Anim Behav Sci* (1990) 26:243–52. doi:10.1016/0168-1591(90)90140-9
27. Wathan J, Proops L, Grounds K, McComb K. Horses discriminate between facial expressions of conspecifics. *Sci Rep* (2016) 6:38322. doi:10.1038/srep38322
28. Erhard HW, Elston DA, Davidson GC. Habituation and extinction in an approach–avoidance test: an example with sheep. *Appl Anim Behav Sci* (2006) 99:132–44. doi:10.1016/j.applanim.2005.10.008
29. Nowak R, Porter RH, Blache D, Dwyer CM. Behaviour and the Welfare of the Sheep. In: Dwyer CM, editor. *The Welfare of Sheep*. Dordrecht, Netherlands: Springer (2008). p. 81–134.
30. Romeyer A, Bouissou MF. Assessment of fear reactions in domestic sheep, and influence of breed and rearing conditions. *Appl Anim Behav Sci* (1992) 34:93–119. doi:10.1016/S0168-1591(05)80060-7
31. Erhard HW, Fàbrega E, Stanworth G, Elston DA. Assessing dominance in sheep in a competitive situation: level of motivation and test duration. *Appl Anim Behav Sci* (2004) 85:277–92. doi:10.1016/j.applanim.2003.09.013
32. Doyle RE, Lee C, Deiss V, Fisher AD, Hinch GN, Boissy A. Measuring judgement bias and emotional reactivity in sheep following long-term exposure to unpredictable and aversive events. *Physiol Behav* (2011) 102:503–10. doi:10.1016/j.physbeh.2011.01.001
33. Gellermann LW. Chance orders of alternating stimuli in visual discrimination experiments. *Pedagog Semin J Genet Psychol* (1933) 42:206–8. doi:10.1080/08856559.1933.10534237
34. Siegel S, Castellan NJ. *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw Hill Book Co. (1988).
35. Mogg K, McNamara J, Powys M, Rawlinson H, Seiffer A, Bradley BP. Selective attention to threat: a test of two cognitive models of anxiety. *Cognit Emot* (2000) 14:375–99. doi:10.1080/026999300378888
36. Delplanque S, Lavoie ME, Hot P, Silvert L, Sequeira H. Modulation of cognitive processing by emotional valence studied through event-related potentials in humans. *Neurosci Lett* (2004) 356:1–4. doi:10.1016/j.neulet.2003.10.014
37. Yang E, Zald DH, Blake R. Fearful expressions gain preferential access to awareness during continuous flash suppression. *Emotion* (2007) 7:882–6. doi:10.1037/1528-3542.7.4.882
38. Vögeli S, Wolf M, Wechsler B, Gygax L. Housing conditions influence cortical and behavioural reactions of sheep in response to videos showing social interactions of different valence. *Behav Brain Res* (2015) 284:69–76. doi:10.1016/j.bbr.2015.02.007
39. Bellegarde LGA, Haskell MJ, Duvaux-Ponter C, Weiss A, Boissy A, Erhard HW. Face-based perception of emotions in dairy goats. *Appl Anim Behav Sci* (2017) 193:51–9. doi:10.1016/j.applanim.2017.03.014
40. Nawroth C, Ebersbach M, von Borell E. Are juvenile domestic pigs (*Sus scrofa* domestica) sensitive to the attentive states of humans? The impact of impulsivity on choice behaviour. *Behav Processes* (2013) 96:53–8. doi:10.1016/j.beproc.2013.03.002
41. Proops L, McComb K. Attributing attention: the use of human-given cues by domestic horses (*Equus caballus*). *Anim Cogn* (2010) 13:197–205. doi:10.1007/s10071-009-0257-5
42. Sugnaseelan S, Prescott NB, Broom DM, Wathes CM, Phillips CJC. Visual discrimination learning and spatial acuity in sheep. *Appl Anim Behav Sci* (2013) 147:104–11. doi:10.1016/j.applanim.2013.04.012
43. Destrez A, Deiss V, Lévy F, Calandreau L, Lee C, Chaillou-Sagon E, et al. Chronic stress induces pessimistic-like judgment and learning deficits in sheep. *Appl Anim Behav Sci* (2013) 148:28–36. doi:10.1016/j.applanim.2013.07.016
44. Doyle RE, Freire R, Cowling A, Knott SA, Lee C. Performance of sheep in a spatial maze is impeded by negative stimuli. *Appl Anim Behav Sci* (2014) 151:36–42. doi:10.1016/j.applanim.2013.11.009
45. Bovet D, Vauclair J. Picture recognition in animals and humans. *Behav Brain Res* (2000) 109:143–65. doi:10.1016/S0166-4328(00)00146-7

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Looking on the Bright Side of Livestock Emotions—the Potential of Their Transmission to Promote Positive Welfare

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Emotions can be defined as an individual's affective reaction to an external and/or internal event that, in turn, generates a simultaneous cascade of behavioral, physiological, and cognitive changes. Those changes that can be perceived by conspecifics have the potential to also affect other's emotional states, a process labeled as "emotional contagion." Especially in the case of gregarious species, such as livestock, emotional contagion can have an impact on the whole group by, for instance, improving group coordination and strengthening social bonds. We noticed that the current trend of research on emotions in livestock, i.e., investigating affective states as a tool to assess and improve animal welfare, appears to be unbalanced. A majority of studies focuses on the individual rather than the social component of emotions. In this paper, we highlight current limitations in the latter line of research and suggest a stronger emphasis on the mechanisms of how emotions in livestock are transmitted and shared, which could serve as a promising tool to synergistically enhance the welfare of all individuals within a group.

Keywords: affective states, emotions, emotional contagion, farm animals, wellbeing

WHAT ARE EMOTIONS?

Emotions have been defined as short-term affective states elicited by internal and/or external events and are associated with synchronized physiological, behavioral, and cognitive components (1, 2). The framework proposed by Mendl et al. (3) suggests that different aspects of the emotional experience (e.g., neurophysiological, behavioral, and cognitive components) of non-human animals can be assessed along two dimensions, namely valence (negative or positive) and arousal [from low to high (4–6)]. One of the main functions of emotions is to prepare an individual to quickly select an appropriate response (i.e., approach or avoidance depending on the positive or negative valence of the emotion) in order to cope efficiently with its environment (1, 2). Moreover, affective states, i.e., emotions and longer-term affective states (mood), can alter the way in which an individual perceives its environment, e.g., making it more cautious (pessimistic-like) after a fear-inducing event or more optimistic-like after a positive event [so-called cognitive bias; (7)]. Considering the evolutionary importance of affective states, scientists' most widely held opinion is that these states occur across different taxa including invertebrates (1, 8–11). Although the lack of verbal communication in non-human animals precludes our access to the subjective component of emotions (i.e., feelings), there is an array of quantifiable parameters that allow us to assess their physiological, behavioral, and cognitive components. For instance, an emotion experienced by an individual can lead to changes in its body posture or expressions [e.g., facial or vocal signals/cues; (11–14)]. Since these expressions can be easily detected by other individuals, it is plausible that emotions do not only operate at the individual level, but also at the group level.

THE SOCIAL COMPONENT IN EMOTIONS

The communication of emotions to conspecifics can play a key role in the regulation of social interactions (e.g., for group defense, play, agonistic behavior, maternal nursing, mating competition). Moreover, the expression of emotions can lead to the sharing of affective states between individuals (15). In gregarious species, synchronized emotional states within a group of individuals can be highly adaptive (16–18) and this phenomenon has been suggested to be a crucial element in the evolution of empathy (19, 20). The benefits of sharing emotional states between individuals include improved group coordination and strengthening bonds between individuals (21–24).

