



HOKKAIDO UNIVERSITY

Title	Development and function of biological motion preference:behavioral study in domestic chicks
Author(s)	三浦, 桃子
Degree Grantor	北海道大学
Degree Name	博士(生命科学)
Dissertation Number	甲第12273号
Issue Date	2016-03-24
DOI	https://doi.org/10.14943/doctoral.k12273
Doc URL	https://hdl.handle.net/2115/64871
Type	doctoral thesis
File Information	Momoko_Miura.pdf



Development and function of biological motion preference:
behavioral study in domestic chicks
(生物的運動選好性の発達と機能：ニワトリ雛を用いた行動学的研究)

A DISSERTATION
submitted to the Graduate School of Life Science,
Hokkaido University
in partial fulfillment of the requirements for the degree of
DOCTOR OF LIFE SCIENCE

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2015

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General Introduction: Scientific Backgrounds of the Thesis

“Throughout animal evolution, valid information about other animals' motion has achieved a very high survival value.” (Johansson, 1973)

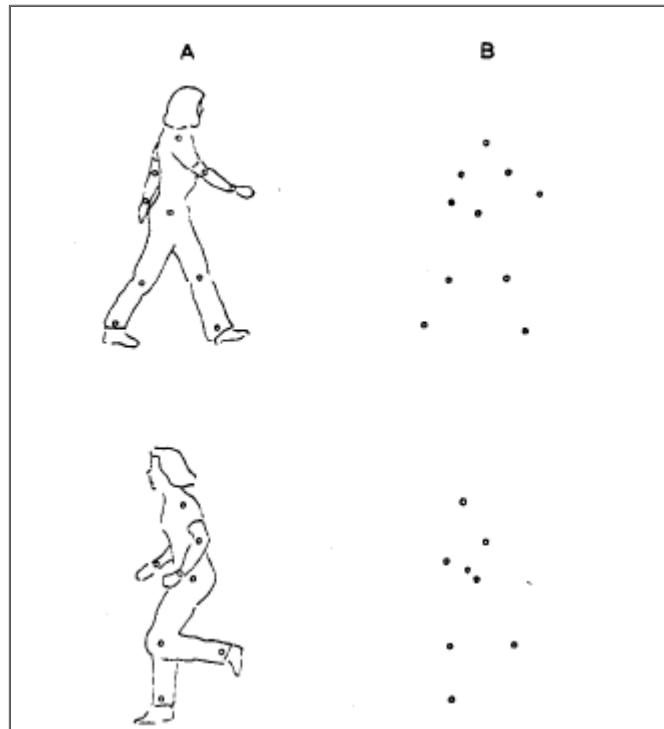
Animations composed of moving light points, if these points were appropriately arranged to represent the major joints of a walking human, generate a vivid perception of human locomotion. This phenomenon is known as the perception of biological motion (BM), since it was reported by Johansson in his pioneering paper published in 1973 (see the figure below). Following his finding, intensive studies have been accomplished in human subjects. Even though the animation is highly simplified, human observers detect not only the direction and type of the movement (Cutting et al., 1988), but also infer various features associated with the animation such as gender (Kozlowski and Cutting, 1977), age (Pavlova et al. 2002) and emotion (Dittrich et al., 1996), and can even identify the individual (Cutting and Kozlowski, 1977). For a long time after the Johansson's finding, the cognitive capability for BM perception has been supposed to be limited only to human adults that have mature visual processing mechanisms, because the capacity develops gradually and slowly in juveniles (Pavlova et al., 2001, Blake et al., 2003, Freire et al., 2006).

In non-human animals, on the other hand, the ability to perceive BM has been supposed to be limited. Point light animations can be discriminated by a variety of animals after intensive training sessions, and the list of these animals includes pigeons (Dittrich et al., 1998), cats (Blake, 1993), rats (MacKinnon et al., 2010), baboons (Parron et al., 2007), and chimpanzees (Tomonaga, 2001). However, these animal

studies commonly report that many training trials were needed for animals to reach the criteria of successful discrimination. It is thus highly possible that the learned discrimination is based on the animals' memorizing elementary parts of the animation, rather than that animals really perceived the biological motion just as human adults do.

Interestingly enough, recent studies have shown that BM perception might arise much earlier in life and occur in taxonomically remote animals. Pioneering behavioural studies by Vallortigara and his colleagues reported that newly-hatched domestic chicks show a preference to the point-light animation that depicts a walking hen (BM) over non-BM alternatives (Vallortigara et al. 2005, 2006). BM preference in the early stage of life is also reported in humans, as 2-day-old new-born babies prefer the walking hen animation in preference looking tests (Simion et al. 2008). Although it is not clear whether chicks or new-born human babies have cognitive processes similar to those employed by human adults, the BM preference in the early post-natal (post-hatch) period may be based on an evolutionarily ancient and common mechanism that is shared by birds and humans.

These results raise a series of questions about the development and the function of the BM preference. In chapter 1 of my thesis, I focused on the development, and investigated; (1) whether the BM preference arises innately, i.e., without specific and instructive visual experiences, (2) how could the BM preference be modified by post-hatch experiences. In the following chapter 2, with an assumption that the BM preference has a functional role in imprinting, I examined two possible scenarios; (1) chicks would selectively learn the colour associated with BM animations, and (2) if pre-treated with non-specific visual stimuli to show a BM preference, chicks would show a higher score in learned colour preference by imprinting.



