UNIVERSITY OF PADOVA

Department of General Psychology

Bachelor's Degree Course in Psychological Science

Final dissertation

A judgement bias task: implications for our understanding of emotional lateralisation in chicks (*G. gallus domesticus*)

Supervisor
Professor Lucia Regolin

Co-supervisor
Luna Dudine

Candidate: Franca Zaccaria
Student ID number: 2067414

Academic year 2024/2025

Table of Contents

Abstract

The field of animal cognition has shown increasing interest in the development of reliable ways to assess animals' emotional states. Cognitive biases, including judgement biases, seem to be a promising solution, as they allow to assess the animal's emotional state through observable and quantifiable behaviour. In this experimental study, we investigated the effects of a positive or a negative emotional treatment on 3-day-old domestic chicks (*G. gallus domesticus*; n=92, 46 in the positive treatment condition and 46 in the negative treatment condition). After having tested chicks' motivation, we observed their approach behaviour towards familiar stimuli before the emotional treatment and towards an ambiguous one after it. Emotional treatments involved either affiliative or distress-inducing conditions. We expected the emotional treatment to influence how they would interpret the ambiguous stimulus. To examine potential lateralised brain activation associated with affect, chicks were simultaneously presented with the same stimulus on the right and left of their visual fields, and their approach behaviour towards both was analysed. Behavioural data were recorded and analysed using ANOVA. Results revealed consistent differences in latencies and in approach behaviour between pre- and post-treatment conditions, indicating that the treatment was potentially effective in inducing a positive or negative emotional state, determining a judgment bias. However, the absence of significant differences in some behavioural indexes and a lack of discrimination between high- and low-value stimuli raise questions about training efficacy and treatment validity. Notably, no effect was observed on chicks' behaviour in terms of side preferences depending on the treatment and on their emotional state. Possible explanations for these results are presented, along with suggestions for future research.

Key words: animal cognition, domestic chick, judgement bias, brain lateralisation, emotional state.

1. Introduction

1.1 Animal emotions and their ethical implications

In the past decades, animal welfare, defined as a state of harmony between the animal and its environment that determines complete mental and physical health (Désiré et al., 2002), has become a topic of significant interest and concern in both ethical discourse and practical application. The evident limitation of this definition is the lack of specificity regarding the processes contributing to animal welfare and its measurable outcome (Fraser, 1995). On the other hand, what clearly emerges is that the science of animal welfare lays its roots in the assumption that non-human animals can experience suffering and pleasure, not only on a strictly physical level. Researchers generally agree that welfare consists of the absence of negative emotional experiences (e.g., pain) as much as of the presence of positive experiences (e.g., pleasure). According to Boissy et al. (2007), the latter is actually the predominant determinant of well-being, as the lack of positive affects could be considered, in itself, a sign of emotional discomfort. However, scientific investigation has primarily focused on negative emotions, neglecting the assessment of positive affects (Boissy et al., 2007).

It is generally believed that animals do experience a range of different emotions, both positive and negative, but it is noteworthy to mention that no definitive method exists that provides a clear indication of the animal's emotional state, regardless of its valence (i.e., positive or negative). Therefore, scientists have been increasingly interested in developing reliable techniques to obtain proxy measures of the affective states of animals. This has now become the primary goal of animal welfare research (Mendl et al., 2009).

Although an incredibly extensive literature exists on the psychology of emotions, no universally agreed-upon definition of emotion exists in the literature, with a large number of different definitions being proposed over time emphasizing different aspects of it (Kleinginna & Kleinginna, 1981). Paul et al. proposed that

> "Emotions refer to processes which are likely to have evolved from basic mechanisms that gave animals the ability to avoid harm/punishment and seek valuable resources/reward" (2005, p. 470).

They are, therefore, adaptive states. Furthermore, emotions are recognised to be multifaceted, comprising physiological, behavioural, subjective, and cognitive components (Paul et al., 2005). The terms ‘emotion’ and ‘affect’ are hereafter, as in most part of the literature, used interchangeably.

Humans can verbally report the emotions elicited by a given situation, providing a direct measure of their subjectively experienced affective state. On the contrary, non-human animals clearly cannot do so and, as a consequence, behavioural and physiological indicators were considered for a long time to be the only available ones. Examples of those include respectively measures of approach and avoidance behaviour (Hedlund et al., 2021) and measures of the hypothalamic-pituitary-adrenal (HPA) activity (Weiss et al., 2004).

More recently, a number of researchers pointed out the utility of adopting a cognitive approach to assess emotional states (e.g., Désiré et al., 2002; Mendl et al., 2009; Paul et al., 2005). This approach is based on the demonstrated premise from human psychology that cognitive processes influence and are influenced by an individual’s emotional state, proving the existence of links between them that occur in both causal directions (Dolcos & Denkova, 2015). Because of this close interrelationship existing between cognition and emotion, objective changes in cognitive function can be used as an allegedly accurate tool for the detection and assessment of animal emotional states (Paul et al., 2005).

1.2 Cognitive bias

Cognitive processes that have been proven to be affected by emotional states in humans include attention, memory, and judgement. The emotional influences on these, considered to be the cognitive outputs of emotions (Paul et al., 2005), are collectively known as cognitive biases, with the possibility of making a further distinction between the three different main subtypes: attention biases, memory biases, and judgement biases. The impact of affective states on cognitive processes (i.e., the cognitive biases) is reflected in observable behaviours.

A significant number of studies have been conducted to address cognitive biases in humans. Attention biases are the result of an anxious state that produces a shift in attention towards threatening information (Kindt & Hout, 2001). Memory biases can be observed in storage, consolidation, and retrieval of information, as events with emotional valence are more easily remembered (Cahill & McGaugh, 1996). As described in greater details in 1.2.1 Judgement bias, judgement bias refers to the effect of emotions on judgement in various areas, including interpretation of ambiguous stimuli, risk-taking, and expectations about the future (e.g., Eysenck et al., 1991).

In evolutionary terms, despite apparently being design flaws, cognitive biases appear to have an adaptive value and to have been shaped by evolutionary forces (Haselton et al., 2015). For example, Haselton et al. (2015) proposed that cognitive biases may be caused by processing limitations that pose the necessity to develop shortcuts that "work well in most circumstances, but are prone to break down in systematic ways" (p. 726). Although the evolutionary processes underlying cognitive biases are not fully known, researchers agree that these biases are more functional than they initially seem and that it is therefore plausible that such effects are also extended to non-human animals. Indeed, the existence of cognitive biases has been tested and proved in various animal species using different experimental protocols (for example, attention bias in sheep (Monk et al., 2018) and in psittacines (Cussen & Mench, 2014); memory bias in rats (O. H. P. Burman & Mendl, 2018); judgement bias in bumblebees (Solvi et al., 2016) and in dogs (O. H. P. Burman et al., 2011)).

1.2.1 Judgement bias task

Most of the research conducted on cognitive biases focuses specifically on the process of judgement and on how it is influenced by the animal's emotional state – that is, it focuses on judgement bias, i.e., "a relative reaction (or 'interpretation') to an ambiguous stimulus" (Boleij et al., 2012, p. 23). The animal behaviourally responds to the ambiguous stimulus in a way that indicates whether they are anticipating relatively positive or negative outcomes. Generally, an animal experiencing a negative affective state classifies an ambiguous stimulus as predicting negative outcomes (a propensity termed 'pessimism'), while the opposite response is expected from animals experiencing positive emotions ('optimism') (Bateson & Matheson, 2007).

The first experimental protocol to assess the existence of a judgement bias in animals was developed to test whether a pessimistic response bias could be induced in rats as a result of unpredictable housing conditions (Harding et al., 2004). They trained the rats to make an operant discrimination between different tones, associated with either a positive or a negative event. After reaching a criterion performance, the animals were exposed to ambiguous tones that were intermediate between the training tones in order to measure their response. A mild negative affective state was induced in the experimental group of rats through the implementation of unpredictable housing conditions, characterised by negative interventions such as unfamiliar or tilted cages and reversed light/dark cycles. By comparing the responses to ambiguous stimuli of the rats in the experimental group to those of the rats in the control condition (i.e., predictable housing with no negative interventions), Harding et al. (2004) concluded that rats with an induced negative affective state responded less and, when they did, they were slower to respond to the ambiguous tones close to the positive one and to the positive one itself.

Following the publication of Harding et al.'s study in 2004, researchers have tested judgement bias in different species using the same protocol or variations of it. For example, studies have proven the existence of a pessimistic judgement bias in broiler chickens due to experimentally elevated levels of glucocorticoid stress hormone (Iyasere et al., 2017) and of an optimistic judgement bias in restrained and isolated sheep after release (Doyle et al., 2010). The findings of these studies suggest that judgment bias is a particularly promising indicator of affective states in animals and that, therefore, assessing it has significant implications for animal welfare.

According to Mendl et al. (2009), the decision-making process that leads to the animal's judgement of ambiguous stimuli is constituted by various components which can all be differently affected by the animal's affective state. The first component is the sensory registration of the stimulus, determined by perceptual and attentional processes on which the affective state does indeed have an influence as the concept of attentional bias proves. Secondly, the evaluation of the ambiguous stimulus occurs, probably by comparing it with the representations of the training stimuli stored in the animal's memory. The expected utility (EU) model, firstly proposed by Bernoulli in 1738, is the dominant theory used to explain how decisions are made under risk and uncertainty (Loewenstein et al., 2008). It postulates that the evaluation of the ambiguous stimulus is determined by the outcome value and desirability, by its probability of occurring, or by its EU, i.e., the integrated combination of utility and probability. The EU of a decision is calculated as:

$$EU = \sum_{i=1}^{n} p_i \cdot u(x_i)$$

where p_i is the probability of outcome x_i and $u(x_i)$ is the utility of that outcome. However, irregularities can be observed in the decision-making process due to the experienced emotional state, which can modulate outcome value, probability, and EU leading to judgement biases (Mendl et al., 2009). Lastly, the third component in the decision-making process is that of action selection, whose mechanisms can change based on affective information. It is evident, therefore, that a range of processes are involved in making a judgment in a situation of ambiguity and the animal's affective state may modulate some or all of them.

1.3 *Gallus gallus domesticus* as model species

For our experiment, we decided to use chicks as our subjects; in particular, *Gallus gallus domesticus* was our model species.

As explained in 1.1 Animal emotions and their ethical implications, finding reliable ways to assess animals' emotional states and to contribute to their welfare is one of the primary goals of animal research. Among all animal species, that of domestic chicks is perhaps the one most in need of urgent interventions. Indeed, they are the world's most numerous bird species, with a population of over 27.2 billion in 2023 (*FAOSTAT*, n.d.). Considering the ongoing ascending trend (15.9 billion in 2003 and 20.9 billion in 2013, *FAOSTAT*, n.d.), this number is expected to further increase. According to Bennett et al. (2018), this surge is largely driven by the expanding chicken-meat consumption –while the world population showed a 145% increase from 1961 to 2018, chicken-meat production increased by 1404% in the same period (Uzundumlu & Dilli, 2022). Although welfare concerns differ between broiler chickens (e.g., Meluzzi & and Sirri, 2009) and laying hens (e.g., Hemsworth & Edwards, 2020 and Bryden et al., 2021), generally investigating how to contribute to *G. gallus domesticus*' welfare is of pivotal importance.

Additionally, choosing domestic chicks was particularly suitable for various other reasons, ranging from the practical one that they are easily handled and quickly habituate to human presence to the more technical one regarding the extended literature existing on them and their abilities. Indeed, chicks exhibit a range of advanced cognitive and sensory abilities, which make them ideal for this study.