Different levels in the transmission of emotions have been proposed, with mechanisms requiring less cognitive load such as emotional contagion, and more cognitively sophisticated processes such as perspective taking and targeted helping (20, 25). Emotional contagion occurs when the affective state of an individual is influenced by the perception of the affective state of another individual (20). It results in state-matching between two individuals (e.g., distress with distress), without necessarily requiring conscious and effortful processing or self-other distinction (26). In the case of emotional contagion, the response of the subject should be in line with (i.e., match) the emotional state of the observed individual. By contrast, in the case of cognitive forms of empathy ("cognitive empathy"; e.g., perspective taking), the result will not necessarily be a matching state between the observer and observed individuals, because this phenomenon will result in the observer regulating its own emotional response in order to efficiently interact with the latter (27).

According to the Perception-Action Model of empathy proposed by Preston and de Waal (20), and as shown by neurobiological studies in humans (28), the attentive perception of the observed individual's emotional state automatically activates the observer's representation of this state. These representations trigger associated autonomic and somatic responses and allow the observer to connect with the internal state and situation of the observed individual through the activation of the neural representations of similar internal states that the observer has previously experienced (29–31).

EVIDENCE FOR TRANSMISSION OF EMOTIONS IN LIVESTOCK

Empathic responses occur widely within the mammalian taxon, with emotional contagion being the most common phenomenon investigated (24, 32). But since the field of non-human emotion research started to expand a few decades ago (33), research on farm animal welfare has focused mostly on the expression of emotions at the individual level, as an indicator of animals' welfare state [i.e., their physical but also psychological wellbeing; (24)]. By contrast, only a few studies have focused on emotional contagion and how the expression of emotions affects the welfare of the group (26, 34). The limited available evidence, however, suggests that this phenomenon might have a crucial impact on animal wellbeing (24). For instance, when pigs were restrained in a dispenser without access to food, other pigs later avoided the system/dispenser, especially when the reaction of being restrained was very aversive and associated with urination (35). In another study, untrained pigs showed a higher rate of defecation or higher levels of play when they observed conspecifics that were trained to anticipate an aversive or rewarding event, respectively (36). In addition, piglets showed a stronger reaction (higher proximity, decreased locomotion, and increase freezing behavior) toward distressed conspecifics when they had previously experienced the same stressor themselves (i.e., being restrained) compared with piglets that had not been restrained (34). In cattle, the presence of a stressed companion animal led to an increase in cortisol, a longer latency to feed and slower feeding rates in the tested subject, indicating increased fearfulness (37). Additionally, cattle showed a longer lasting approach response when a novel object was impregnated with urine of stressed conspecifics compared to urine of non-stressed conspecific. The increased fearfulness thus seemed to be at least partly mediated by olfactory cues present in the distressed animals' urine (37).

Vocalizations in particular have been shown to reflect emotional states in many species, and might therefore serve as a crucial channel for emotional contagion (15, 38). For example, pig vocalizations elicited during different stressful situations were related to the specific type of stress (14). Similarly, pig vocalizations, but also physiology, were affected by the induced emotional valence of repeated moderate aversive and rewarding events and are linked to emotional reactivity within and across different contexts (39). However, a similar experiment showed that when pigs heard recordings of distress calls from unfamiliar pigs of the same age and sex, the emotional valence of the calls did not induce a comparable state of distress (40). More recently, a study investigating the behavioral, physiological, and acoustic correlates of emotions in goats showed that parameters differed in each of these categories according to the valence and/or arousal of the emotions experienced by the animals (41). Subjects in high-arousal situations (such as food frustration), compared to low-arousal ones (such as isolation), showed lower heart-rate variability, higher respiration rates, increased body and head movements and vocalizations, and spent more time with their ears pointing forwards. In the positive situation (anticipation for food), compared to the negative ones (food frustration and isolation), goats spent less time with their ears oriented backwards and more time with their tails up. In addition, several acoustic parameters were identified as reliable indicators of arousal and valence. The fundamental frequency contour and energy quartiles of their vocalizations increased, while the first formant decreased, with arousal, whereas the fundamental frequency variation decreased from negative to positive valence (42). These results strongly support the notion that the vocal domain in group-living animals, such as goats, could be a potential way of emotion transmission. Moreover, playback experiments in goats indicated that subjects exposed to emotional-linked calls (food anticipation, food frustration, and isolation) from conspecifics preferentially showed a lateralized head-turning response to the right side (43). This right side head-turning bias suggests the involvement of the left hemisphere for processing calls conveying emotional contents (44, 45). In addition, it was demonstrated that the acoustic structure of domestic horses' whinnies varies between positive and negative contexts, and that familiar conspecifics are able to perceive this information (46, 47).

It is possible that lifetime experience could affect empathic responses. Very young subjects, or subjects housed in environmentally and/or socially deprived housing conditions, have shown impaired abilities to display emotional contagion (34, 48). In addition, the transmission of emotions can be enhanced by several factors, including familiarity and relatedness between the observer and the observed individuals, as well as former experience of a similar emotional situation by the observer (20). These context-specific ontogenetic differences warrant further investigations.

To date, the question of to what extent farm animals are able to perceive the emotions of conspecifics through behavioral, but also acoustic and olfactory cues and whether emotional contagion occurs as a result is still debated. However, considering the evidence for perception and contagion of emotions in several non-livestock taxa (15), farm animals are likely to also share these empathic capacities. More studies on emotional contagion in livestock are needed in order to extend our understanding of the impact of this phenomenon on animal welfare, and of the mechanisms underlying it.

DISENTANGLING MODALITIES AND LIMITATIONS OF THE TRANSMISSION OF EMOTIONS

Emotional states in farm animals have been shown to be transmitted via olfactory cues (37), vocalizations (15), and direct observation of conspecific behavior (49). However, interpretations of current research on emotional contagion in livestock are often limited by two factors. First, the use of live animals as signalers does not control for other modalities than the ones primarily investigated (36). On the other hand, the restriction to only one signaling modality (e.g., acoustic cues through playbacks, visual cues through images or videos) does not provide a holistic view on the underlying mechanisms of emotional contagion. We thus encourage a multi-modal approach including controlled stimuli in the study of non-human emotional contagion, for example, by using signals produced by conspecifics experiencing fully validated positive and negative emotional states. A future step must be to investigate whether controlled visual (e.g., images or movies) or auditory cues (e.g., playbacks) alone and/or in combination can lead to the spread of affective states within the group. By providing signals of two or more modalities simultaneously, one could estimate whether cues simply add up or have synergistic effects, i.e., if and how this might enhance the transfer of emotions by making it more salient and/or relevant. In addition, violation of expectation experiments (i.e., providing cues that do not match an observer's expectations, such as displaying playbacks of positive valence and videos/images of negative valence simultaneously) could identify whether a subject forms a mental cross-modal representation not just about the features/appearance of another subject, but also about its emotional state (50, 51).

For a long time, the main aim of animal welfare research has been to reduce, and hence also to assess, negative emotions and to lower stress during an animal's life. Recent views, however, have pointed toward an effort to also explore and promote positive emotions (52, 53). Similarly, most studies investigating emotional contagion in livestock have focused solely on the transfer of negative emotional states. Yet, although the function of contagion of positive and negative emotions may differ, it is likely that the mechanisms underlying emotional contagion are independent of the valence (26). The bias toward research in negative emotions might be a result of the increased availability of parameters indicating negative compared to positive emotions (8, 9, 53). The increased set of tools developed to investigate negative emotions could be explained by the fact that many positive emotions are less intense in their expression compared with negative ones, and often the expression and perception of negative emotions (e.g., distress, need, pain) plays a substantial role for survival, making them more prominent and easily detectable (19, 54, 55). In contrast, the consequences of not being responsive to positive emotions expressed by conspecifics might be less severe regarding immediate survival.