Outline contours of a walking and a running subject (A) and the corresponding dot configurations (B). Adapted from Johansson, (1973) *Perception & Psychophysics* 14: 202

Chapter I

*Preference for biological motion in domestic chicks:
sex-dependent effect of early visual experience*

Highlights

- BM preference is innate in domestic chicks.
- Early visual experience of any point-light animations induces the BM preference in males.
- The induced BM preference is correlated with the locomotor activity in training.

1. INTRODUCTION

Johansson (1973) first reported that point light animations can create a vivid and immediate percept of human locomotion if they are strategically placed on the joints of a walking human. This phenomenon is now widely used to study the perception of biological motion (BM). Recently, a preference for BM has been reported in visually inexperienced animals, such as newly hatched domestic chicks (Vallortigara et al., 2005, 2006). Similarly, in humans, 2-day old newborn babies have been reported to show a similar BM preference in tests using the preference looking technique (Simion et al., 2008). Adult marmosets (particularly females) have been found to attend more to BM without specific training using the BM animation (Brown et al., 2010). These studies suggest that BM perception is based on a common mechanism, involving innately predisposed BM preferences in a variety of animals of different taxa.

BM discrimination is, however, subject to change by experience. Point light animations can be discriminated by learning in a variety of non-human animals, including pigeons (Dittrich et al., 1998), cats (Blake, 1993), rats (MacKinnon et al., 2010), baboons (Parron et al., 2007), and chimpanzees (Tomonaga, 2001). These animal studies commonly report that many training trials were needed to reach the criteria, suggesting that the learned discrimination may be based on memorized elements of the animation. Furthermore, in humans, the ability to discriminate motion in BM animations is specifically enhanced by acquiring the novel motor patterns that correspond to the BM (Casile & Giese, 2006). This indicates that the human capability to discriminate motion in BM images can be enhanced without visual stimulation.

These results raise a series of questions about how strongly BM perception relies on innate factors, how modifiable BM preference is, and whether locomotor activity can

specifically enhance BM preference. Vallortigara et al. (2005) placed newly hatched chicks on a treadmill to walk for 30 min in complete darkness just before testing, because such motor activities are thought to be crucial for the predisposition to develop (Johnson et al., 1985, Johnson & Horn, 1988). The innate preference for BM reported by Vallortigara et al. (2005) could have been caused by the locomotor activity.

In addition, some evidence suggests that sex-related differences in BM perception may occur at a young age. Female chicks have been reported to lose sight of the mother hen less frequently than males (Workman & Andrew, 1989). Furthermore, females stay longer near a familiar object than males, even at 3 days old (Vallortigara, 1992). Similar sex differences were found by Regolin et al. (2000), who reported that male chicks were neophilic compared to females, approaching novel animation that had not been used in training (Regolin et al., 2000).

To address these questions, I examined BM preference using a filial imprinting procedure with a particular focus on sex differences. During the imprinting period, chicks learn about visual features such as colour and shape in a process of actively following the imprinting object (Matsushima et al., 2003 and Horn, 2004 for reviews; also see Izawa et al., 2001). In the present study, male and female chicks were individually exposed to one of the five animations of different attributes without any pretreatment, and were tested for their preference of a walking hen (BM) to a rotating hen (non-BM).

2. MATERIALS AND METHODS

Subjects

Newly hatched domestic chicks (109 males and 111 females) of the Leghorn (Julia)

strain (*Gallus domesticus*) were used; the hatching day was assigned day 1. Fertilized eggs were supplied from a local hatchery (Hokuren Co., Iwamizawa, Japan) and incubated at 37-38°C in darkness. Hatchlings were housed in another incubator kept in complete darkness. Experiments were performed between 09:00 and 18:00, but the circadian cues (e.g., photoperiod) were not given. After hatching, chicks were separately housed and kept in complete darkness except during training and testing. A light-reflecting small plastic ball was attached to the head for off-line analysis of the walking trajectories using Move-tr/2D software (Library, Tokyo, Japan). Chicks tested on day 1 or 2 did not receive food or water. Chicks tested on day 5 were fed from day 2 with a mixture of baby food (3 ml) and powdered milk (1 ml) per day, which was supplied to the crop directly with a syringe, in a dark room. After the experiment, feathers and blood were sampled to determine sex, based on the CHD genes of sex chromosomes (Fridolfsson & Ellegren, 1999). All experiments were conducted in accord with the guidelines and approval of the Committee on Animal Experiments of Hokkaido University. These guidelines are based on the national regulations for animal welfare in Japan (Law for Humane Treatment and Management of Animals; after partial amendment No.68, 2005). After the experiments, chicks were sacrificed using carbon dioxide according to the guidelines.

Apparatus

I used a dark chamber (37 × 20 × 40 cm) illuminated by infra-red LEDs. The inner temperature was kept at 27-30°C. The chamber was equipped with two LCD monitors (10.4", 800 × 600 pixels, Logitech LCM-T102, Japan), one on each side (Fig. I-1A). An electric shutter (liquid crystal film on transparent Plexiglass partition) was placed on

each of the LCD screens to turn the visual stimulation on/off. Chicks' behaviour was recorded using an infra-red CCD camera (250k pixels with NTSC output, placed at the ceiling), and stored with a video recorder (DCR-SR60, Sony, Japan) for offline analysis.

