



Filial Imprinting and Social Predispositions in Chicks (*Gallus gallus domesticus*)

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List of Publications

Included in this thesis:

Chapter 1

Lemaire, B.S., Rucco, D., Josserand, M., Vallortigara, G. & Versace, E. Stability and individual variability of social attachment in imprinting. *Sci Rep* 11, 7914 (2021). <https://doi.org/10.1038/s41598-021-86989-3>

Chapter 2

Lemaire, B.S. No evidence of spontaneous preference for slowly moving objects in visually naïve chicks. *Sci Rep* 10, 6277 (2020).

<https://doi.org/10.1038/s41598-020-63428-3>

Chapter 3

Lorenzi E, Lemaire BS, Versace E, Matsushima T and Vallortigara G (2021) Resurgence of an Inborn Attraction for Animate Objects via Thyroid Hormone T3. *Front. Behav. Neurosci.* 15:675994.

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Rosa-Salva, O., Mayer, U., Versace, E., Hébert, M., Lemaire, B.S., & Giorgio Vallortigara. Sensitive periods for social development: Interactions between predisposed and learned mechanisms. *Cognition*, 104552 (2021).

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Chiandetti C., Lemaire B.S, Versace E., & Vallortigara G. Early- and Late-Light Embryonic Stimulation Modulates Similarly Chicks' Ability to Filter out Distractors. *Symmetry*. 2017; 9(6):84. <https://doi.org/10.3390/sym9060084>

Zanon, M., Lemaire, B. S., & Vallortigara, G. (*under revisions*). Steps towards a computational ethology: an automatized, interactive setup to investigate filial imprinting and biological predispositions. *Biological Cybernetics*.

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The domestic chick became a model for understanding memory, learning and the onset of social behaviours. Just after hatching and for a limited period, the naïve bird seeks a suitable object to imprint. Thanks to laboratory studies, the filial imprinting has been well documented in the very first few hours. However, how the filial imprinting preferences develop and evolve over time remained relatively unexplored. Therefore, we built an automated setup allowing us to follow the animal behaviour across prolonged durations and investigate the stability and variability of filial imprinting preferences. We demonstrated that three days of exposure to artificial objects produce lasting and robust imprinting preferences. With lower imprinting duration, we found that the animal predispositions strongly influence the filial imprinting preferences. Those social predispositions guide the domestic chicks towards living creatures – or at least, towards stimuli conveying animacy. To complete this general pattern, we performed two experiments manipulating motion dynamics. We showed that chicks prefer quickly rotating objects and agents moving with unpredictable temporal sequences: two cues probably used to detect living animals' presence. Both imprinting and social predispositions influence each other, but whether they share a neurophysiological ground was yet to be described. Such as for filial imprinting, we showed that the thyroid hormone T3 strongly affects the sensitive period for animacy preference. T3-inhibition closes the sensitive period for animacy preference and T3-injections re-opens it. Altogether, the present thesis complete previous research on filial imprinting and social predispositions: two distinct but interconnected mechanisms that can help to better understand the mind foundations at the onset of life.

For the greatest part, all starts when it is time. For precocial species such as domestic chicks, it starts as soon as they step out from their shell; ready to explore their world.

Precocial species - mostly nidifugous birds - hatch with developed senses, motoric and cognitive abilities (Rose, 2000; Vallortigara, 2012) and can impress anyone patient enough to observe them. The British ethologist Douglas Spalding did watch domestic chicks and noticed they could form a strong attachment toward 'abnormal' objects (Spalding, 1873). Later on, Oskar Heinroth (1910) and Konrad Lorenz (1937) described this phenomenon and called it 'filial imprinting'. A mechanism that leads most young of precocial species to form a solid and lasting bond toward the first individual or conspicuous stimulus they encounter. Lorenz's geese imprinted on his boots (Lorenz, 1937), but in optimal conditions, the young birds should imprint and narrow their social preference towards their parent to survive and thrive.

Since then, precocial birds such as domestic chicks and ducklings entered the laboratories, and filial imprinting went under scientific scrutiny. It became a model for understanding memory, learning and the onset of social behaviours in neonates animals (Di Giorgio et al., 2017; Rose, 2000, 2003; Solomon & McCabe, 2015; Versace & Vallortigara, 2015).

Filial imprinting

Filial imprinting is a form of learning through which the social behaviour of a neonate becomes restricted to a particular individual (Hess, 1958; Bateson, 1966; Bolhuis, 1991; Versace and Vallortigara, 2018; McCabe, 2019). Just after hatching, and for a limited period, these neonate birds are attracted by the most conspicuous stimulus in their environment and therefore learn its characteristics through mere exposure – they imprint on it. In only a few minutes, imprinting occurs and leads the animals to prefer their imprinting stimulus afterwards (Bolhuis, 1991). Although imprinting is directed

toward the mother hen in natural condition, chicks can imprint on a plethora of stimuli (Bolhuis, 1991; McCabe, 2019; Figure 1).

Imprinting in the lab

Chicks and ducklings can imprint on static and moving objects (Bolhuis, 1991; Martinho & Kacelnik, 2016) as well as flashing lights and virtual stimuli displayed on monitor screens (Bateson & Jaeckel, 1976; Versace et al., 2017; Figure 1). This ability paved the way for systematic studies of filial imprinting in the laboratory.

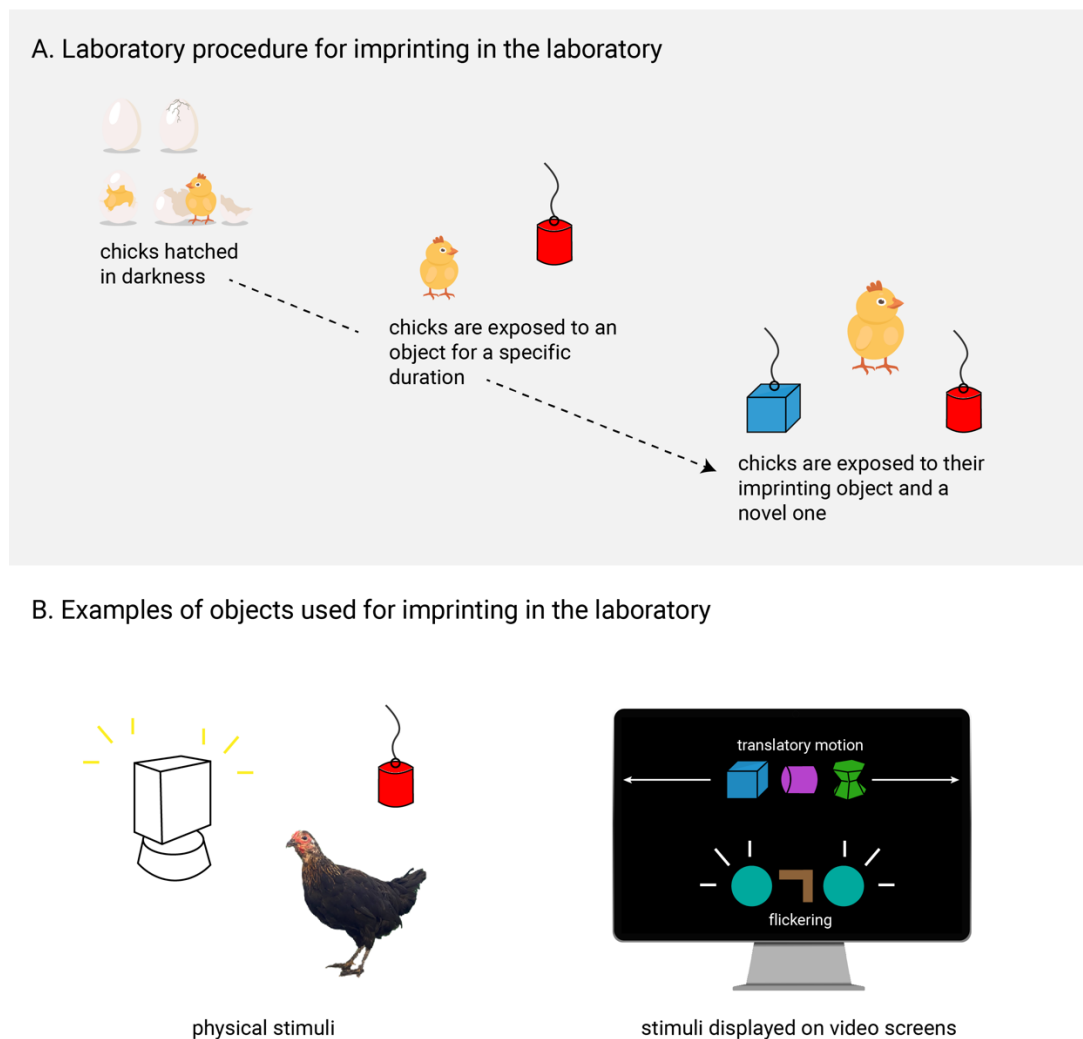


Figure 1: Standard procedure (A) and objects used for imprinting (B) investigation in the laboratory.

Over the years, imprinting investigations used a standard procedure (Figure 1). Chicks or ducklings hatched in darkness (to prevent any visual stimulation) are exposed to a stimulus for a couple of minutes/hours (Bolhuis, 1991; Izawa et al., 2001). They are then

returned to their incubator in complete darkness before their preference for the imprinting stimulus is tested. The preference towards an object can be measured in a single stimulus presentation (where the animal's motivation to reach this object is scored) or in a choice test (where the animals can choose between the imprinting stimulus and a novel one) (Andrew, 1991). In the latest, researchers can easily score the imprinting strength by looking at the time spent close to the imprinting stimulus. This procedure is still commonly used. However, a totally automated setup was missing to investigate imprinting and studies taking advantage of it.

In the general method of this thesis, I describe the automated setup (Figure 4) we developed (Zanon, Lemaire, & Vallortigara, *in prep*). It can be used for different research purposes. In the work presented here, we used the automated setup to investigate filial imprinting (Chapter 1) and social predispositions (Chapter 2; Study 1). Combined with an experimental design taking advantage of imprinting, the automated setup can help scientists to investigate several different aspects of cognition using imprinting as a key to mind, such as number (Lemaire et al., 2020; Rugani, et al., 2009; Rugani, Loconsole, & Regolin, 2017; Rugani, Regolin, & Vallortigara, 2011; Rugani, Vallortigara, & Regolin, 2013; Rugani, Regolin, & Vallortigara, 2010), space (Vallortigara, 2015; Vallortigara et al., 2010), object permanence (Regolin & Vallortigara, 1995; Regolin, Vallortigara, & Zanforlin, 1995; Vallortigara et al., 1998) and others (for a review see Chiandetti & Vallortigara, 2018; Marino, 2017; Vallortigara, 2012).

Preference for the imprinting stimulus

Imprinting's early work associated the exposure duration to a stimulus with imprinting strength. In 1959, Hess described that the more a chick is exposed to a stimulus after hatching, the stronger its preference. Bateson and Jaekel (1976) confirmed this finding by imprinting chicks with an object for different durations (0, 15, 30, 45 or 60 minutes) and offered the animals a choice between their imprinting object and a novel one. Chicks showed a stronger preference for their imprinting stimulus when exposed for 60 minutes compared to lower durations. Nonetheless, the correlation between imprinting duration and strength was irregular. Indeed, chicks exposed for 15 or 45 minutes had a higher preference for their imprinting object than the group exposed for 30 minutes

(Bateson & Jaeckel, 1976). This result suggests a transient preference for novelty. Jackson and Bateson (1974) had already described this curious drop in preference in a previous study. They imprinted chicks for 15, 30 or 60 minutes with a rotating flashing light of a specific colour. At test, chicks were located in an operant conditioning box where they could see their imprinting stimulus. A pedal located inside the box could change the colour of the imprinting stimulus. Chicks exposed to their imprinting stimulus for 15 minutes and 30 minutes pressed significantly more the pedal, exposing themselves more often to the unfamiliar colour than the other group. This preference to seek slight novelty at an early stage of imprinting was described as an adaptive behaviour (Bateson & Jaeckel, 1976). In nature, seek slight novelty might allow the chicks to build a complete representation of their mother from different perspectives. Honey and Bateson (1996) supported the former hypothesis by imprinting chicks successively on the side and back views of a hen. The group of chicks imprinted with a fast succession of back and side views took longer to differentiate than the group imprinted with a slower succession. Chantrey (1974) also manipulated the interval duration between stimuli presentation and demonstrated that when stimuli are presented in close temporal proximity (under 30 seconds), chicks failed to dissociate them.

The duration of exposure to an imprinting stimulus influences the preference, but the relationship between imprinting strength and exposure time can be unsteady and unforeseen. How filial imprinting preferences develop over long duration (days) remain to be explored. At the neural level, converging evidence implicates the intermediate and medial mesopallium (IMM; Horn, 2004; McCabe, 2013; Rose, 2000; Solomon & McCabe, 2015). During and after imprinting, IMM undergoes biochemical-learning related changes with an accurate temporal profile. Up to fifteen hours after the start of an imprinting procedure, researchers observed adjustments at the synaptic level into the IMM (see Solomon & McCabe, 2015 for review).

In the first chapter of this thesis, we investigate the development of filial preferences for up to six days using our automated setup. We manipulate the imprinting duration up to

three days and use different imprinting objects to understand their effect on the filial imprinting stability.

Predispositions

In the mid 50', Fabricius and Boyd (1954) reported that ducklings prefer to follow objects larger than a matchbox. In the 70', Schuman, Hale and Graves (1970) observed that chicks prefer to approach circular objects around 10-20 cm. Half of a century later, scientists discovered many more predispositions in domestic chicks. They canalise the precocial bird's attention towards features shared by living creatures: animacy features (Rosa-Salva, Mayer, & Vallortigara, 2015; Vallortigara, 2021). This leads the animals to approach stimuli that are more likely to be social partners (Di Giorgio et al., 2017; Vallortigara, 2012).

Colours are unequally attractive for domestic chicks. While blue and orange are preferred over yellow and green, red is the colour chicks like the most (Ham & Osorio, 2007; Kovach, 1971; Salzen, Lily, & McKeown, 1971; Schaefer & Hess, 2010). Interestingly, red is predominant on the fowl's faces - a zone the chicks are drawn to. In experiments carried out by Johnson and Horn (1988; Figure 2), chicks had to choose between an intact model of a jungle fowl and increasingly degraded versions of the same model. Overall, the chicks showed a preference for the unchanged model except when the degraded version of the jungle fowl maintained the head-neck structure. This was also true when a stuffed gadwall duck and a polecat model was used (Figure 2). Rosa-Salva et al. (2019) recently reproduced a similar version of this experiment and showed similar results except that no preference was found to approach the polecat (Figure 2). Altogether, these results suggest that the head region is relevant to the chick's predispositions. In fact, two days old naïve chicks spontaneously prefer a set of three dots arranged as a face (Rosa-Salva, Regolin, & Vallortigara, 2010; Figure 2).

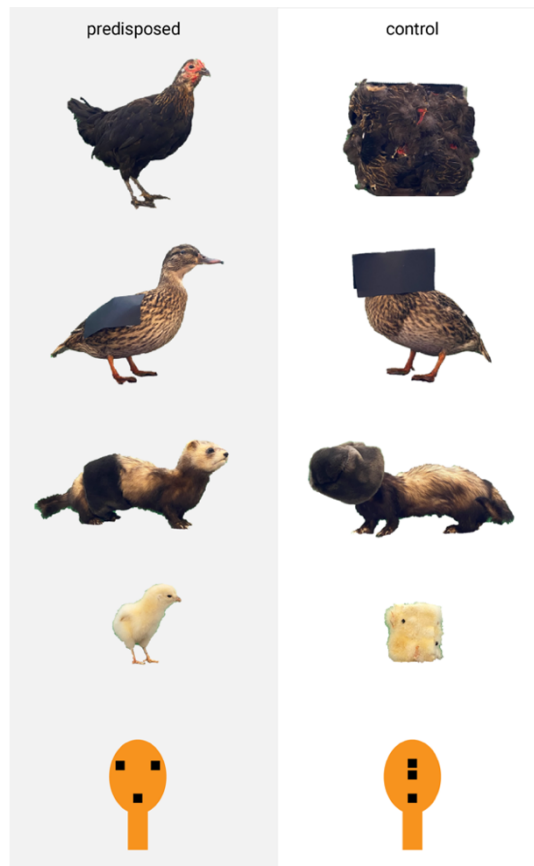


Figure 2: Pairs of physical stimuli used to test for social predispositions. For each pair of stimuli, the object spontaneously preferred by visually naïve chicks is presented in the left column.

Motion is very effective in attracting the attention of naïve domestic chicks (Cate, 1989; Hoffman, 1978). As for the arrangements of static features (e.g. face-like configuration), dynamic ones can convey animacy perception and make simple objects look alive (Heider & Simmel, 1944).

Over the recent years, many spontaneous preferences for motion dynamics have been described in domestic chicks (Mascalzoni, Regolin, & Vallortigara, 2010; Rosa-Salva et al., 2016, 2018; Vallortigara, Regolin, & Marconato, 2005). Vallortigara et al. (2005) initially discovered that naïve chicks prefer point-light displays representing semi-rigid biological motion (Figure 3). In such patterns, points located on the top of the animal move according to others and perfectly mimic the animal's activity. Moreover, the preference observed is not species-specific. Chicks exhibit a spontaneous preference for the biological motion of other vertebrates, even when it mimics the motion pattern of a potential predator. Miura and Matsushima (2012) reported a spontaneous preference for biological motion too, but sex differences were apparent. Males

spontaneously preferred biological motion, unlike females. A slight difference that could be explained by the use of a different chick strain.

Simplified motion arrangements and stimuli can convey animacy perception. This is the case of changing-state objects – objects that can accelerate, decelerate and rotate autonomously as if they had inner energy. Chicks prefer to imprint on objects that start to move on their own rather than with objects being pushed in motion after a collision (Mascalzoni et al., 2010). Several other experiments demonstrated that naïve domestic chicks exhibit a spontaneous preference for a self-propelled object rather than an object moving at a constant speed (Lorenzi et al., 2021; Rosa-Salva et al., 2016; Versace, Ragusa, & Vallortigara, 2019; Figure 3). Domestic chicks also favour objects that rotate with their main body axis (Rosa-Salva et al., 2018; Figure 3). The velocity of an object itself is an important cue. In humans, objects that move faster are more often considered animate than slower objects (Szego & Rutherford, 2007). By looking at imprinting's early works, chicks tend to prefer objects that move (or flicker) faster (James, 1959, 1960a, 1960b; Sluckin & Salzen, 1961). This fits with the idea that chicks spontaneously prefer motion patterns that look more animate, but a recent study showed the opposite (Wood, 2017). In this study, chicks exhibited a strong preference for slowly rotating objects. Therefore, in the second chapter, we replicate Wood's experimental design and re-investigate whether domestic chicks prefer slow- rather than fast-rotating objects.

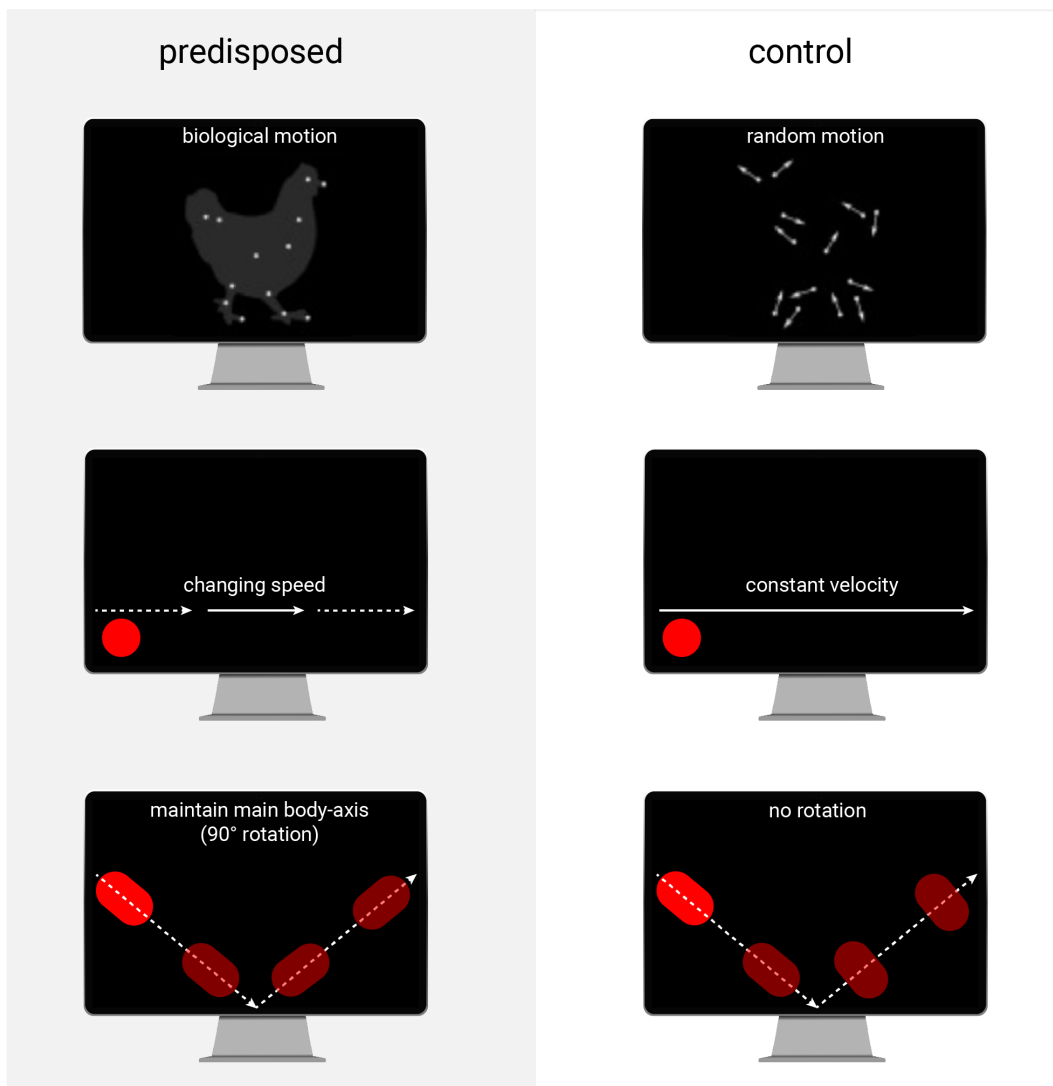


Figure 3: Pair of dynamic stimuli displayed on monitor screens and used to test for social predispositions. For each pair of stimuli, the object spontaneously preferred by visually naïve chicks is presented in the left column.