Overall, we believe that there are several limitations in most of the existing studies on livestock emotions and emotion transfer, including a lack of validated and accurate assessments of the emotional state of both the producer (observed individual) and receiver (observer) of the emotional signal. Such validation could be done by using neuro-physiological, cognitive, or behavioral indicators of emotions (8, 42). In addition, there is a general lack of detailed evidence showing that the change in emotions observed in the receiver is due to the signal to which it was exposed to, and not due to other environmental cues that were not controlled for. To ensure that the changes observed in the animals are due to the signal, subjects should ideally be tested in a neutral environment (such as their home pen); an environment that does not induce an emotion by itself. Alternatively, the emotional state of the animal before exposing it to the signal should be assessed and controlled for.

IMPLEMENTATION IN APPLIED SETTINGS AND FUTURE DIRECTIONS

Emotional contagion can lead to the spread of both positive and negative emotions in groups of animals (35). This phenomenon is of strong importance for the welfare of group-housed domestic and/or captive animals. Indeed, emotional contagion could potentially be used as a tool to improve welfare by facilitating the spread of positive emotions as well as by reducing negative high-arousal emotions, or, at least, by preventing the spread of such emotions. Therefore, knowledge about the primary modalities that livestock species use to perceive emotional cues from conspecifics or even from humans, in case of cross-species contagion of emotions (56), would help us to better comply with their emotional needs and thus provide them with a better quality of life.

Vocalizations are a potent modality to express emotions as shown above. It would be interesting to test how conspecifics perceive emotion-linked calls and how these modulate the emotional state of the receiver. This knowledge could be used to design tools for improving welfare. For instance, playbacks of positive low-arousal vocalizations (or other sensory cues) could have the potential to decrease the impact of stressful events, such as transport, rehoming in an unfamiliar environment or veterinary practice, on the animal. We argue that positively valenced emotional stimuli (e.g., vocal or olfactory cues) could be used as a tool to promote positive emotions in receivers, or alternatively, to reduce negative ones. It would thus be valuable to investigate whether negative high-arousal states can be counteracted by using playbacks of positive low-aroused calls (or other sensory cues). Additionally, one of the hypothesized functions of positive emotions and their contagion is to strengthen social bonds (57). Tools such as social network analyses, which provide us with the quality (e.g., affiliative or agonistic) and quantity (number of interaction) of social relationships, could inform us on if and how negative and/or positive emotional stimuli spread within a group.

Given the widespread occurrence of emotional contagion in a diverse set of animal taxa, livestock species surely are no exception to the rule. The investigation of shared emotional states in livestock and its interactions with other social phenomena, such as social buffering (58), however, remains an underdeveloped field. In addition, several methodological limitations (e.g., the use of live animals as signalers or the restriction to one signaling modality in playback experiments) still have to be addressed, and there is a need for research to move away from negative emotions in order to include positive ones as well. Indeed, in order to harness the full potential of empathic responses in livestock and to transfer it into an applied setting, we must first identify the mechanisms and modes involved in the transmission of affective states. Understanding the perceptual mechanisms of the social dimension of animal emotions will open new ways to reduce high-arousal negative emotions and, in the long-term, promote positive welfare in livestock.

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

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REFERENCES

1. Mendl M, Burman OHP, Paul ES. An integrative and functional framework for the study of animal emotion and mood. *Proc R Soc B* (2010) 277:2895–904. doi: 10.1098/rspb.2010.0303
2. Paul ES, Harding EJ, Mendl M. Measuring emotional processes in animals: the utility of a cognitive approach. *Neurosci Biobehav Rev.* (2005) 29:469–91. doi: 10.1016/j.neubiorev.2005.01.002
3. Mendl M, Brooks J, Basse C, Burman O, Paul E, Blackwell E, et al. Dogs showing separation-related behaviour exhibit a "pessimistic" cognitive bias. *Curr Biol.* (2010) 20:R839–40. doi: 10.1016/j.cub.2010.08.030
4. Bradley MM. Emotion and motivation. *Handb Psychophysiol.* (2000) 2:602–42. doi: 10.1017/CBO9780511546396.025
5. Kuppens P, Tuerlinckx F, Russell JA, Barrett LF. The relation between valence and arousal in subjective experience. *Psychol Bull.* (2013) 139:917–40. doi: 10.1037/a0030811
6. Russell JA. Core affect and the psychological construction of emotion. *Psychol Rev* (2003) 110:145–72. doi: 10.1037//0033-295X.110.1.145
7. Harding EJ, Paul ES, Mendl M. Animal behaviour: cognitive bias and affective state. *Nature* (2004) 427:312. doi: 10.1038/427312a
8. Perry CJ, Baciadonna L, Chittka L. Unexpected rewards induce dopamine-dependent positive emotion–like state changes in bumblebees. *Science* (2016) 353:1529–31. doi: 10.1126/science.aaf4454
9. Perry CJ, Baciadonna L. Studying emotion in invertebrates: what has been done, what can be measured and what they can provide. *J Exp Biol.* (2017) 220:3856–68. doi: 10.1242/jeb.151308
10. Anderson DJ, Adolphs R. A framework for studying emotions across species. *Cell* (2014) 157:187–200. doi: 10.1016/j.cell.2014.03.003
11. LeDoux J. Rethinking the emotional brain. *Neuron* (2012) 73:653–76. doi: 10.1016/j.neuron.2012.02.004
12. Proctor HS, Carder G. Nasal temperatures in dairy cows are influenced by positive emotional state. *Physiol Behav.* (2015) 138:340–4. doi: 10.1016/j.physbeh.2014.11.011