Animations

I used six types of point-light animations in training and testing. These animations (except the **pendulum** and **stationary pattern**) were identical to those used in a previous report (Vallortigara et al., 2005). All animations were constructed from identical yellow light points on a black background. The walking hen stimulus (**W-hen**) consisted of 13 points and the animation was constructed based on a real hen walking leftward. The rotating hen stimulus (**R-hen**) consisted of a similar arrangement of 13 light points that mimicked a rigid hen rotating around its vertical axis. The **pendulum** stimulus consisted of 13 fixed but randomly arranged light points, periodically swinging around a center point like a pendulum. The **random motion** stimulus consisted of 13 randomly arranged light points that moved independently of each other. The **stationary pattern** was a single frame arbitrarily chosen from the **random motion** animation. The walking cat stimulus (**W-cat**) was a 13 light-point animation based on a walking cat.

Initial pilot experiments suggested that the chicks trained with **W-hen** exhibited a preference toward **W-hen** at test. Untrained control chicks did not show such a preference. I therefore sought to determine the critical features of the **W-hen** stimulus using a systematic series of animations (Table I-1). Both the **W-hen** and **W-cat** stimuli were characterized as biological motion (*BM*). **W-hen** and **R-hen** stimuli were constructed based on a video-recording of a walking hen. All stimuli except the **random motion** and **stationary pattern** stimuli were composed of repeated short video clip (1-3

sec in duration), thus these were denoted as periodic animation. All of the videos were composed of identical light points. I assumed that the **W-hen** animation was the closest to real hens that chicks may encounter, and that the other animations were further removed from the **W-hen** in the order of **R-hen** < **pendulum** < **random motion** < **stationary pattern**. Statistical analyses (see below) were conducted according to the systematic arrangement of the animations.

Procedures

Chicks were individually trained and tested in the experimental chamber. Chicks received two training trials with a 1 hr interval (Fig.I-1C). During the interval, chicks were separately kept in the dark incubator. Each training trial lasted for 1 hr, in which the same animation was displayed alternately on the right and left monitor every 1 min using shutters on each screen (Fig. I-1A, B), i.e., a total of 30 min for each monitor. In training, I counted the cumulative number of approaches to the monitor, measured as the number of times the subject crossed imaginary lines placed 6-7 cm from the shutter (dashed lines in Fig. I-1B). The number of times the line was crossed while stepping away from the monitors was not counted.

Thirty minutes after the second training trial, preference was examined in a binary choice test in two 5-min trials repeated with a 30 min interval. In this test, each chick was carefully placed at the center of the chamber, in which two different animations were simultaneously and continuously displayed on both monitors; the side of presentation was changed in the two test trials. I recorded the amount of ‘stay time’ (i.e., the cumulative sum of the duration, sec) for which the chick stayed in the area close to each screen (within the imaginary lines).

Experiment 1

Eight groups of chicks were compared; six groups were tested on day 2, and the other two groups on day 5. Untrained naïve control chicks were kept in complete darkness until testing. **W-hen** and **R-hen** stimuli were simultaneously presented at testing, and the difference in stay time (**W-hen** – **R-hen**) was used as the preference score.

Experiment 2

Four groups of chicks were compared; two groups were tested on day 2, and the other two groups on day 5. On each day, one group was trained with **random motion**, and the other group served as untrained naïve controls. **W-hen** and **W-cat** stimuli were simultaneously presented at testing, and the difference in stay time (**W-hen** – **W-cat**) was used as the preference score.

Statistical analysis

I used R (computer language developed for statistical computations, version 2.12.0) to construct a series of generalized linear models (GLMs), which were evaluated using Akaike information criteria (AICs). As the response variable (X) denoting the preference as a linear predictor, I analyzed the difference in stay time; i.e., **W-hen** minus **R-hen** in experiment 1, and **W-hen** minus **W-cat** in experiment 2, respectively. The link function was assumed to be linear. Factors such as *sex* (male or female) and *age* (day 2 or day 5) were analyzed, as well as their interactions with the *group* factor. In addition, the correlation between the *count of approaches* in training and the testing preference were analyzed using the Spearman rank correlation test at the significance level of $p < 0.05$.