Motion is powerful at vehiculating animacy perception because it drives action (Blakemore & Decety, 2001). Therefore, specific motion pattern can mimic the action of living animals. This is the case when a stimulus follows another one as a predator would pursue its prey. In this context, the predator adapts its trajectory in relation to the prey trying to escape. At this moment, both the predator and the prey motion are temporally and spatially linked. Being predisposed towards motion patterns with spatiotemporal contingencies could therefore be an important animacy cue. Children around 8 to 10 months are particularly interested with spatiotemporal contingencies (Rochat, Striano, & Morgan, 2004). When observing two dots engaged in a chasing pattern – one dot following another – children expect the chaser to continue chasing the target and are

very surprised if the chaser becomes chased by the target (Rochat et al., 2004). Dogs, pigeons and squirrel monkeys can also use spatiotemporal contingencies to take decisions (Abdai, Baño Terencio, & Miklósi, 2017; Atsumi & Nagasaka, 2015; Goto, Lea, & Dittrich, 2002). Whether this ability is available at birth or develops through experience remain to be determined. In the second chapter, we investigate whether domestic chicks spontaneously prefer motion patterns where the agent's movements are temporally and spatially linked.

Most of the preferences for animacy features described above are shared with other species. Human neonates exhibit preferences for face-like configuration (Buiatti et al., 2019; Di Giorgio et al., 2016; Goren, Sarty, & Wu, 1975; Johnson et al., 1991; Simion & Di Giorgio, 2015), biological motion (Simion, Regolin, & Bulf, 2008) and changing-state objects (Hernik, Fearon, & Csibra, 2014). Monkeys and tortoise hatchlings also show a preference for faces (Sugita, 2008; Versace, Damini, & Stancher, 2020). Those common preferences suggest shared mechanisms and demonstrate the importance of studying precocial species such as the domestic chick in a comparative perspective (Di Giorgio et al., 2017; Versace & Vallortigara, 2015).

Chicks show spontaneous preferences for both static and dynamic features shared by living animals. Those predispositions direct the chick's attention toward the right kind of stimuli from which the animal would benefit the most. Hence, it is anticipated that chicks might prefer to approach novel stimuli to explore their characteristics/features. Versace et al. (2017) found that while in the first five minutes of visual experience, three different breeds had a preference for a stuffed hen, five minutes later, two breeds had already started to explore the other stimulus. The predispositions canalise the chicks' attention and affect the acquisition of the imprinting memory (Miura & Matsushima, 2016; van Kampen, de Haan, & de Vos, 1994). Miura and collaborators (2020) found evidence that imprinting was more robust with a specific arrangement of stimuli. While exposed to a walking-hen animation in red (point-light) and a linear one in yellow, chicks developed a learned preference for the former. When the motion-colours were swapped, the chick did not form any preference. When exposed to multiples objects, secondary imprinting can occur. Usually, chicks prefer the first stimulus they are exposed to, but

they can change their imprinting preferences if exposed to another object for a prolonged duration (Salzen & Meyer, 1968). In contrast, imprinting is irreversible if chicks are exposed to a stimulus more relevant for their predispositions (such as a live hen) in the first place (Boakes & Panter, 1985; Bolhuis & Trooster, 1988). This suggests a close relationship between filial preferences, predispositions and the closing of the imprinting phenomenon: the topic of the next section.

Sensitive periods for imprinting and social predispositions

Sensitive periods for imprinting

The capacity of the brain to adapt and change with its environment for a given time window is defined as a sensitive period (Dehorter & Del Pino, 2020; Hensch, 2005).

Filial imprinting occurs within a sensitive period and is a self-terminating process (Bolhuis, 1991). At the behaviour level, it begins when the young birds start following an object and terminates when they fear novel ones (Bolhuis, 1991). The duration of the sensitive period varies greatly (Bateson, 1966; Bolhuis, 1991; Sluckin, 1972). While Yamaguchi et al. (2012) reported that 4-day-old domestic chicks could not imprint, other studies reported imprinting up to 7 days after hatching (Case & Graves, 1978; Sluckin, 1972). The use of different strains and different experimental conditions can explain this variability. Moreover, both pre-hatch and post-hatch experiences influence the timing of the sensitive period for imprinting (Dimond, 1968; Landsberg, 1976; Simner, 1973).

Several changes occur at the neurophysiological level, leading to the opening and closing of the sensitive period for imprinting (Bateson, 1983; Knudsen, 2004). The thyroid hormone T3 – 3,5,3'-triiodothyronine – strongly affects imprinting and its sensitive period. In domestic chicks, thyroid hormones (T4 and T3) peak around hatching and decrease gradually (Lu, McMurtry, & Coon, 2007; McNabb, 2006). T3 controls the timing of the sensitive period and has a quick action. Inhibiting it impairs visual imprinting 30 minutes later, whereas injecting it re-opens and even extends its sensitive period up to 8 days after hatching (Yamaguchi et al., 2012). Moreover, T3 brain

level - converted from plasma thyroxine (T4) by Dio2 - correlates with imprinting strength (Yamaguchi et al., 2012). Imprinting leads to an increase in T3 in the IMM after - a brain region implicated in filial imprinting (Horn, 2004; Horn, Bradley, & McCabe, 1985; McCabe, 2013; Solomon & McCabe, 2015). Within IMM, the expression of GABA_A-GABA_B receptors directs the opening and closing of the sensitive period downstream to T3 (Aoki et al., 2018). While GABA_B initiates imprinting, GABA_A contributes to its termination.

Steroid hormones influence the sensitive period too. Testosterone and its metabolites are implicated in closing the sensitive period in domestic chicks (Gvoryahu et al., 1986). Corticosterone seems to have a similar effect in ducklings (Weiss, Köhler, & Landsberg, 1977). The sensitive period for imprinting is also influenced by pharmacological agents such as sodium pentobarbital, ketamine and meprobamate (MacDonald, 1968; Parsons & Rogers, 1997, 2000). Those anaesthetic drugs either affect GABA_A or NMDA receptors which are implicated in the development of the imprinting memory (Solomon & McCabe, 2015).

Sensitive periods of predispositions

Filial imprinting is not the only phenomenon to occur during a specific timing (Gervain et al., 2013; Hensch & Quinlan, 2018). Predispositions are observed for a limited period too.

Similar to filial imprinting, predispositions are not present throughout the young bird's life. Predispositions seem to appear and disappear with precise timing (Rosa-Salva et al., 2015). The predispositions often emerge once the chicks have received activating experiences (exposure to visual pattern, motoric activity, handling, acoustic stimulation). For example, chicks visually and or motorically stimulated between 24 and 36 hours after hatching will express a spontaneous preference for a stuffed hen between 2 and 24 hours after the activating experience (Bolhuis & Horn, 1997; Bolhuis, Johnson, & Horn, 1985, 1989; Davies, Johnson, & Horn, 1992; Johnson, Davies, & Horn, 1989). Preference for biological motion also requires activating experience (Miura & Matsushima, 2012; Vallortigara et al., 2005), but it is detectable 30 minutes after

receiving the activating experience. The preference for biological motion persists one day after being activated (post-hatching day 2) but fades away on post-hatching day 5 (Miura et al., 2020). A sensitive period for a self-propelled object has also been described: the preference is observed on the hatching day and disappears three days after (Versace et al., 2019).

While there is evidence that noradrenaline antagonist or the anaesthetic equithisin delays the activating experiences' onset (Bolhuis & Horn, 1997; Davies et al., 1992), the neurophysiological mechanisms controlling the sensitive period for predispositions remain unexplored. In the previous section, I described how predispositions and filial imprinting are interconnected mechanisms. They strongly influence each other, and both have a sensitive period around hatching. It is therefore conceivable that their sensitive periods share molecular grounds. In the last chapter of this thesis, we investigate whether the sensitive period for predispositions is controlled by the thyroid hormone T3, such as filial imprinting.

Aim of the thesis

The present work aimed to investigate filial preferences and social predispositions across time using behavioural techniques in domestic chicks. I start by describing the general methods and the two experimental setups used across all the studies presented here. One setup was built from scratch and fully automated to allow behavioural measurements across long testing duration, while the other is commonly used to investigate social predispositions. The first chapter explores the stability and the individual variability of filial imprinting preferences across several days. The second chapter investigates social predispositions in domestic chicks using moving stimuli. I first replicate a study where Wood (2017) described a preference for slowly rotating object: quite an unexpected finding considering the general pattern of predispositions found in domestic chicks until now. Then I describe a second study where I investigate whether domestic chicks have a spontaneous preference for a motion pattern that mimics social aggregation: a motion dynamic that contains spatiotemporal contingencies. Finally, in the third chapter, I describe a study investigating whether the

thyroid hormone (T3) controls the sensitive period for social predispositions as in filial imprinting.

GENERAL METHODS

I employed similar methods and apparatuses for all the studies of this thesis. Here, I describe the general methods and the two setups used.

Ethics & Animals

All the experiments were carried out in compliance with the European Union and the Italian law on the treatment of animals. The experimental procedures were approved by the Ethical Committee of the University of Trento and licenced by the Italian Health Ministry (permit number 53/2020 and 1139/2015). When possible, chicks were donated to local farms at the end of the experiments.

We used domestic chicks of the strain Ross 308 as it shows a sexual dimorphism located on the feathers at birth and allowed sexing. Sexing is necessary as the difference in the direction of responses of male and female chicks have been reported in several laboratories (Miura & Matsushima, 2012; Vallortigara et al., 1990; Vallortigara, 1992; Versace et al., 2017). The eggs were collected from a commercial hatchery (Azienda Agricola Crescenti) and incubated at the laboratory in complete darkness. From day 1 to day 19, the eggs were kept at 37.7°C and 40 % of humidity. From day 19 to 21, the eggs were kept at 37.7°C and 60 % of humidity. All the animals were visually naïve before starting the experiments.

Automated setup

The automated setup allowed to keep the animal in a testing environment for long duration (up to 6 days). Nine automated setups (Figure 4) were built to allow simultaneous testing. Each apparatus had a rectangular shape (90 cm x 60 cm x 60 cm, Figure 5) with a high frequency screen located on both smaller extremities (ASUS MG248QR, 120 Hz). A Microsoft life camera overhung each apparatus and recorded the animal location. As the chicks remained in the apparatus for long duration, food and water were available ad libitum in the middle of each apparatus.

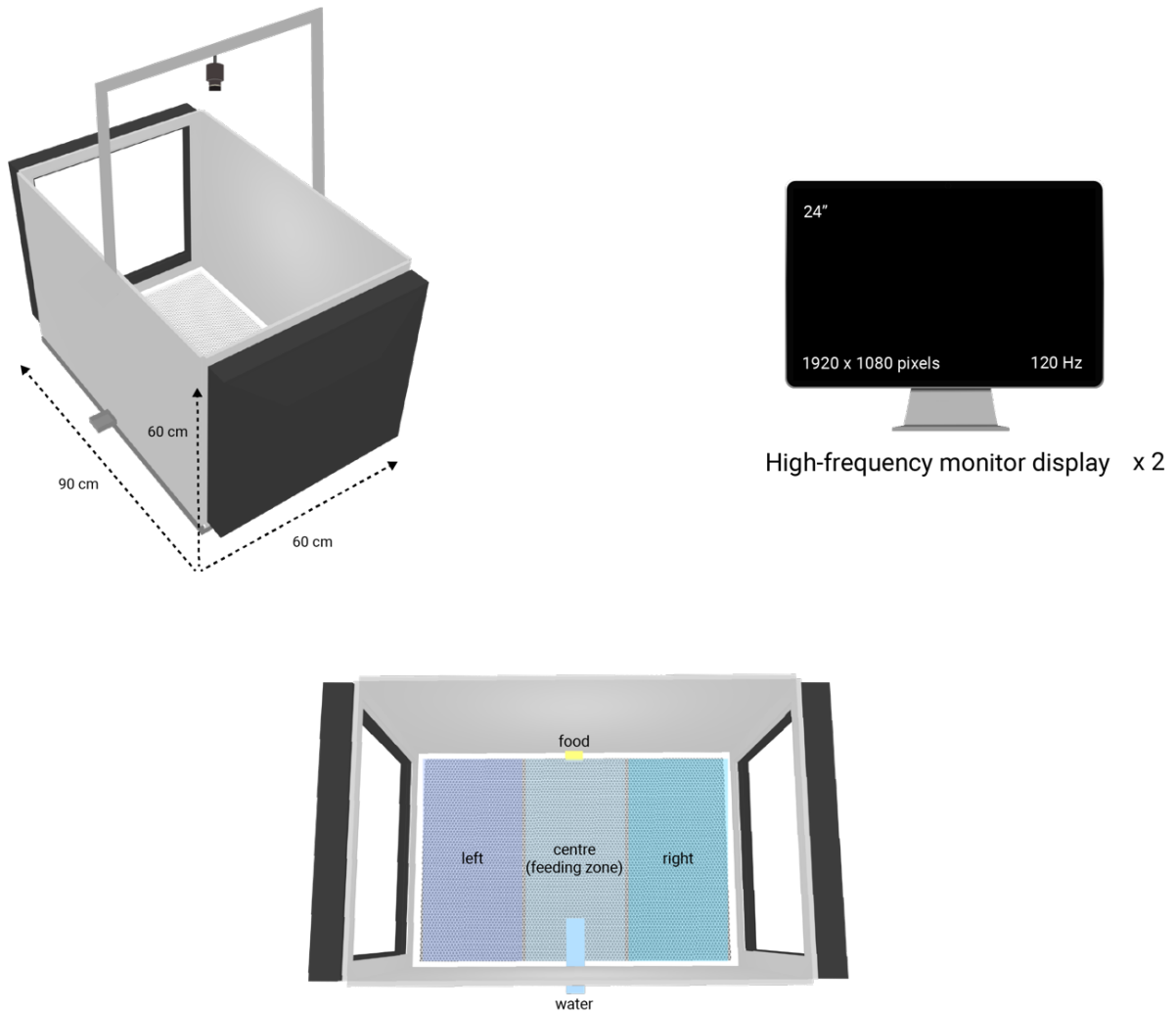


Figure 4: Three-dimensional representation of the automated setup. Two high-frequency screens are located at both ends and are used to display stimuli. The animal behaviour is tracked with an overhung camera which allows to follow the animal position within the automated setup. The time [seconds] spent by the animal in three equivalent zones (left, centre and right) is extracted computationally and translated into a preference index for a specific stimulus. Nine apparatus were used at the same time.

Traditional setup

The 'traditional' setup was built to test the animals for short duration (up to 10 minutes; Figure 5). It had been already used in several studies investigating the spontaneous preferences of domestic chicks (Lorenzi et al., 2020; Rosa-Salva et al., 2016; Versace, Ragusa, & Vallortigara, 2019). It consisted of a simple runway composed of a central zone and two sur-elevated platforms facing high-frequency screens (Asus MG248QR, 120 Hz, Figure 5). As the automated setup, a video overhung the apparatus to record the animal's location.

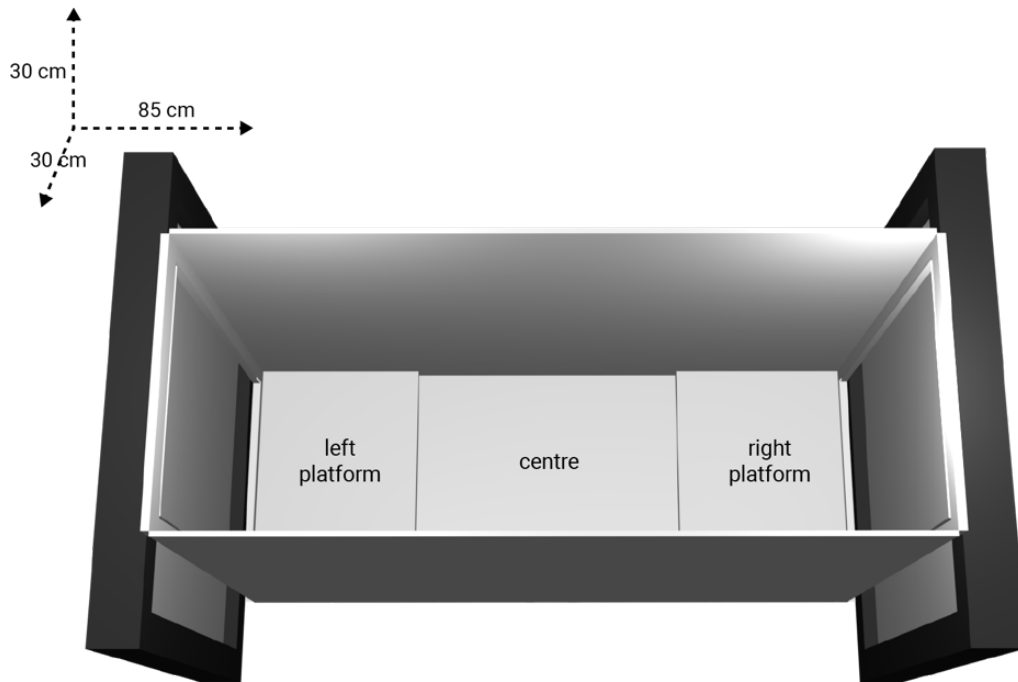


Figure 5: Three-dimensional representation of the traditional setup. Two elevated platforms (20 cm long and 1.5 cm high) give on two high-frequency screens displaying the stimuli. As in the automated setup, a camera overhung the apparatus and allows to follow the animal behaviour. The time [seconds] on each platform is scored by an experimenter as soon as the chicks climbed on the platform and translated into a preference index for a specific stimulus.

Data extraction

Both setups aimed at measuring the animal's preference toward a particular stimulus located on a screen. The position of the stimuli on the screens was counterbalanced across animals and repeated testing sessions (such as usually performed in the automated setup). We used the animal location within the cage to determine its preference toward a specific stimulus. Therefore, both setups were divided into different zones: one in the centre and two on the sides close to the screens where the stimuli were displayed.

In the automated setup, we computationally extracted the data and virtually defined the zones delimitations (Figure 4). In contrast, the zones of the traditional setup were physically designed by the lateral platforms (Figure 5). During the test, an experimenter measured the time spent by the chicks on each platform using a Matlab chronometer.

Both data extraction methods provided us with the time [seconds] spent by the animal in the different zones. The time spent in the central zone was considered as no choice. Therefore, chicks remaining in the apparatus centre for the whole test duration were removed from further analyses. The time spent on the lateral zones was used to calculate a ratio indicating a preference for a particular stimulus.

Statistical analyses

All statistical analyses were performed using RStudio (RStudio Team, 2015). The following packages were used: *gofstest* (Faraway, Marsaglia, Marsaglia, & Baddeley, 2019), *nlme* (Pinheiro et al., 2020), *lme* (Bates et al., 2015), *tidyr* (Wickham & Lionel, 2020), *plyr* (Wickham, 2011), *dplyr* (Wickham et al., 2020), *reshape* (Wickham, 2007), *lsr* (Navarro, 2015), *ggplot2* (Wickham, 2016), *reshape* (Hadley Wickham, 2007), *cowplot* (Wilke, 2020), *ez* (Lawrence, 2016), *emmeans* (Lenth, 2021), *broom* (Robinson, Hayes, & Couch, 2021), *purrr* (Henry & Wickham, 2020), *pupillometryR* (Forbes, 2020). Cohen's *d* (*d*) was reported when possible. The number of subjects required in each experiment was a priori determined with a power analysis (Champely, 2020). Details are provided in the statistical analyses section of each experiment. All the datasets used for the data analyses are available on [figShare](#).