13. Proctor HS, Carder G. Can ear postures reliably measure the positive emotional state of cows? *Appl Anim Behav Sci.* (2014) 161:20–7. doi: 10.1016/j.applanim.2014.09.015
14. Dupjan S, Schon PC, Puppe B, Tuchscherer A, Manteuffel G. Differential vocal responses to physical and mental stressors in domestic pigs (*Sus scrofa*). *Appl Anim Behav Sci.* (2008) 114:105–15. doi: 10.1016/j.applanim.2007.12.005
15. Briefer EF. Vocal contagion of emotions in non-human animals. *Proc R Soc B* (2018) 285:20172783. doi: 10.1098/rspb.2017.2783
16. Nakahashi W, Ohtsuki H. When is emotional contagion adaptive? *J Theor Biol.* (2015) 380:480–8. doi: 10.1016/j.jtbi.2015.06.014
17. Padilla De La Torre M, Briefer EF, Ochocki BM, Mcelligott AG, Reader T. Mother–offspring recognition via contact calls in cattle, *Bos taurus*. *Anim Behav.* (2016) 114:147–54. doi: 10.1016/j.anbehav.2016.02.004
18. Briefer EF, Padilla de la Torre M, McElligott AG. Mother goats do not forget their kids' calls. *Proc Biol Sci.* (2012) 279:3749–55. doi: 10.1098/rspb.2012.0986
19. Fredrickson BL, Branigan C. Positive emotions broaden the scope of attention and thought- action repertoires. *Cogn Emot.* (2011) 19:1–19. doi: 10.1080/02699930441000238
20. Preston SD, de Waal FBM. Empathy: Its ultimate and proximate bases. *Behav Brain Sci.* (2002) 25:1–20. doi: 10.1017/S0140525X02000018
21. Barsade SG. The ripple effect : emotional contagion and its influence on group behavior. *Adm Sci Q.* (2002) 47:644–75. doi: 10.2307/3094912
22. Lakin JL, Jefferis VE, Cheng CM, Chartrand TL. The chameleon effect as social glue: Evidence for the evolutionary significance of nonconscious mimicry. *J Nonverbal Behav.* (2003) 27:145–62. doi: 10.1023/A:1025389814290
23. Spoor JR, Kelly JR. The evolutionary significance of affect in groups: communication and group bonding. *Gr Proc. Integr Relat.* (2004) 7:398–412. doi: 10.1177/1368430204046145
24. Spinka M. Social dimension of emotions and its implication for animal welfare. *Appl Anim Behav Sci.* (2012) 138:170–81. doi: 10.1016/j.applanim.2012.02.005
25. Panksepp J, Panksepp JB. Toward a cross-species understanding of empathy. *Trends Neurosci.* (2013) 36:489–96. doi: 10.1016/j.tins.2013.04.009
26. Edgar JL, Nicol CJ, Clark CCA, Paul ES. Measuring empathic responses in animals. *Appl Anim Behav Sci.* (2012) 138:182–93. doi: 10.1016/j.applanim.2012.02.006
27. de Waal FB. Putting the altruism back into altruism: the evolution of empathy. *Annu Rev Psychol.* (2008) 59:279–300. doi: 10.1146/annurev.psych.59.103006.093625
28. Marsh AA. The neuroscience of empathy. *Curr Opin Behav Sci.* (2018) 19:110–15. doi: 10.1016/j.cobeha.2017.12.016
29. Bastiaansen JACJ, Thioux M, Keysers C. Evidence for mirror systems in emotions. *Philos Trans R Soc B* (2009) 364:2391–404. doi: 10.1098/rstb.2009.0058
30. Decety J, Chen C, Harenski C, Kiehl KA. An fMRI study of affective perspective taking in individuals with psychopathy: imagining another in pain does not evoke empathy. *Front Hum Neurosci.* (2013) 7:489. doi: 10.3389/fnhum.2013.00489
31. Panksepp JB, Lahvis GP. Rodent empathy and affective neuroscience. *Neurosci Biobehav Rev.* (2011) 35:1864–75. doi: 10.1016/j.neubiorev.2011.05.013
32. de Waal FBM, Preston SD. Mammalian empathy: behavioural manifestations and neural basis. *Nat Rev Neurosci.* (2017) 18:498–509. doi: 10.1038/nrn.2017.72
33. Fraser D. Animal behaviour, animal welfare and the scientific study of affect. *Appl Anim Behav Sci.* (2009) 118:108–17. doi: 10.1016/j.applanim.2009.02.020
34. Goumon S, Špinka M. Emotional contagion of distress in young pigs is potentiated by previous exposure to the same stressor. *Anim Cogn.* (2016) 19:501–11. doi: 10.1007/s10071-015-0950-5
35. Vieuille-Thomas C, Signoret JP. Pheromonal transmission of an aversive experience in domestic pig. *J Chem Ecol.* (1992) 18:1551–1557.
36. Reimert I, Bolhuis JE, Kemp B, Rodenburg TB. Indicators of positive and negative emotions and emotional contagion in pigs. *Physiol Behav.* (2013) 109:42–50. doi: 10.1016/j.physbeh.2012.11.002
37. Boissy A, Terlouw C, Le Neindre P. Presence of cues from stressed conspecifics increases reactivity to aversive events in cattle: evidence for the existence of alarm substances in urine. *Physiol Behav.* (1998) 63:489–95.
38. Briefer EF. Vocal expression of emotions in mammals: mechanisms of production and evidence. *J Zool.* (2012) 288:1–20. doi: 10.1111/j.1469-7998.2012.00920.x
39. Leliveld LMC, Düpjan S, Tuchscherer A, Puppe B. Vocal correlates of emotional reactivity within and across contexts in domestic pigs (*Sus scrofa*). *Physiol Behav.* (2017) 181:117–26. doi: 10.1016/j.physbeh.2017.09.010
40. Düpjan S, Tuchscherer A, Langbein J, Schön PC, Manteuffel G, Puppe B. Behavioural and cardiac responses towards conspecific distress calls in domestic pigs (*Sus scrofa*). *Physiol Behav.* (2011) 103:445–52. doi: 10.1016/j.physbeh.2011.03.017
41. Briefer EF, Oxley JA, McElligott AG. Autonomic nervous system reactivity in a free-ranging mammal: effects of dominance rank and personality. *Anim Behav.* (2015) 110:121–32. doi: 10.1016/j.anbehav.2015.09.022
42. Briefer EF, Tettamanti F, McElligott AG. Emotions in goats: mapping physiological, behavioural and vocal profiles. *Anim Behav.* (2015) 99:131–43. doi: 10.1016/j.anbehav.2014.11.002
43. Baciadonna L, Nawroth C, Briefer EF, McElligott AG. Perceptual lateralization of vocal stimuli in goats. *Curr Zool.* (2018) zoy022. doi: 10.1093/cz/zoy022
44. Demaree HA, Everhart DE, Youngstrom EA, Harrison DW. Brain lateralization of emotional processing: historical roots and a future incorporating "dominance." *Behav Cogn Neurosci Rev.* (2005) 4:3–20. doi: 10.1177/1534582305276837
45. Leliveld LMC, Langbein J, Puppe B. The emergence of emotional lateralization: evidence in non-human vertebrates and implications for farm animals. *Appl Anim Behav Sci.* (2013) 145:1–14. doi: 10.1016/j.applanim.2013.02.002
46. Briefer EF, Mandel R, Maigrot AL, Briefer Freymond S, Bachmann I, Hillmann E. Perception of emotional valence in horse whinnies. *Front Zool.* (2017) 14:8. doi: 10.1186/s12983-017-0193-1
47. Briefer EF, Maigrot AL, Mandel R, Freymond SB, Bachmann I, Hillmann E. Segregation of information about emotional arousal and valence in horse whinnies. *Sci Rep.* (2015) 4:9989. doi: 10.1038/srep09989
48. Buchli C, Raselli A, Bruckmaier R, Hillmann E. Contact with cows during the young age increases social competence and lowers the cardiac stress reaction in dairy calves. *Appl Anim Behav Sci.* (2017) 187:1–7. doi: 10.1016/j.applanim.2016.12.002
49. Wascher CAF, Scheiber IBR, Kotrschal K. Heart rate modulation in bystanding geese watching social and non-social events. *Proc Biol Sci.* (2008) 275:1653–9. doi: 10.1098/rspb.2008.0146
50. Nakamura K, Takimoto-Inose A, Hasegawa T. Cross-modal perception of human emotion in domestic horses (*Equus caballus*). *Sci Rep.* (2018) 8:8660. doi: 10.1038/s41598-018-26892-6
51. Albuquerque N, Guo K, Wilkinson A, Savalli C, Otta E, Mills D. Dogs recognize dog and human emotions. *Biol Lett.* (2016) 12:20150883. doi: 10.1098/rsbl.2015.0883
52. Boissy A, Erhard HW. How studying interactions between animal emotions, cognition, and personality can contribute to improve farm animal welfare. *Genet Behav Domes Anim.* (2014) 81–113. doi: 10.1016/B978-0-12-394586-0.00003-2
53. Boissy A, Manteuffel G, Jensen MB, Moe RO, Spruijt B, Keeling LJ et al. Assessment of positive emotions in animals to improve their welfare. *Physiol Behav.* (2007) 92:375–97. doi: 10.1016/j.physbeh.2007.02.003
54. Fredrickson BL. What good are positive emotions. *Rev Gen Psychol.* (1998) 2:300–19.
55. Brudzynski SM. Ultrasonic calls of rats as indicator variables of negative or positive states: acetylcholine-dopamine interaction and acoustic coding. *Behav Brain Res.* (2007) 182:261–73. doi: 10.1016/j.bbr.2007.03.004

56. Huber A, Barber ALA, Faragó T, Müller CA, Huber L. Investigating emotional contagion in dogs (Canis familiaris) to emotional sounds of humans and conspecifics. *Anim Cogn.* (2017) 20:703–15. doi: 10.1007/s10071-017-1092-8
57. Young C, Majolo B, Heistermann M, Schülke O, Ostner J. Responses to social and environmental stress are attenuated by strong male bonds in wild macaques. *Proc Natl Acad Sci USA*. (2014) 111:18195–200. doi: 10.1073/pnas.1411450111
58. Rault JL. Friends with benefits: social support and its relevance for farm animal welfare. *Appl Anim Behav Sci* (2012) 136:1–14. doi: 10.1016/j.applanim.2011.10.002