Similarly to most other bird species, chicks significantly rely on their visual ability, that allows them to see stimuli presented both at a short and at a long distance and to distinguish a range of colours vaster than that of humans (Marino, 2017). Moreover, researchers have uncovered the lateralised mechanisms controlling their right and left eyes' functioning, as will be explained in greater details later in this section. Chicks' audition is also well-developed, as they can detect low- and high-frequency sounds, including infrasound (< 20 Hz) that are below the human hearing threshold (Garnham & Løvlie, 2018). Early studies (e.g., Collias & Joos, 1953) have also proved that different chicks' vocalisations can be distinguished based on, for example, duration and frequencies, while later studies have corroborated the idea that different types of vocalisations are associated with different emotional states and that changes in acoustic parameters indicate differences in emotional arousal and valence (Collins et al., 2024).

Since in our experimental protocol we used, among other things, recordings of tweets or distress calls produced by other unfamiliar chicks to induce a change in the affective state of our subjects, it was indispensable that they not only have the sensory abilities to hear and discriminate them, but that they also show an empathic response. Empathy has been defined as having a similar emotional state to another (in our case, the recorded chicks) as a result of perceiving their situation (Preston & Waal, 2002). Emotional contagion, i.e., having an emotional state as a direct result of perceiving the same state in another, can be said to be encompassed in the broader concept of empathy (Preston & Waal, 2002). Studies have demonstrated the existence of emotional contagion in various complex social species (for a review, see Pérez-Manrique & Gomila, 2022), including birds and, of greater interest to our study, 9-week-old chicks (*Gallus gallus domesticus*), which exhibited a similar emotional state to their stressed brood mates, as measured with behavioural and physiological indices (Edgar & Nicol, 2018).

Another important cognitive ability of chicks that we made use of in our study is imprinting, defined as the behaviour newborn precocial birds show by memorising the properties of the first object they encounter and later showing a preference for it (Lorenz, 1937). Chicks can be imprinted on objects other than their mother, including non-living objects, if they are appropriately displayed (e.g., Rugani et al., 2010).

Finally, the organisation of the avian brain, and specifically that of the chick, has long been a subject of interest for researchers. Brain lateralisation, characterised by hemispheres being specialised in different functions, has been observed in vertebrate and invertebrate species, with the majority of individuals in a species being lateralised in the same direction (Rogers & Kaplan, 2019). Having a lateralised brain seems to be associated with a number of advantages, including the enhanced ability to perform two tasks simultaneously by carrying out qualitatively different types of processing in the two hemispheres at the same time (Rogers et al., 2004). Rogers and Anson (1979) have been the first to discover lateralisation of function in the chicken forebrain, more specifically lateralised processing of visual information and control of visual behaviour, by experimentally proving that most visual information entering one eye is processed contralaterally. Since the initial study, domestic chick's brain lateralisation has been widely investigated and the functions of the two hemispheres have been determined mainly by testing the animal monocularly. The left hemisphere ('right eye system' (RES) due to contralateral visual processing) is specialised in the categorisation of stimuli and, consequently, in their discrimination, including between types of conspecifics (Rogers &

Kaplan, 2019). An appropriate response is then associated to the categories formed and strategies of behaviour are established, that allow the chick to respond only to pertinent cues and inhibit response to irrelevant stimuli (Rogers, 2008). It is particularly involved in the discrimination of stimuli associated with reinforcement, either positive or negative, (McKenzie et al., 1998) and it is active in conditions in which focused attention on the task being performed is required and in which the animal is in a relaxed state (Rogers & Kaplan, 2019). On the other hand, the right hemisphere ('left eye system' (LES)) is active in emotionally charged situations, such as agonistic interactions and escape responses (Rogers & Kaplan, 2019), as will be explained in greater details in 1.3.1 Emotional lateralisation. It is interested in and detects visual novelty and changes in a previously experienced stimulus, manifesting broad attention used in global processing (Rogers, 2008; Rogers & Anson, 1979). Finally, it seems to be the dominant hemisphere in the response to both acute and chronic stress (Rogers, 2010).

1.3.1 Emotional lateralisation

Functional lateralisation in non-human animals' brains has also been studied in relation to the animal's emotional state. In particular, two major hypotheses regarding lateralised emotional processing have been proposed: the 'right-hemisphere hypothesis' suggests a dominance of the right hemisphere in all emotional processing; and the 'emotional-valence hypothesis' posits a right-hemisphere dominance in the processing of negative emotions, such as fear and aggression, and a left-hemisphere dominance in the processing of positive emotions, such as those associated with food rewards (Leliveld et al., 2013). No definitive support for either of the two hypotheses is provided. Nevertheless, in their review Leliveld et al. (2013) propose that the 'emotional-valence hypothesis' may be the best one to explain findings from the literature, as the general pattern that emerges is that right-hemisphere dominance is associated with processing of emotions of negative valence and left-hemisphere dominance with processing of emotions of positive valence. Therefore, accepting the 'emotional-valence hypothesis', both hemispheres are involved in emotional processing and hemispheric dominance may be used as an indicator of emotional valence, also in cognitive bias tasks.

1.4 The present study

Different variants of the judgement bias task have already been used to assess welfare in *Gallus gallus domesticus*. Salmeto et al. (2011), for example, have experimentally demonstrated that chicks in an induced anxiety-like state exhibit more pessimistic behaviour. Their paradigm involved the presentation of ecologically-relevant stimuli (i.e., silhouettes of a conspecific chick, of an owl, and three intermediate ambiguous cues morphing chick and owl characteristics to varying degrees) and the measurement of chicks' approach/avoidance responses.

The aim of our experimental protocol was to assess the presence of a pessimistic or optimistic judgement bias in domestic chicks using a paradigm based on the spontaneous judgement of an ambiguous stimulus. In order to induce the bias, we used a negative or a positive emotional treatment, designed to alter the affective state of the animal and, therefore, their judgement.

The environmental manipulations that we used included listening to recordings of conspecifics either producing distress calls (negative treatment) or tweeting (positive treatment). As outlined in greater details in 1.3 *Gallus gallus domesticus* as model species, chicks can hear and distinguish different vocalizations and each of these is associated with a specific affective state. Furthermore, for our treatment to work it was essential that our subjects could discriminate emotional arousal and valence from the recordings used and that, as already mentioned in 1.3 *Gallus gallus domesticus* as model species, they showed emotional contagion. The effect of recordings of conspecifics' vocalisations on chicks in a novel environment was tested by Vallortigara (1988), who found that in 2-day-old chicks conspecific calls, regardless of the emotional valence, seem to have no specific effect in ambulation latency and that in 7-day-old chicks the effects of conspecific calls are the same regardless of the specific type of vocalisation. The literature on the topic is, however, controversial, as others have proved that alarm calls can increase tonic immobility in hens (Jones, 1986) and chicks (Thompson & Liebreich, 1987) as compared to other types of vocalisations, suggesting that recordings of conspecifics may have an effect.

Light intensity manipulation was also used to induce changes in the affective state of chicks, which underwent the emotional treatment either in the dark (negative treatment) or in the same bright light they were tested in for the rest of the experiment (positive treatment). It has been proposed that for nocturnal animals, such as rats, bright light may be a danger signal, evoking various fear behaviours (Godsil & Fanselow, 2004), while sudden darkness may diminish fear and anxiety (Nasello et al., 1998). The effects of abrupt changes in light intensity on diurnal species have not been as extensively investigated yet. A reduction in the light exposure period in egg-laying hens is associated with behavioural and physiological changes that are attributable to an experienced state of stress (Khalil et al., 2010). We can, therefore, suppose that diurnal species are also affected by light intensity manipulation, similarly to nocturnal ones.

Finally, the emotional treatment also involved the presence (positive treatment) or absence (negative treatment) of the social object chicks had imprinted on. Chicks in a stressful or fear inducing situation without the imprinted object, as our negative emotional treatment is, may freeze, squeal, produce distress calls or jump at the walls (Salzen, 1967). This suggests a negative effect of the situation on their emotional state that is not alleviated by the presence of the familiar object. On the other hand, when the imprinted object is restored, chicks not only show a desire to be reunited with it with a strong approach behaviour, but also produce pleasure instead of distress calls, indicating that the familiar object may be used to ease fear and that its presence is pleasurable (Berns & Bell, 1979; Salzen, 1967). Behavioural responses in the same direction were expected in the present study after, respectively, the negative and the positive emotional treatment. No reunion with the imprinted object after the negative emotional treatment was planned.

After having induced a positive or negative emotional state in chicks, the approach behaviour towards an ambiguous stimulus was measured, following the typical judgement bias task paradigm. The ambiguous stimulus fell between a positive one, associated with a food with high affordability, and a negative one, associated with a food with lower affordability.

Our experimental design, besides inducing and measuring a judgement bias in chicks, also aimed at uncovering the brain's lateralised activation depending on the emotional state of the animal. Indeed, as mentioned in 1.3.1 Emotional lateralisation, lateralised emotional processing can be observed in the *Gallus gallus domesticus*. The chick's choices in approaching the same stimulus (positive, negative, or ambiguous) presented simultaneously on the right and on the left on its visual field were considered to investigate how different emotional states are associated with dominant activation in one hemisphere or the other. Observing the lateralised behaviour of the animal when presented with the ambiguous stimulus may serve as an accurate indicator of whether the chick categorises it as closer to a positive or a negative stimulus and, therefore, of the chick's affective state.

1.4.1 Hypotheses

For the stimuli presented before the emotional treatment, we hypothesized that left hemisphere activation would occur with the presentation of the positively conditioned stimulus, as it is associated with positive emotions and food reward. Therefore, we expected the chick to choose the stimulus presented on the right. The opposite pattern of behaviour (i.e., choice of the stimulus on the left due to right hemisphere activation) was expected for the negatively conditioned stimulus due to its association with negative emotions.

We further hypothesized that different patterns of lateralisation and, therefore, approach behaviour could be observed after the emotional treatment based on its emotional valence. Right hemisphere dominance and a left choice were expected after the negative emotional treatment, as it is the hemisphere associated with both novelty and negative emotions. In contrast, the positive emotional treatment was expected to produce left hemisphere dominance and a right choice.

2. Methods

2.1 Subjects and rearing conditions

During the time of the experiment, we tested 92 domestic chicks (*Gallus gallus domesticus*), 46 randomly assigned to the positive emotional treatment condition (23M and 23F) and 46 to the negative emotional treatment condition (23M and 23F). When tested, the chicks were 3 days old. Before hatching, the eggs were incubated at a controlled temperature of 37-38°C and a humidity of 60%. After hatching, newborn chicks were sexed, given an identificative name code and weighted. Every following day, the subjects were weighted to ensure they were properly gaining weight, as the contrary is considered a sign of possible health problems in the animal. In the days preceding testing, all subjects were housed individually in the rearing room in standard metal cages (28cm x 32cm wide x 40cm high), with controlled conditions of temperature (between 28 and 31°C), humidity (68%), and lighting (with lights on from 7 a.m. to 7 p.m., and alternating light/dark cycles every 2 to 3 hours during the night) and a glass of water.

Before the test day (i.e., in the chicks' first two days of life), a dish of high-affordability food (large grains) was also placed behind a panel with either a black or white stimulus to form a positive association between the stimulus and food. In contrast, a dish containing food with lower affordability (small grains) was placed behind the stimulus of the other colour to form a negative association. As described in greater details in 2.2 Apparatus and data collection, stimuli consisted of rectangles of different shades of achromatic grey. The placement of the food dishes was changed every four hours during daylight hours. In this way, both stimuli assumed either a high value (stimulus associated with the high-affordability food) or a low value (stimulus associated with the low-affordability food) for the animal. The colour of the high value stimulus was randomly chosen for each chick. The presence of the high-affordability food was shown to the animal by moving a larva in the cage in such a way to attract the chick close to the dish. Consequently, not only big grains of food, but also larvae were associated with the high value stimulus.