Two types of formulation (type A and B) were made for the linear predictor (X) in

the data from day 2 of experiment 1; day 5 data were not included.

$$A \quad X = \alpha_0 + \alpha_1 * group + \alpha_2 * sex$$

Thus, I examined two explanatory variables (*group* and *sex*). *Group* constitutes a variable that takes natural numbers (1 to 6) for each of the six experimental groups; W-hen (*group* = 1), R-hen (= 2), pendulum (= 3), random motion (= 4), stationary pattern (= 5) and untrained control (= 6), respectively. *Sex* constitutes a categorical variable that is either *male* or *female*.

$$B \quad X = \beta_0 + \beta_1 * super_group$$

Here, I have one variable *super_group* that takes a natural number, 1 or 0, depending on how the six groups are further allocated to two super-groups. For example, a model [1,2,3/4,5,6] denotes a situation in which chicks of the first super-group (W-hen (1), R-hen (2) and pendulum (3); *super_group* = 1) behaved similarly, and chicks of the second super-group (random motion (4), stationary pattern (5) and untrained control (6); *super_group* = 0) also behaved similarly, but a difference occurred between the two super-groups. I thus constructed five models ([1/2,3,4,5,6], [1,2/3,4,5,6], [1,2,3/4,5,6], [1,2,3,4/5,6] and [1,2,3,4,5/6]). Therefore, 25 (=5x5) models were constructed after considering all possible combinations for males and females. Note that 20 of these 25 models represented sex differences, and the other five did not. AICs were calculated for each model, so that I could exhaustively search the pattern of super-groups that most closely matched the observed preference.

Effects of day (*age*) were examined together with the factor *sex* in both experiments 1 and 2 by constructing the following models (type C). Data obtained in the **random motion** and **untrained** groups were included.

$$C \quad X = \gamma_0 + \gamma_1 * sex + \gamma_2 * age + \gamma_3 * training + \gamma_4 * sex : training + \gamma_5 * sex : age$$

Here, I have three explanatory variables (*sex*, *training* and *age*) together with two interaction terms (*sex:training* and *sex:age*). *Sex* denotes a categorical variable (either *male* or *female*), whereas *training* represents whether chicks were trained or not (**random motion**; *training* = 1, **untrained**; *training* = 0). *Age* represents whether chicks were tested at day 2 (*age* = 0) or day 5 (= 1).

3. RESULTS

Experiment 1: BM preference

Following training with animation, day 2 males preferred **W-hen** to **R-hen** in test, irrespective of whether they had been exposed to **W-hen** or other stimulus types (**R-hen**, **pendulum** and **random motion**) (Fig. I-2). On the other hand, among females, only day 2 chicks that had been trained with **W-hen** stimuli showed a preference for **W-hen**. In the following, I will show two lines of statistical computation for these conclusions.

Effects of visual experiences were supported by the GLM analysis based on the type A formulation (Table I-2). The full model (composed of *group* and *sex*) gave rise to the smallest AIC (1237.8). In contrast, the partial models (composed only of *group* or *sex*) exhibited large AICs, and the AIC for the null model (*sex* and *group* variables not included) was even larger (1257.2). I therefore examined if the effects of visual experiences differed between males and females in the following analysis using the type B formulation.

Differences between males and females were confirmed (Table I-3). Four models were chosen for the smallest AICs, and compared with the five models that did not assume sex differences, i.e., the models with identical super-grouping between males and females. The combination of super-groups ([1,2,3,4/5,6] for males and [1/2,3,4,5,6]

for females) exhibited substantially smaller AIC (1222.3) than the second (1232.9), the third (1235.2) and the fourth models (1235.9). On the other hand, all five models without sex differences gave rise to larger AICs (1241.9 – 1254.2), and the AIC of the null model (no super-grouping included) was largest (1257.2).

In males (but not females), individuals with higher approach scores during training exhibited a stronger preference for BM. In Fig. I- 3, data obtained in four groups (**W-hen**, **R-hen**, **pendulum** and **random motion**) are plotted against the number of approaches (open circles). A Spearman rank test revealed a significant correlation among males ($r=0.45$, $t=2.626$, $0.01 < p < 0.05$, $n=29$) but not females ($r=-0.11$, $t=0.635$, $0.05 < p$, $n=34$). **Stationary pattern** group data are shown as gray discs (Fig. I-3). It should be noted that the number of approaches (ranging from 13-130 in seven males, and from 36-109 in seven females) overlapped with those in the four groups, although the preference of these chicks was distributed around 0 sec, as in the **untrained** chicks (data not plotted). I therefore conclude that the locomotor activity involved in training alone does not cause the BM preference I observed in testing.

The effects of *age* were examined in groups trained with random motion. A GLM analysis based on type C formulation revealed a clear contribution of *age*, but interaction terms with *sex* were not included among the best three models with the smallest AIC (Table I-4).

Experiment 2: Hen preference

When trained with **random motion**, females (but not males) preferred the **W-hen** to the **W-cat** stimuli in both ages (day 2 and 5, Fig. I-4). No preference was found in trained males or naïve chicks of both sexes. GLM analysis based on the type C

formulation (Table I-5) revealed a clear contribution of the interaction term (*sex:training*), but *age* was not included among the best 3 models with the smallest AIC.

4. DISCUSSIONS

BM preference is biologically predisposed and not learned

The present results revealed two major findings: (1) BM preference has an innate basis, but (2) it can be permissively induced for functional expression in the early post-hatch period, particularly in males. In experiment 1, males trained with any animation stimulus preferred the **W-hen** to the **R-hen** stimuli at testing (Fig. I-2). It should also be noted that males trained with the pendulum or random animations preferred the **W-hen** stimulus, even though they had never seen it before. The results of the present study thus differ from previous reports, in which animals memorized elements of the point-light animations.