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Lemaire, B.S., Rucco, D., Josserand, M. et al. Stability and individual variability of social attachment in imprinting. *Sci Rep* 11, 7914 (2021).
<https://doi.org/10.1038/s41598-021-86989-3>

Introduction

The development of filial preferences has been well described at an early stage (Bolhuis, 1991; McCabe, 2019). However, how these preferences develop and vary within days have been poorly documented. Filial imprinting preferences are influenced by two main factors: the predispositions and experiences. In this study, we investigated how these two variables affect the development and stability of filial preferences over time. For this purpose, we used the automated setup described in the previous section. We followed the animal's behaviour for six entire days and manipulated the objects with which the animals were imprinted. We used objects different in colours and shapes (a green hourglass and a blue cube) so that chicks will find them differently attractive. From the literature, chicks seem to prefer blue over green (Kovach, 1971; Salzen et al., 1971; Schaefer & Hess, 2010). Therefore, we used a blue and a green object as imprinting stimuli. The exposure duration to those objects was manipulated from one (experiment 1, 3 and 4) to three days (experiment 2), and the preferences for the imprinting object against a novel one monitored from three (experiment 2, 3 and 4) to five days (experiment 1). The automated setup (Figure 4) combined with the longitudinal aspect of this study allowed us to investigate how filial preferences developed in time at the group and individual level.

General procedure

After hatching, the visually naïve chicks were sexed in darkness and transported in the automated setups for six days in a day-night cycle (14-10 hours). During the night period, the screens were turned black. During the day, chicks were exposed to different stimuli. In this experiment series, we used three-dimensional virtual visual stimuli created, animated and rendered on Blender (v 2.79). The objects were different in term of colours and shapes. We created a green hourglass (hex: 30B619), a blue cube (hex: 2EBAFF) and a pink cylinder (hex: C33CDB). The objects had similar sizes (5 cm x 5 cm, see Figure 6; Figure 8; Figure 10; Figure 12) and were exported with a high frame frequency (120 frames per second, fps) as they were crossing the screen linearly from left to right (4.5 seconds). The exposure duration to these stimuli was scattered into sessions of different duration depending on the experimental design (from 7 sessions of 2 hours to 24 sessions of 30 minutes).

Experiment 1

Methods

Procedure

This experiment was divided into two phases (Figure 6). Chicks were first exposed to the blue cube (blue condition) or green hourglass (green condition) for one day (7 sessions of 2 hours, imprinting phase). We used different imprinting objects (condition) to test the effect of predispositions on the development and stability of filial preferences. Following the imprinting phase, we tested the animal preference toward its imprinting object for five consecutive days (24 sessions of 30 minutes per day, testing phase). In this phase, the chicks were exposed to two stimuli, their imprinting object and

an unfamiliar one (in the blue condition, the imprinting stimulus was the blue cube, and the unfamiliar stimulus was the green hourglass; vice versa for the green condition).

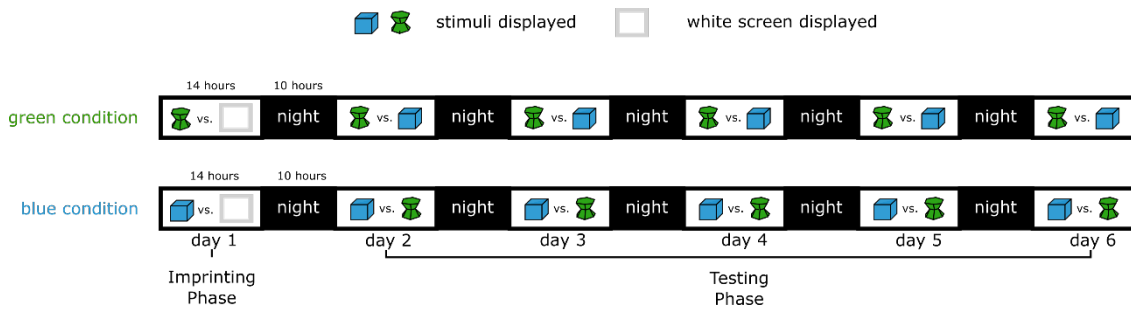


Figure 6: Experimental timeline of experiment 1. Chicks of both conditions are exposed to their imprinting object for one day. After that, chicks are exposed to their imprinting and a novel object for five days.

Animals

We imprinted 16 animals (8 females, 8 males) with the green hourglass (green condition) and 16 animals (8 females, 8 males) with the blue cube (blue condition).

Data analyses

During the imprinting phase, we analyse the number of seconds [s] spent close to the stimulus.

During the testing phase, the Preference for the imprinting stimulus [%] was calculated using the following formula:

$$\text{Preference for imprinting object} = \frac{\text{time spent by the imprinting object}}{\text{time spent by the two objects}} \times 100$$

A score higher than 50% indicated a preference for the imprinting object. A score lower than 50 % indicated a preference for the unfamiliar stimulus. A score of 50 % indicated no preference for either stimulus.

Statistical analyses

The number of subjects required in each group was a priori determined with an effect size of 1.5 and an alpha of 0.05. Eight individuals per group were required to achieve a power of 0.80.

To assess the time spent by the chicks close to the imprinting stimulus during the imprinting phase, we used an ANOVA with time (seconds) spent close to the imprinting stimulus as a dependent variable and Condition (imprinted with green, imprinted with blue) and Sex (female, male) as between-subjects factors; Day was a within-subjects factor. The parametric analyses assumptions were met.

At test, to determine whether chicks had different preferences for the imprinting stimulus between Condition (imprinted with green, imprinted with blue), Sex (female, male) and Day (day 2, 3, 4, 5, 6), we performed a mixed-design ANOVA for each testing phase. To meet parametric analysis assumptions (visualised using Q-Q plots), we arcsin transformed the data. To check whether chicks had a significant preference for the imprinting stimulus or unfamiliar stimulus, we performed two-tailed one-sample t-tests to check for significant departures from chance level (50%). Since the chicks underwent several imprinting and testing sessions across testing days, it was possible to test their preference individually. Therefore, individual preferences were assessed and compared from chance-level (50%) using two-tailed one-sample t-tests. Levene's test was conducted to explore chicks' variability between conditions.

Results & discussion

Imprinting

The ANOVA did not reveal any effect of Condition (imprinted with a green hourglass or imprinted with a blue cube; $F_{(1, 28)} = 0.57, p = 0.46$), Sex ($F_{(1, 28)} = 0.18, p = 0.67$) or interaction Sex x Condition, $F_{(1, 28)} = 0.14, p = 0.71$) on the time spent close to the imprinting stimulus. The chicks significantly remained closer as they spent 96% of their time (± 0.56 SEM) close to the imprinting stimulus ($t_{(31)} = 83.25, p < 0.001$, Cohen's $d = 14.72$).

All chicks (32) followed and remained significantly more on the side of the arena in which the imprinting stimulus was displayed.

Testing

The results are shown in Figure 7. The ANOVA revealed a significant interaction between Day and Condition on the Preference for the imprinting stimulus ($F_{(4, 112)} = 2.69, p < 0.05$) but no effect of Condition ($F_{(1, 28)} = 0.89, p = 0.37$), Sex ($F_{(1, 28)} = 0.50, p = 0.49$), Day ($F_{(4, 112)} = 1.06, p = 0.38$) or other interactions (Sex x Condition $F_{(1, 28)} = 0.009, p = 0.93$; Sex x Day, $F_{(4, 112)} = 0.28, p = 0.89$; Sex x Condition x Day, $F_{(4, 112)} = 0.40, p = 0.81$). The Post hoc analysis (Tukey) showed that the preference for the imprinting stimulus observed on day 2 was significantly different from the preference observed on day 4 in the green condition ($t_{(112)} = 3.52, p < 0.05$, Cohen's $d = 0.74$). On day 2, chicks had a significant preference for the imprinting stimulus ($t_{(15)} = 4.45, p < 0.001$, Cohen's $d = 1.12$). They spent 65% (+/- 3.31 SEM) of their time close to it. However, on day 4, chicks had no preference ($t_{(15)} = 0.33, p = 0.75$, Cohen's $d = 0.082$) and spent 52% (+/- 5.26 SEM) of their time close to their imprinting stimulus. The post hoc test did not reveal other differences. Chicks imprinted with the blue stimulus had a significant and stable preference for the imprinting stimulus ($t_{(15)} = 3.83, p < 0.01$, Cohen's $d = 0.96$) and spent 62% (+/- 3.23 SEM) of their time close to it.

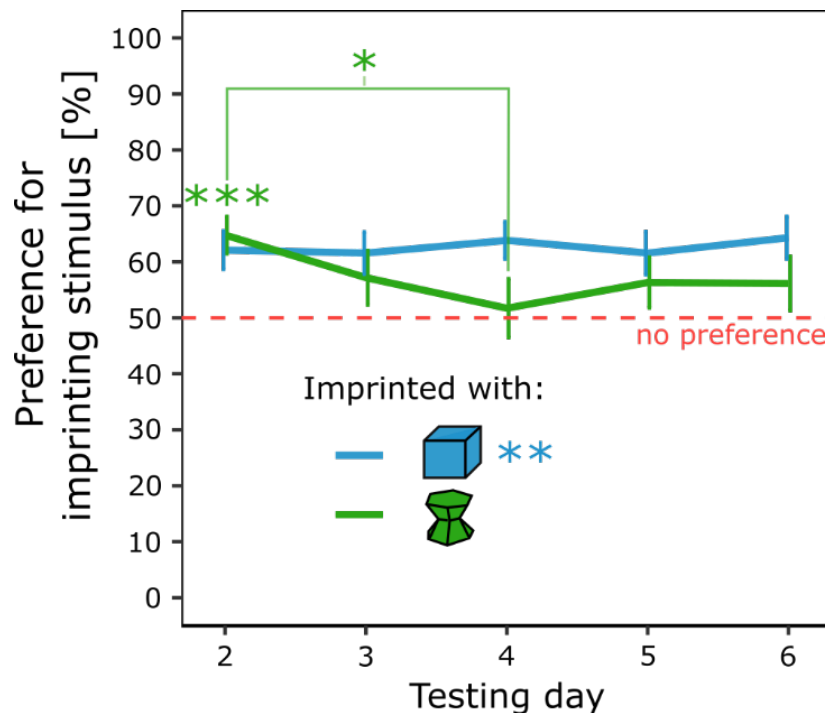


Figure 7: Preference for the imprinting stimulus (vs. novel object) after 1 day of exposure to it between conditions and testing days. The blue line represents the chicks imprinted with the blue cube (the blue asterisk shows the statistical

*significance of this group; $p < 0.01$, **). The green line represents the chicks imprinted with the green hourglass (the green asterisks show the statistical significance of this group; $p < 0.05$, *; $p < 0.001$, ***).*

In the blue condition, 10 chicks (63%) preferred the imprinting stimulus, 5 (31%) had no preference, and 1 (6%) preferred the unfamiliar stimulus. In the green condition, 7 chicks (44%) preferred the imprinting stimulus, 6 (37%) had no preference, and 3 (19%) preferred the unfamiliar stimulus. Levene's test showed that the variances of the two conditions were similar ($F_{(1, 30)} = 0.32, p = 0.86$).

As shown by the results, the filial preferences were disparate between conditions and time, although the chicks received equal exposure to their imprinting object and spent a similar amount of time close to it. While chicks of the green condition lost their preference for their imprinting object and explored more the unfamiliar object with time, chicks of the blue condition had a robust and stable preference.

Our result confirms previous reports of an advantage of blue over green imprinting stimuli (Kovach, 1971; Salzen et al., 1971; Schaefer & Hess, 2010). It also demonstrates that 1 day of exposure with an imprinting object is insufficient to produce a lasting imprinting preference using artificial stimuli. After one day of imprinting, the predispositions still influence the animals' filial preferences and lead the chicks of the green conditions to approach the unfamiliar stimulus more often: the blue stimulus. In such condition – with repeated exposure to stimuli – secondary imprinting might take place with the blue stimulus in the green-imprinted group.

The difference between blue and green-imprinted chicks is also apparent looking at their individual performances. In the blue condition, more than half of the chicks preferred the imprinting stimulus, and only one chick preferred the novel stimulus. In contrast, in the green condition, less than half of the chicks preferred the imprinting stimulus, and three chicks preferred the novel stimulus. Moreover, the analysis of individual behaviours revealed that some chicks had consistent preferences for unfamiliar stimuli not only at the very beginning of imprinting, as hypothesised by Bateson's model (Bateson, 1973).

Fifteen hours after the start of imprinting, several biochemical changes are still at play at neural level (Solomonica & McCabe, 2015). Here, after 14 hours of exposure to a stimulus, the imprinting memories appear available but not fully consolidated yet and probably more plastic when imprinted with less predisposed stimuli. Miura et al. (2016, 2020) already described that the animal predispositions strongly affect the imprinting memory. We confirm this here by showing that the same experience (with different stimuli) produces different learning outcomes. The predispositions affected both learning and the between-subjects variability in learning. Learning appears faster, stronger and less variable with predisposed stimuli.

In a second experiment, we increased the imprinting duration to three days and investigated whether the predispositions still affect the filial imprinting.

Experiment 2

Similar methods and procedure were used in this experiment except that the imprinting duration was increased to three days (7 sessions of 2 hours on the first imprinting day and 13 sessions of 1 hour on the second and third imprinting day; Figure 8).

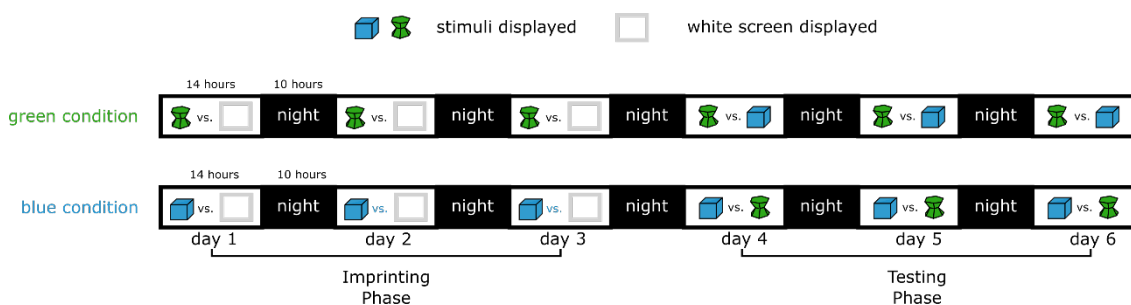


Figure 8: Experimental timeline of experiment 2. Chicks of both conditions are exposed to their imprinting object for three days. After that, chicks are exposed to their imprinting and a novel object for three days.

We imprinted 16 animals (8 females, 8 males) with the green hourglass (green condition) and 16 animals (8 females, 8 males) with the blue cube (blue condition) and performed a similar statistical analysis.

Results & discussion

Imprinting

The ANOVA did not reveal any effect of Condition ($F_{(1, 28)} = 1.15, p = 0.29$), Sex ($F_{(1, 28)} = 0.002, p = 0.97$) or interaction (Sex x Condition, $F_{(1, 28)} = 3.3, p = 0.08$) on the time spent close to the imprinting stimulus. The trend revealed above was induced by an opposite pattern between males and females within each condition with small variances. Nonetheless, the time spent close to the imprinting stimulus between each group was similar. Overall, the chicks significantly remained 93% of their time (± 0.46 SEM) close to the imprinting stimulus ($t_{(31)} = 49.92, p < 0.001$, Cohen's $d = 8.82$).

All chicks (32) chose significantly more the side of the arena, where the imprinting stimulus was displayed.

Testing

The results are shown in Figure 9. The ANOVA did not reveal any effect of Condition ($F_{(1, 28)} = 2.90, p = 0.10$), Sex ($F_{(1, 28)} = 2.12, p = 0.16$), Day ($F_{(2, 56)} = 0.63, p = 0.54$) or interactions (Sex x Condition, $F_{(1, 28)} = 0.003, p = 1.0$; Sex x Day, $F_{(2, 56)} = 0.05, p = 0.95$, Condition x Day, $F_{(2, 56)} = 0.46, p = 0.63$; Sex x Condition x Day, $F_{(2, 56)} = 1.52, p = 0.23$) on the Preference for the imprinting stimulus. The preference for the imprinting stimulus was significantly different from chance-level ($t_{(31)} = 6.58, p < 0.001$, Cohen's $d = 1.16$). The chicks spent 69% (± 2.90 SEM) of their time close to their imprinting stimulus.

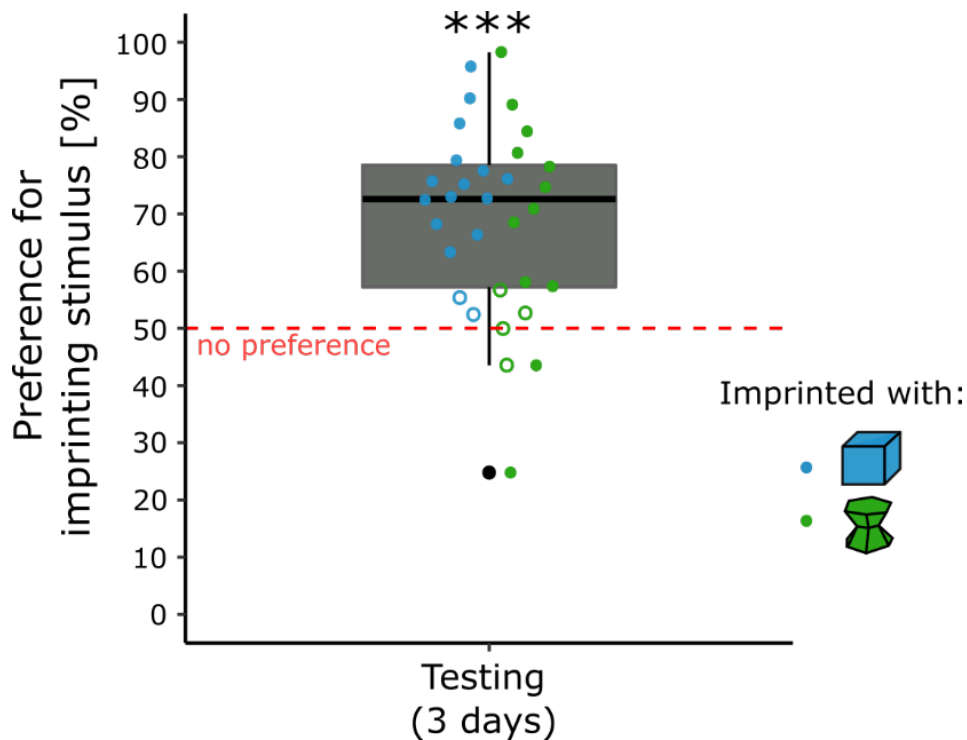


Figure 9: Overall preference for the imprinting stimulus (vs. novel object) after 3 days of exposure to it ($p < 0.001$, ***). The blue dots represent the individual preference score of the chicks imprinted with the blue cube. The green dots represent the individual preference score of the chicks imprinted with the green hourglass. Filled dots show the individual having a significant preference. Empty dots show the individual with no preference.

In the blue condition, 14 chicks (87.5%) preferred the imprinting stimulus, 2 (12.5%) had no preference, and none preferred the unfamiliar stimulus. In the green condition, 10 chicks (62.5%) had a significant preference for the imprinting stimulus, 4 (25%) had no preference, and 2 (12.5%) had a significant preference for the unfamiliar stimulus. Levene's test showed that the variances of the two conditions were significantly different ($F(1, 30) = 6.14, p < 0.05$). Chicks imprinted with the green stimulus showed higher variability in their preferences for the imprinting stimulus during testing ($\sigma^2 = 380.85$) than chicks imprinted with the blue stimulus ($\sigma^2 = 129.91$).

By increasing the imprinting duration up to three days, the differences observed between conditions in the first experiment faded away. Here the chicks of both conditions showed a robust and stable preference for their imprinting objects. In the literature, it was already demonstrated that longer imprinting duration leads to stronger filial preference scores. However, we show that longer duration also influences the steadiness of the filial preferences. After three days of exposure to their respective

objects, the imprinting memory of both conditions appears consolidated. Nonetheless, animals' spontaneous preferences are still influencing chicks' filial preferences to a lower degree. In the green condition, the variability was three-time higher than in the blue condition. In the blue condition, almost all chicks showed a strong preference for their imprinting objects. In the green condition, more than a third (6 chicks out of 16) did not prefer their imprinting stimulus.