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A Framework to Assess the Impact of New Animal Management Technologies on Welfare: A Case Study of Virtual Fencing

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To be ethically acceptable, new husbandry technologies and livestock management systems must maintain or improve animal welfare. To achieve this goal, the design and implementation of new technologies need to harness and complement the learning abilities of animals. Here, from literature on the cognitive activation theory of stress (CATS), we develop a framework to assess welfare outcomes in terms of the animal's affective state and its learned ability to predict and control engagement with the environment, including, for example, new technologies. In CATS, animals' perception of their situation occurs through cognitive evaluation of predictability and controllability (P/C) that influence learning and stress responses. Stress responses result when animals are not able to predict or control both positive and negative events. A case study of virtual fencing involving avoidance learning is described. Successful learning occurs when the animal perceives cues to be predictable (audio warning always precedes a shock) and controllable (operant response to the audio cue prevents receiving the shock) and an acceptable welfare outcome ensues. However, if animals are unable to learn the association between the audio and shock cues, the situation retains low P/C leading to states of helplessness or hopelessness, with serious implications for animal welfare. We propose a framework for determining welfare outcomes and highlight examples of how animals' cognitive evaluation of their environment and their ability to learn relates to stress responses. New technologies or systems should ensure that predictability and controllability are not at low levels and that operant tasks align with learning abilities to provide optimal animal welfare outcomes.

Keywords: animal welfare, cattle, cognition, cognitive activation theory of stress, sheep

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INTRODUCTION

The development of new husbandry systems and management technologies has increased the complexity of the environment farmed animals must learn to engage with. For example, cattle may need to learn how to interact with automated milking systems and virtual fences, and laying hens need to learn temporal and spatial design features of new free range and aviary systems. Cognitive and learning abilities vary between individuals and contribute to fitness and survival within wild populations (1, 2). For farmed animals, variation in learning ability (speed to learn and ability to master new tasks) may influence the impact of new technologies on individuals and thus have welfare consequences for introduction of new livestock management systems.

A theory proposed by Ursin and Eriksen (3), termed the cognitive activation theory of stress (CATS), describes concepts that are relevant when considering how animals learn to interact with new farming technologies and systems. The CATS describes the relationship between cognitive evaluation (appraisal) and the stress responses based on studies in rats and humans. Specifically, the stress response relates to what the animal has learned to expect in response to a stimulus. Whether a stimulus is positive or negative depends on the individual appraisal of the situation, which is based on previous experience and expectations of the outcomes of stimuli. Expectancy occurs when the animal registers, stores, and uses information about what stimulus precedes a following stimulus (learning). Ursin and Eriksen (3) describe two stages of learning. The first is termed classical conditioning and is stimulus-stimulus learning involving acquisition of stimulus expectancies, and the second is operant conditioning involving acquisition of response expectancies. The CATS is an activation theory as the stimuli may induce arousal that is indicated through a measurable stress response, such as activation of the hypothalamic pituitary adrenal axis. In general, the welfare outcomes of the two stages of learning are higher for positive expectations (e.g., positive reinforcement) and lower for negative or uncertain expectations (e.g., negative reinforcement) (4, 5).

Both stress and welfare are linked and depend on how an animal perceives its environment (6). Cognitive evaluation of the predictability and controllability (P/C) of a situation are important elements that influence how animals learn and determine whether welfare outcomes will be positive or negative. A classic study in rats conducted by Weiss (7) demonstrates how a lack of P/C can induce stress responses and impact health. When individual rats were placed in identical cages where treatments A and B received identical electric shocks but treatment A rats received a light signal to indicate when a shock was forthcoming (i.e., the shocks were predictable), the stress response in the A rats was similar to controls that did not receive any electric shocks. The B rats displayed high stress responses as evidenced by high corticosterone levels and stomach wall lesions. This same response was seen when A rats were able to prevent an electric shock by turning a wheel (i.e., the shocks were controllable). Surprisingly, the ability to either control or predict the occurrence of the electric shock was equally effective at reducing the stress response, which was explained by the fact that the animals knew they were experiencing a safe period if they hadn't received a warning signal (7).

Bringing together the concepts described above, this paper will propose a framework for determining welfare outcomes based on two dimensions: (1) affective state and (2) predictability and controllability (P/C). We will highlight examples of how animals' cognitive evaluation of their environment and their ability to learn relates to stress responses and animal welfare.

PREDICTABILITY AND CONTROLLABILITY AND ANIMAL WELFARE

The framework (**Figure 1**) describes the relationship between affective state (as a continuum between positive and negative) and predictability and controllability (low to high). Affect is a core psychological state that modulates neural, behavioral, physiological, and immune functions and hence influences health and productivity (8). For simplicity, we do not decompose affect into its components of valence and arousal. Similarly, we do not separate predictability and controllability in our framework, however, animals may experience situations of high controllability and low predictability and vice versa (9). Cognitive evaluation of environmental characteristics such as predictability and controllability have been demonstrated to trigger emotions in animals (10). Appraisal of other criteria, including suddenness, novelty, and pleasantness have been proposed by Desire et al. (9) as an indicator of emotions in animals. The previously described classical experiments performed on rats by Weiss (7) provide a clear example of how cognitive elements influence stress responses based on predictability and controllability. This has also been demonstrated in livestock species, including sheep where exposure to aversive events that occurred unpredictably and uncontrollably induced chronic stress and negative affective states (11, 12).

Each quadrant of the framework describes an example of a welfare state as a result of the level of P/C and affective states. The examples discussed in relation to the framework do not account for all possible states in each quadrant and as with all frameworks, there are limitations to its use. Not all situations of high predictability and controllability result in good welfare outcomes (see bottom right quadrant; **Figure 1**). An example is barren environments that are generally highly predictable and not always controllable with animals experiencing negative

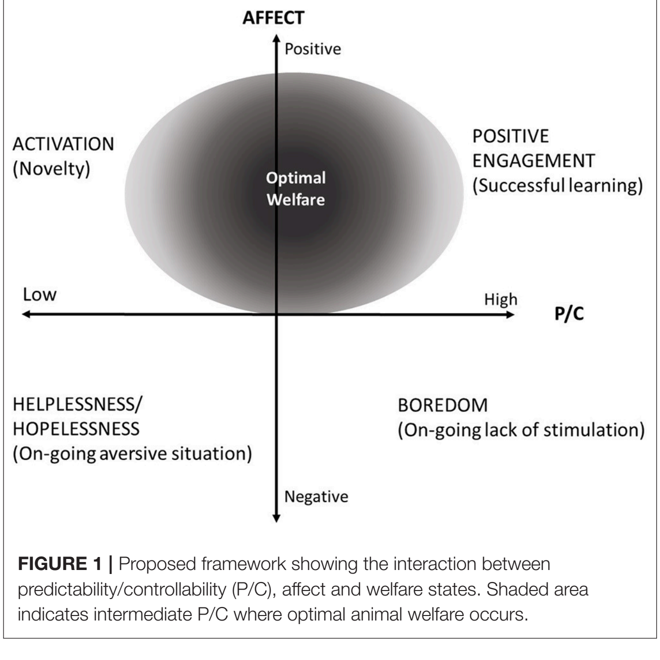

FIGURE 1 | Proposed framework showing the interaction between predictability/controllability (P/C), affect and welfare states. Shaded area indicates intermediate P/C where optimal animal welfare occurs.

affective states such as boredom from a lack of stimulation on a daily basis. Barren environments can result in chronic stress as demonstrated by increased ACTH-induced cortisol responses in tethered pigs (13) and stereotypies may result from the animals attempting to cope with the lack of environmental stimulation. There is potential to enhance welfare of animals kept in barren environments by signaling the arrival of a food reward that would increase P/C and improve affective states thereby moving welfare states to the top right quadrant of **Figure 1**. This presents potential for welfare improvement in intensive systems that may not offer the opportunity to perform a full range of natural behaviors. The top left quadrant is termed novelty. An example of low P/C and positive affect is shown in captive orange-winged Amazon parrots where the frequent rotation of enrichment objects reduced neophobia when compared to provision of new enrichments alone (14). However, individual differences in fearfulness may influence the affective state resulting from this procedure and could create for some individuals a negative welfare outcome.