Two objects were constantly present in the cage, a yellow square (2.5 x 2.5cm) and a red cylinder (4.5cm high x 3cm diameter), so that the animal could imprint on them. Indeed, we then made use of the imprinted objects during the positive emotional treatment (since its presence is perceived as pleasurable, as illustrated in 1.4 The present study) and during other phases of the experimental procedure, as explained in 2.3.2 Familiarisation with the arena and

2.3.3 Trainings. After the experiment was concluded, chicks were randomly paired and kept in the same mental cages together, providing them with water and food *ab libitum*, until they were donated to licensed breeders – that is, usually one or two days after being tested and, in any case, by their second week of life.

2.2 Apparatus and data collection

The testing area consisted of an arena made of green plastic boards and shaped as a triangle (oblique side: 62 cm, base: 68 cm) with a rectangular base (68 cm x 14.5 cm). The walls were ~25 cm high (Fig. 1). The triangle and the rectangle areas were clearly distinguished through the presence of a small step between them. The vertex of the triangle was the starting point for the subject during the experimental procedure. In some phases of the experiment, a clear plexiglass wall was placed at 16 cm from the vertex in front of the chick, that had to peck it for it to be removed, as described in greater details in 2.3.2 Familiarisation with the arena.

The stimuli (rectangles of 6.6 cm x 11 cm of different shades of achromatic grey (RGB 231, 231, 231; RGB 25, 25, 25; RGB 128, 128, 128), from now on referred to respectively as 'white', 'black', and 'grey') were placed on the wall opposite to the vertex, so that the chick could see them when placed into the arena (Fig. 1). Only one stimulus was attached to the wall during familiarisation and trainings, while two identical stimuli were presented during pre- and post-treatment tests. The stimulus was considered to be approached by the subject when this entered the rectangular part of the arena, as explained in 2.5.2 Pre- and post-treatment test analysis. When two stimuli were presented (that is, during the pre- and post-treatment tests), an opaque green plastic panel was placed exactly in the middle of the wall in order to divide the choice area into a left and a right part, as shown in the example of Figure 1. This panel stretched out towards the inside of the arena for 14.5 cm. When in the left choice area, the chick could not see the right stimulus, and *vice versa*.

Figure 1

A picture of the experimental arena

Note. The picture shows the position of the clear plexiglass wall, in front of the triangle vertex, i.e., the starting point for the chick during the experimental procedure, and of the small step distinguishing the triangular and rectangular areas. In this case, two white stimuli are presented to the subject on the wall opposite to the vertex and are separated by a green vertical panel.

A video camera (Sony HDR-CX405) was positioned above the arena, in a way that would not create any visible shadow for the chick. Video recordings were made to collect data, with a setting of 25 frames per second.

2.3 Pre-testing experimental procedures

2.3.1 Food deprivation and motivation test

In order to increase the chicks' motivation during the experimental procedure, for at least 2 hours the usually-provided large grains of food were substituted with smaller grains of food, that are less appetising for chicks. In this way, we ensured that they were not completely food deprived and still had constant access to food, but, at the same time, we increased their motivation to participate in the experimental procedure, as they were rewarded with food with a higher affordability and palatability during it.

After 2 hours, a test was performed to assess the chick's motivation before starting the actual experiment. The subject was placed in a metal cage with a white floor, similar to the ones in which they were housed, with two petri dishes, one containing larvae (food later also used as reinforcement during trainings, as explained in 2.3.3 Trainings) and the other containing grains (food previously used to create a positive association with the high value stimulus, as explained in 2.1 Subjects and rearing conditions). The animal was let free to move in the cage and peck at the dishes for two minutes. After this time, the position of the two dishes was switched and the chick had another two minutes in the cage to freely peck at the food dishes. All subjects were later tested and the test was used to assess the chick's general motivation towards food by measuring how much time they spent pecking at the dishes. Using these data, we calculated their motivation score, as defined in 2.5.1 Motivation test analysis. As explained in greater details in 2.5.3 Identification and exclusion of outliers, motivation scores were then used to identify under- and over-motivated subjects.

2.3.2 Familiarisation with the arena

A familiarisation procedure with the arena was included at the beginning, so that any reaction caused by the novelty of the environment could be observed at this point and not later. In the first familiarisation trial, the chick was placed at 16 cm from the vertex, facing the opposite wall of the arena, on which the high value stimulus (black or white) and the yellow imprinting square were attached exactly in the middle, with a larva placed in front of them (Fig. 2A). The animal was let free to explore the arena for 2 minutes and was only removed after the time was over, even if it had already approached the stimulus and eaten the larva. If the subject had not done so after 2 minutes, it was helped by the experimenter who moved the yellow imprinting object in the arena in such a way to attract the chick to the stimulus. It was then removed from the arena after having eaten the larva.

All subsequent trails did not include the presence of the yellow imprinting square attached to the wall and of a larva already placed in the arena, and lasted a maximum of 30 seconds or until the chick had approached the stimulus, in which case it was removed from the arena before the time ended. The trials were considered successful if the subject approached the stimulus (regardless of its positive or negative valence) on its own, while the need for the chick to be helped by the experimenter (by moving the imprinting object in the environment) was considered as indication of a failed trial. When the stimulus presented was the high value one, the chick was rewarded with a larva before being removed, regardless of the success or failure of the trial. In this case, the chick was always rewarded, even in case of failure, because we wanted the animal to maintain the positive association between the stimulus and the reward. On the other end, when the negative stimulus was presented, no reward was ever given, regardless of the success or failure of the trial. Contrary to what said before, in this case the chick was never rewarded because we did not want it to form a positive association between the negative stimulus and the larvae.

In the second and third trials, similarly to the first one, the chick was placed at 16cm from the vertex (Fig. 2B). In the following three trials, it was placed right at the vertex of the arena, always facing the opposite wall (Fig. 2C). After these first six trials, a clear plexiglass wall was introduced in the arena at 16cm from the vertex and the chick was placed at the vertex, facing the clear wall (Fig. 2D). For the clear wall to be removed and for the animal to be able to approach the stimulus, the subject had first to peck it.

Figure 2

The familiarisation procedure

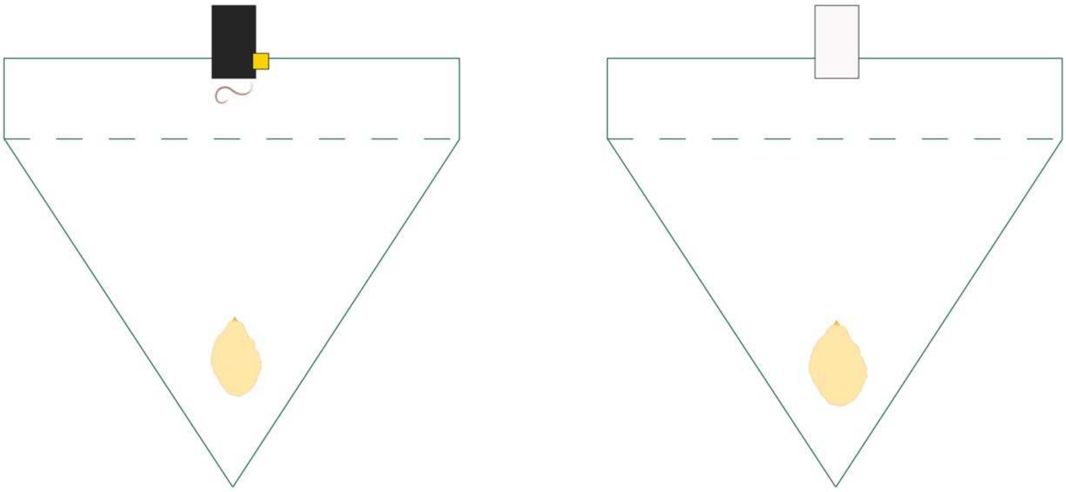


Fig. 2A In the first trial of the familiarisation, the chick was placed 16cm from the vertex and a larva and a social stimulus were present in the arena. The high value stimulus was attached to the wall opposite the vertex. The chick had 2 minutes to explore the arena, approach the stimulus and eat the larva.

Fig. 2B In the second and third trails of the familiarisation, the chick was placed 16cm from the vertex of the arena. The stimulus was attached to the wall opposite the vertex. The chick had 30 seconds to explore the arena and approach the stimulus.

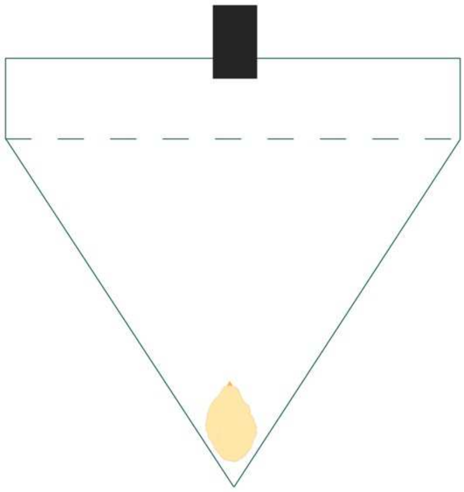


Fig. 2C In the fourth, fifth, and sixth trials of the familiarisation, the chick was placed at the vertex of the arena. The stimulus was attached to the wall opposite the vertex. The chick had 30 seconds to explore the arena and approach the stimulus.

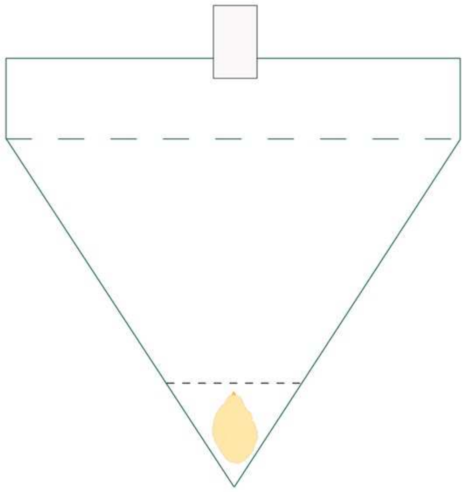


Fig. 2D In the seventh and in all subsequent trials, the chick was placed at the vertex and a clear plexiglass wall was introduced in the arena 16cm from the vertex. The clear plexiglass wall is here represented by a dashed line in front of the chick. The stimulus was attached to the wall opposite the vertex. The chick had 30 seconds to explore the arena and approach the stimulus.

Note. The colours of the stimuli (black or white) have been randomly chosen in these figures for graphic purposes. In the experiment, during each trial the colour of the stimulus presented depended on which colour had been associated with the high value reward for each chick.

The familiarisation included no more than ten trials after the introduction of the clear wall and was considered completed when the chick performed at least three consecutive successful trials – which meant that only eight failed trials with the clear wall were sufficient to consider the familiarisation failed for some chicks. The number of presentations of the positive and negative stimuli were carefully counterbalanced, so that each was shown the same number of times. When a failing animal showed clear signs of distress in the form of, e.g., prolonged freezing and distress calls, it was excluded from the study. On the other hand, when no signs of distress were present, the failing chick underwent familiarisation again and with at least 30 minutes of rest between each familiarisation procedure. The familiarisation was repeated for a maximum of three times (but one or two were already enough if successful), after which, in case of repeated failure, the chick was excluded and no longer tested. 25 subjects failed to complete familiarisation and were excluded.