Our finding suggests that prolonged exposure to an object leads to more stable preferences. This is convincing and in line with previous evidence (Bateson & Jaeckel, 1976; Bolhuis et al., 2000). Nevertheless, the ontogenetic stage at which the preferences were tested could have influenced filial preferences.

Experiment 3

To investigate whether the ontogenetic stage at which the animals were tested influenced the filial preferences described in the previous experiment, we imprinted our subjects for one day as in experiment 1. Then, we tested their preference on day 4, 5 and 6 such as in experiment 2. To prevent a complete social deprivation from day 2 to day 3, the animals were exposed to a new object (13 sessions of 1 hour per day; Figure 10) different in colour and shape: a pink cylinder (hex: C33CDB). For clarity, the first exposure to an object (blue or green) will now be called primary imprinting, and the second exposure to the pink cylinder will now be called secondary imprinting.



Figure 10: Experimental timeline of experiment 3. Chicks of both conditions are exposed to their primary imprinting object for one day. After that, both conditions are exposed to a secondary object for two days before being exposed to their primary imprinting and a novel object for three days.

We imprinted 16 animals (8 females, 8 males) with the green hourglass (green condition) and 17 animals (8 females, 9 males) with the blue cube (blue condition).

Results & discussion

Primary imprinting

The ANOVA did not reveal any effect of Condition ($F_{(1, 29)} = 0.52, p = 0.48$), Sex ($F_{(1, 29)} = 0.17, p = 0.69$) or interaction (Sex x Condition, $F_{(1, 29)} = 1.62, p = 0.21$) on the time spent close to the primary imprinting stimulus. The chicks significantly remained close the primary imprinting stimulus ($t_{(32)} = 87.18, p < 0.001$, Cohen's $d = 15.18$) 97% of their time (+/- 0.54 SEM).

All chicks (33) remained significantly more on the side of the arena, where the primary imprinting stimulus was displayed.

Secondary imprinting

The ANOVA did not reveal any effect of Condition on the time spent close to the secondary imprinting stimulus ($F_{(1, 29)} = 0.14, p = 0.72$), Sex ($F_{(1, 29)} = 0.49, p = 0.49$) or interaction (Sex x Condition, $F_{(1, 29)} = 0.70, p = 0.41$) on the time spent close to the secondary imprinting stimulus. The chicks significantly remained close the secondary imprinting stimulus ($t_{(32)} = 34.72, p < 0.001$, Cohen's $d = 6.04$) 93% of their time (+/- 1.25 SEM).

All the chicks (33) remained significantly more on the side of the arena, where the secondary imprinting stimulus was displayed.

Testing

The results are shown in Figure 11. The ANOVA revealed a significant effect of Condition ($F_{(1, 29)} = 70.35, p < 0.001$) but non-significant effects of Sex ($F_{(1, 28)} = 2.98, p = 0.095$), Day ($F_{(2, 58)} = 0.54, p = 0.59$) or interactions (Sex x Condition, $F_{(1, 29)} = 1.21, p = 0.28$; Sex x Day, $F_{(2, 58)} = 0.072, p = 0.93$, Condition x Day, $F_{(2, 58)} = 0.41, p = 0.67$; Sex x Condition x Day, $F_{(2, 58)} = 0.010, p = 0.10$) on the preference for the primary imprinting stimulus.

The preference for the primary imprinting stimulus was significantly different from chance level for the chicks imprinted with the blue stimulus ($t_{(16)} = 12.27, p < 0.001$, Cohen's $d = 2.98$, Bonferroni correction). Blue-imprinted chicks spent 83 % (+/- 2.66 SEM) of their time close to the primary imprinting stimulus. The Preference score was non-significantly different from chance level for the chicks imprinted with the green stimulus ($t_{(15)} = -1.94, p = 0.14$, Cohen's $d = 0.48$, Bonferroni correction). Green-imprinted chicks spent 42 % (+/- 3.90 SEM) of their time close to the primary imprinting stimulus.

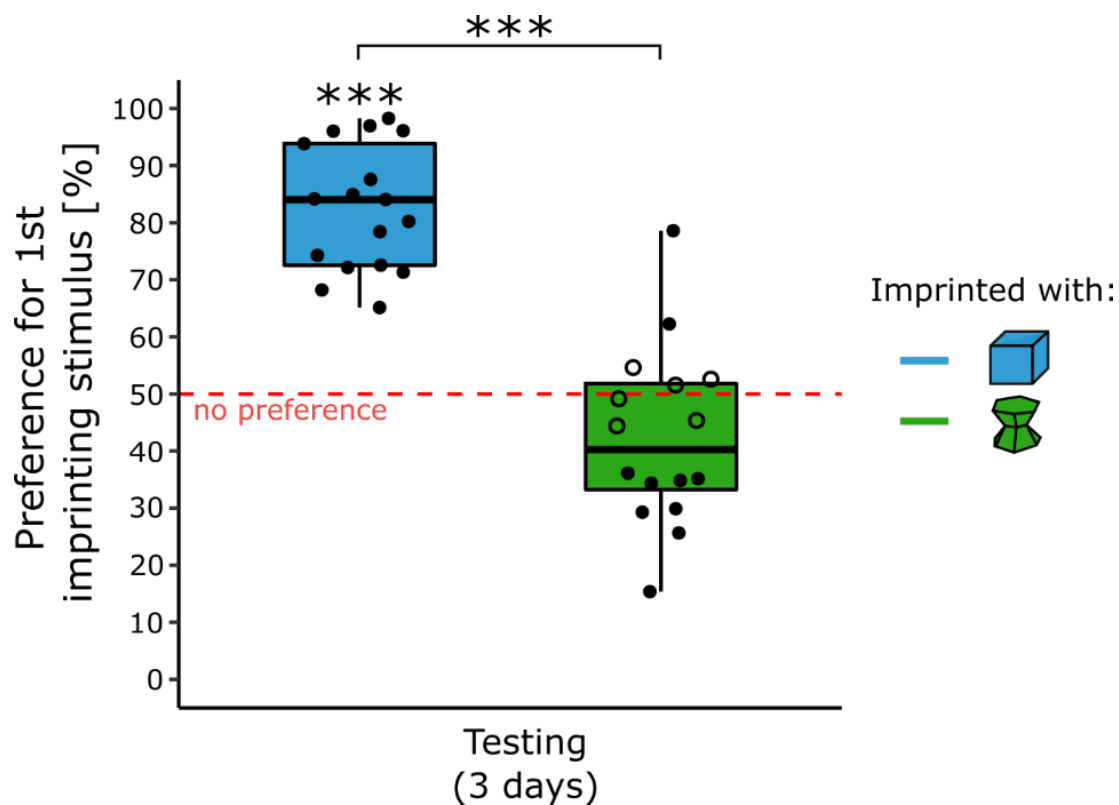


Figure 11: Overall preference for the primary imprinting stimulus (vs. novel object) after 1 day of exposure to it and 2 days of exposure to a secondary imprinting object between conditions. The blue box plot ($p < 0.001$, ***) represents the chicks imprinted with the blue cube. The green boxplot represents the chicks imprinted with the green hourglass. Filled dots show the individual having a significant preference. Empty dots show the individual with no preference.

All the chicks (17) preferred the imprinting stimulus while primary imprinted with the blue stimulus. Whereas for the chicks primarily imprinted with the green stimulus, 2 (13%) preferred their primary imprinting stimulus, 6 (37%) had no preference and 8 (50%) preferred the unfamiliar stimulus. Levene's test showed that the variances of the two conditions were similar ($F_{(1, 31)} = 1.45, p = 0.24$).

Similarly to what we observed in the first experiment (short imprinting duration), the filial preferences differ between conditions. In the blue condition, all the individuals preferred their primary imprinting object, showing that the memory of the primary imprinting stimulus lasted although chicks had been detached by the initial stimulus for days. At the same time, preferences among individuals of the green conditions were disparate with 2 individuals preferring the imprinting object, 6 showing no preferences and even 8 showing a preference for the novel object. The preferences observed here were not wholly similar to the first experiment. As the preferences were stable in time for both conditions one could argue that the filial preferences observed resulted from a lack of memory. However, the results showed two distinct patterns depending on the primary imprinting stimulus used. In the case of a memory loss, chicks would have either approached the more attractive stimulus (the blue cube here) or not chosen any but this is not what we observed. Furthermore, in the literature, chicks going through successive imprinting always showed recall of the primary imprinting object (Bolhuis & Bateson, 1990; Salzen & Meyer, 1968). To investigate whether chicks had imprinted on the secondary imprinting object, we ran another experiment.

Experiment 4

This time, we tested the animals' preferences for their primary vs. secondary imprinting object. We used a similar procedure and method to the third experiment but replaced the unfamiliar object displayed at testing with the secondary imprinting object (Figure 12).

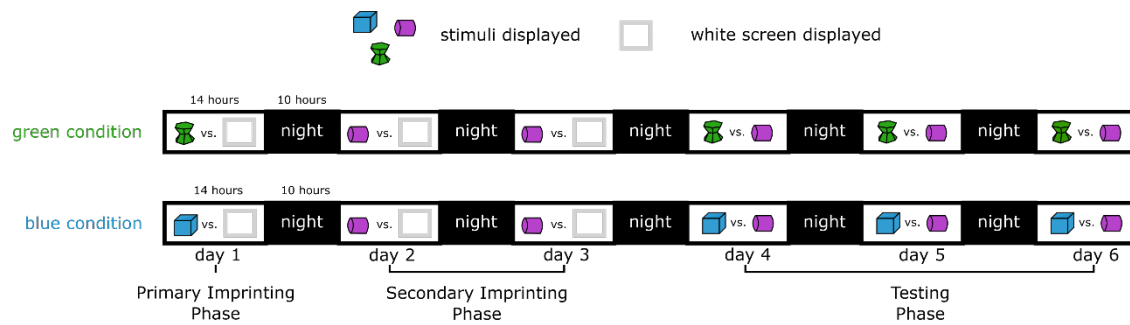


Figure 12: Experimental timeline of experiment 4. Chicks of both conditions are exposed to their primary imprinting object for one day. After that, both conditions are exposed to a secondary object for two days before being tested with their primary and secondary imprinting objects for three days.

We imprinted 16 animals (8 females, 8 males) with the green hourglass (green condition) and 17 animals (8 females, 9 males) with the blue cube (blue condition).

Results & discussion

Primary imprinting

The ANOVA did not reveal any effect of Condition ($F_{(1, 29)} = 3.44, p = 0.074$), Sex, ($F_{(1, 29)} = 0.50, p = 0.23$) or interaction (Sex x Condition, $F_{(1, 29)} = 0.10, p = 0.75$) on the time spent close to the primary imprinting stimulus. The chicks significantly remained close the primary imprinting stimulus ($t_{(32)} = 45.53, p < 0.001$, Cohen's $d = 7.93$). They spent 95% of their time (+/- 0.99 SEM) close to it.

Most of the chicks (32) chicks remained significantly more on the side of the arena, where the primary imprinting stimulus was displayed; only 1 chick did not.

Secondary imprinting

The ANOVA did not reveal any effect of Condition on the time spent close to the secondary imprinting stimulus ($F_{(1, 29)} = 0.27, p = 0.61$), Sex ($F_{(1, 29)} = 0.002, p = 0.96$) or interaction (Sex x Condition, $F_{(1, 29)} = 0.30, p = 0.59$) on the time spent close to the secondary imprinting stimulus. The chicks significantly remained close the secondary imprinting stimulus ($t_{(32)} = 40.27, p < 0.001$, Cohen's $d = 7.01$) 93% of their time (+/- 1.07 SEM).

All chicks (33) chose significantly more the side of the arena where the secondary imprinting stimulus was displayed.

Testing

Two males of the blue condition were removed from the following analyses because the video recordings of their last testing day went missing (camera crash). The results are shown in Figure 13. The ANOVA did not reveal any effect of Condition ($F_{(1, 27)} = 0.11, p = 0.74$), Sex ($F_{(1, 27)} = 2.22, p = 0.15$), Day ($F_{(2, 54)} = 0.14, p = 0.87$) or interactions (Sex x Condition, $F_{(1, 27)} = 0.16, p = 0.69$; Sex x Day, $F_{(2, 54)} = 0.21, p = 0.81$, Condition x Day, $F_{(2, 54)} = 0.38, p = 0.68$; Sex x Condition x Day, $F_{(2, 54)} = 0.50, p = 0.61$) on the preference for the primary imprinting stimulus. The preference for the primary imprinting stimulus was significantly different from chance-level ($t_{(30)} = -4.24, p < 0.001$, Cohen's $d = 0.76$). Overall, the chicks preferred the secondary imprinting object and spent 63 % (+/- 3.05 SEM) of their time close to it.

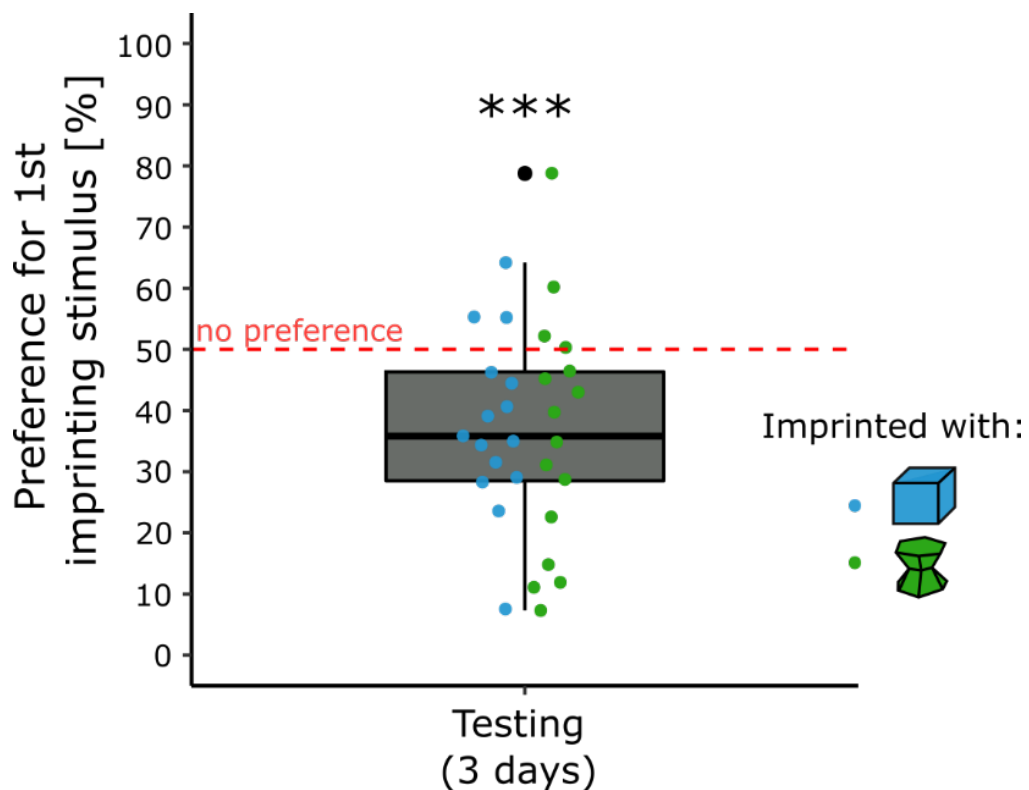


Figure 13: Overall preference for the primary imprinting stimulus (vs. secondary imprinting stimulus) after 1 day of exposure to it and 2 days of exposure to a secondary imprinting object ($p < 0.001, ***$). The blue dots represent the individual preference score of the chicks imprinted with the blue cube. The green dots represent the individual

preference score of the chicks imprinted with the green hourglass. Filled dots show the individual having a significant preference. Empty dots show the individual with no preference.

In the blue condition, 1 chick (7%) preferred the primary imprinting stimulus, 9 (60%) had no preference, and 5 (33%) preferred the unfamiliar stimulus. In the green condition, 2 chicks (13%) preferred the primary imprinting stimulus, 9 (56%) had no preference, and 5 (31%) preferred the unfamiliar stimulus. Levene's test showed that the variances of the two conditions were similar ($F_{(1, 29)} = 2.15, p = 0.15$).

Both conditions showed a similar preference for the secondary imprinting stimulus. It is already known that chicks can imprint on multiple objects (Boakes & Panter, 1985; Bolhuis & Trooster, 1988). However, the preference for a primary imprinting stimulus can be reversed after prolonged exposure with a secondary imprinting object (Cherfas & Scott, 1981). Our results align with the literature as the secondary imprinting phase was longer than the primary imprinting one. The filial bond formed through secondary imprinting has then likely influenced the chicks' original imprinting preference. On top of it, chicks may be more predisposed toward pink than blue or green objects.

Conclusion

Thanks to our automated setup, we followed the animal's behaviour in an imprinting context for several days. In all experiments, the filial imprinting preferences were all pointing in the same direction. Although chicks received the same amount of experience, chicks imprinted with the blue stimulus (where blue is a more predisposed colour) had a more robust and stable preference in time for their imprinting stimulus compared to the chicks imprinted with the green stimulus (where green is a less predisposed colour). We also showed that chicks can have steady and robust distinctive differences in their preferences for familiar vs. novel stimuli. Some chicks consistently preferred to approach their imprinting stimulus, while others preferred the novel one. Moreover, the animals' spontaneous preferences modulated the inter-individual variability. Further studies should clarify whether these differences stem from genetic variability and/or derive from stochasticity in the course of development (Mitchell, 2018) and their neurobiological basis. Altogether, our findings strongly suggest that some

features of the objects (e.g. colour) are more efficient for forming filial imprinting preferences – features to which animals are instinctively attracted to.

CHAPTER 2: Social predispositions

Many predispositions have been discovered using domestic chicks. Overall, the young birds instinctively pick up features displayed by living animals: animacy features. This is striking when looking at the spontaneous preferences expressed for specific motions. Chicks spontaneously prefer point-light display representing biological motion (Miura & Matsushima, 2012, 2016; Miura et al., 2020; Vallortigara et al., 2005), objects maintaining their main body axis while changing direction (Rosa-Salva et al., 2018) and self-propelled objects (Mascalzoni et al., 2010; Rosa-Salva et al., 2016).

In this chapter, I tested whether domestic chicks spontaneously preferred two motion cues representing animacy. In a first study, we manipulated a straightforward variable – the speed of rotating objects – and showed that chicks prefer faster than slower rotating objects. In a second study, I manipulated multiple variables to investigate whether chicks prefer spatially and temporally linked agents. More specifically, we investigated whether domestic chicks spontaneously preferred motions that mimic ‘social aggregation’ – when one agent moved, others reached out.

Study 1: Spontaneous preference for quickly rotating objects in domestic chicks

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Lemaire, B.S. No evidence of spontaneous preference for slowly moving objects in visually naïve chicks. Sci Rep 10, 6277 (2020).

<https://doi.org/10.1038/s41598-020-63428-3>

Introduction

Motion has been commonly used to attract chick’s attention in imprinting paradigms (Bolhuis, 1991; Cate, 1989; Fabricius & Boyd, 1954; Hess, 1959b). For successful

imprinting, the faster an object moves, the better (Sluckin & Salzen, 1961). In such context, it was demonstrated that chicks prefer to approach fast- rather than slow-flickering lights (James, 1959, 1960a, 1960b). Nonetheless, this view was recently challenged by Wood (2017), who reported a spontaneous preference for slowly moving objects in naïve domestic chicks. According to the author, exposure to slowly moving objects would help the young chicks build up an accurate object representation, whereas exposure to quickly moving objects would lead to an inaccurate representation.

The speed is an important motion feature that can be used to categorize animate from non-animate objects. When two dots are moving with different speeds, human adults categorize the faster dot as more animate than the slower one (Szego & Rutherford, 2007). Based on the filial imprinting literature and the pattern of social predispositions described in domestic chicks, the spontaneous preference for slowly moving objects reported by Wood (2017) is quite unexpected and thus theoretically important. In this study, I duplicated Wood's experimental design and re-investigated whether newly-hatched chicks do spontaneously prefer slowly-rotating objects. I performed three different experiments and manipulated the objects used and the frame frequency at which they were displayed.

Experiment 1

In this experiment, I tested whether chicks preferred a slow- or a fast-rotating cube.

Methods

The methods used were similar to the first experiment of the original study (Wood, 2017). Soon after hatching, visually naïve chicks were placed in the automated setups described in the general method. Eleven chicks (7 females) were stimulated with two versions of a stimulus, a slow- and fast-rotating stimulus.

Procedure

After hatching, chicks were sexed (using night-vision goggles) and singly placed in their apparatus for five days in a day-night cycle (LD 13:11 hr). During the day, the chicks underwent 13 sessions of 59 minutes of test with the stimuli displayed on the screens

(fast-moving stimulus displayed on one screen and slow-moving stimulus on the other). Between sessions, the displaying of the stimuli was interrupted by 1 minute of dark screens. The position of the stimuli on the screens was balanced across sessions. During the night, dark screens were displayed.