Even positive events have the ability to compromise welfare if they are not controllable or predictable, for example removal of control by lambs over food delivery induced stress (15). Failure to reward pigs that were taught to perform an operant task to obtain a food reward induced frustration and aggressive behavior (16). In comparison, providing predictability of a food reward enhanced positive emotions as demonstrated in rats (17). This is further supported by evidence that the announcement of the arrival of enrichment (access to a hallway containing mixed grains) to pigs induced more positive emotions than providing enrichment alone (18). The importance of controllability for positive events was demonstrated by pigs that received cognitive enrichment (learning an operant task to access a food reward) displaying positive emotions (less fearful and more exploratory and lower sympathetic activation during feeding), compared to pigs that did not experience cognitive enrichment (19). In these examples, provision of predictability over the arrival of the positive event provides an improved welfare state and animals would move into the top right quadrant of the framework. We have termed this quadrant "Positive engagement" and this aligns with concepts described by Mellor (20). Wechsler and Lea (21) highlight the opportunity to develop enrichment tasks that take into account the learning abilities of animals that may result in improved welfare. Predictable scheduling of feeding in broiler breeder pullets was associated with improved welfare (22). Learning in itself can be motivating and induce emotional responses in animals, for example, heifers showed increased heart rate and more vigorous movement down a race when they made improvements in their learning (23). It has been suggested that a capacity to acquire prediction and control over the environment through learning contributes to resilience of the animal to environmental change (24). Together, evidence of inducing positive affect by providing opportunities for animals to be able to predict and control positive experiences shows much promise for improving welfare of animals kept in confined conditions.

Situations where an individual is exposed to unpredictable and uncontrollable negative events occur when there is an acquired expectancy that no relationships exist between responses and reinforcement (**Figure 1**: bottom left quadrant). In this situation, the individual perceives that there is no relationship between any action they can do and the outcome and this state is termed helplessness. Hopelessness is similar but with the learned expectancy that all responses lead to a negative result. States of helplessness and hopelessness occur when the animal is not coping and may lead to somatic disease through sustained arousal (3), which has serious implications for animal welfare. Overall, for welfare to be optimal in livestock farming systems, predictability and controllability should not be too high or too low, i.e., it should be at an intermediate level (see circle area in **Figure 1**). This represents a level that provides stimulation and prevents boredom through providing opportunities to learn that are within the animals cognitive ability.

A CASE STUDY: AVOIDANCE LEARNING IN VIRTUAL FENCING

To highlight the concepts developed in the framework, a relevant example of a new technology being applied to livestock management is presented. Traditional fences are physical barriers that contain animals by obstructing their passage across a boundary. The traditional barriers can be strengthened by inclusion of aversive stimuli such as spikes (e.g., barbed wire) or electric shocks (e.g., conventional electric fencing). In contrast, virtual fences replace the physical barrier with a benign cue (audio) that heralds the imminent imposition of an aversive stimulus (electric shock) if the animal proceeds across the virtual barrier. Virtual fencing has the potential to reduce labor and material costs associated with moving and maintaining physical fences, enable more efficient pasture management and better protection of environmentally sensitive areas. As the virtual fence is not visible and is more complex for the animal to learn than a conventional electric fence, there may be more interactions with the fence and therefore more shocks received by the animal, however, to date no comparative studies have been reported. For a conventional electric fence, the number of shocks received is highest in the first hour on the first day of exposure (25). With a virtual fence, half the cattle learned to respond to the audio cue after ∼6 interactions with the fence (26). Direct contrasts are needed to better understand the comparative impact of the technologies on behavior and welfare. With a commercial system being developed (Agersens®) and a strong demand for the product, it is expected that application of virtual fence technology on farm is imminent. The virtual fencing system utilizes the animal's capacity for avoidance learning through operant conditioning so that the animal learns to respond to an audio cue (conditioned stimulus) to avoid receiving an electric shock (response stimulus). **Figure 2** shows the process for assessing welfare outcomes of new technologies such as virtual fencing. With avoidance learning, on the initial approaches to the virtual fence, we suggest that animals are in a situation of low predictability and controllability as they do not know what the audio warning means and are unable to avoid receiving the shock. We interpret this as a negative welfare state as the animals show an acute stress responses to the electric shock (27). After the initial learning period [∼6 approaches for 50% of cattle to learn (26)] and with coupling of the application of the audio warning consistently at every approach event, responses indicate that cattle learn the situation has high predictability. If the animal continues to travel further toward the virtual fence line, it receives an aversive electric shock, however, if the animal stops in response to the audio cue and does not proceed across the virtual boundary, it avoids the electric shock. Thus the animal learns to control its exposure to the aversive response stimulus by responding behaviorally to the conditioned audio cue (by turning back from the virtual fence). Avoidance learning by the animal confers high predictability and controllability to the temporal sequence and spatial relationship between the benign conditioned cue and the aversive response stimulus. Through acquisition of the ability to predict and control, the animal's agency improves (28, 29); the resulting welfare state is more positive (**Figure 2**) and the animal is classified as "coping" (30). Importantly, in avoidance learning, animals that learn and perform well do not display signs of stress, even though avoidance is linked to fear (5).

In contrast, some animals may not have the ability to learn the association between the audio and shock cue, resulting in a situation of low predictability and controllability as described in the studies conducted in rats (5, 7). Whether learning will occur depends on the properties of the events, the consistency of their presentation and the pairing of the stimuli. Ursin and Eriksen (3) termed this predictive value. How an individual perceives the probability of an expected event is termed the perceived probability (31). Predictability is where the perceived probability of stimulus expectancies is high and control occurs when the perceived probability of response expectancies is high (3). As the perceived probability is subjective and dependent on the individual's ability to learn, some animals may not have the ability to learn the appropriate response and therefore the situation for them would be one of low P/C. Individual differences in personality may influence cognitive and learning abilities (32–34). For example, indoor-preferring birds in free range laying systems are more fearful (35, 36) and slower to learn a T-maze test than outdoor-preferring birds (37). More fearful birds use different strategies to learn a task and are less flexible in their learning (38). Further understanding of the relationship between individual differences in personality and cognition will provide guidance in developing operant learning tasks that match learning abilities or for selecting animals better suited to specific production systems. Importantly, for species exhibiting group behaviors such as flocking, motivation to stay close to conspecifics may provide an additional suite of cues to animals that lack an ability to learn the specific conditioned stimulus/responses stimulus paradigm.