After completing the familiarisation successfully, the subject was carefully brought back to its cage, where it stayed for at least 30 minutes to rest.

2.3.3 Trainings

Before starting the pre-treatment test, a training phase was included to ensure that the chick remembered what it had learned during the familiarisation – that is, to peck the clear wall for it to be removed and to quickly approach the stimulus presented on the wall opposite to the vertex. During the training trials, the vertex of the arena was the starting point for the chick, placed to face the clear wall. A trial was considered successful when the chick pecked the clear wall, which was consequently removed, and then approached the stimulus on its own in a maximum time of 30 seconds. Failing to do so resulted in a failed trial and, in this case, the chick was guided to the stimulus by showing it the yellow imprinting square. A larva was given when the high value stimulus was presented, while no reward was given when the low value stimulus was presented.

A training included no more than ten trials and was considered completed when the chick performed three consecutive successful trials. The number of presentations of the positive and negative stimuli were carefully counterbalanced, so that each was shown the same number of times. Therefore, the minimum possible number of trials was four – that is, three consecutive successful trials and a fourth (successful or failed) one, with two presentations of the positive stimulus and two of the negative one.

A training was performed not only before the pre-treatment test, but also during it, as explained in 2.4.1 Pre-treatment test, and before the emotional treatment, as explained in 2.4.2 Emotional treatment. At any point during the experimental procedure, when the subject failed to complete a training, it was excluded from the experiment and no longer tested.

2.4 Test

2.4.1 Pre-treatment tests

After the first training described in 2.3.3 Trainings, the first pre-treatment test began. The chick was put into the arena, positioned at the vertex and facing the clear wall, that it had to peck for it to be removed. On the wall opposite to the vertex, two identical stimuli of the same colour (white or black) were attached, divided by a green opaque panel placed vertically, exactly in the middle of the wall and extending towards the inside of the arena for 14.5 cm (Fig. 3A, Fig.

3B). Four minutes were timed since the removal of the clear plexiglass wall, during which the chick was free to explore the arena and approach the stimuli.

Figure 3

The pre-treatment tests

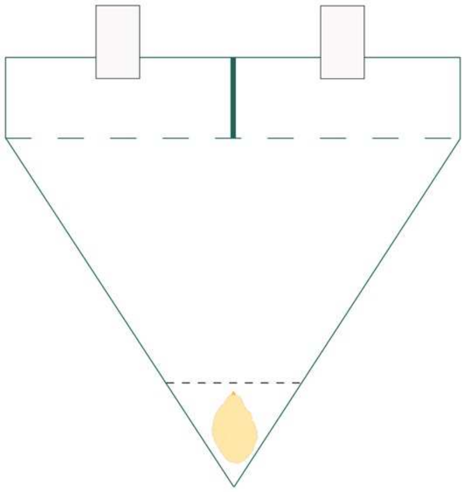


Fig. 3A The pre-treatment apparatus when two white stimuli were presented.

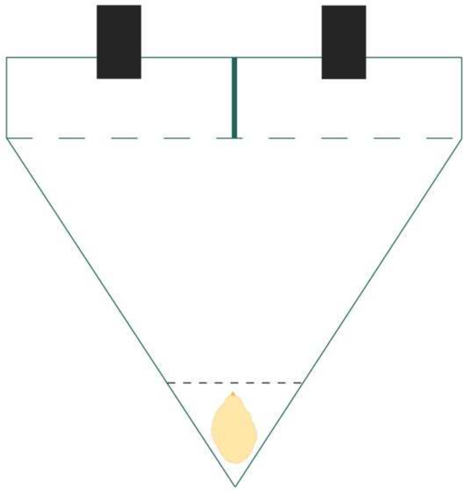


Fig. 3B The pre-treatment apparatus when two black stimuli were presented.

Note. During the pre-treatment tests, two identical stimuli (either white (Fig. 3A) or black (Fig. 3B)) were attached to the wall opposite to the chick. The stimuli were divided by a green opaque panel stretching towards the inside of the arena for 14.5 cm. This panel is here represented with a continuous vertical between the two stimuli. The chick was placed at the vertex facing the stimuli and a clear plexiglass wall was placed in front of the chick, 16 cm from the vertex.

At the end of the 4 minutes, the chick was removed from the arena, which was prepared for a second training, performed right after. If the training, identical to the first one described in 2.3.3 Trainings, was completed, the chick underwent the second pre-treatment test. It was the same as the first one, the only difference being the stimuli presented: if two white stimuli had been presented in the first test, two black ones were presented in the second and *vice versa*. We made sure that the same number of subjects was firstly presented with the stimulus of positive valence as those firstly presented with the stimulus of negative valence.

The chick was subsequently brought back to its metal cage to rest for at least 30 minutes.

2.4.2 Emotional treatment

After the ~30 minutes of rest, a third training, identical to the previous ones described in 2.3.3 Trainings, was performed. If it was completed, the subject underwent the positive or negative emotional treatment. The number of chicks in the positive treatment condition and in the negative treatment condition was counterbalanced. Both kinds of treatments occurred with the chick placed in a white plastic container (the same one used to carry the animal during the experiment and in which it was kept while the arena was manually adjusted and modified for the different parts of the experimental procedure) with a green floor.

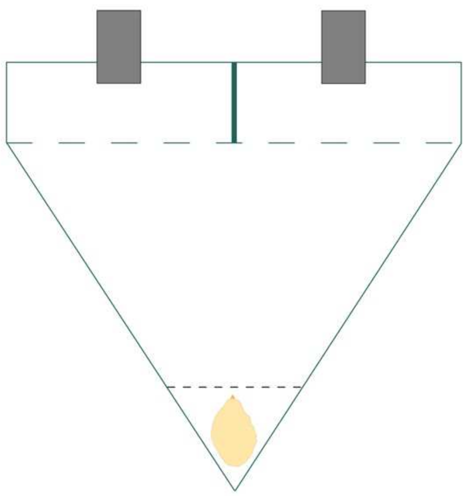
As already described in 1.4 The present study, in the case of the positive emotional treatment, the light in the room was kept on and the red cylinder (i.e., one of the social objects the chick had imprinted on) was added in the container, while the chick listened to a recording of other chicks tweeting. On the other hand, the negative emotional treatment, which occurred in the dark, involved a recording of distress calls produced by other unfamiliar chicks, with no imprinting object present in the container. Both audio tracks lasted 5 minutes and consisted of 20-second-long recordings looped repeatedly. Three chicks – unfamiliar to the experimental subjects – were recorded at the University of Padova either producing distress calls or tweeting. The audio was played from a Galaxy Tab S6 Lite at a volume of approximately 75 dB.

2.4.3 Post-treatment test

Immediately after the emotional treatment, the chick was put back at the vertex of the arena, facing the clear wall to peck and two ambiguous grey stimuli attached on the opposite wall and separated by a green opaque panel (Fig. 4). Similarly to the pre-treatment test, once the clear wall was removed the chick had 4 minutes to freely explore the arena and approach the stimuli. At the end of the 4 minutes, the chick was removed from the arena.

Figure 4

The post-treatment test



Note. During the post-treatment test, two identical grey stimuli were attached to the wall opposite to the chick. The stimuli were divided by a green opaque panel stretching towards the inside of the arena for 14.5 cm. This panel is here represented with a vertical line between the two stimuli. The chick was placed at the vertex facing the stimuli and a clear plexiglass wall was placed in front of the chick, 16 cm from the vertex.

A summary of the entire experimental procedure is presented in Table 1.

Table 1

Summary of the experimental procedure

	Description	Duration/Number of trials
1. Food deprivation	Large grains of food were substituted with smaller grains.	At least 2 hours.
2. Motivation test	The chick was let free in a cage with a dish containing larvae and one containing grains of food. Its pecking behaviour was registered.	2 minutes + 2 minutes. (The position of the dishes was switched in the second part).
3. Familiarisation with the arena	The chick was let free to explore the arena and approach the stimulus. Its behaviour was registered. In the first three trials, the starting position of the chick was 16 cm from the vertex. In all other trials it was the vertex. Starting from the seventh trial, a clear plexiglass wall for the chick to peck was introduced at 16 cm from the vertex.	2 minutes for the first trial and 30 seconds for all others. Maximum 10 trials after the introduction of the plexiglass wall (i.e., 16 trials in total); minimum 3 consecutive successful trials. The whole procedure could be repeated for maximum 3 times. The number of presentations of the stimuli was counterbalanced.
4. First training	The chick was placed at the vertex of the arena and let free to explore it and approach the stimulus on the opposite wall. A plexiglass wall to peck was present. A reward was given when the stimulus presented was the high value one.	30 seconds for all trials. Maximum 10 trials; 3 consecutive successful trials needed to consider the training completed. The number of presentations of the stimuli was counterbalanced.

5. First pre-treatment test	The chick was placed at the vertex of the arena and let free to explore it. A plexiglass wall to peck was present. Two identical familiar stimuli (either the high or the low value one) were attached on the end wall, separated by a panel. The approach behaviour of the subject was recorded.	4 minutes
6. Second training	The chick was placed at the vertex of the arena and let free to explore it and approach the stimulus on the opposite wall. A plexiglass to peck wall was present. A reward was given when the stimulus presented was the high value one.	30 seconds for all trials. Maximum 10 trials; 3 consecutive successful trials needed to consider the training completed. The number of presentations of the stimuli was counterbalanced.
7. Second pre-treatment test	The chick was placed at the vertex of the arena and let free to explore it. A plexiglass wall to peck was present. Two identical familiar stimuli (either the high or the low value one, different from the one presented in the first test) were attached on the end wall, separated by a panel. The approach behaviour of the subject was recorded.	4 minutes
8. Third training	The chick was placed at the vertex of the arena and let free to explore it and approach the stimulus on the opposite wall. A plexiglass to peck wall was present. A reward was given when the stimulus presented was the high value one.	30 seconds for all trials. Maximum 10 trials; 3 consecutive successful trials needed to consider the training completed. The number of presentations of the stimuli was counterbalanced.
9. Emotional treatment	It was used to induce a change in the affective state of the chick. - Positive treatment: a recording of unfamiliar conspecifics tweeting was played, the light was kept on, an imprinting object was present in the cage.	5 minutes

	- Negative treatment: a recording of unfamiliar conspecifics producing stress calls was played, the light was turned off, no imprinting object was present.	
10. Post-treatment test	The chick was placed at the vertex of the arena and let free to explore it. A plexiglass wall to peck was present. Two identical ambiguous stimuli were attached on the end wall, divided by a panel. The approach behaviour of the subject was recorded.	4 minutes

2.5 Data analysis

As already mentioned in 2.2 Apparatus and data collection, video recordings of the tests were made for each subject. The videos were then analysed using the free program Boris (v. 8.25; Friard & Gamba, 2016) to extrapolate data from them. Furthermore, the video analysis was conducted in a blind condition, with scorers not knowing in which condition (positive or negative emotional treatment) the chick was, and which stimulus colour (black or white) was the high value one for that subject. For the sake of this study, we considered and analysed the video recordings of the motivation test and of the pre-treatment and post-treatment tests.

2.5.1 Motivation test analysis

For what concerns the analysis of the motivation test videos, we used Boris (Friard & Gamba, 2016) to score for each subject the choice time (sec), i.e., the total time they spent choosing, regardless of their choice. Then, we calculated their motivation score, i.e., the arithmetic average of the ratios between the choice time and 120 sec in the first and in the second part of the motivation test – such that, a score of 0 would mean that they never chose and a score of 1 that they always chose.