Stimulus

The stimulus used was a 3-dimensional tilted cube (5 cm) coloured in blue (Figure 14).

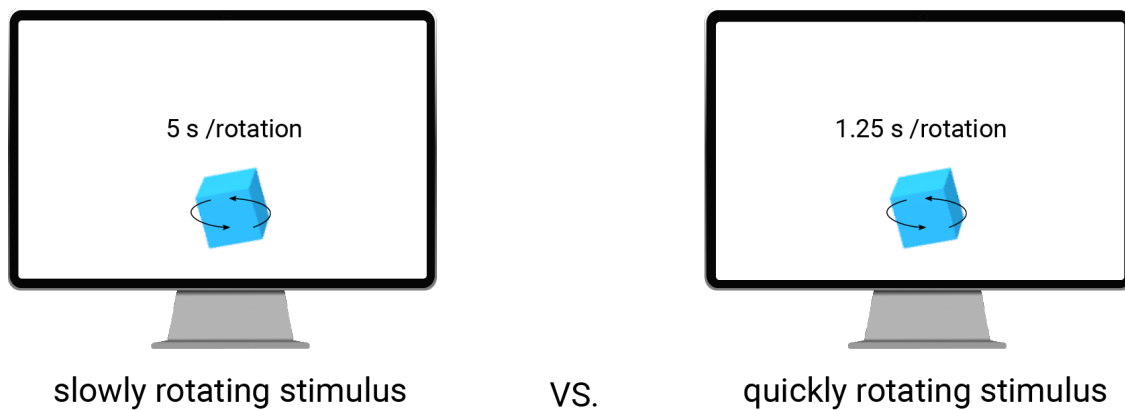


Figure 14: Stimuli used in experiment 1. One stimulus rotates slowly (5 seconds per rotation), and the other quickly (1.25 seconds per rotation).

Two versions of the stimulus were created. On the first version, the blue cube was rotating on itself quickly (one rotation every 1.25s). On the second version, the cube was rotating slowly (one rotation every 5s). The use of these rotatory speeds has been described to produce a preference for slow motion in Wood's second experiment (Wood, 2017). The videos were exported with a high frame rate (120 fps) to ensure a smooth display of the stimuli. The stimuli were created, animated and rendered using Blender (version 2.79). The screen frequency was set at 120 Hz to avoid flicker perception.

Statistical analysis

The number of subjects required in each group was based on the replicated study (Wood, 2017) with an effect size of 1.25 and an alpha of 0.05. 11 individuals were required per group to achieve a power of 0.80.

A preference score had been calculated using the following formula:

$$\text{Preference for slow} = \frac{\text{time spent by the slow – moving stimulus}}{\text{time spent by the two stimuli}} \times 100$$

A score of 50% indicated no preference for either stimulus. A score higher than 50% indicated a preference for the slow-rotating stimulus. A score lower than 50% indicated a preference for the fast-rotating stimulus.

To determine whether chicks' preferences were influenced by sex and testing day, I performed a mixed ANOVA. The distribution of the residuals was checked using Q-Q plots and validated as normally distributed. Sphericity was checked using Mauchly's test, and corrections were performed when the assumptions were not met.

The preference toward a stimulus (different from chance-level) was evaluated using two-tailed one-sample t-tests and by estimating the relative likelihood ratio or Bayes factor (BF_{10}). The latest demonstrates how likely the overall preference is different from the null hypothesis (H_0), such as in the original study (Wood, 2017).

Results & discussion

The results are shown in Figure 15. The ANOVA did not reveal any effect of sex ($F_{(1, 9)} = 0.17, p = 0.90, \eta^2_G = 0.001$), day ($F_{(4, 36)} = 1.12, p = 0.36, \eta^2_G = 0.044$) or interaction (sex x day, $F_{(4, 36)} = 1.37, p = 0.26, \eta^2_G = 0.054$). Overall, the preference score was not significantly different from chance level ($t_{(10)} = -1.50, p = 0.16, \text{Cohen's } d = 0.45$). Furthermore, the BF_{10} for the overall preference, which is 0.72, confirmed that the preference observed is not different from the null hypothesis. There was however a trend for a preference for the fast-rotating object driven by 3 subjects showing strong preference (chick 5: $t_{(64)} = -3.64, p < 0.001, \text{Cohen's } d = 0.45$; chick 6: $t_{(64)} = -5.48, p < 0.001, \text{Cohen's } d = 0.68$; chick 8: $t_{(64)} = -5.68, p < 0.001, \text{Cohen's } d = 0.70$).

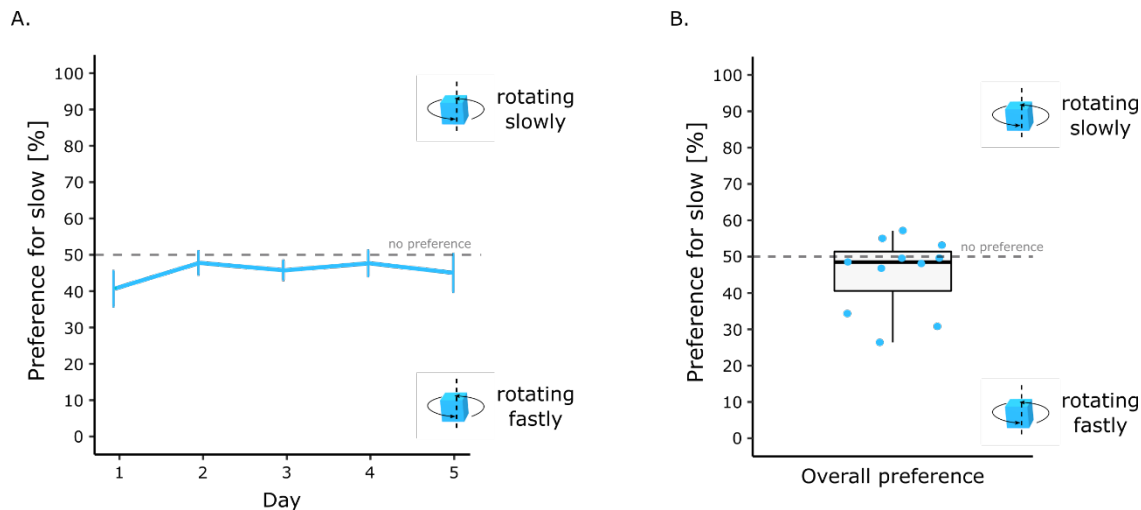


Figure 15: Preference for the slow-rotating cube across days (A) and overall (B). The blue dots show the individual preferences.

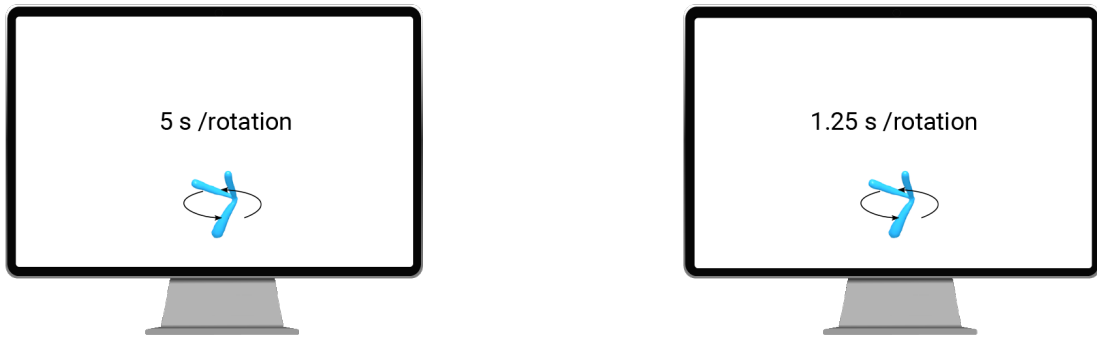
The results of the first experiment did not reveal any clear preference. This could be due to the shape of the stimulus used. For the human vision, the number of corners and contour structure affect motion perception of rotatory objects (Porter et al., 2011). Besides, the object's colour might have influenced the approach behaviours of the animals towards the stimuli. Colours are unequally attractive in domestic chicks (Bolhuis, 1991; Kovach, 1971; Salzen et al., 1971; Schaefer & Hess, 2010) and red was the chosen colour of the original study (Wood, 2017). To control for both effects, the shape and the colour of the object used, we conducted a second experiment using stimuli similar in shape and colour to those used in the original study (Wood, 2017).

Experiment 2

Methods

The methods were the same as in the first experiment except for the stimulus used. The new stimulus (Digital Embryo, Figure 16) had been generated using a program called Digital Embryo Workshop ([DEW](#)). The stimulus was then imported, animated and rendered in two versions (red and blue, Figure 16) using Blender (v2.79).

A. Blue pair of digital embryo



A. Red pair of digital embryo

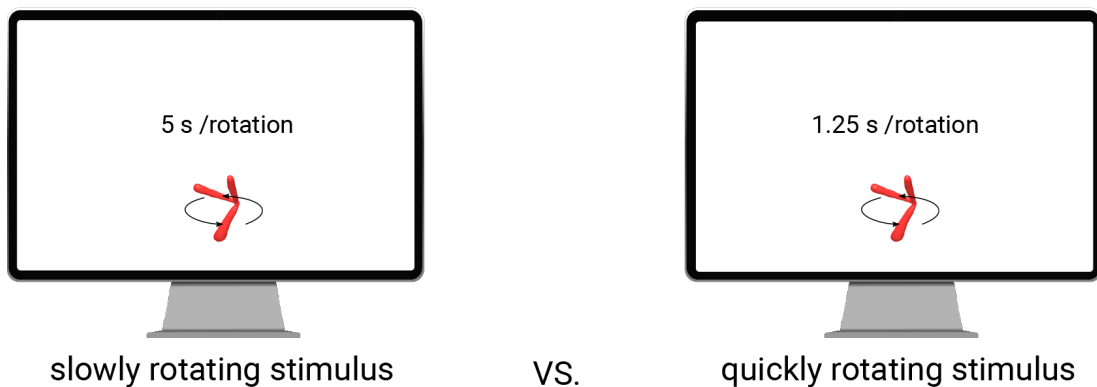


Figure 16: Digital Embryos used in experiment 2 and 3.

We tested ten chicks (four females) the red digital embryo and eleven chicks (four females) using the blue digital embryo.

Results & discussion

The results are shown in Figure 17. The ANOVA did not reveal any effect of sex ($F_{(1, 17)} = 0.18, p = 0.89, \eta^2_G = 0.0004$), colour ($F_{(1, 17)} = 0.017, p = 0.25, \eta^2_G = 0.027$), day ($F_{(4, 68)} = 1.10, p = 0.36, \eta^2_G = 0.042$) or interactions (sex x colour, $F_{(1, 17)} = 0.43, p = 0.52, \eta^2_G = 0.0083$; sex x day, $F_{(4, 68)} = 0.26, p = 0.90, \eta^2_G = 0.010$; colour x day, $F_{(4, 68)} = 0.23, p = 0.92, \eta^2_G = 0.0090$; sex x colour x day, $F_{(4, 68)} = 0.28, p = 0.89, \eta^2_G = 0.011$). The preference score was significantly different from chance level ($t_{(20)} = -5.26, p < 0.001$, Cohen's $d = 1.15$). The chicks' majority chose the fast-rotating object and spent in average 58% (+/- 1.50 SEM) of their time close to it (Figure 17). Furthermore, the BF_{10} revealed that the overall preference score is 653 times more likely than the null hypothesis. The BF_{10} provides

extreme evidence that the chicks possess a strong preference for the fast-rotating digital embryos (Lee & Wagenmakers, 2013).

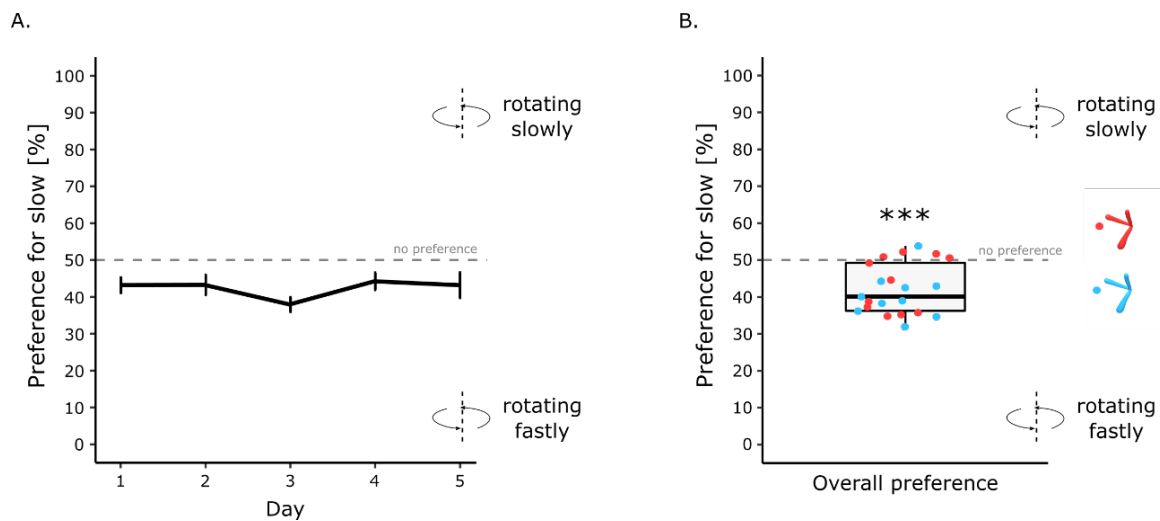


Figure 17: Preference for the slow-rotating digital embryos (blue and red) across testing days (A) and overall (B, $p < 0.001$, ***). The blue dots show the individual preferences of the chicks exposed to the blue digital embryo. The red dots show the individual preferences of the chicks exposed to the red digital embryo.

Although a similar trend was also observed in Exp. 1, our results confirmed that the object shape likely influenced the perception of rotatory motion. Interestingly, the preference observed is opposite to that originally reported (Wood, 2017). One reason for this discrepancy could be associated with the frame frequency of the videos displaying the stimuli. In the replicated study (Wood, 2017), videos with a low frame rate (24 fps) were used, whereas videos with a high frame rate (120fps) were used in this study. Birds possess highly developed visual systems, which make them perceive the environment in slow motion compared to what humans perceive (Healy et al., 2013). In chicks, the temporal perception (quantified using critical flicker fusion, CFF) is higher than in humans and can reach 115 Hz in some individuals (Lisney et al., 2011, 2012). Therefore, the videos displayed at 24 fps might have been perceived as a series of still images rather than a smooth motion, thereby altering their motion perception.

To test this hypothesis, in the third experiment, we used the same stimuli employed in the first and second experiment but changed their frame rate (fps) from a high to low frequency value (such as that used in Wood, 2017).

Experiment 3

Methods

Similar methods than in previous experiments were used except for the frame rate of the displaying of the stimuli, which decreased from 120 to 24 fps using Blender.

Twelve chicks (six females) were tested using the blue cube. Eleven chicks (five females) were tested using the red Digital Embryo. Eleven chicks (five females) were tested using the blue Digital Embryo.

Results & discussion

The results are shown in Figure 18. First, I checked for any effect of colour within the group of chicks exposed to the Digital Embryos. There were no significant effects associated with colour ($F_{(1, 18)} = 1.04, p = 0.32, \eta^2_G = 0.035$), sex ($F_{(1, 30)} = 0.13, p = 0.72, \eta^2_G = 0.050$) or interactions (sex x colour, $F_{(1, 18)} = 1.05, p = 0.32, \eta^2_G = 0.035$; colour x day, $F_{(4, 72)} = 0.34, p = 0.20, \eta^2_G = 0.0072$; sex x colour x day, $F_{(4, 72)} = 0.45, p = 0.77, \eta^2_G = 0.0094$) on the preference score.

Since, there were no effect of colour, chicks exposed to the blue and red Digital Embryos were grouped together for the subsequent analyses. The ANOVA did not reveal any effect of stimulus ($F_{(1, 30)} = 0.51, p = 0.48, \eta^2_G = 0.0098$), sex ($F_{(1, 30)} = 0.13, p = 0.72, \eta^2_G = 0.0025$) or interactions between sex and stimulus ($F_{(1, 30)} = 1.40, p = 0.20, \eta^2_G = 0.032$).

Mauchly's test indicated that the assumption of sphericity had been violated for one factor (day, $W = 0.46, p < 0.01$) and several interactions (day x sex, $W = 0.46, p < 0.01$; day x stimulus, $W = 0.46, p < 0.01$; day x sex x stimulus, $W = 0.46, p < 0.01$). Therefore, degrees of freedom were corrected using Huynh-Feldt estimates of sphericity. There was a significant interaction between testing day and stimulus ($F_{(1.82, 54.66)} = 6.07, p < 0.001, \eta^2_G = 0.078$) but no significant effect of day ($F_{(1.82, 54.66)} = 0.44, p = 0.76, \eta^2_G = 0.0060$) or other interactions (day x sex, $F_{(1.82, 54.66)} = 2.13, p = 0.10, \eta^2_G = 0.029$; day x sex x stimulus, $F_{(1.82, 54.66)} = 0.10, p = 0.97, \eta^2_G = 0.0014$).

The preference scores were significantly different from chance-level on each testing day with the cube (day 1, $t_{(11)} = -5.82, p < 0.001$, Cohen's $d = 1.68$, day 2, $t_{(11)} = -3.45, p < 0.01$, Cohen's $d = 0.99$, day 3, $t_{(11)} = -6.95, p < 0.001$, Cohen's $d = 2.01$, day 4, $t_{(11)} = -2.85, p < 0.05$, Cohen's $d = 0.82$, day 5, $t_{(11)} = -3.12, p < 0.01$, Cohen's $d = 0.91$). Similar results were observed with the Digital Embryos (day 1, $t_{(21)} = -2.58, p < 0.05$, Cohen's $d = 0.55$, day 2, $t_{(21)} = -2.91, p < 0.01$, Cohen's $d = 0.62$, day 3, $t_{(21)} = -2.60, p < 0.05$, Cohen's $d = 0.55$, day 4, $t_{(21)} = -4.14, p < 0.001$, Cohen's $d = 0.88$, day 5, $t_{(21)} = -3.55, p < 0.01$, Cohen's $d = 0.76$).

Furthermore, the BF_{10} revealed that the overall preference score is 41204 times more likely than the null hypothesis. The BF_{10} provides extreme evidence that the chicks possess a strong preference for the fast-rotating stimuli when displayed at a low frame rate (Lee & Wagenmakers, 2013).

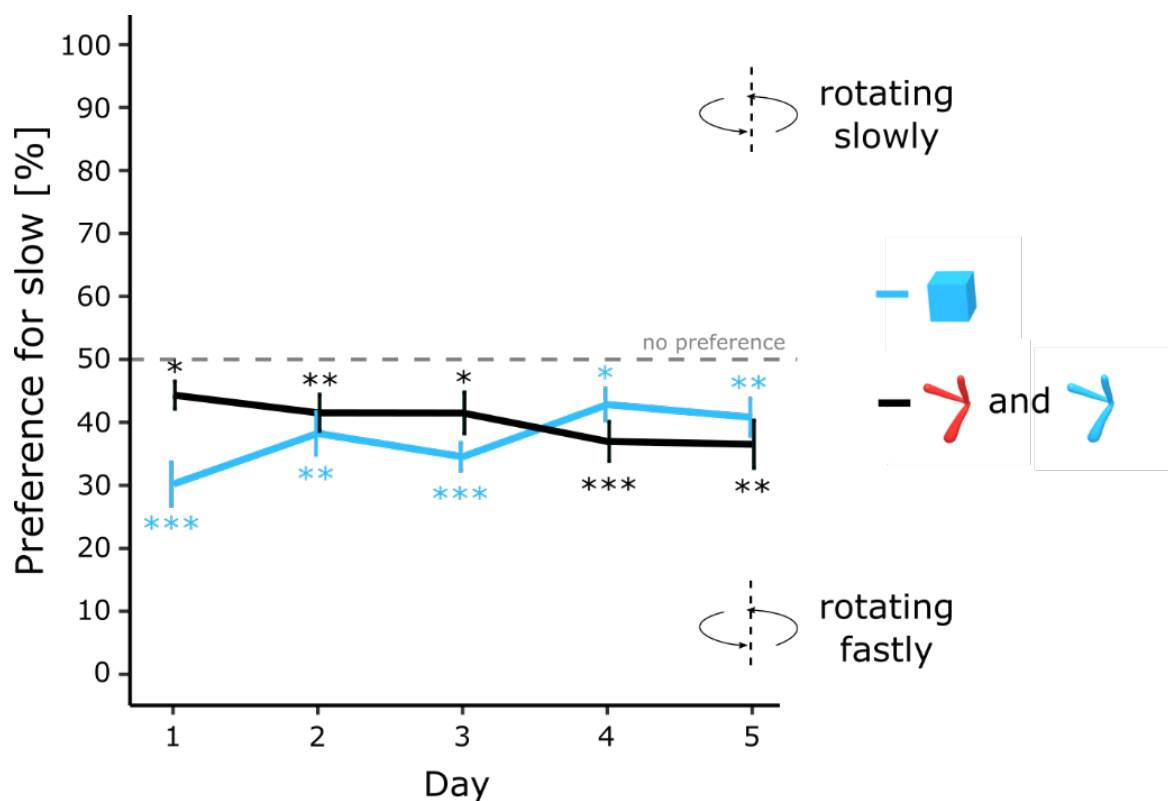


Figure 18: Preference for the slow-rotating digital embryos and the blue cube across testing days. The black line shows the preference of the chicks exposed to the digital embryos (blue and red together; $p < 0.05$, *, $p < 0.01$, **, $p < 0.001$, ***). The blue line shows the preference of the chicks exposed to the blue cube ($p < 0.05$, *, $p < 0.01$, **, $p < 0.001$, ***).