LEARNING ABILITIES AND ANIMAL WELFARE

An acceptable welfare outcome for virtual fencing requires the system to be designed and implemented in a manner that enables animals to learn through prediction and control to avoid the

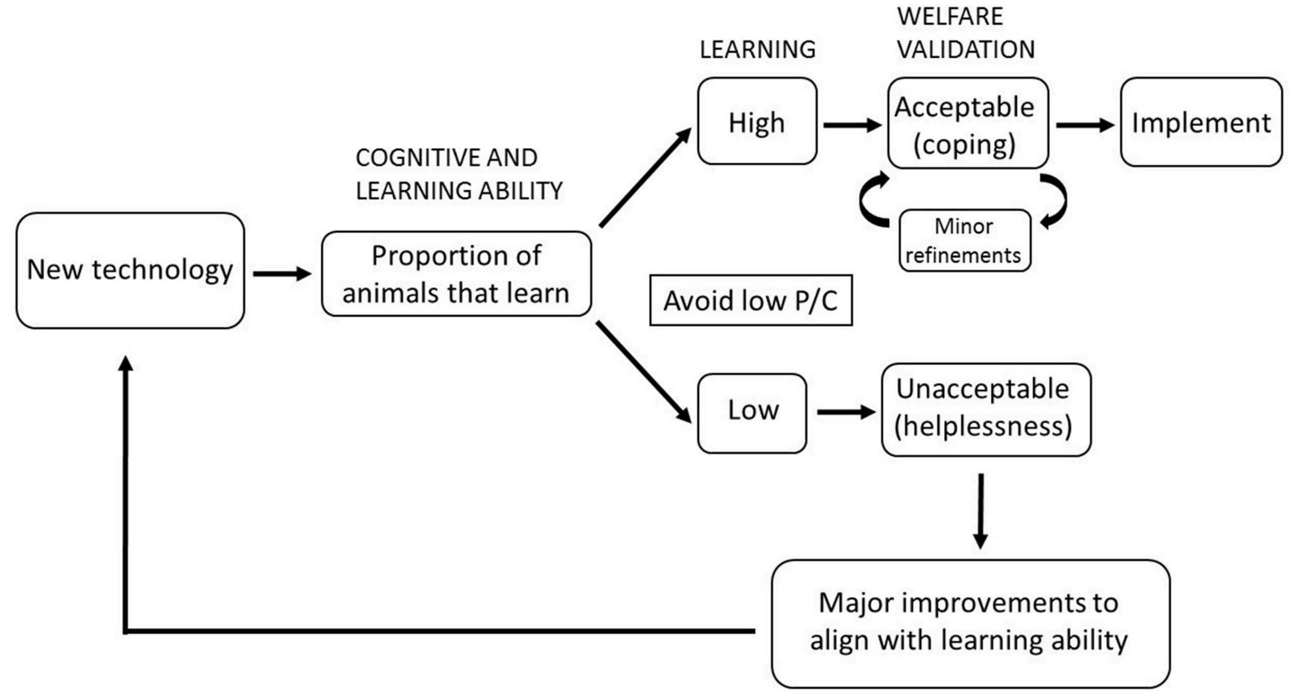

FIGURE 2 | A proposed process to assess welfare outcomes of new technologies or systems applied to livestock. Situations of low Predictability and Controllability (P/C) should be avoided.

electric shock. While it has been demonstrated in several studies that cattle (26, 39–41) and sheep (42, 43) readily learn to respond to the audio cue, these studies report a large variation between animals in learning speed and task competency. Further research is needed to determine the influence of the duration of training and social environment in which training occurs on learning outcomes. For instance, initial studies suggest that when cattle (26) and sheep (43) were trained individually there was more variation between animals in learning to respond to the audio cue than when trained in groups (41, 42). This is in accord with the potential for social cues to influence learning and task acquisition (44). Further, while acute stress is expected to result when the animals are undergoing avoidance learning, the stress response should be minimal once animals learn to avoid the shock and the situation becomes predictable and controllable.

Animals attempt to learn and adapt to the environment using behavioral and physiological responses, however if the limits to their adaptation or learning ability are reached then chronic stress can occur. Determination of the physiological and behavioral consequences of long-term exposure to virtual fencing in cattle and sheep is needed to ensure that welfare is not compromised. This will include assessment of behavioral patterns, as disturbances in normal time budgets can indicate welfare issues, for example lying time has been demonstrated to indicate comfort of lying surfaces in cattle (45). Over a short-term virtual fencing study in cattle, changes in behavioral time budgets were minor (41), however further research is needed over longer time periods. Learned helplessness may occur if the situation is one of low P/C. Helplessness is associated with chronic stress, and could be assessed for instance through corticotrophin releasing hormone (CRH) or adrenocorticotrophic hormone (ACTH) challenges (13) to monitor welfare impacts of virtual fencing. Increasing complexity of the virtual fencing system such as its use for herding large groups of animals (e.g., mustering dairy cows) and creep feeding young stock will need to be considered in relation to the proposed framework. With increased complexity comes a greater chance of more animals not learning to avoid the shock (i.e., a situation of low predictability and controllability) and a greater potential for poor welfare outcomes. Evidence of animals not coping with a virtual fencing system will require that either the system be altered to ensure learning occurs or that virtual fencing is not implemented for certain groups of animals.

CONCLUSIONS

This paper provides a framework for understanding animal welfare in terms of the animal's affective state and its learned ability to predict and control engagement with its environment. Stress responses occur when animals are unable to predict or control both positive and negative events. This is usually not a welfare issue if the situation of low P/C is short-term, as a normal acute stress response will be observed. However, if the situation is on-going, then chronic stress can be induced, the animal may not be able to cope and welfare outcomes will be poor. There are also potential issues with situations of high and long lasting P/C, which may provide certainty but lack stimulation and lead to boredom. It is recommended that P/C should be intermediate to be of optimal value (5). This intermediate level should be one where the operant task aligns with the learning ability of the animal so that it is predictable and controllable to ensure that welfare is not compromised.

ETHICS STATEMENT

This is a review that did not involve any animal experimentation.

AUTHOR CONTRIBUTIONS

CL conceived the idea and prepared the review. IC contributed to the content, wrote parts, and edited the manuscript. DC contributed to the content, wrote parts, and edited the manuscript.

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REFERENCES

1. Lee C, Colegate S, Fisher AD. Development of a maze test and its application to assess spatial learning and memory in Merino sheep. *Appl Anim Behav Sci.* (2006) 96:43–51. doi: 10.1016/j.applanim.2005.06.001
2. Hirata M, Tomita C, Yamada K. Use of a maze test to assess spatial learning and memory in cattle: can cattle traverse a complex maze? *Appl Anim Behav Sci.* (2016) 180:18–25. doi: 10.1016/j.applanim.2016.04.004
3. Ursin H, Eriksen HR. The cognitive activation theory of stress. *Psychoneuroendocrinology* (2004) 29:567–92. doi: 10.1016/S0306-4530(03)00091-X
4. Wiepkema PR. Behavioural aspects of stress. *Biol. Stress Farm Anim.* (1987) 113–33. doi: 10.1007/978-94-009-3339-2_9
5. Wiepkema PR, Koolhaas JM. Stress and animal welfare. *Anim Welfare* (1993) 2:195–218.
6. Veissier I, Boissy A. Stress and welfare: two complementary concepts that are intrinsically related to the animal's point of view. *Physiol Behav.* (2007) 92:429–33. doi: 10.1016/j.physbeh.2006.11.008
7. Weiss JM. Psychological factors in stress and disease. *Sci Am.* (1972) 226:104–13. doi: 10.1038/scientificamerican0672-104
8. Mendl M, Burman OHP, Paul ES. An integrative and functional framework for the study of animal emotion and mood. *Proc R Soc B Biol Sci.* (2010) 277:2895–904. doi: 10.1098/rspb.2010.0303
9. Desire L, Boissy A, Veissier I. Emotions in farm animals: a new approach to animal welfare in applied ethology. *Behav Proc.* (2002) 60:165–80. doi: 10.1016/S0376-6357(02)00081-5
10. Boissy A, Lee C. How assessing relationships between emotions and cognition can improve farm animal welfare. *Rev Sci Tech.* (2014) 33:103–10. doi: 10.20506/rst.33.1.2260
11. Doyle RE, Lee C, Deiss V, Fisher AD, Hinch GN, Boissy A. Measuring judgement bias and emotional reactivity in sheep following long-term

exposure to unpredictable and aversive events. *Physiol Behav.* (2011) 102:503–10. doi: 10.1016/j.physbeh.2011.01.001