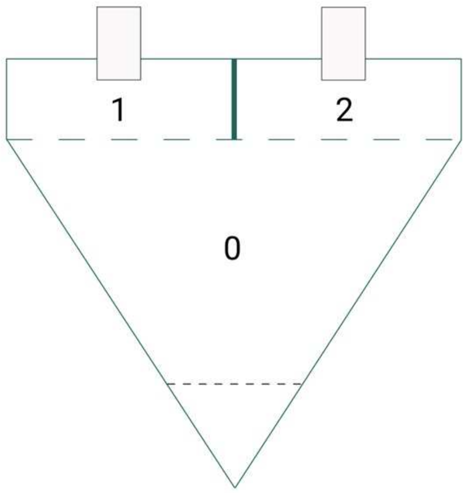
2.5.2 Pre- and post-treatment test analysis

For what concerns the analysis of the pre-treatment and post-treatment test videos, Boris (Friard & Gamba, 2016) allowed us to register the timing of all relevant events, including when the chick was inserted into the arena, when it pecked the clear wall, when the clear wall was removed and the chick could freely move in the arena, every time it made a choice (or no choice), and when it was removed from the arena.

As illustrated in Figure 5, three different areas of the arena were indeed distinguished: a no-choice area in which the chick could see both stimuli without approaching a specific one (zone 0), a left-choice area (zone 1), and a right-choice area (zone 2). A choice, either left or right, was considered to be made when the chick entered either zone 1 or zone 2.

Figure 5

No-choice, left-choice, and right-choice area distinction



Note. Zone 0 represents the no-choice area and includes the area from the vertex to the little step. Zone 1 represents the left-choice area. Zone 2 represents the right-choice area. Zones 1 and 2 are clearly separated by a green opaque panel, here represented by a continuous vertical line between the two stimuli. The white colour of the stimuli was here randomly chosen for representative purposes, but the same distinction between the different zones was also made when the stimuli presented were black or grey.

As soon as the clear wall was removed, the subject was considered to be in the no-choice area and the choice behaviour in the following 4 minutes was analysed. For each subject we scored (i) their first choice (binomial variable, left or right), (ii) the exit latency (sec), i.e., the time it took them to peck the clear wall after being inserted into the arena, and (iii) the first-choice latency (sec), i.e., the time it took them to make their first choice after the wall was removed.

Furthermore, by keeping track of when each choice was made, Boris (Friard & Gamba, 2016) measured (i) the no-choice time (sec), i.e., the time spent in Zone 0, (ii) the left-choice time (sec), i.e., the time spent in Zone 1, and (iii) the right-choice time (sec), i.e., the time spent in Zone 2. By summing up left- and right-choice times, we obtained (iv) the overall choice time (sec), i.e., the time spent in either Zone 1 or 2.

Finally, for each chick, we calculated:

- their choice score in the pre-treatment and in the post-treatment tests, i.e., the ratio between the choice time (sec) and the sum of choice and no-choice times (sec) – such that, a score of 0 would mean that they never chose and a score of 1 that they always chose, and
- their side score in the pre-treatment in the post-treatment tests, i.e., the ratio between the right-choice time (sec) and the choice time (sec) – such that, a score of 0 would mean that they always made a left choice and a score of 1 that they always made a right choice. Assigning 0 to a consistent left choice and 1 to a consistent right choice was an arbitrary decision.

All the data collected and indexes calculated, along with the codes to recognise the condition and the stimuli colour, and additional notes, were organized in an excel spreadsheet, to be further analysed with JASP (Version 0.18.1; JASP Team, 2023).

2.5.3 Identification and exclusion of outliers

Before conducting any statistical analysis, we decided to identify the over- and under-motivated subjects and consider them outliers to be excluded. In order to do so, we firstly analysed the motivation scores calculated from the video analysis of the motivation tests. Since we found a highly significant difference between the motivation scores of the first and second motivation test ($F (1, 100) = 60.535$, $p < .01$) but no effect of the position of the two different petri dishes ($F (1, 100) = 0.019$, $p = 0.892$), we decided to further consider only the motivation scores of the first test. The average score was 0.469 ($SD = 0.276$), with a minimum of 0.007 and a maximum of 0.960. Due to the non-normal distribution of data, to identify the outliers we adopted an approach that makes use of the median absolute deviation (*MAD*) – that is, we considered outliers those outside of the range $Median \pm c \cdot MAD$. We assigned a value of 2 to c, adopting a poorly conservative approach as suggested by Leys et al. (2013). This led us to exclude 9 subjects with a score smaller than 0.088, while no subject had a score higher than 0.968. A motivation score outside the $Median \pm c \cdot MAD$ range was the only criterion used to identify outliers, as all other subjects were considered in the following analyses, including those that did not complete the experiment. If, for instance, a chick completed the familiarisation, the first training, and the first pre-treatment test, but failed to complete the second training and, therefore, did not complete the experiment, the data obtained from the pre-treatment test were still considered.

2.5.4 Inferential statistics

To check the statistical significance of our results, we conducted various analyses, including between-subjects ANOVAs (ANOVA on JASP) and between- and within-subjects mixed ANOVAs (Repeated Measures ANOVA on JASP). In each of them we considered different tests, including the pre-treatment test in which the high-value stimulus was presented, the pre-treatment test in which the low-value stimulus was presented, the post-treatment test after undergoing the positive emotional treatment, and the post-treatment test after undergoing the negative emotional treatment. In the between-subjects ANOVAs, the variables considered were: (i) the chick's sex (M or F), (ii) the positively reinforced colour (black or white), and (iii) the order of presentation of high- and low-value stimuli in the pre-treatment test (H-L or L-H). Furthermore, when taking the post-treatment test into consideration in the analyses, split analyses were conducted for chicks in the positive treatment condition and chicks in the negative treatment one.

3. Results

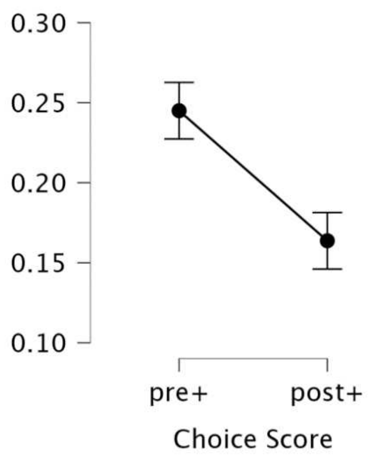
3.1 Mixed between-subjects ANOVA between pre- and post-treatment tests

To understand whether the emotional treatments (both the positive and the negative one) produced the expected judgement bias, for all the indices we conducted a between-subject mixed ANOVA comparing each pre-treatment test to the post-treatment one, distinguishing between treatments. The term 'pre+' will be used hereafter to refer to the pre-treatment test during which the high-value stimulus was presented, while 'pre-' to refer to the pre-treatment test during which the low-value stimulus was presented. Similarly, 'post+' will refer to the post-treatment test after the positive emotional treatment and 'post-' to the test after the negative treatment. The between-subjects variables considered were those previously described in 2.5.4 Inferential statistics.

3.1.1 Choice score

Concerning the choice score, when comparing the pre+ to the post+, a highly significant difference was observed (F (1, 39) = 10.593, $p < .01$). Chicks chose significantly more during the pre+ (M = 0.245, SD = 0.140) than during the post+ (M = 0.164, SD = 0.137), as Graph 1 illustrates.

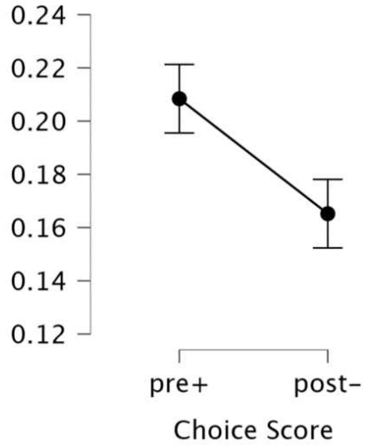
Graph 1



Note. Graphical representation of choice score (*y* axis) in the pre-treatment test with the presentation of the high-value stimulus (*x* axis, *pre+*) and in the post-treatment test after the positive emotional treatment (*x* axis, *post+*). Bars represent standard errors.

As shown in Graph 2, similar significant effect was found when comparing the pre+ to the post- (F (1, 43) = 5.620, $p < .05$), with chicks choosing more in the pre+ (M = 0.208, SD = 0.107) than in the post- (M = 0.165, SD = 0.138).

Graph 2



Note. Graphical representation of choice score (*y* axis) in the pre-treatment test with the presentation of the high-value stimulus (*x* axis, *pre+*) and in the post-treatment test after the negative emotional treatment (*x* axis, *post-*). Bars represent standard errors.

A highly significant effect of the variable 'Order' on choice score was found when considering the pre- and the post+ (F (1, 39) = 8.934, *p* = .005). Notably, the choice score was lower both in the pre- and post+ for chicks that were presented firstly with the high-value stimulus and secondly with the low-value stimulus in the pre-treatment tests, as compared to those to which stimuli were presented in the opposite order (Table 2, Graph 3).

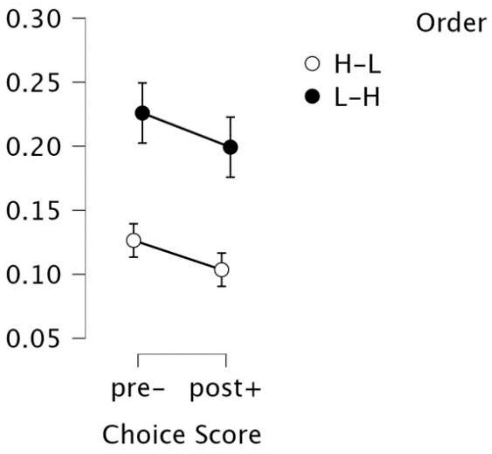
Table 2

Descriptives – Choice Score

Choice Score	Order	N	Mean	SD	SE	Coefficient of variation
pre-	H-L	41	0.176	0.121	0.019	0.690
	L-H	45	0.222	0.144	0.021	0.650
post+	H-L	15	0.113	0.086	0.022	0.759
	L-H	27	0.199	0.151	0.029	0.756

Note. Concerning the variable *Order*, *H-L* is used to indicate the presentation of the high-value stimulus (*H*) first and of the low-value stimulus (*L*) later, while *L-H* is used to indicate the opposite order.

Graph 3



Note. Graphical representation of choice score (*y* axis) in the pre-treatment test with the presentation of the low-value stimulus (*x* axis, *pre-*) and in the post-treatment test after the positive emotional treatment (*x* axis, *post+*). Separate lines indicate the two different possible orders of stimuli presentation, with *H-L* representing chicks that have lastly seen the low-value stimulus (*L*) and *L-H* representing chicks who have lastly seen the high-value stimulus (*H*). Bars represent standard errors.

When comparing the pre- and the post-, no significant effect with *p* < .05 was found for any variable.

3.1.2 Side score

Concerning the side score, no significant effect with p < .05 was found in any comparison. Table 3 reports the descriptive statistics of the side score in the different tests.

Table 3

Descriptive Statistics – Side Score

	Pre +	**Pre -**	**Post**	
			Neg	**Pos**
Valid	86	86	44	42
Mean	0.470	0.436	0.460	0.470
Std. Deviation	0.243	0.270	0.327	0.322
Minimum	0.000	0.000	0.000	0.000
Maximum	1.000	0.940	1.000	1.000

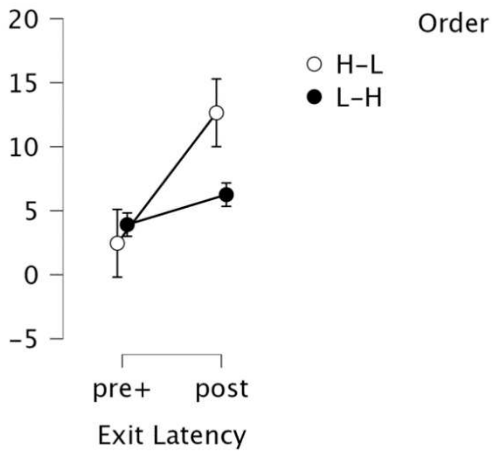
Note. Concerning the column '*Post'*, descriptive statistics are reported separately based on the emotional treatment valence, with *Neg* indicating chicks who underwent the negative emotional treatment and *Pos* indicating chicks who underwent the positive emotional treatment.