Both chicks tested with the cube and with the Digital Embryo showed a preference for the fast-rotating stimulus. On day 1, the preference was more robust in the group exposed to the cube than in the group exposed to the Digital Embryos. On day 1, chicks tested with the rotating cubes spent 70% (+/- 3.41 SEM) of their time close to the fast-rotating object, while chicks tested with the rotating Digital Embryos spent 56% (+/- 2.20 SEM) of their time close to the fast-rotating object. Moreover, the preference toward the fast-rotating cube decreased with time until it reached 59% (+/- 2.95 SEM) on day 5. In contrast, the preference toward the fast-rotating Digital Embryos increased with time until it reached 64% (+/- 3.80 SEM) on day 5.

Discussion

This study aimed to re-investigate the spontaneous preference for slow- (compared to fast-) rotating objects described in Wood, 2017. We tried to duplicate the preference for slow-moving objects using two different objects and colours. Then, we explored whether the frame frequency of the videos displayed influenced the animal preference. In all experiments, we were unable to find a preference for slowly rotating objects. Instead, we found a preference for quickly rotating ones.

The preference I observed agrees well with previous literature on filial imprinting. Soon after hatching, chicks seek for an appropriate stimulus to imprint on. As mentioned in this chapter, they possess many predispositions to help them narrow their choice toward specific stimuli. Among all the predispositions described concerning motion, chicks have a general preference for movements associated with animate stimuli (Di Giorgio, Loveland, et al., 2017; Mascalzoni et al., 2010; Miura & Matsushima, 2016; Miura & Matsushima, 2012; Rosa-Salva et al., 2018; Vallortigara et al., 2005; Vallortigara, 2012). The results described in this study may fit into this preference pattern since fast-moving objects are more likely to be categorized as animate than slow-moving objects; at least in humans (Szego & Rutherford, 2007). Classical imprinting literature from the 1960s has also reported that the faster an object moves (or flicker), the more appealing for the young birds, so that they would readily approach and imprint on it (James, 1959, 1960b, 1960a; Sluckin & Salzen, 1961).

Given that the frequency of frames used for stimuli presentation did not affect the direction of the preference for quickly moving objects in my experiments, it could be that the discrepancy between my results and Wood's (2017) results is due to the monitor screen themselves. The monitors used in Wood's study were problem refreshing with a frequency below the flicker frequency fusion threshold of birds (Lisney et al., 2012, 2011). Further research would be needed to understand how it could influence the animal's behaviour. Meanwhile, these results demonstrate the necessity of controlling the temporal video and monitor frequency when displaying moving stimuli to avian species. The differences between the studies might be related to the chicks' strain and sex. In some strains, male and female chicks show opposite preferences (Miura & Matsushima, 2012). In our strain, however, no sex differences were observed on this particular task. Unfortunately, Wood (Wood, 2017) did not sex his animals, and this might have influenced the direction of the preference observed.

Furthermore, in the literature, spontaneous preferences have been described by focusing on the first minutes and first choices after exposure to some stimuli (Rosa-Salva et al., 2016, 2018; Vallortigara et al., 2005; Versace et al., 2016). In this way, the attraction observed toward a particular stimulus cannot be explained by learning. Therefore, the term 'spontaneous' is used appropriately if a preference is observed. In contrast, the preference observed in prolonged exposure paradigms – such as those used here and in the replicated study – cannot be defined as a spontaneous preference since it results from at least two mechanisms: predispositions and learning through filial imprinting.

Introduction

Most spontaneous preferences representing animacy focused on the motion characteristics of a simple object; its speed, its rotation/orientation. However, when multiples agents are in motion in a social context, new features might be used to disentangle animate from inanimate entities. This is the case for objects which present spatiotemporal contingencies and trigger the impression of interactive events (Bassili, 1976). With such a relationship, objects move in relation to others and appear to be goal-directed – a feature that is shared by living animals. When object X goes in a particular direction, object Y follows. Human infants appear to be very attracted by such chasing motion patterns (Gao, Newman, & Scholl, 2009; Gao & Scholl, 2011; Rochat et al., 2004), and adult even interpret them in terms of goals (Heider & Simmel, 1944; Rochat & Hespos, 1997; Scholl & Gao, 2013). Interestingly, dogs show a strong interest in chasing-like motion (Abdai et al., 2017), and trained pigeons and squirrel monkeys discriminate a chasing pattern from a random one (Atsumi & Nagasaka, 2015; Goto et al., 2002).

The findings harvested suggest that the spatiotemporal contingencies between moving agents can be used to differentiate between different motion patterns. More than that, the spatiotemporal contingencies could be used to detect the presence of living entities as it implies goal-directedness (Bassili, 1976). This second study aimed to investigate whether domestic chicks notice and prefer to approach motion patterns with spatiotemporal contingencies. For this purpose, we created a ‘social aggregating’ animation (with spatiotemporal contingencies) and a random pattern (without spatiotemporal contingencies).

Methods

We conducted a series of 6 experiments using the same procedure but changing stimuli. In this study, we used the traditional setup (Figure 5) described in the General Methods part of this thesis.

Stimuli creation

The stimuli were video clips of 30 seconds manually generated with Blender 2.8 and contained two kinds of moving agents (Figure 19).

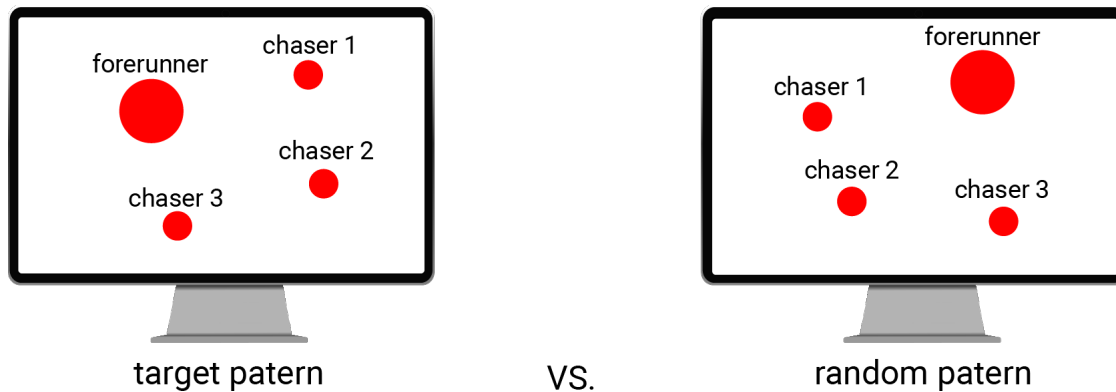


Figure 19: Agents used to build the motion patterns.

The stimuli were composed of an agent called the forerunner (5 cm diameter, Figure 19). It had a simple motion sequence: moving straight (in random directions) for 1 second and stopping for 2.75 seconds. The stimuli were also composed of three other agents called the chasers (3 cm diameter each, Figure 19). They always moved simultaneously (although scattered randomly on the screens). Their motion was composed of five sequences within each scene: moving randomly for 1 second twice, having a small break (stop 1) for 0.25 second, accelerating for 1 second and having a longer break (stop 2) for 0.5 seconds (Figure 20). Each video clip was composed of eight scenes containing five sequences each (Figure 20). Within each experiment, we controlled that the distance travelled, and velocity of the agents were identical for each stimulus. From one experiment to another, we manipulated the arrangement of the motion sequences. The stimuli arrangements are detailed at the beginning of each experiment section.

Example of motion sequences composing a scene

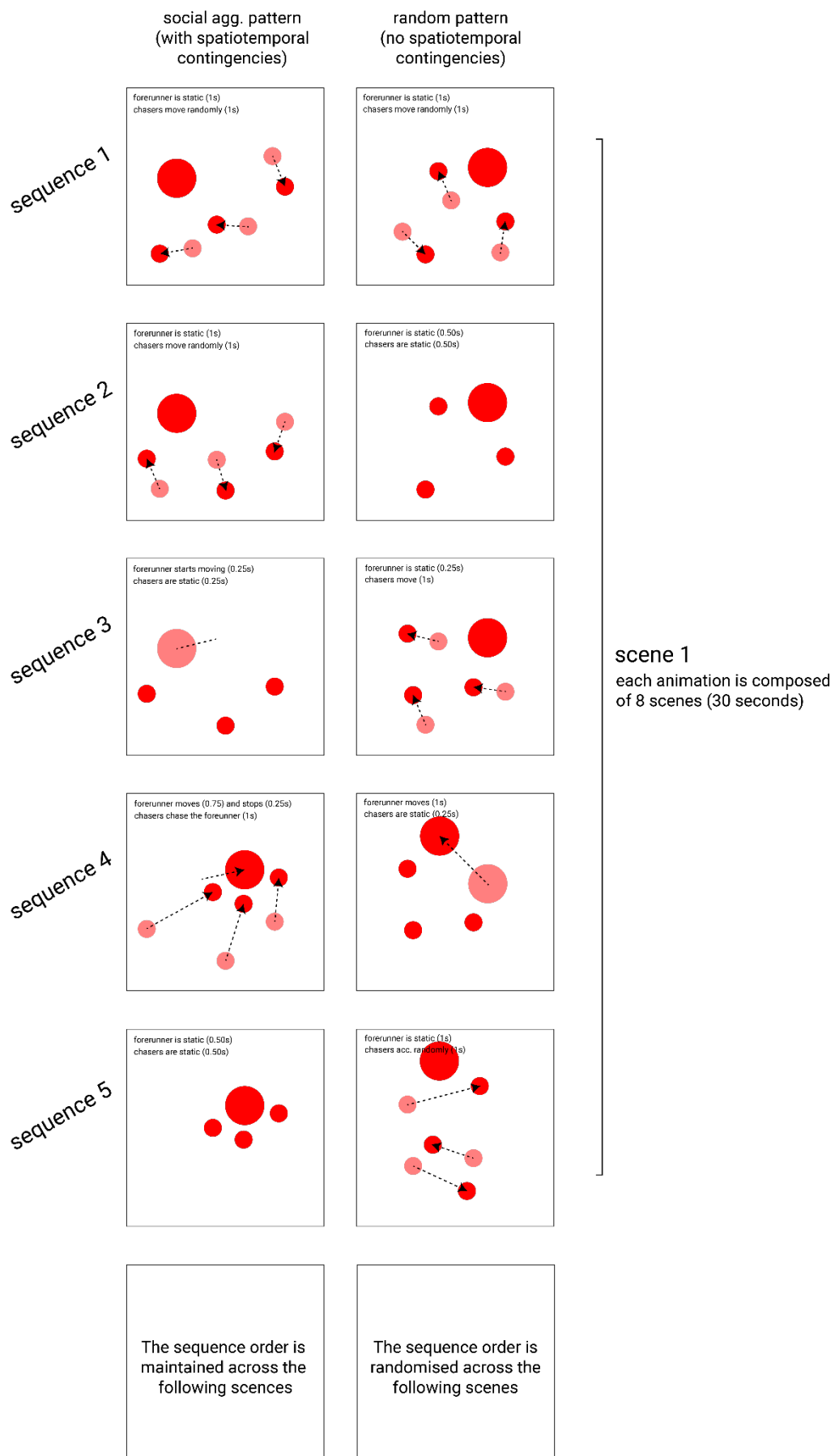


Figure 20: Example of 5 motion sequences manually generated to create two distinct motion patterns: the social aggregating pattern (left column) and the random pattern (right column). For each animation generated, eight scenes of 5 motion sequences were rendered. We controlled for the velocity and the distance travelled by the agents for each animation pair rendered.

General procedure

Just after hatching, the visually naïve animals were singly placed in the centre of the testing apparatus for 6 minutes. As the generated stimuli lasted 30 seconds, they were looped across the whole test duration.

Like previous studies, we calculated the animals' preference for the target stimulus (the stimulus that contains social aggregating events in most of the experiment) using the following formula:

$$\text{Preference for target stimulus} = \frac{\text{time spent by the target stimulus}}{\text{time spent by the two stimuli}} \times 100$$

To complete our analysis, we also investigated the animal first choice. The first choice is defined as the first stimulus approached: the target or the random stimulus.

Statistical analysis

We calculated the number of animals required in each group using a power analysis (Champely, 2020) with an effect size (d) of 0.60 and an alpha of 0.05. The power analysis revealed that 23 individuals were required per group to achieve a power of 0.80. In total, we used 276 chicks (46 chicks per experiment).

The data distribution normality was assessed by looking at the residuals' distribution (Q-Q plot). Parametric assumptions were not met. Therefore, non-parametric tests were used.

For each experiment, we performed a permutation test with F-probabilities to investigate the effect of Sex on the animals' preference. To examine whether chicks had a

significant preference for either stimulus, we performed one-sample Wilcoxon tests against chance level (50%).

To determine whether the first choice of the chicks to approach a stimulus was different from chance, we performed a binomial test.

Experiment 1:

This experiment aimed to investigate whether chicks have a spontaneous preference for a motion pattern that mimics social aggregation: a pattern with spatiotemporal contingencies.

In the target stimulus, we represented social aggregation using two different kinds of agents (Figure 19). A forerunner moves in a random direction from time to time, and three chasers move around when the forerunner is static and aggregate around the forerunner when it moves (left column of the Figure 20).

In the random stimulus, all the agents were moving in random directions, and there was no spatiotemporal contingency between the movement of the agents – in other words, the forerunner action did not trigger the chasers' acceleration. Both the direction and the temporal sequence arrangements of the agents were randomised (right column of the Figure 20).

Results & discussion

As shown in Figure 21, we observed an unexpected and strong preference for the random stimulus ($V_{(45)} = 270$, $p < 0.01$, Cohen's $d = 0.56$). The first-choice analysis confirmed this preference. The binomial test revealed a proportion of 0.24 with 11 subjects approaching the social aggregating pattern and 35 subjects approaching the random pattern (95 % CI = 0.13 – 0.39, $p < 0.001$; Figure 21). The permutation test did not reveal any effect of sex ($F_{(1,41)} = 0.19$, $p = 0.89$) on the animals' preference.

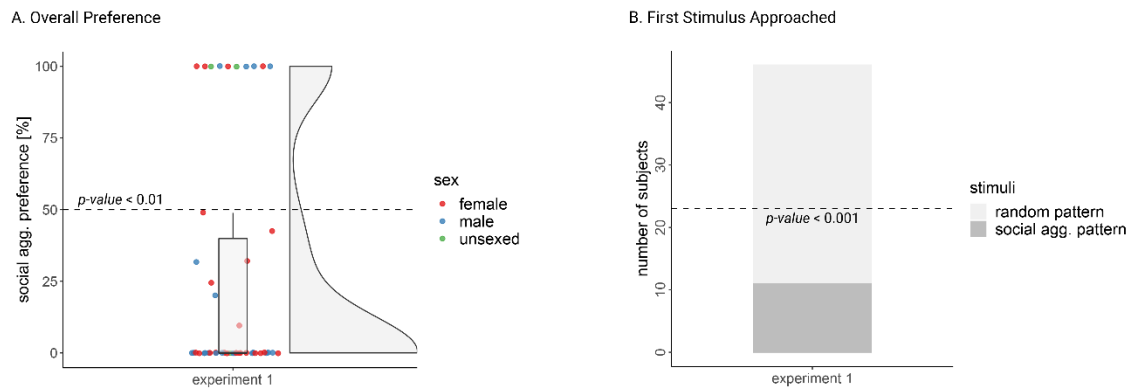


Figure 21: Overall preference for the motion pattern containing social aggregating events vs. the random pattern without temporal contingency between the agents' motion (A) and first stimulus approached by the subjects (B). The dots represent the individual preference (females in red, males in blue, three subjects were not sexed; A).

We found a strong preference for the random pattern. Yet, as the animations were created manually and the trajectories of the forerunners were different, it is possible that the preference observed is influenced by this difference. In the next experiment, we tested whether the forerunners' trajectories influenced the animal choice.

Rather than preferring the random pattern, it is possible to imagine that chicks avoided the social aggregating one. Perceptually, while the chasers aggregate around its forerunner, the moving stimuli create some sort of looming effect. This kind of motion pattern evokes fear responses in chicks and might have driven them away from the social aggregating pattern (Hébert, Versace, & Vallortigara, 2019). We tested this hypothesis in experiment 4.

Experiment 2:

In this second experiment, we investigated whether the forerunners' trajectories influenced the animal preferences. We used the animation of the first experiment but removed the chasers from the scenes.

Results & discussion

As shown in Figure 22, none of the two forerunners' trajectories was preferred by the animals ($V_{(45)} = 634.5$, $p = 0.28$, Cohen's $d = 0.16$). The first-choice analysis confirmed

this absence of preference. The binomial test indicated a proportion of 0.52 with 24 subjects approaching the trajectory of the target stimulus and 22 subjects approaching the trajectory of the random stimulus (95 % CI = 0.37 - 0.67, $p = 0.88$; Figure 22). Again, the permutation test did not reveal any effect of sex ($F_{(1, 44)} = 0.05$, $p = 0.83$) on the animals' preference.

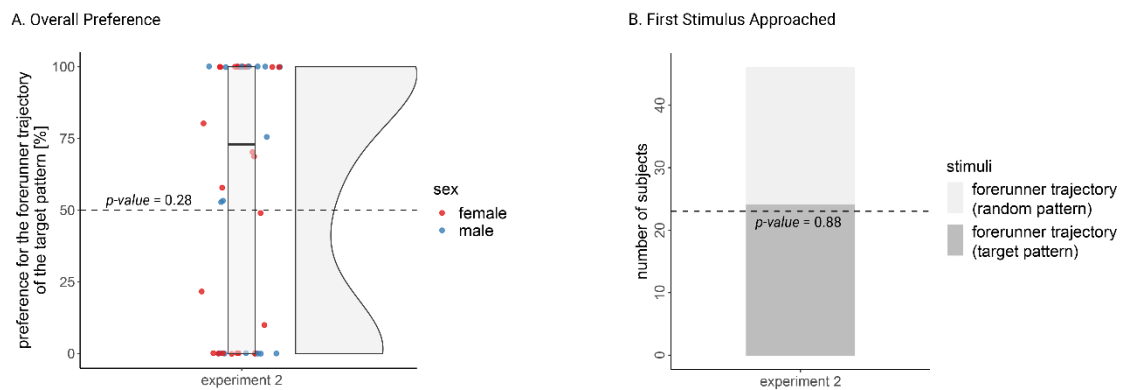


Figure 22: Overall preference for the forerunner of the pattern containing social aggregating events vs. the random pattern (A) and first stimulus approached by the subjects (B). The dots represent the individual preference (females in red, males in blue; A).

The forerunners trajectories alone did not cause the preference for the random stimulus observed in the first experiment. Similarly, we wondered whether the trajectories of the chasers alone did influence the animal preference. This also allowed us to test whether spatiotemporal contingencies within the same kind of agents (chasers of the target animation) is preferred over temporal contingencies (chasers of the random animation).

Experiment 3:

As in the second experiment, we took the animations of the first experiment but removed the forerunners. We simplify the stimuli but still have some sort of social aggregation events in the target stimulus. Indeed, the chasers moved around for some time and then grouped. The chasers were spatially and temporally linked. In the random pattern, the chasers were temporally but not spatially linked with one another.

Results & discussion

As shown in Figure 23, none of the chasers' patterns was preferred by the animals ($V_{(45)} = 540.5$, $p = 1$, Cohen's $d = 0$). The first-choice analysis confirmed this absence of preference. The binomial test indicated a proportion of 0.5 with 23 subjects approaching the grouping pattern and 23 subjects approaching the random pattern (95 % CI = 0.35 - 0.65, $p = 1$; Figure 23). Again, the permutation test did not reveal an effect of sex ($F_{(1, 4)} = 0.34$, $p = 0.56$) on the animals' preference.