The preference for BM may occur via specific learning through the chick's own locomotion, similarly to a report in humans (Casile & Giese, 2006). However, this is not plausible in the present case, because the males trained with random motion walked a considerable distance but did not show a preference for **W-hen** stimuli (Fig. I-3). However, no clear preference was found in the untrained chicks, in contrast to the previous report by Vallortigara et al. (2005). The discrepancy may be ascribed to the much smaller sample sizes of the present study, different genetic backgrounds of the subject chicks, or the pretreatment of the chicks tested in the previous report (Vallortigara et al., 2005). Further studies using domestic chicks of different strains, and studies using different species of Galliformes are needed.

Possible involvement of induced BM preference in imprinting

The current results suggest that imprinting is a complex phenomenon involving multiple processes, in which innate preference and the memory formation interact. In the present study, chicks that actively moved between the two opposing monitors tended to show a stronger preference for BM (Fig. I-3, males). Such an activity-dependence has been documented in imprinting since Hess (1958, 1959) reported “the law of effort,” which states that the further a chick runs, the more intensively it is imprinted.

Furthermore, the BM preference appeared only in day-2 chicks, and was not found in day-5 chicks (Figure I-2 and Table I-4), similarly to the sensitive period in imprinting (Hess, 1958, 1959, Bateson, 1979). This finding suggests that, in nature, the induced BM preference may help chicks learn the visual features of their mother hen more effectively, as Vallortigara et al. (2005) previously discussed.

Ecological accounts of sex differences

The sex differences in BM preference found in this study may have been caused by differences in reproductive strategies. In mate choice, females are generally choosier than males, and choices are often based on motion perception. For example, in wild red jungle fowls (*Gallus gallus*, the ancestor of domestic chickens), females choose males based on their external traits (e.g., morphology of cockscomb) and courtship displays (Zuk et al., 1995).

The notion that females are choosier is also supported by the results of experiment 2, in which females preferred the **W-hen** to the **W-cat** stimulus at both days 2 and day 5. Since both **W-hen** and **W-cat** are BM animations, females would be expected to exhibit a preference based on more specific attributes than those examined in experiment 1.

Future studies should examine whether a similar sex difference also occurs in sexually mature females and males.

Alternatively, the sex difference may be explained in terms of the dispersion from the home nest. Male Belding's ground squirrels disperse further than females to avoid inbreeding (Holekamp & Sherman, 1989). In the case of domestic chickens, on the other hand, a dominant male maintains and patrols a large territory where a number of females reside (McBride et al., 1969). These ecological contexts are in accord with the notion that male chickens are likely to seek novelty, to actively disperse. The sex difference found in experiment 1 might thus be explained by a difference in novelty-seeking behaviour. However, this cannot account for the sex difference found in experiment 2.

A third alternative explanation for the sex differences I observed is the influence of genetic differences in domesticated chickens. The strain of chickens used in the present study were bred specifically to exhibit that the flight feathers of female chicks grow faster than those of males, as a cue for determining the sex of hatchlings. A series of sex-linked alleles are reported to influence various other traits such as growth rate, sexual maturity, and the rate of survival (Dunnington et al., 1986; Tamura et al., 1987). It is therefore critically important to examine whether similar sex differences also occur in other birds of the order Galliformes such as quails, which have been less selectively bred than domesticated chickens.

FIGURES

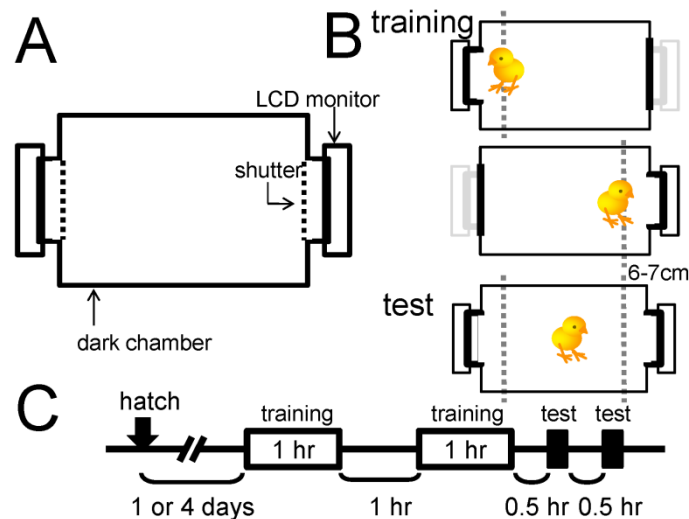


Figure I-1 Experimental apparatus and experimental procedures. (A) A dark chamber equipped with two LCD monitors. A shutter was placed over each LCD monitor to control visual stimulation. (B) In training trials (upper), two monitors were alternately turned on by shutters, so that the chick was exposed to the same training animation displayed on either of the monitors every 1 min. In test trials lasting 5 min each (lower), both monitors were turned on at the same time. I measured the preference between the two animations displayed simultaneously. (C) Schedule of training and test trials. Chicks were trained twice, with an interval of 1hr. At 30 mins post-training, chicks received two test trials, with a 30 min interval.