3.1.3 Exit latency

Concerning the exit latency, a significant effect with *p* < .05 was found in all comparisons: F (1, 39) = 5.162, *p* = .029 for pre+ with post+; F (1, 43) = 6.131, *p* = .017 for pre+ and post-; F

(1, 40) = 4.296, *p* = .045 for pre- with post+; and F (1, 43) = 7.102, *p* = .011 for pre- with post-. When compared to the pre-treatment tests, exit latencies were always significantly longer in the post-treatment tests. Furthermore, the ANOVA demonstrated a significant effect of the variable 'Order' on exit latency when comparing the pre+ (but not the pre-) to the post-treatment test regardless of its valence – that is, regardless of whether the emotional treatment was positive or negative (F (1, 82) = 4.090, *p* = .046). Notably, as shown in Graph 4, the biggest difference in exit latencies could be observed in the post-treatment test, with chicks who had lastly been presented with the low-value stimulus in the pre-treatment tests (H-L) taking longer to exit (*M* = 12.650, *SD* = 23.932) than those which had lastly seen the high-value stimulus (L-H, *M* = 6.257, *SD* = 8.783).

Graph 4

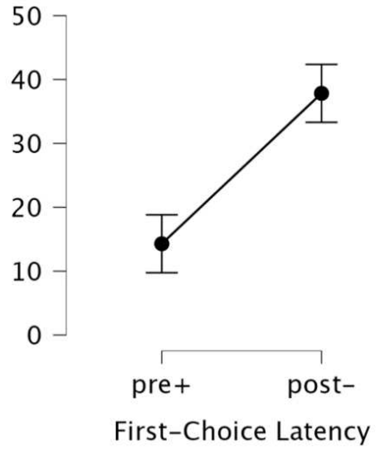


Note. Graphical representation of exit latency (*y* axis) in the pre-treatment test with the presentation of the high-value stimulus (*x* axis, *pre+*) and in the post-treatment test regardless of the emotional treatment valence (*x* axis, *post*). Separate lines indicate the two different possible orders of stimuli presentation, with *H-L* used to indicate the presentation of the high-value stimulus (*H*) first and of the low-value (*L*) later and *L-H* used to indicate the opposite order. Bars represent standard errors.

<u>3.1.4 First-choice latency</u>

Concerning the first-choice latency, the comparison between pre+ and post+ revealed no significant difference between the two tests (F (1, 41) = 2.917, *p* = .095), despite, on average, the latency was bigger in the post+ (*M* = 20.891, *SD* = 38.037) than in the pre+ (*M* = 10.073, *SD* = 17.978). The same tendency was also observed in the comparison between pre- and post- (F (1, 43) = 3.951, *p* = .053), with an average latency of 18.354 (*SD* = 31.264) in the former and 37.833 (*SD* = 56.735) in the latter. The mixed ANOVA of first-choice latencies in the pre+ and post- was, on the other hand, highly significant (F (1, 43) = 13.491, *p* < .001), as times were consistently shorter in the pre+ (*M* = 14.293, *SD* = 38.008) than in the post- (*M* = 37.833, *SD* = 56.735) (Graph 5).

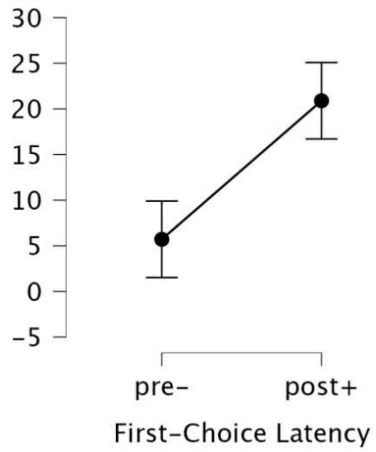
Graph 5



Note. Graphical representation of first-choice latency (*y* axis) in the pre-treatment test with the presentation of the high-value stimulus (*x* axis, *pre+*) and in the post-treatment test after the negative emotional treatment (*x* axis, *post-*). Bars represent standard errors.

Similarly, as shown in Graph 6, the comparison between pre- and post+ was significant (F (1, 41) = 6.570, *p* = .014), with latencies being smaller in the former (*M* = 5.710, *SD* = 5.296) than in the latter (*M* = 20.891, *SD* = 38.037).

Graph 6



Note. Graphical representation of first-choice latency (*y* axis) in the pre-treatment test with the presentation of the low-value stimulus (*x* axis, *pre-*) and in the post-treatment test after the positive emotional treatment (*x* axis, *post+*). Bars represent standard errors.

3.2 Mixed within-subjects ANOVA between pre-treatment tests

To check whether chicks had learned to discriminate between the stimulus colour with a high value (associated with food with high affordability) and the stimulus colour with a low value (associated with food with lower affordability), we used a within-subjects mixed ANOVA to compare each subject's two pre-treatment tests.

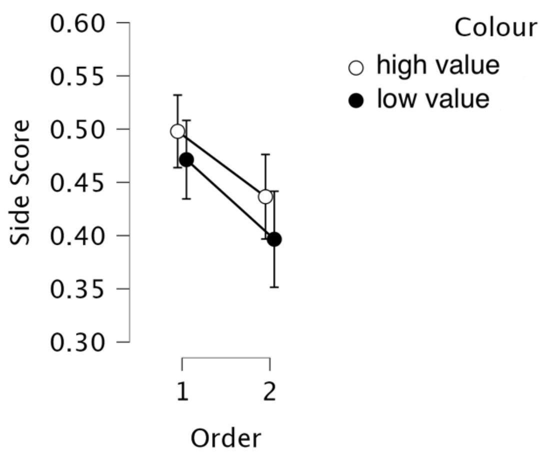
As for the choice score, the ANOVA revealed a non-significant ($F(1, 83) = 3.715$, $p = .057$) tendency for the chicks to choose more when the high-value colour was presented ($M = 0.224$, $SD = 0.124$) as compared to the low-value one ($M = 0.193$, $SD = 0.128$). As for the side score, no significant effect ($F(1, 83) = 0.985$, $p > .05$) was found when comparing the two tests. However, the interaction between the side score and the order of presentation of the reinforced colours ($F(1, 82) = 3.710$, $p = .058$) almost reached the significance level. Notably, chicks had higher side scores (i.e., chose more the stimulus on the right) in the first presentation, regardless of the stimulus value, than they did in the second presentation, as shown in Table 4 and Graph 7.

Table 4

Descriptives - Side Score

Order	Colour	N	Mean	SD	SE	Coefficient of variation
1	high value	43	0.498	0.223	0.034	0.499
	low value	45	0.471	0.247	0.037	0.522
2	high value	43	0.437	0.260	0.040	0.599
	low value	41	0.397	0.289	0.045	0.691

Graph 7



Note. Graphical representation of side score (*y* axis) in the pre-treatment tests (*x* axis, where 1 is the first pre-treatment test and 2 is the second pre-treatment test). Separate lines indicate the value of the two different possible colours of presented stimuli, either high or low. Bars represent standard errors.

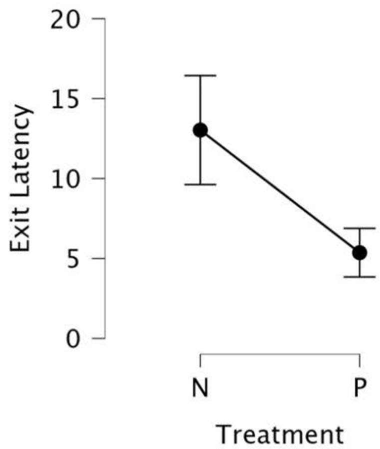
As for the exit latency and the first-choice latency, the mixed ANOVA revealed no statistically significant effect or interaction with $p < .05$.

3.3 Between-subjects ANOVA between post-treatment tests

Finally, we compared the post-treatment test of chicks which underwent the positive emotional treatment to that of chicks which underwent the negative one by conducting a between-subjects ANOVA, considering all the variables previously described in 2.5.4 Inferential statistics.

Considering all the indexes, no significant difference was found in terms of choice score (F (1, 84) = 0.013, p = .910), side score (F (1, 84) = 0.021, p = .884), and first-choice latency (F (1, 84) = 2.620, p = .109). The ANOVA only demonstrated a statistically significant difference between the post-treatment tests of chicks in the two different conditions with respect to the exit latency (F (1, 83) = 4.015, p = .048). Notably, chicks which underwent the negative treatment took on average 13.030 seconds (*SD* = 22.615) to exit, while those in the positive treatment condition took 5.365 seconds (*SD* = 9.728) to do so (Graph 9).

Graph 9



Note. Graphical representation of exit latency (*y* axis) in the post-treatment test of chicks who underwent the negative emotional treatment (*x* axis, *N*) and in the post-treatment test of chicks who underwent the positive emotional treatment (*x* axis, *P*). Bars represent standard errors.

4. Discussion

With the present study, we attempted to induce a judgement bias in domestic chicks (*G. gallus domesticus*) by means of positive or negative emotional treatments and to uncover how the different emotional states are reflected in lateralised brain activation, inferred from preferential approach to stimuli presented in the left or right of their visual field.

Regardless of the significance of results, there was a clear tendency for chicks to choose more in the pre-treatment tests as compared to the post-treatment one. Similarly, they tended to make their first choice in a shorter time when presented with the familiar black and white stimuli. Finally, the time it took them to peck the clear wall (i.e., the exit latency) was always significantly shorter in both pre-treatment tests as compared to the post-treatment one. This is not surprising given that animals were familiar with the pre-treatment test stimuli (both the low- and the high-value one) and unfamiliar with the ambiguous one and it proves that they did perceive the latter as different from what they had seen before.

Besides confirming that they distinguish the ambiguous stimulus from the familiar ones, we also wanted to determine whether they discriminated between the two familiar stimuli (black and white), depending on the affordability of the associated food. A tendency for chicks to choose more the high-value stimulus than the low-value one emerged, but no result reached the significance level. Therefore, it is possible to suppose that the trainings were not effective and that chicks had not learned to discriminate between the high- and the low-value stimuli. Without strong evidence that chicks learned the association between colour and food value, interpretations of responses to the ambiguous stimulus become less reliable.

One of the key questions was whether the emotional manipulation affected how chicks judged ambiguity. Results showed no significant difference in choice score between the pre-treatment test with presentation of the low-value stimulus and the test with the presentation of the ambiguous stimulus after the negative emotional treatment. This is in line with what we expected to find, as it proves that, after the negative treatment and, supposedly, when having a negative emotional state, chicks classify the ambiguous stimulus as close to the negative one and, therefore, respond to it in a similar way. The significant difference between chicks' choice score when presented with the high-value stimulus and when presented with the ambiguous one after the negative emotional treatment also supports the existence of a judgement bias, since it shows, as expected, that chicks categorise these stimuli differently.