12. Destrez A, Deiss V, Leterrier C, Boivin X, Boissy A. Long-term exposure to unpredictable and uncontrollable aversive events alters fearfulness in sheep. *Animal* (2013) 7:476–84. doi: 10.1017/S1751731112001796
13. Janssens C, Helmond FA, Wiegant VM. Increased cortisol response to exogenous adrenocorticotrophic hormone in chronically stressed pigs - influence of housing conditions. *J Anim Sci.* (1994) 72:1771–7. doi: 10.2527/1994.7271771x
14. Fox RA, Millam JR. Novelty and individual differences influence neophobia in orange-winged Amazon parrots (*Amazona amazonica*). *Appl Anim Behav Sci.* (2007) 104:107–15. doi: 10.1016/j.applanim.2006.04.033
15. Greiveldinger L, Veissier I, Boissy A. Behavioural and physiological responses of lambs to controllable vs. uncontrollable aversive events. *Psychoneuroendocrinology* (2009) 34:805–14. doi: 10.1016/j.psyneuen.2008.10.025
16. Dantzer R, Arnone M, Mormede P. Effects of frustration on behavior and plasma corticosteroid levels in pigs. *Physiol Behav.* (1980) 24:1–4. doi: 10.1016/0031-9384(80)90005-0
17. Van Der Harst JE, Fermont PCJ, Bilstra AE, Spruijt BM. Access to enriched housing is rewarding to rats as reflected by their anticipatory behaviour. *Anim Behav.* (2003) 66:493–504. doi: 10.1006/anbe.2003.2201
18. Dudink S, Simonse H, Marks I, De Jonge FH, Spruijt BM. Announcing the arrival of enrichment increases play behaviour and reduces weaning-stress-induced behaviours of piglets directly after weaning. *Appl Anim Behav Sci.* (2006) 101:86–101. doi: 10.1016/j.applanim.2005.12.008
19. Zebunke M, Puppe B, Langbein J. Effects of cognitive enrichment on behavioural and physiological reactions of pigs. *Physiol Behav.* (2013) 118:70–9. doi: 10.1016/j.physbeh.2013.05.005
20. Mellor DJ. Enhancing animal welfare by creating opportunities for positive affective engagement. *N Z Veter J.* (2015) 63:3–8. doi: 10.1080/00480169.2014.926799
21. Wechsler B, Lea SEG. Adaptation by learning: its significance for farm animal husbandry. *Appl Anim Behav Sci.* (2007) 108:197–214. doi: 10.1016/j.applanim.2007.03.012
22. Lindholm C, Johansson A, Middelkoop A, Lees JJ, Yngwe N, Berndtson E, et al. The quest for welfare-friendly feeding of broiler breeders: effects of daily vs. 5:2 feed restriction schedules. *Poult Sci.* (2018) 97:368–77. doi: 10.3382/ps/pex326
23. Hagen K, Broom DM. Emotional reactions to learning in cattle. *Appl Anim Behav Sci.* (2004) 85:203–13. doi: 10.1016/j.applanim.2003.11.007
24. Colditz IG, Hine BC. Resilience in farm animals: biology, management, breeding and implications for animal welfare. *Anim Prod Sci.* (2016) 56:1961–83. doi: 10.1071/AN15297
25. Martiskainen P, Tuomisto L, Huuskonen A, Mononen J. Training dairy bull calves to stay within light-built electric fences. *Agric Food Sci.* (2008) 17:121–6. doi: 10.2137/145960608785328189
26. Campbell DLM, Lea JM, Haynes SJ, Farrer WJ, Leigh-Lancaster CJ, Lee C. Virtual fencing of cattle using an automated collar in a feed attractant trial. *Appl Anim Behav Sci.* (2018) 200:71–7. doi: 10.1016/j.applanim.2017.12.002
27. Lee C, Fisher AD, Reed MT, Henshall JM. The effect of low energy electric shock on cortisol, beta-endorphin, heart rate and behaviour of cattle. *Appl Anim Behav Sci.* (2008) 113:32–42. doi: 10.1016/j.applanim.2007.10.002
28. Spinka M, Wemelsfelder F *Environmental Challenge and Animal Agency. Animal welfare.* Wallingford: CABI International (2011).
29. Colditz IG. Objecthood, agency and mutualism in valenced farm animal environments. *Animals* (2018) 8:50. doi: 10.3390/ani8040050
30. Weiss JM. Effects of coping responses on stress. *J Comp Physiol Psychol.* (1968) 65:251–60. doi: 10.1037/h0025562
31. Bolles RC. Reinforcement, expectancy and learning. *Psychol Rev.* (1972) 79:394–409. doi: 10.1037/h0033120
32. De Haas EN, Lee C, Hernandez CE, Naguib M, Rodenburg TB. Individual differences in personality in laying hens are related to learning a colour cue association. *Behav Proc.* (2017a) 134:37–42. doi: 10.1016/j.beproc.2016.11.001
33. Guillette LM, Naguib M, Griffin AS. Individual differences in cognition and personality. *Behav Proc.* (2017) 134:1–3. doi: 10.1016/j.beproc.2016.12.001
34. Nawroth C, Prentice PM, Mcelligott AG. Individual personality differences in goats predict their performance in visual learning and non-associative cognitive tasks. *Behav Proc.* (2017) 134:43–53. doi: 10.1016/j.beproc.2016.08.001
35. Campbell DLM, Hinch GN, Downing JA, Lee C. Fear and coping styles of outdoor-preferring, moderate-outdoor and indoor-preferring free-range laying hens. *Appl Anim Behav Sci.* (2016) 185:73–7. doi: 10.1016/j.applanim.2016.09.004
36. Hartcher KM, Hickey KA, Hemsworth PH, Cronin GM, Wilkinson SJ, Singh M. Relationships between range access as monitored by radio frequency identification technology, fearfulness, and plumage damage in free-range laying hens. *Animal* (2016) 10:847–53. doi: 10.1017/S1751731115002463
37. Campbell DLM, Talk AC, Loh ZA, Dyall TR, Lee C. Spatial cognition and range use in free-range laying hens. *Animals* (2018) 8:E26. doi: 10.3390/ani8020026
38. De Haas EN, Lee C, Rodenburg TB. Learning and judgment can be affected by predisposed fearfulness in laying hens. *Front Veter Sci.* 4:113. doi: 10.3389/fvets.2017.00113
39. Lee C, Prayaga K, Reed M, Henshall J. Methods of training cattle to avoid a location using electrical cues. *Appl Anim Behav Sci.* (2007) 108:229–38. doi: 10.1016/j.applanim.2006.12.003
40. Lee C, Henshall JM, Wark TJ, Crossman CC, Reed MT, Brewer HG, et al. Associative learning by cattle to enable effective and ethical virtual fences. *Appl Anim Behav Sci.* (2009) 119:15–22. doi: 10.1016/j.applanim.2009.03.010
41. Campbell DLM, Lea JM, Farrer WJ, Haynes SJ, Lee C. Tech-savvy beef cattle? How heifers respond to moving virtual fence lines. *Animals* (2017) 7:72. doi: 10.3390/ani7090072
42. Marini DLR, Belson S, Lee C. Controlling within-field sheep movement using virtual fencing. *Animals* (2018) 8:31. doi: 10.3390/ani8030031
43. Marini DM, Belson S, Rodenburg TB, Llewellyn R, Lee C. Developing an ethically acceptable virtual fencing system for sheep. *Animals* (2018) 8:33. doi: 10.3390/ani8030033
44. Nicol CJ. The social transmission of information and behavior. *Appl Anim Behav Sci.* (1995) 44:79–98. doi: 10.1016/0168-1591(95)00607-T
45. Haley DB, Rushen J, De Passille AM. Behavioural indicators of cow comfort: activity and resting behaviour of dairy cows in two types of housing. *Can J Anim Sci.* (2000) 80:257–63. doi: 10.4141/A99-084

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