Experiment 1: walking hen vs. rotating hen

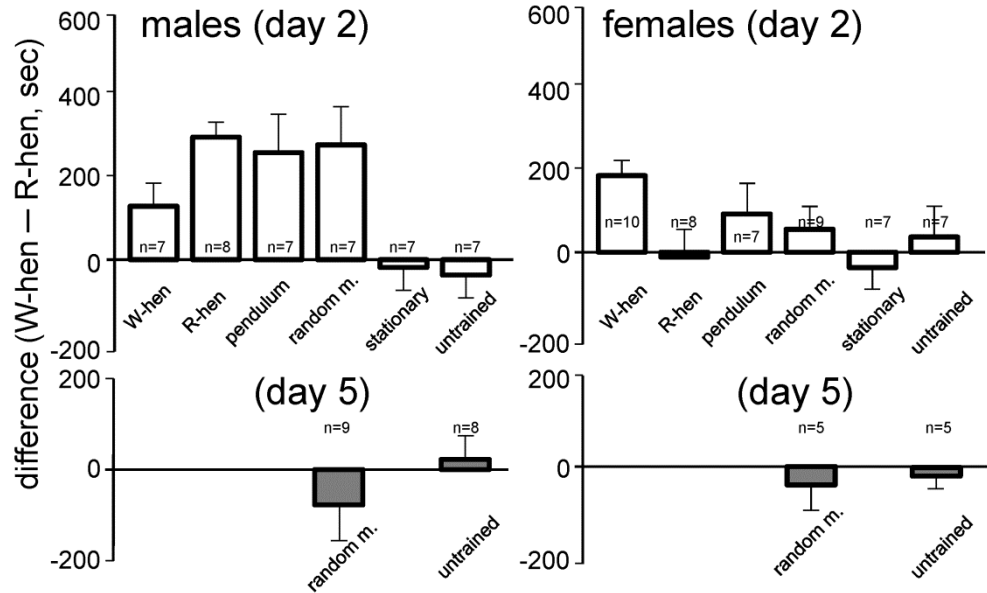


Figure I-2 Induced preference to biological motion in newly-hatched males (experiment 1). Difference in stay time (walking hen (W-hen) minus rotating hen (R-hen), sec, mean $\pm$ SEM) in test trials are shown for each group of chicks. Open and filled columns indicate data obtained in day-2 and day-5 chicks, respectively. Groups differed in the point-light animations used in the training trials; walking hen (W-hen), rotating hen (R-hen), pendulum, random motion (random motion), stationary pattern (stationary). Data were compared together with those obtained in untrained control chicks (untrained). For statistics, see text and tables 2, 3 and 4.

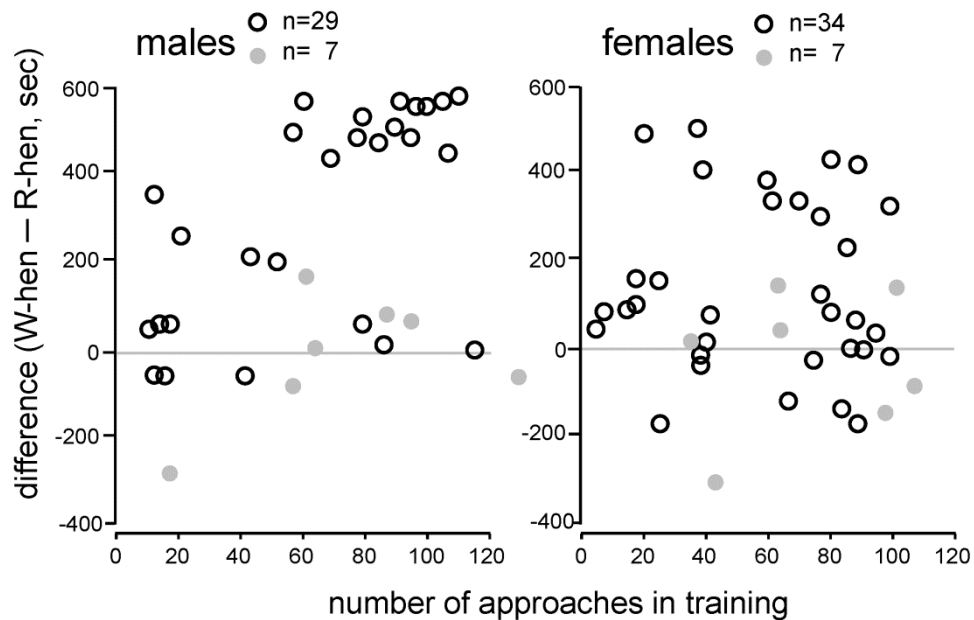


Figure I-3 Influences of approach count in training on biological motion preference (experiment 1). Difference in stay time (y-axis) was plotted against the number of approaches to the monitors in training trials (x-axis). Symbols represent individual chicks. Open circles indicate chicks in the “W-hen,” “R-hen,” “pendulum” and “random motion” groups. Gray circles indicate chicks in the group “stationary” (stationary pattern); note that these chicks also showed a considerable number of approaches in training, but failed to show distinct preferences in testing. Day-2 data were merged, and day-5 data and untrained data (control at both ages) were not included.