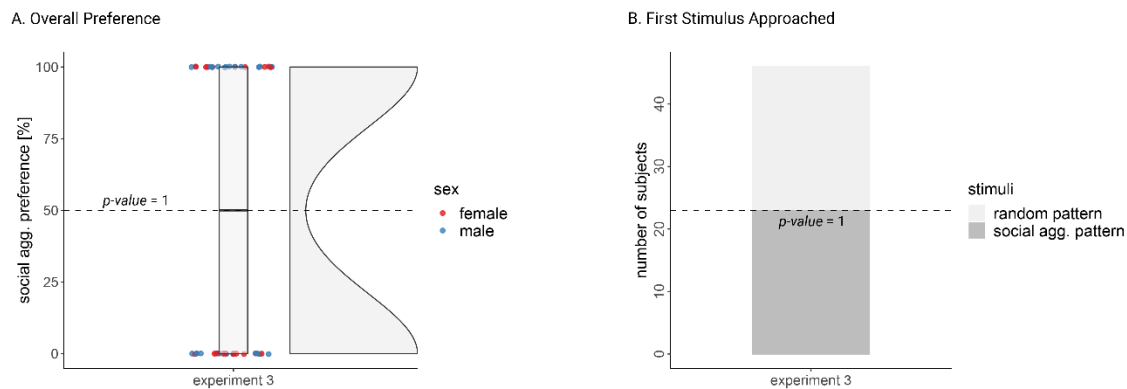


Figure 23: Overall preference for the chasers of the pattern containing social aggregating events vs. the chasers of the random pattern (A) and first stimulus approached by the subjects (B). The dots represent the individual preference (females in red, males in blue; A).

The results showed that the trajectories of the chasers alone did not reproduce the preference observed in the first experiment. This suggests that two kinds of agents (a forerunner with three chasers) are necessary to direct the animal attention toward the random pattern of the first experiment. The spatial and temporal contingencies of the chasers here did not seem to matter as both patterns were equally approached. Furthermore, this experiment seemed to refute the hypothesis that chicks avoid the social aggregating stimulus rather than preferring the random stimulus. In this experiment, a looming effect – to a lower degree – could still be perceived in the target animation. Nevertheless, the chicks did not avoid it at all. In the next experiment, we refute the hypothesis that chicks avoid the social aggregating animation.

Experiment 4:

Again, we used the same stimuli as the first experiment but unfilled the chasers to reduce the looming effect created by the aggregating events. If chicks previously avoided the social aggregating pattern, then the preference obtained in the first experiment should disappear or at least be strongly reduced.

Results & discussion

As shown in Figure 24, we observed a preference for the random stimulus similar to what we observed in the first experiment ($V_{(45)} = 360$, $p < 0.05$, Cohen's $d = 0.35$). The first-choice analysis confirmed this preference. The binomial test revealed a proportion of 0.33, with 15 subjects approaching the social aggregating pattern and 31 approaching the random pattern (95 % CI = 0.20 – 0.48, $p < 0.05$; Figure 24). Again, the permutation test did not reveal any effect of sex ($F_{(1, 44)} = 0.69$, $p = 0.41$) on the animals' preference.

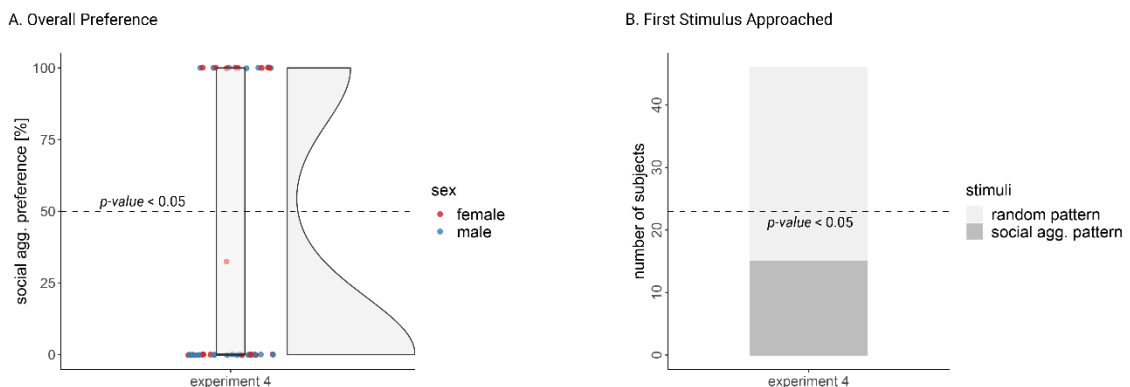


Figure 24: Overall preference for the motion pattern containing social aggregating events vs. the random pattern without temporal contingency between the agents (A) and first stimulus approached by the subjects (B). In comparison to experiment 1, the perceptual features of the chasers were changed (dots unfilled). The dots represent the individual preference (females in red, males in blue; A).

In this experiment, we replicated the findings of the first experiment while changing the perceptual features of the chasers. By doing so, we strongly reduced the looming effect allowing us to refute the hypothesis that chicks avoided the aggregating pattern.

For some reasons, the chicks strongly preferred the random pattern. By replicating the finding, we also emphasise the need of having two different kinds of agents together in motion – the forerunner and the chasers – to produce the preference. The spatiotemporal contingencies of the chasers were not crucial for the chicks (see results of experiment 3), but the spatiotemporal contingencies between the agents appeared to be important. Chicks preferred to approach the pattern without (or at least unpredictable) spatiotemporal contingencies between two kinds of agents. In the random pattern, the chasers and forerunners sequences were randomised. Therefore, we wondered whether the absence of an evident temporal link between the forerunner and the chasers attracted the chicks' attention and led them to approach the random pattern.

Experiment 5:

This experiment aimed to investigate whether the preference observed in experiment 1 was influenced by the temporal contingency of the agents' motion. We used the target animation of the first experiment and generated a new random animation. This time, both kinds of agents of the random animation were temporally linked. In this way, in the random pattern, the forerunner's action triggered the chasers' motion too. This time, the only difference between the target and the random animations lied in the spatial contingencies. While the chasers followed and aggregated around the forerunner in the target pattern, the chasers moved in random directions in the random pattern.

Results & discussion

As shown in Figure 25, we did not find a significant preference for the random stimulus looking at the time spent ($V_{(45)} = 400$, $p = 0.11$, Cohen's $d = 0.24$) or the first choice analysis. The binomial test revealed a probability of 0.39 with 18 subjects approaching the chasing pattern and 28 subjects approaching the random pattern (95 % CI = 0.25 – 0.55, $p = 0.18$; Figure 25). Again, the permutation test did not reveal any effect of sex ($F_{(1, 44)} = 0.14$, $p = 0.72$) on the animals' preference.

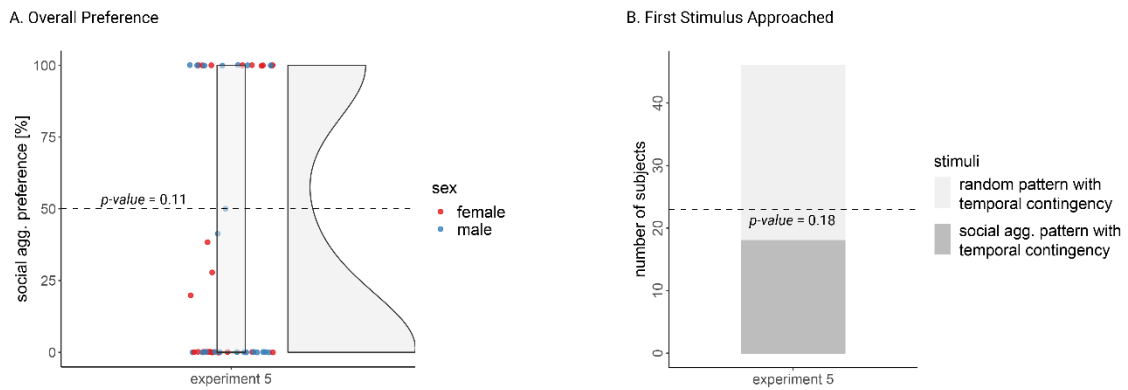


Figure 25: Overall preference for the motion pattern containing social aggregating events vs. the random pattern with temporal contingency between the agents' motion (A) and first stimulus approached by the subjects (B). The dots represent the individual preference (females in red, males in blue; A).

The absence of any significant preference confirms that the spatial contingencies do not influence the animal's choice and supports the idea that the temporal contingencies of agents' motion are important. Chicks spontaneously preferred motion pattern with agents that have unpredictable temporal relationships. To confirm this effect, we ran one last experiment.

Experiment 6:

This time we tested the animals with the random pattern of the first experiment and the random pattern of the fifth experiment. In this case, both stimuli had no social aggregating events (no spatial contingency) but differed in the temporal contingency of their motion sequences. In one pattern, the agents' motion was temporally linked. In the other pattern, there was no evident relationship.

Results & discussion

As shown in Figure 26, our hypothesis was confirmed as the animals preferred to approach the motion pattern where the agents had no temporal link between the two agents ($V_{(45)} = 292$, $p < 0.01$, Cohen's $d = 0.50$). The first choice confirmed this preference. The binomial test revealed a proportion of 0.35 with 30 subjects approaching the pattern without temporal contingency and 16 subjects approaching the contingent pattern (95 % CI = 0.21 – 0.50, $p = 0.054$; Figure 26). Again, the permutation test did not reveal any effect of sex ($F_{(1, 44)} = 0.03$, $p = 0.86$) on the animals' preference.

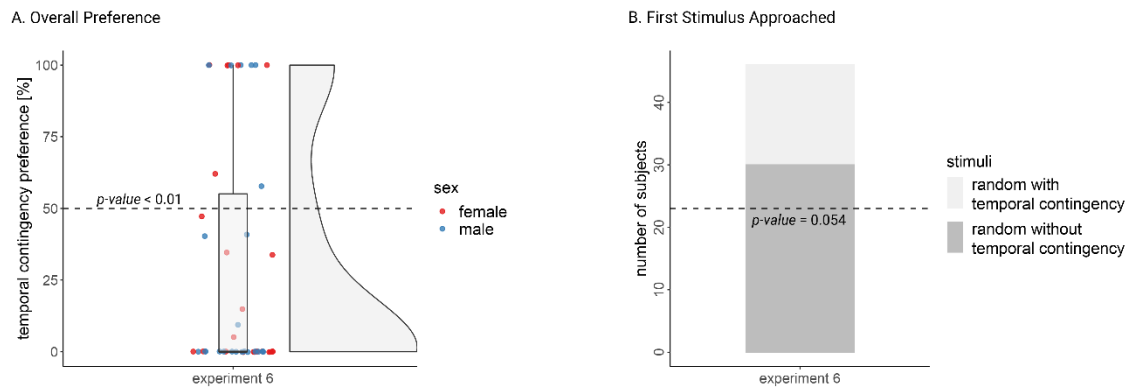


Figure 26: Overall preference for the random pattern with temporal contingency between the agents' motion vs. the random pattern without temporal contingency between the agents' motion (A) and first stimulus approached by the subjects (B). The dots represent the individual preference (females in red, males in blue; A).

This experiment confirmed that chicks preferred animation with unpredictable temporal contingencies between two kinds of agents.

Our findings are opposite to our initial hypothesis. At first sight, the preferred direction is even surprising. However, contingencies also create mechanical motion (Blakemore, 2003). In this case, the motion does not drive animacy but quite the opposite. It creates a very unnatural motion pattern. Therefore, the preference observed could be interpreted as a preference for a motion pattern with more variability, which would indicate the presence of animate agents.

In the auditory domain, bobwhite quail chicks exposed to contingent calls of Japanese quail eliminate their species-specific preference for their own species calls (Harshaw, Tourgeman, & Lickliter, 2008). Interestingly, adding variability to the contingency even produced higher preferences for the Japanese quail calls. Moreover, at 3-months of age, human infants are more attentive to imperfect contingencies of an adult interacting with another infant than perfect or absent contingency (Bigelow, 1998; Muir, Hains, Cao, & D'Entremont, 1996; Rochat, 1999). Merging the findings from different species highlights the temporal contingency's role in a social context and suggests a preference for imperfect/unpredictable contingencies.

Overall, our findings demonstrate that chicks can extract and use temporal contingencies to take a decision without receiving any sort of experience. A capacity that could then be used to detect goal-directedness and, probably, later on, to infer intentions (agency perception; Johnson, Alpha Shimizu, & Ok, 2007; Leslie, 1994). The latest remains to be investigated.

Conclusion

Among many other things, the domestic chicks excel at detecting animacy from motion pattern. This is visible by looking at their spontaneous preferences from simple to more complex motion cues. This chapter first investigated whether domestic chicks have a spontaneous preference for fast over slow rotating objects. Unlike a previous report, we found that chicks spontaneously prefer fast-rotating objects and emphasise the importance of controlling the frame frequency of the animations displayed on screens. In a second study, we investigated whether chicks possess one of the possible rudiments to detect goal-directedness, i.e., whether they can detect and prefer spatiotemporal contingencies from moving agents. Remarkably, we discovered that chicks do prefer to approach motion pattern with unpredictable temporal contingencies.

Both findings complete the general pattern of spontaneous preferences described in domestic chicks and are consistent with the idea that chicks prefer to approach stimuli that could be potential interactive partners. A pattern shared across species and for which the neurophysiological mechanisms remain to be explored.

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Lorenzi E, Lemaire B.S, Versace E, Matsushima T and Vallortigara G (2021)
Resurgence of an Inborn Attraction for Animate Objects via Thyroid Hormone
T3. *Front. Behav. Neurosci.* 15:675994. doi: 10.3389/fnbeh.2021.675994

Introduction

Social predispositions and filial imprinting are interconnected mechanisms. The social predispositions canalise the animal attention towards specific stimuli (Rosa-Salva et al., 2015, 2021) and filial imprinting leads to a strong attachment through exposure to those stimuli (Bolhuis, 1991; McCabe, 2019). Both processes occur for a limited period and happen in close temporal proximity. In domestic chicks, filial imprinting occurs within the first four days after hatching (Yamaguchi et al., 2012). Similarly, the spontaneous preference for animacy (using a self-propelled object) disappears on the third post-hatching day (Versace et al., 2019). One could then ask whether the control of the sensitive periods for imprinting and social predispositions share a neurophysiological ground.

The thyroid hormone (T3) – which concentration peaks around hatching and decrease gradually afterwards in chicks – controls the opening and closing of the sensitive period for imprinting. In this study, we investigated whether the same mechanisms influence the sensitive period for animacy preference. We ran three experiments. In the first one, we replicated previous findings in a different strain of chicks and highlight the existence of a sensitive period for animacy preference. In the second experiment, we investigated whether a potent inhibitor of the thyroid hormone (IOP) abolishes the spontaneous preference for animacy on the hatching day (day 1). Finally, in the third experiment, we

investigated whether the thyroid hormone T3 could re-establishes the spontaneous preference for animacy at a later stage.

Experiment 1

In this first experiment, we replicated the experimental design of Versace et al. (2019), who demonstrated the existence of a sensitive period for animacy preference. Different chick strains showed a spontaneous preference for a self-propelled object (a reliable cue to animacy) on the hatching day (day 1) but no apparent preference on the third post-hatching day (day 3).

Methods

We used the traditional setup (Figure 5) described in the General Method part of this thesis. We tested the animals' spontaneous preference on two different days: 35 chicks (18 females) were tested just after hatching (day 1), and 36 chicks (18 females) were tested on the third post-hatching day (day 3).

Stimuli

The stimuli used have been initially described by Rosa-Salva et al. (2016). Each stimulus contains a red circle (3 cm diameter) crossing the screen horizontally back and forth (Figure 27). One stimulus crossed the screen at a constant speed (≈ 4.64 cm/s), while another crossed the screen while changing speeds (the slower speed being ≈ 3.37 cm/s and the faster one being ≈ 19.64 cm/s).

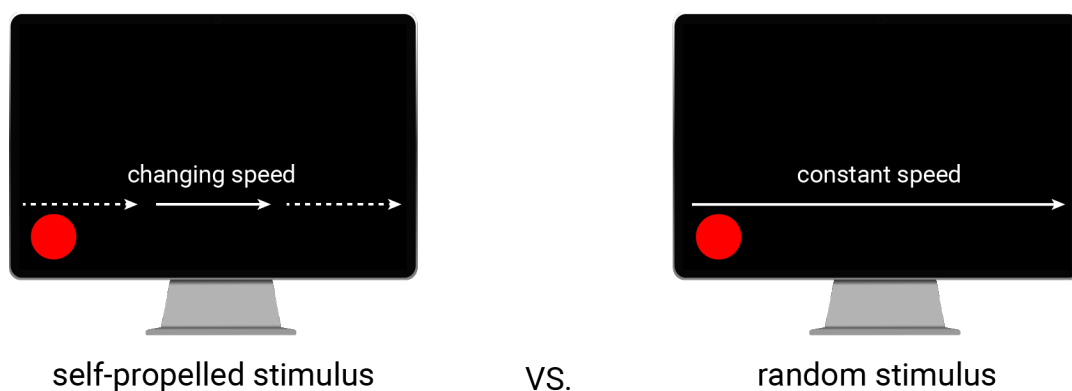


Figure 27: Stimuli displayed to visually naïve domestic chicks. One stimulus accelerates and decelerates autonomously while the other moves at a constant speed.

Procedure

Chicks were placed in the traditional setup for 10 minutes. For each animal tested, a score demonstrating the animals' preference for the animate stimulus was calculated using the following formula:

$$\text{Preference for animacy} = \frac{\text{time spent close to animacy}}{\text{time spent close to both stimuli}} \times 100$$

A score higher than 50 % indicated a preference for the animate stimulus (self-propelled). A score lower than 50 % indicated a preference for the non-animacy stimulus (moving at a constant speed). A score of 50 % indicated no preference.

Statistical analysis

The number of subjects required in each group was a priori determined with an effect size of 0.96 and an alpha of 0.05. Eighteen individuals were required per group to achieve a power of 0.80.

To assess the normality of the data distribution, we looked at the residuals' distribution (Q-Q plot). As parametric assumptions were not met, we used non-parametric tests. Outliers were detected by looking at the Cook's distance using a multivariate approach. When the distance was four times greater than the group mean, the animal behaviour was observed, and chicks were discarded if necessary (Kannan & Manoj, 2015). We performed a permutation test using F-test probabilities to understand the effect of Sex and Testing day (1 or 3) on the animacy preference. We conducted one-sample Wilcoxon tests against chance level (50%) to determine whether the chicks demonstrated a significant preference for either stimulus. We conducted two-sample Wilcoxon tests to determine whether the preference was statistically different between testing days (1 or 3).

Results & discussion

The results are shown in Figure 28. The permutation test revealed a significant effect of testing day ($F_{(1, 67)} = 5.40, p < 0.05$). Chicks tested on day 1 had a significant preference for the animacy stimulus ($V_{(35)} = 458, p < 0.05, d = 0.44$) while chicks tested on day 3 had no preference ($V_{(36)} = 310, p = 0.72, d = 0.08$). The permutation test did not reveal any effect of sex ($F_{(1, 67)} = 1.17, p = 0.28$) or interaction ($F_{(1, 67)} = 0.05, p = 0.83$).

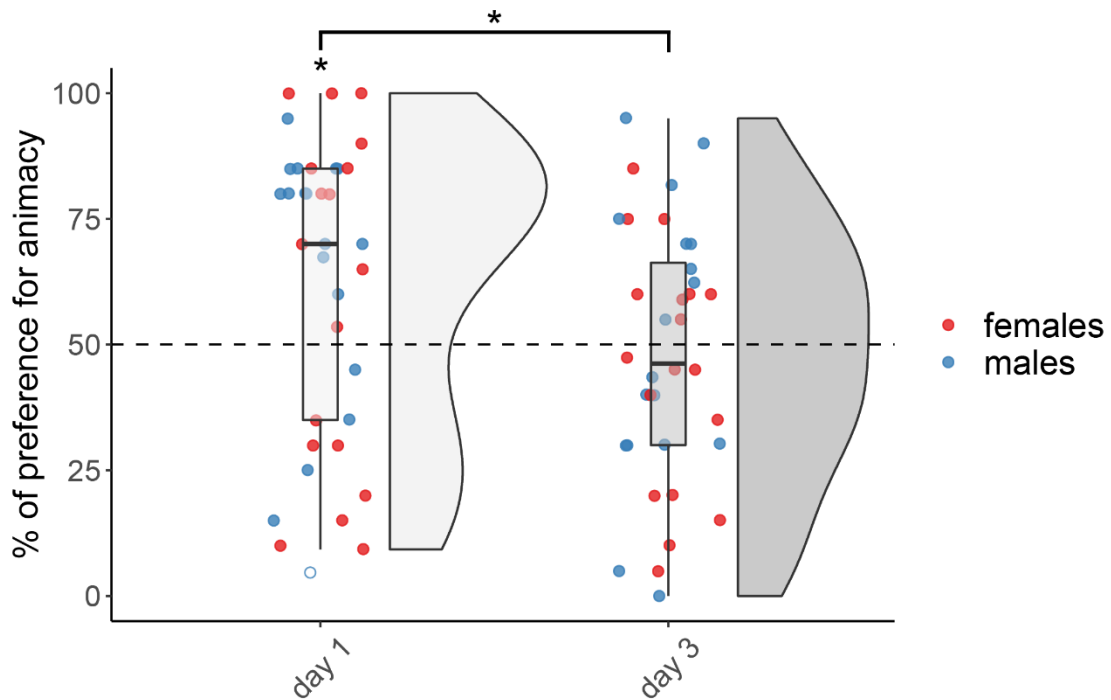


Figure 28: Animacy preference for chicks tested on the first hatching day (day 1; $p < 0.05, *$) and on the third post-hatching day (day 3). The dots represent the individual preference (females in red, males in blue).