Other findings that seem to support the efficacy of the emotional treatments in influencing chicks' judgements include the significantly different first-choice latency after the negative treatment compared to the pre-treatment test with the high-value stimulus and after the positive treatment compared to the pre-treatment test with the low-value stimulus. As a matter of fact, we expected to find these differences in case of a judgement bias, as they show that the ambiguous stimulus was interpreted negatively after the negative treatment and positively after the positive treatment.

To test the effect of the different emotional manipulations, we also compared the post-treatment test of chicks which underwent the positive treatment and that of chicks which the negative one. Results were significant only for the exit latency, proving the existence of a difference based on the emotional treatment. As for the other indexes (choice score, side score, and first-choice latency), no difference was found to be significant. Therefore, despite some of the results seem to suggest that the emotional treatment produced a judgement bias, exit latency is the only index for which a clear and significant difference is observed depending on the valence of the emotional treatment.

Inconsistent results temper these findings, as they suggest that the emotional treatments did not produce the expected judgement bias. For example, despite hypothesizing a tendency for chicks to choose more when presented with the high-value stimulus than in the post-treatment test after inducing a positive state due to the unfamiliarity of the ambiguous stimulus, we did not expect such a highly significant difference ($F(1, 39) = 10.593$, $p < .01$). Indeed, this goes against the core expectation of a judgement bias, that is that a positive emotional state leads to the classification of the ambiguous stimulus as predicting positive outcomes, while a negative state produces pessimistic behaviour.

Similarly, a significant difference between the choice scores calculated when chicks were presented with the low-value stimulus and with the ambiguous one after the positive emotional treatment was expected. This was not found but, curiously, the order of presentation of the stimuli (high and low value) in the pre-treatment tests had a significant effect on choice score. Being presented with the low-value stimulus during the second pre-treatment test determines significantly lower choice scores in the latter. A possible explanation is that these chicks, which have just seen the high-value stimulus and have received no reward despite approaching it (as no reward was given during pre-treatment tests), were less prone to choose when presented with the low-value stimulus because their expectations were previously not met. Perhaps, unmet expectations reduced motivation or altered cognitive strategies in subsequent tests. When the order of presentation was the opposite, choice scores were higher in the pre-treatment test with the low-value stimulus (that is, the first pre-treatment test in this case), as their expectations about the high-value one had not yet been failed. Choice scores were also significantly lower in the post-treatment test of chicks who underwent the positive emotional treatment if they had lastly seen the low-value stimulus, as compared to those that had seen it before the high-value one. Indeed, the order of presentation of stimuli in the pre-treatment tests affected the behaviour of these chicks that were supposedly in a positive emotional state, possibly because they interpreted the ambiguous stimulus as being close to the low-value one simply due to having been lastly presented with the latter. These order effects possibly confounded the intended emotional manipulation.

Similarly, the variable ‘Order’ was proven to have a significant effect on exit latency when comparing the pre-treatment tests to the post-treatment one, regardless of the positive or negative treatment valence. Having lastly seen the low-value stimulus is linked to longer exit latency in the post-treatment test. Comparably to what suggested before about the effect of the order of stimuli presentation on the choice score of the post-treatment test, perhaps chicks categorised the ambiguous stimulus as the low-value one (and, consequently, took longer to exit) because it was the last one they had seen before the emotional treatment.

4.1 Lateralised brain activation

The side score is the variable of interest to understand how an induced positive or negative emotional state is linked to lateralised activation in the chick’s brain. Indeed, we hypothesised that, during the pre-treatment tests, when presented with the high-value stimulus chicks would have higher side scores (i.e., choose more the stimulus on the right) due to increased left hemisphere activation, while the opposite pattern of activation (right hemisphere) would produce lower side scores (i.e., choose more the stimulus on the left) when presented with the low-value stimulus. In addition, we expected increased left hemisphere activation (higher side scores) after the positive treatment and increased right hemisphere activation (lower side scores) after the negative one. The side score was used as a proxy for hemispheric activation.

To understand whether our hypothesis was correct, we firstly compared the pre-treatment tests. Even though the difference in side scores was not significant *per se*, the interaction with the variable ‘Order’ (i.e., the order of presentation of the high- and low-value stimuli in the pre-treatment tests) almost reached the significance level ($F (1, 82) = 3.710$, $p = .058$). There was indeed an interesting tendency for chicks to choose more the stimulus on the right in the first pre-treatment test, regardless of whether the stimulus presented had a low or high value. Lower side scores, and therefore increased right hemisphere activation, in the second test may be an indicator of a negative emotional state induced by the experimental procedure. On the other hand, the higher side scores (closer to the chance level 0.5) observed in the first presentation may be due to the fact that the animal's emotional state had not yet been significantly affected. We can therefore suppose that, after the first pre-treatment test, the long experimental procedure induced a negative emotional state that persisted for the whole experiment. For this reason, no clear effect of the emotional treatment was found on chicks' brain activation in terms of lateralisation, as the non-significant results of the ANOVAs between pre- and post-treatment tests and between post-treatment tests prove. Chicks who underwent the positive treatment did not show any difference in side scores from those in the negative treatment condition perhaps because they were also in an induced negative emotional state.

4.2 Limitations and future research

The first possible limitation of this study, linked to chicks possibly failing to learn to discriminate between high- and low-value stimuli, is that during trainings there was no threshold to be reached for them to be considered effective, besides approaching the stimulus within a limited time and for multiple consecutive times as described in 2.3.3 Trainings. In future studies it could be useful to establish a threshold for choosing time and latencies depending on the value of the stimulus presented, in order to be sure that the animals do not only learn to approach both stimuli but also discriminate them and recognise their different values depending on the affordability of the associated food.

It is also possible that the stimuli used were not appropriate, as they are all naturally neutral for the animal. Using ecologically-relevant stimuli (as Salmeto et al. (2011) did using silhouettes of a conspecific chick, an owl, and intermediate ambiguous cues created morphing chick and owl characteristics) may be more effective, instead of trying to create a conditioned reaction to stimuli that are normally irrelevant for the animal.

A further possible limitation of this study is the length of the experimental procedure, which in its entirety lasted multiple hours. Despite trying to make the procedure less stressful as possible for the animals, for example by including multiple resting periods during which they were brought back to their cages between the different phases, it was perhaps too long and tiring for them. As mentioned before, this may have affected their emotional state and, consequently, also their behaviour. Notwithstanding the importance of every phase of the procedure, in the future reducing its length may be useful to exclude the possibility of it being too stressful for chicks.

It could also be that the emotional treatment was ineffective in altering animals' emotional state for a number of reasons. The literature is indeed controversial on the effects of the manipulations used in the emotional treatments, including the effects of recordings of conspecifics and of light manipulations. No studies have yet proven the existence of emotional contagion in 3-day-old domestic chicks and, as mentioned in 1.4 The present study, Vallortigara (1988) actually demonstrated that in young chicks conspecific calls have no effect on ambulation latency. Our subjects were perhaps too young for the recordings used to have a clear effect on their behaviour. Furthermore, it has not been experimentally proven that light intensity manipulations affect 3-day-old chicks in the same way they affect older hens or nocturnal species. It is therefore possible that the manipulations we used to induce a negative emotional state did not have an effect, which would explain why for most of the indexes considered no difference was found between the tests after the positive treatment and after the negative one. Further studies should be conducted to assess the effectiveness of every manipulation we included in our treatments on 3-day old chicks. If they are confirmed not to have a consistent effect, it may be interesting to use older subjects and to understand how old chicks have to be to be affected by them. Additionally, in that case different manipulations in the emotional treatments should be considered.

Finally, considering that the order of presentation of stimuli in the pre-treatment tests had a significant or almost-significant effect on various index and in various analyses, further exploring them in future research designs could be insightful.

In conclusion, while our findings contribute to our understanding of how emotional states may influence judgement in young chicks, they also highlight important methodological challenges that must be addressed. Although lateralised brain activation was not observed in response to emotional treatments, this study lays the groundwork for future research into the neural correlates of emotion in chicks and the development of more sensitive behavioural proxies for hemispheric engagement.

5. Conclusion

In summary, this study aimed to investigate whether emotional treatments of positive or negative valence could induce a judgment bias in 3-day-old domestic chicks, and whether such bias would be reflected in lateralised brain activation. Using a judgment bias task, we assessed how chicks categorised an ambiguous stimulus—as more similar to a previously reinforced high-value or low-value stimulus—following emotional manipulation. Additionally, drawing on the 'emotional-valence hypothesis' (Leliveld et al., 2013), we examined whether emotional state influenced hemispheric dominance, as inferred from lateralised approach behavior.

Behavioral changes following treatment, particularly increased latency in response to ambiguous stimuli, suggest that negative affect may bias decision-making in a pessimistic direction. However, the lack of consistent discrimination between familiar high- and low-value stimuli and the limited number of significant effects point to methodological shortcomings, particularly in training efficacy and emotional induction procedures. Notably, the lack of clear evidence for lateralised activation based on affective state, implies that the emotional treatments may not have reliably altered chicks' emotional state.

These findings highlight the importance of refining and validating emotional manipulation protocols in young chicks. Future research should explore age effects, optimise training and treatment efficacy, and consider additional behavioural or physiological markers of emotional state.

Overall, this study contributes to the growing field of animal emotion research by identifying both the potential and the current limitations of using cognitive bias tasks in very young chicks, paving the way for more refined approaches in future investigations.

6. References

Bateson, M., & Matheson, S. (2007). Performance on a categorisation task suggests that removal of environmental enrichment induces 'pessimism' in captive European starlings (*Sturnus vulgaris*). *Animal Welfare*, *16*(S1), Article S1. https://doi.org/10.1017/S0962728600031705

Bennett, C. E., Thomas, R., Williams, M., Zalasiewicz, J., Edgeworth, M., Miller, H., Coles, B., Foster, A., Burton, E. J., & Marume, U. (2018). The broiler chicken as a signal of a human reconfigured biosphere. *Royal Society Open Science*, *5*(12), 180325. https://doi.org/10.1098/rsos.180325

Berns, P. V., & Bell, L. M. (1979). Tonic immobility in chicks during presentations and withdrawals of an imprinting stimulus. *Animal Learning & Behavior*, *7*(3), 383–386. https://doi.org/10.3758/BF03209689

Bernoulli, D. (1738). Exposition of a new theory on the measurement of risk. Transl. L Sommer, 1954. *Econometrica, 22(1)*, 23-36.