Experiment 2: walking hen vs. walking cat

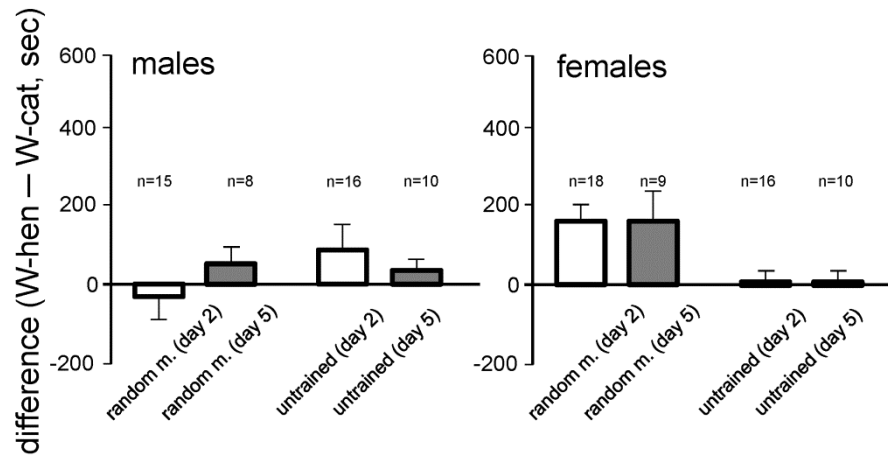


Figure I-4 Induced preference to hen-like animation in females (experiment 2).

Differences in stay time (W-hen minus W-cat) are shown as mean $\pm$ SEM. Open and filled columns indicate data obtained in day-2 and day-5 chicks, respectively. Data obtained from chicks trained with random motion were compared with untrained control data. See table 5 for statistics.

TABLES

Experiment 1					
animations	<i>BM</i>	<i>hen-based animation</i>	<i>periodic animation</i>	<i>motion</i>	<i>light points</i>
W-hen (walking hen)	○	○	○	○	○
R-hen (rotating hen)		○	○	○	○
pendulum			○	○	○
random motion				○	○
stationary					○
Experiment 2					
animations	<i>BM</i>	<i>hen-based animation</i>	<i>periodic animation</i>	<i>motion</i>	<i>light points</i>
W-hen (walking hen)	○	○	○	○	○
W-cat (walking cat)	○		○	○	○
random motion				○	○

Table I-1 Attributes of animations used for training and testing in groups in experiments 1 and 2.

Experiment 1: walking hen vs. rotating hen

Models	AIC	α_0 (<i>intercept</i>)	α_1 (<i>group</i>)	α_2 (<i>sex=male</i>)
Null model ($X=\alpha_0$)	1257.2	139.8	-	-
$X=\alpha_0 + \alpha_2 \cdot \text{sex}$	1254.6	90.3	-	104.8
$X=\alpha_0 + \alpha_1 \cdot \text{group}$	1242.3	332.8	-56.8	-
$X=\alpha_0 + \alpha_1 \cdot \text{group} + \alpha_2 \cdot \text{sex}$	1237.8	284.0	-58.1	112.4

Table I-2 General linear models (GLMs) were constructed for analyzing the differences among groups of different training conditions in experiment 1. AICs (Akaike Information Criteria) and estimated coefficients were compared among the four models; null model, model with *group* as explanatory variable, model with *sex* variables, and full model. Bold letters indicate that the coefficients could include 0 at a low probability of $p < 0.05$, meaning that these variables should be taken into account.

Experiment 1: walking hen vs. rotating hen

Models	super-grouping		AIC	β_0 (intercept)	β_1 (super_group)
	males	females			
Null model	(no super-groups)		1257.2		-
Models of the smallest AICs	[1,2,3,4 / 5,6]	[1 / 2,3,4,5,6]	1222.3	21.6	275.9
	[1,2,3 / 4,5,6]	[1 / 2,3,4,5,6]	1232.9	52.6	247.9
	[1,2,3,4 / 5,6]	[1,2 / 3,4,5,6]	1235.2	22.1	227.8
	[1,2,3,4 / 5,6]	[1,2,3 / 4,5,6]	1235.9	26.2	224.8
Models with identical super-grouping between males and females	[1 / 2,3,4,5,6]		1254.2	113.7	139.7
	[1,2 / 3,4,5,6]		1248.9	81.5	160.8
	[1,2,3 / 4,5,6]		1246.4	51.4	171.2
	[1,2,3,4 / 5,6]		1241.9	-7.6	213.1
	[1,2,3,4, 5 / 6]		1253.6	5.2	159.1

Table I-3 GLMs were constructed for analyzing the differences among groups of different training conditions in experiment 1. Of 25 possible models with different or identical super-grouping in males and females, four models with the smallest AICs are compared with the null model in the upper lines; note that the best model has a substantially smaller AIC (=1223.9) than the other models. In the lower lines, five models with identical super-grouping are shown. Smaller italic letters indicate that the probability is higher than 0.05, meaning that the contributions of these variables are likely to be moderate.

Experiment 1: walking hen vs. rotating hen

Models	AIC	γ_0 (intercept)	γ_1 (sex=male)	γ_2 (age)	γ_3 (training)
Null model	773.4				
1	771.0	85.0	—	-130.0	—
2	772.3	61.8	—	-111.3	43.6
3	772.5	66.8	39.1	-118.3	—
Full model	776.5				

Table I-4 GLMs were constructed for analyzing the effects of sex and age in experiment 1. Only those data obtained in the random motion and untrained groups were included. The top three models with the smallest AICs (1-3) are shown together with the estimated coefficients (γ_0 to γ_3).