In this first experiment, we replicated previous findings (Versace et al., 2019). Chicks showed a spontaneous preference for animacy at the onset of life (day 1) which disappeared in the subsequent day (day 3). As already suggested in the Introduction, the sensitive period for animacy happens in close temporal proximity with filial imprinting. Imprinting occurs during the first days of life (Yamaguchi et al., 2012), and the active thyroid hormone T3 controls its sensitive period. Imprinting leads to an increase of T3 in the brain and specifically into the intermediate medial mesopallium (IMM), a region implicated in the filial imprinting memory (Horn, 2004; McCabe, 2013; Solomon & McCabe, 2015). In the vascular endothelial cells of the brain, the enzyme

Dio₂ (type 2 iodothyronine deiodinase) converts the circulating inactive form T4 into the active form T3 (Yamaguchi et al., 2012). Injection of iopanoic acid (IOP) inhibits Dio₂, prevents T4 conversion, and impairs visual imprinting (Pascual, Montiel, & Aranda, 1987; Yamaguchi et al., 2012).

In the second experiment, we investigated whether IOP impairs the animacy preference observed on the hatching day (day 1).

Experiment 2

Methods

We tested chicks on the hatching day (day 1). Thirty chicks (16 females) were injected with IOP (IOP-injected), and twenty-eight chicks (14 females) were injected with a saline solution (vehicle-injected). The chicks were tested one hour after receiving their injections. Similar statistical analyses than the first experiment were performed.

Injections

The injections were performed to the thigh (intramuscular). The animals were taken from the incubator and placed with a black hood on the head to prevent visual stimulation. After the injections, chicks were located in individual compartments situated in an incubator. Chicks of the first experiment underwent a similar procedure, although they did not receive injections.

We dissolved Iopanoic acid (IOP 10mM, TCI I0300, Tokyo Chemical Industry co. Ltd., Tokyo, Japan) in 0.05M NaOH solution at 1mM and rebuffered to pH=8.5 by 6M HCl. The vehicle solution was a 0.05M NaOH solution buffered to pH=8.5 by 6M HCl and allowed to control for the effect of the injection.

Results & discussion

The results are presented in Figure 29. The permutation test revealed a significant effect of treatment ($F_{(1, 54)} = 5.74, p < 0.05$). As expected, the vehicle-injected chicks had a significant preference for animacy ($V_{(28)} = 347, p < 0.001, d = 1.01$) whereas IOP-injected

chicks did not show any preference for either stimuli ($V_{(30)} = 268.5, p = 0.46, d = 0.14$). The permutation test did not reveal any effect of sex ($F_{(1, 54)} = 1.10, p = 0.30$) or interaction ($F_{(1, 54)} = 0.66, p = 0.42$).

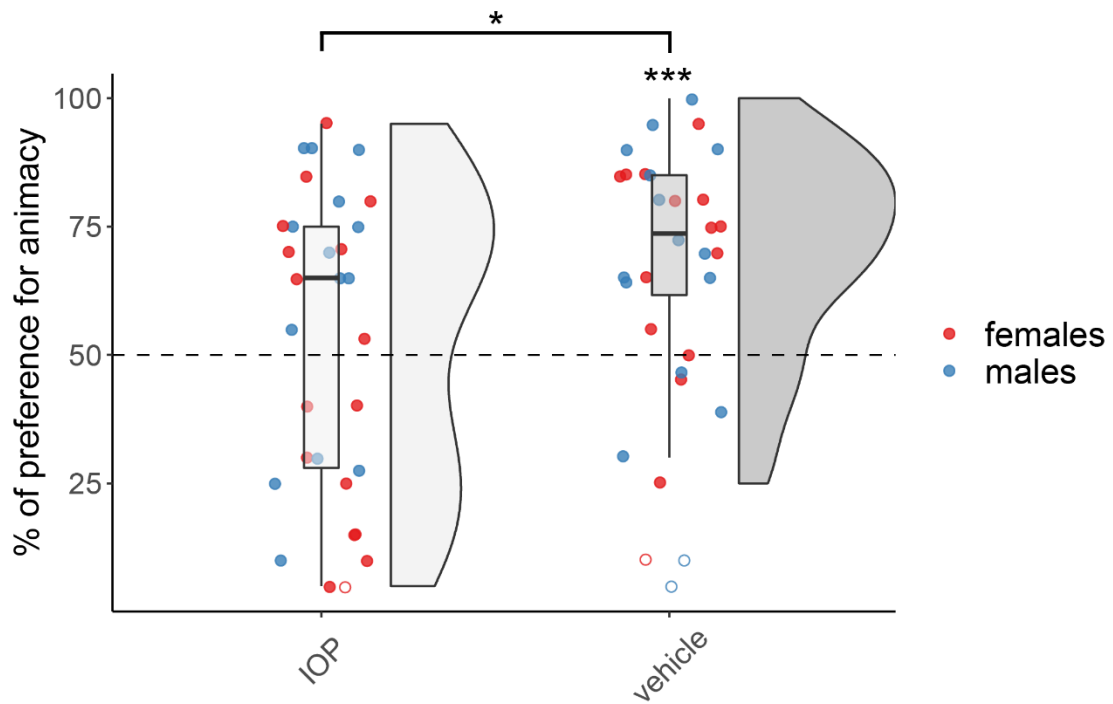


Figure 29: Animacy preference for chicks tested on the first hatching day (day 1) and injected with IOP or a vehicle solution ($p < 0.001$). The dots represent the individual preference (females in red, males in blue).

As we hypothesised, IOP abolished the animacy preference on the first hatching day. Such as for imprinting, T3 seems to control the sensitive period for animacy preference through a quick neurophysiological mechanism. In the third experiment, we tested whether the thyroid hormone restore the animacy preference.

Experiment 3

Methods

Similar methods were used, but this time we tested chicks on day 3 - when the animacy preference has disappeared - and injected 29 chicks (14 females) with T3 and 32 chicks (15 females) with a vehicle solution. We dissolved T3 (3,3',5-Triiodo-L-thyronine, 100 μ M, Sigma Aldrich, T-2877) in 0.002M NaOH and 0.9% NaCl. The vehicle solution was a 0.9%

NaCl and 0.002M NaOH solution. Similar statistical analyses than the previous experiments were performed.

Results & discussion

The results are presented in Figure 30. The permutation test revealed a significant interaction between sex and treatment ($F_{(1, 57)} = 25.02, p < 0.001$) but no main effects of treatment ($F_{(1, 57)} = 1.27, p = 0.26$) or sex ($F_{(1, 57)} = 0.20, p = 0.65$). Females and males showed an opposite pattern within each treatment (T₃-injected: $W_{(29)} = 166.5, p < 0.01, d = 1.17$; vehicle-injected: $W_{(32)} = 39.5, p < 0.001, d = 1.40$). T₃- and vehicle-injected females showed opposite preferences ($W_{(29)} = 183.5, p < 0.001, d = 1.59$). T₃-injected females showed a significant preference for animacy ($V_{(14)} = 85, p < 0.05, d = 0.63$), whereas vehicle-injected females showed a significant preference for the non-animacy stimulus ($V_{(15)} = 11, p < 0.01, d = 1.0$). T₃- and vehicle-injected males also showed opposite preferences ($W_{(32)} = 61, p < 0.05, d = 0.98$). However, in spite of a trend for an inverted pattern with respect to females, injected males did not show any significant preference for either stimuli (T₃-injected: $V_{(15)} = 27, p > 0.05, d = 0.53$; vehicle-injected: $V_{(17)} = 116, p > 0.05, d = 0.47$).

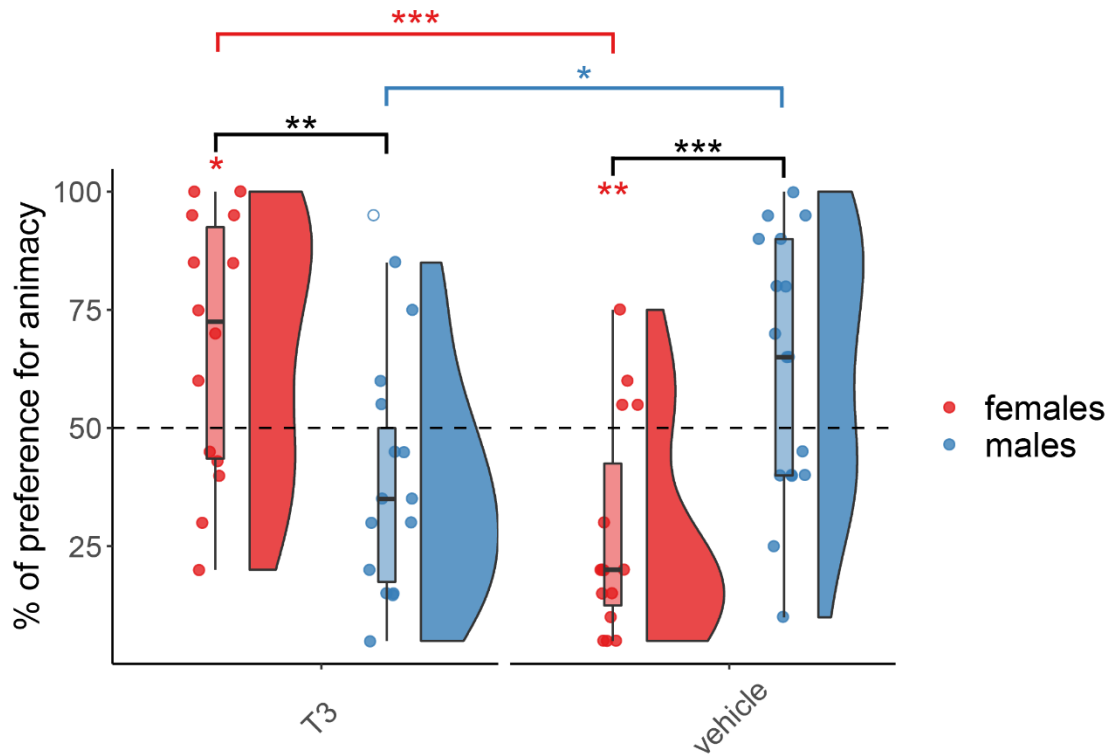


Figure 30: Animacy preference for chicks tested on the third hatching day (day 3) and injected with T3 or a vehicle solution ($p < 0.001$). The dots represent the individual preference (females in red, males in blue).

The thyroid hormone T3 restored the animacy preference in females and confirmed an active role of the thyroid hormone in controlling the sensitive period for animacy preference. This behavioural difference between sexes may result from upregulation of the Dio_2 gene in males compared to females (Yamaguchi et al., 2012) or a difference in T3 transport from muscle to the brain. The latest may point towards a possible limitation of our injection's method.

However, this is not the first time that sex differences are associated with sensitive periods regulation by thyroid hormones (Batista & Hensch, 2019; Miura & Matsushima, 2012; Miura et al., 2020; Yamaguchi et al., 2012). Previous evidence already suggested the implication of sex hormones in the expression of social predispositions (Bolhuis, McCabe, & Horn, 1986; see Rosa-Salva et al., 2015 for review). In our study, T3 probably affected both sexes but led the animal to express different behavioural responses because of an interaction between sex and thyroid hormones. Interestingly, the brain

regions implicated in the animacy preference – part of the so-called Social Behaviour Network (Goodson & Kingsbury, 2013; Lorenzi et al., 2017; Newman, 1999; O’Connell & Hofmann, 2011) – are rich in steroid hormone receptors (Lorenzi et al., 2017; Mayer et al., 2016, 2017; Mayer, Rosa-Salva, & Vallortigara, 2017). Further studies should be performed to understand better the exact mechanisms and the interactions of these hormones in relation to animacy preference and its sensitive period.

Sex differences are also apparent in the vehicle-injected group. Females showed a strong preference for the non-animacy stimulus, while males tend to prefer the animacy one. Considering that no sex differences were found in experiment 1 and 2, it is very likely that the fear induced by the injections, together with the difference in sex hormones, influenced the chicks’ behaviour. As chicks grew older, avoidance responses increase with different timing between sex (Schaller & Emlen, 1962). Males show weaker avoidance than females until post-hatching day 4 (Schaller & Emlen, 1962). We could then expect that the fear provoked by the injections led the females to avoid the animacy pattern while males were more explorative in the vehicle-injected group. This is consistent with the general sex differences observed in domestic chicks (Cailotto, Vallortigara, & Zanforlin, 1989; Vallortigara, 1992; Vallortigara et al., 1990) and the feral history of the species (McBride & Foenander, 1962; McBride, Parer, & Foenander, 1969).

Conclusion

Such as in imprinting, the thyroid hormone plays a crucial role in controlling the sensitive period for animacy preference. Our findings suggest that both mechanisms share molecular ground. This makes sense from an evolutionary perspective as chicks are driven by their spontaneous preference to first approach stimuli and then imprint on them. Opening one sensitive period and not the other would appear worthless.

This study shows that the animacy preference observed in visually naïve chicks disappeared when we injected a T3 inhibitor but could be re-established by injecting T3.

Sensitive period for other spontaneous preferences have been described in chicks (biological motion: Miura & Matsushima, 2012; stuffed jungle fowl: Johnson et al., 1989) and human new-borns (face-like stimuli: Buiatti et al., 2019; Johnson et al., 1991; Shultz, Klin, & Jones, 2018; Simion & Di Giorgio, 2015; Tomalski, Csibra, & Johnson, 2009). Whether those sensitive periods are also regulated by thyroid hormone remain to be determined.

Most animacy preferences described until now seem to be shared across species (Di Giorgio et al., 2017). Then, one could hypothesise that the neurophysiological mechanisms involved are similar. Thyroid hormones are key regulators to brain development (Van Herck et al., 2013) and influences genes expression (Harvey & Williams, 2002). Thyroid hormones disruption can cause attention deficit-hyperactivity (Hauser et al., 1993), mood disorders (Bauer et al., 2008) and has been linked to autistic spectrum disorders (Getahun et al., 2018; Hoshiko et al., 2011; Lyall et al., 2017). Although the levels of thyroid hormones appear normal in autistic children of 10-14 years old (Cohen et al., 1980), post-mortem analyses of autistic brains (ranging from 4 to 16 years old humans) revealed a deficit of T3 in specific cortical regions and expression alteration of several thyroid hormones dependent-genes (Khan et al., 2014). Hoshiko et al. (2011) even suggested a link between low levels of T4 at birth and the presence of subsequently diagnosed autism spectrum disorder. However, this was not confirmed by Lyall et al. (2017). Interestingly, the disruption of animacy preference has also been linked to autism in domestic chicks (Lorenzi et al., 2019; Sgadò et al., 2018) and human neonates (Di Giorgio et al., 2016) and whose sensitive period appears to be controlled by the thyroid hormone T3. Further investigations should be performed to better understand the link between thyroid abnormalities and opening/closing periods of brain plasticity which could lead to disorders such as autism.

GENERAL CONCLUSION

The domestic chick is a fascinating animal model. In a controlled environment (such as in a laboratory), the young bird can help researchers unravel the origin of knowledge and the development of social attachment (Rosa-Salva et al., 2015; Vallortigara, 2012, 2021; Versace & Vallortigara, 2015). This can be done by observing, recording and analysing the animal behaviour in simple dual-choice tasks as we did in the studies presented in the present thesis.

Within all the studies, we used two different but complementary setups. With the automated setup, we investigated behaviour across prolonged duration. In a first study, we investigated filial imprinting preference and observed the development of social attachment for six days at the group and individual level (Lemaire et al., 2021). We also replicated a previous experiment investigating the development of preference for fast-versus slowly-rotating objects (Lemaire, 2020). Contrariwise, the traditional setup allowed us to focus on the first approach and minutes of experience with different stimuli. This approach allows to differentiate the innate from the learned preferences. Using the traditional setup, we investigated the biological priors influencing and canalising the chick's attention towards specific motion characteristics. We also focused on the timing at which those preferences appear and identified that the thyroid hormone T3 controls the opening and closing of the sensitive periods for animacy preference (Lorenzi et al., 2021).

In the first chapter of this thesis, I presented a study where we investigated the development of filial imprinting. We manipulated the chicks' predispositions for specific objects/colours and the exposure duration to those objects. We then tested the animal filial preferences for several days to investigate the stability and variability of social attachment. We found that animal predispositions and experience strongly influence the social attachment formed through imprinting. Three days of imprinting produce robust and long-lasting preference independently of the imprinting object used (more predisposed or not), whereas one day is insufficient. Chicks imprinted with the less predisposed object explored more often the novel object (more predisposed). Novelty

exploration was already described at an early stage of imprinting (after 15-45 minutes of exposure with an imprinting object; Bateson & Jaeckel, 1974, 1976) and was reported to be adaptative. It has been argued that it would allow the animal to explore different views of the mother hen in order to build a complete representation of it (Bateson, 1973). Here we found that novelty exploration occurs at a later stage; when imprinting is well established. At this stage, it is doubtful that chicks explore novelty to build a better representation of their imprinting object. In this chapter, we also showed that the social predispositions strongly influence the chicks' filial preferences. Chicks imprinted with less predisposed objects are more likely to explore the unfamiliar object later. It is then possible that chicks explore novelty, not to build a complete representation of their imprinting object, but rather explore stimuli that could be more suitable to their need, objects with features they instinctively prefer.

In the second chapter of this thesis, I described two studies investigating the chick's social predispositions towards specific motion characteristics. In a first study, we showed that chicks have a solid and lasting preference for fast compared to slow rotating objects. This preference is in line with imprinting's early work (James, 1959, 1960a, 1960b; Sluckin & Salzen, 1961) and the general pattern of predispositions observed until now: chicks spontaneously prefer objects having animacy features (see Rosa-Salva, Mayer, & Vallortigara, 2015 for review). Moreover, we showed the importance of controlling the frame-frequency of videos used to display stimuli and suggest doing the same with the screen frequency used to display stimuli in the laboratory. Chicks have a higher flicker-fusion threshold than humans and therefore can perceive beam of light and unsmooth motion sequences if the frequencies are lower than 100 Hz/fps (Lisney et al., 2012, 2011). In the second study, we investigated whether chicks prefer spatiotemporal contingencies. For this purpose, we created motion sequences that mimic social aggregation – when one agent starts to move in a specific direction, scattered agents follow and aggregate around the former. We hypothesised that chicks would strongly prefer these motion sequences as it shows goal-directedness and therefore might indicate the presence of animate beings. However, chicks did not show any interest in this pattern. Interestingly, chicks preferred the motion pattern with unpredictable temporal sequences. A line can be drawn between our finding and

preference for unperfect contingencies in human children (Bigelow, 1998; Muir et al., 1996; Rochat, 1999) and quail chicks in the acoustical domain (Harshaw et al., 2008). These findings highlight the importance of temporal contingency in a social context (Bassili, 1976) and might be the base to infer intentions to others. When perfect contingencies might look mechanical, unperfect ones appear more animate. Further investigation should clarify how chicks deal with spatial contingencies, which seems essential to define the nature of interactions between individuals in humans (Bassili, 1976).

Such as for filial imprinting (Bolhuis, 1991; McCabe, 2019; Vallortigara & Versace, 2018), the social predispositions occur within a sensitive period (Rosa-Salva et al., 2015, 2021). Chicks can imprint until the fourth post-hatching day (Yamaguchi et al., 2012). Similarly, the spontaneous preference for a self-propelled object is detectable on the hatching day and disappears on the third post-hatching day (Versace et al., 2019). In the third chapter of this thesis, we showed that the neurophysiological mechanisms controlling the opening and closing of the sensitive periods are similar for both processes: filial imprinting and social predispositions. Inhibiting the inactive (T4) conversion to the active thyroid hormone (T3) abolished the animacy preference, whereas supplying chicks with endogenous T3 re-established the animacy preference on the third post-hatching day. A hormonal cocktail – including sex, stress, and thyroid hormones – seems at play at this development stage. How those hormones interact, which mechanisms and how they influence animal behaviour remain to be explored.

Altogether, the present thesis complete previous research on filial imprinting and social predispositions: two distinct but interconnected mechanisms. Studying those research topics using the domestic chick can help better understand the mind foundations at the onset of life.

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