Boissy, A., Manteuffel, G., Jensen, M. B., Moe, R. O., Spruijt, B., Keeling, L. J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissier, I., & Aubert, A. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiology & Behavior*, *92*(3), Article 3. https://doi.org/10.1016/j.physbeh.2007.02.003

Boleij, H., van't Klooster, J., Lavrijsen, M., Kirchhoff, S., Arndt, S. S., & Ohl, F. (2012). A test to identify judgement bias in mice. *Behavioural Brain Research*, *233*(1), 45–54. https://doi.org/10.1016/j.bbr.2012.04.039

Bryden, W. L., Li, X., Ruhnke, I., Zhang, D., & Shini, S. (2021). Nutrition, feeding and laying hen welfare. *Animal Production Science*, *61*(10), 893–914. https://doi.org/10.1071/AN20396

Burman, O. H. P., & Mendl, M. T. (2018). A novel task to assess mood congruent memory bias in non-human animals. *Journal of Neuroscience Methods*, *308*, 269–275. https://doi.org/10.1016/j.jneumeth.2018.07.003

Burman, O., McGowan, R., Mendl, M., Norling, Y., Paul, E., Rehn, T., & Keeling, L. (2011). Using judgement bias to measure positive affective state in dogs. *Applied Animal Behaviour Science*, *132*(3), Article 3. https://doi.org/10.1016/j.applanim.2011.04.001

Cahill, L., & McGaugh, J. L. (1996). Modulation of memory storage. *Current Opinion in Neurobiology*, *6*(2), 237–242. https://doi.org/10.1016/S0959-4388(96)80078-X

Collias, N., & Joos, M. (1953). The Spectrographic Analysis of Sound Signals of the Domestic Fowl. *Behaviour*, *5*(1), 175–188. https://doi.org/10.1163/156853953X00104

Collins, S. A., Herborn, K., Sufka, K. J., Asher, L., & Brilot, B. (2024). Do I sound anxious? Emotional arousal is linked to changes in vocalisations in domestic chicks (*Gallus gallus* dom.). *Applied Animal Behaviour Science*, *277*, 106359. https://doi.org/10.1016/j.applanim.2024.106359

Cussen, V. A., & Mench, J. A. (2014). Personality predicts cognitive bias in captive psittacines, *Amazona amazonica*. *Animal Behaviour*, *89*, 123–130. https://doi.org/10.1016/j.anbehav.2013.12.022

Désiré, L., Boissy, A., & Veissier, I. (2002). Emotions in farm animals: A new approach to animal welfare in applied ethology. *Behavioural Processes*, *60*(2), 165–180. https://doi.org/10.1016/S0376-6357(02)00081-5

Dolcos, F., & Denkova, E. (2015). *Dissociating Enhancing and Impairing Effects of Emotion on Cognition* (pp. 1–18). https://doi.org/10.1002/9781118900772.etrds0079

Doyle, R. E., Fisher, A. D., Hinch, G. N., Boissy, A., & Lee, C. (2010). Release from restraint generates a positive judgement bias in sheep. *Applied Animal Behaviour Science*, *122*(1), Article 1. https://doi.org/10.1016/j.applanim.2009.11.003

Edgar, J. L., & Nicol, C. J. (2018). Socially-mediated arousal and contagion within domestic chick broods. *Scientific Reports*, *8*(1), 10509. https://doi.org/10.1038/s41598-018-28923-8

Eysenck, M. W., Mogg, K., May, J., Richards, A., & Mathews, A. (1991). Bias in interpretation of ambiguous sentences related to threat in anxiety. *Journal of Abnormal Psychology*, *100*(2), 144–150. https://doi.org/10.1037/0021-843X.100.2.144

FAOSTAT. (n.d.). Retrieved 9 June 2025, from https://www.fao.org/faostat/en/#data/QCL

Fraser, D. (1995). Science, Values and Animal Welfare: Exploring the 'Inextricable Connection'. *Animal Welfare Collection*. https://www.wellbeingintlstudiesrepository.org/acwp_awap/39

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-sorce event-logging software for video/audio coding and live observations. *Methods Ecol Evol, 7*, 1325-1330.

Garnham, L., & Løvlie, H. (2018). Sophisticated Fowl: The Complex Behaviour and Cognitive Skills of Chickens and Red Junglefowl. *Behavioral Sciences*, *8*(1), Article 1. https://doi.org/10.3390/bs8010013

Godsil, B. P., & Fanselow, M. S. (2004). Light stimulus change evokes an activity response in the rat. *Learning & Behavior*, *32*(3), 299–310. https://doi.org/10.3758/BF03196029

Harding, E. J., Paul, E. S., & Mendl, M. (2004). Cognitive bias and affective state. *Nature*, *427*(6972), 312–312. https://doi.org/10.1038/427312a

Haselton, M. G., Nettle, D., & Andrews, P. W. (2015). The Evolution of Cognitive Bias. In *The Handbook of Evolutionary Psychology* (pp. 724–746). John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470939376.ch25

Hedlund, L., Palazon, T., & Jensen, P. (2021). Stress during Commercial Hatchery Processing Induces Long-Time Negative Cognitive Judgement Bias in Chickens. *Animals : An Open Access Journal from MDPI*, *11*(4), Article 4. https://doi.org/10.3390/ani11041083

Hemsworth, P. H., & Edwards, L. E. (2020). Natural behaviours, their drivers and their implications for laying hen welfare. *Animal Production Science*, *61*(10), 915–930. https://doi.org/10.1071/AN19630

Iyasere, O. S., Beard, A. P., Guy, J. H., & Bateson, M. (2017). Elevated levels of the stress hormone, corticosterone, cause 'pessimistic' judgment bias in broiler chickens. *Scientific Reports*, *7*(1), 6860. https://doi.org/10.1038/s41598-017-07040-y

JASP Team (2023). JASP (Version 0.18.1)[Computer software].

Jones, R. B. (1986). Conspecific vocalisations, tonic immobility and fearfulness in the domestic fowl. *Behavioural Processes*, *13*(3), 217–225. https://doi.org/10.1016/0376-6357(86)90085-9

Khalil, A., Matsui, K., & Takeda, K.-I. (2010). Responses to abrupt changes in feeding and illumination in laying hens. *Turkish Journal of Veterinary & Animal Sciences*, *34*(5), 433–439. https://doi.org/10.3906/vet-0901-25

Kindt, M., & Hout, M. (2001). Selective Attention and Anxiety: A Perspective on Developmental Issues and the Causal Status. *Journal of Psychopathology and Behavioral Assessment*, *23*, 193–202. https://doi.org/10.1023/A:1010921405496

Kleinginna, P. R., & Kleinginna, A. M. (1981). A categorized list of emotion definitions, with suggestions for a consensual definition. *Motivation and Emotion*, *5*(4), 345–379. https://doi.org/10.1007/BF00992553

Leliveld, L. M. C., Langbein, J., & Puppe, B. (2013). The emergence of emotional lateralization: Evidence in non-human vertebrates and implications for farm animals. *Applied Animal Behaviour Science*, *145*(1), 1–14. https://doi.org/10.1016/j.applanim.2013.02.002

Leys, C., Ley, C., Klein, O., Bernard, P., & Licata, L. (2013). Detecting outliers: Do not use standard deviation around the mean, use absolute deviation around the median. *Journal of Experimental Social Psychology*, *49*(4), 764–766. https://doi.org/10.1016/j.jesp.2013.03.013

Loewenstein, G., Rick, S., & Cohen, J. D. (2008). Neuroeconomics. *Annual Review of Psychology*, *59*(1), 647–672. https://doi.org/10.1146/annurev.psych.59.103006.093710

Lorenz, K. Z. (1937). The Companion in the Bird's World. *The Auk*, *54*(3), 245–273. https://doi.org/10.2307/4078077

Marino, L. (2017). Thinking chickens: A review of cognition, emotion, and behavior in the domestic chicken. *Animal Cognition*, *20*(2), 127–147. https://doi.org/10.1007/s10071-016-1064-4

McKenzie, R., Andrew, R. J., & Jones, R. B. (1998). Lateralization in chicks and hens: New evidence for control of response by the right eye system. *Neuropsychologia*, *36*(1), 51–58. https://doi.org/10.1016/S0028-3932(97)00108-5

Meluzzi, A., & and Sirri, F. (2009). Welfare of broiler chickens. *Italian Journal of Animal Science*, *8*(sup1), 161–173. https://doi.org/10.4081/ijas.2009.s1.161

Mendl, M., Burman, O. H. P., Parker, R. M. A., & Paul, E. S. (2009). Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. *Applied Animal Behaviour Science*, *118*(3), 161–181. https://doi.org/10.1016/j.applanim.2009.02.023

Monk, J. E., Doyle, R. E., Colditz, I. G., Belson, S., Cronin, G. M., & Lee, C. (2018). Towards a more practical attention bias test to assess affective state in sheep. *PLOS ONE*, *13*(1), e0190404. https://doi.org/10.1371/journal.pone.0190404

Nasello, A. G., Machado, C., Bastos, J. F., & Felicio, L. F. (1998). Sudden Darkness Induces a High Activity-Low Anxiety State in Male and Female Rats. *Physiology & Behavior*, *63*(3), 451–454. https://doi.org/10.1016/S0031-9384(97)00462-9

Paul, E. S., Harding, E. J., & Mendl, M. (2005). Measuring emotional processes in animals: The utility of a cognitive approach. *Neuroscience & Biobehavioral Reviews*, *29*(3), 469–491. https://doi.org/10.1016/j.neubiorev.2005.01.002

Pérez-Manrique, A., & Gomila, A. (2022). Emotional contagion in nonhuman animals: A review. *WIREs Cognitive Science*, *13*(1), e1560. https://doi.org/10.1002/wcs.1560

Preston, S., & Waal, F. (2002). Empathy: Its Ultimate and Proximate Bases. *The Behavioral and Brain Sciences*, *25*, 1–20; discussion 20. https://doi.org/10.1017/S0140525X02000018

Rogers, L. J. (2008). Development and function of lateralization in the avian brain. *Brain Research Bulletin*, *76*(3), 235–244. https://doi.org/10.1016/j.brainresbull.2008.02.001

Rogers, L. J. (2010). Relevance of brain and behavioural lateralization to animal welfare. *Applied Animal Behaviour Science*, *127*(1), 1–11. https://doi.org/10.1016/j.applanim.2010.06.008

Rogers, L. J., & Anson, J. M. (1979). Lateralisation of function in the chicken fore-brain. *Pharmacology Biochemistry and Behavior*, *10*(5), 679–686. https://doi.org/10.1016/0091-3057(79)90320-4

Rogers, L. J., & Kaplan, G. (2019). Does Functional Lateralization in Birds Have any Implications for Their Welfare? *Symmetry*, *11*(8), Article 8. https://doi.org/10.3390/sym11081043

Rogers, L. J., Zucca, P., & Vallortigara, G. (2004). Advantages of having a lateralized brain. *Proceedings of the Royal Society B: Biological Sciences*, *271*(Suppl 6), Article Suppl 6.

Rugani, R., Regolin, L., & Vallortigara, G. (2010). Imprinted numbers: Newborn chicks' sensitivity to number vs. continuous extent of objects they have been reared with. *Developmental Science*, *13*(5), 790–797. https://doi.org/10.1111/j.1467-7687.2009.00936.x

Salmeto, A. L., Hymel, K. A., Carpenter, E. C., Brilot, B. O., Bateson, M., & Sufka, K. J. (2011). Cognitive bias in the chick anxiety-depression model. *Brain Research*, *1373*, 124–130. https://doi.org/10.1016/j.brainres.2010.12.007

Salzen, E. A. (1967). Imprinting in Birds and Primates. *Behaviour*, *28*(3–4), 232–254. https://doi.org/10.1163/156853967X00028

Solvi, C., Baciadonna, L., & Chittka, L. (2016). Unexpected rewards induce dopamine-dependent positive emotion–like state changes in bumblebees. *Science*, *353*(6307), Article 6307. https://doi.org/10.1126/science.aaf4454

Thompson, R. K. R., & Liebreich, M. (1987). Adult chicken alarm calls enhance tonic immobility in chicks. *Behavioural Processes*, *14*(1), 49–61. https://doi.org/10.1016/0376-6357(87)90066-0

Uzundumlu, A. S., & Dilli, M. (2022). Estimating Chicken Meat Productions of Leader Countries for 2019-2025 Years. *Ciência Rural*, *53*, e20210477. https://doi.org/10.1590/0103-8478cr20210477

Vallortigara, G. (1988). Chicks in a Novel Environment: Effects of Conspecific Calls. *Ethology*, *78*(4), 341–345. https://doi.org/10.1111/j.1439-0310.1988.tb00243.x

Weiss, I. C., Pryce, C. R., Jongen-Rêlo, A. L., Nanz-Bahr, N. I., & Feldon, J. (2004). Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat.

Behavioural Brain Research, *152*(2), 279–295.
https://doi.org/10.1016/j.bbr.2003.10.015