Experiment 2: walking hen vs. walking cat

Models	AIC	γ_0 (intercept)	γ_1 (sex=male)	γ_4 (sex=male: training)	γ_4 (sex=female: training)
Null model	1369.6				
1	1360.3	28.0	—	—	142.4
2	1361.6	39.4	—	-37.4	130.9
3	1361.7	3.81	71.3	-73.1	166.5
Full model	1366.3				

Table I-5 GLMs were constructed for analyzing the effects of sex and age in experiment 2. Data obtained in all groups were included. The top three models with the smallest AIC (1-3) are shown together with estimated coefficients (γ_0 , γ_1 and γ_4).

Chapter II

Biological motion facilitates filial imprinting

Highlights

- **Walking hen** facilitates both the approach activity and the learned preference, although significant positive correlations do not appear between these at the individual level.
- Local movement features of the BM animation are critical in making chicks approach and learn the associated color.
- Chicks with a high BM preference score show a high learning score in subsequent imprinting.

1. INTRODUCTION

Animations composed of moving light points, if appropriately arranged to represent the major joints of a walking human, generate a vivid perception of locomotion. This phenomenon is known as the perception of biological motion (BM) (Johansson, 1973). Highly simplified though the animation is, human observers detect not only the direction and type of the movement (Cutting et al., 1988), but also infer various features associated with the animation such as gender (Kozlowski and Cutting, 1977), age (Pavlova et al., 2002) and emotion (Dittrich et al., 1996), and can even identify the individual (Cutting and Kozlowski, 1977). The cognitive capability for BM perception has been supposed to be limited only to human adults with mature visual processing mechanisms, as it develop gradually and slowly in juveniles (Pavlova et al., 2001, Blake et al., 2003, Freire et al., 2006).

Recent studies have shown that BM perception might arise much earlier in life and occur in taxonomically remote animals. Pioneering works by Vallortigara and his colleagues reported that newly-hatched domestic chicks show a preference to the point-light animation depicting a walking hen (BM) over non-BM alternatives (Vallortigara et al., 2005, 2006). More recently, however, I found that visually inexperienced chicks failed to show a BM preference, but they did show a preference when they had been pre-treated with any sort of point-light animation (Miura and Matsushima, 2012). The animation did not necessarily have to be a BM picture, and even an assembly of randomly moving light points was effective. I therefore agree with the idea that BM preference is predisposed (Rosa Salva et al., 2015) after a slight modification. Chicks do not learn BM, but the innate BM preference is induced through non-specific visual experiences.

BM preference in the early stage of life is also found in humans, as 2-day-old newborn babies prefer the walking hen animation in preference looking tests (Simion et al., 2008). Though it is unclear whether chicks or newborn human babies have cognitive processes similar to those employed by human adults, the BM preference in the early post-natal (post-hatch) period may be based on an evolutionarily ancient mechanism shared by birds and humans.

What functional roles might the BM preference play in early life? Imprinting, and the social bond formation that follows, might be a target. Chicks of precocial birds follow conspicuous objects and learn their visual features (colour and shape in particular) after a brief exposure in the early post-hatch period, and this process is referred to as imprinting (Lorenz, 1937) (also see reviews (Bolhuis and Honey, 1998, Matsushima et al. 2003, Horn 2004)). Even though chicks can be imprinted with a variety of artifacts such as a rotating cylinder or a toy made of LEGO blocks (Izawa et al., 2001, Yamaguchi et al., 2012), the preference gradually shifts toward more natural objects like a stuffed hen (Johnson & Bolhuis, 1985), suggesting that filial behaviour is also influenced by an innate predisposition (Bolhuis 1991, Rosa Salva et al., 2015). I may assume a possibility that the imprinted chicks gradually shift their preference to those objects with a predisposed nature, such as BM pictures. Alternatively, the BM preference may arise earlier than the filial imprinting, allowing chicks to form a memorised attachment selectively to those objects.

In this study, I investigated the possible contribution of BM preference to imprinting by examining two possible scenarios: (1) chicks would selectively learn the colour associated with BM animations; and (2) if pre-treated with non-specific visual stimuli to show a BM preference, chicks would show a higher score in learned colour

preference by imprinting. To do that, I recorded two behavioural parameters: the number of approaches to a single visual stimulus during imprinting, and the learned colour preference in a binary choice test.

2. MATERIALS AND METHODS

Subjects

Domestic chicks of white Leghorn (*Gallus domesticus*, Julia strain, n=221) were used at 24–48 hours post-hatch. Fertilised eggs supplied from a local hatchery (Iwamura Poultry Co. Ltd., Yubari, Japan) were incubated in the laboratory. The inside of the incubator was kept in darkness. To avoid post-hatch visual experiences, hatchlings were individually housed and kept in another incubator in complete darkness. All experiments were conducted under the guidelines and approval of the Committee on Animal Experiments of Hokkaido University. The guidelines are based on the national regulations for animal welfare in Japan (Law for Humane Treatment and Management of Animals; after a partial amendment No. 68, 2005). After the experiments, chicks were sexed and euthanised by carbon dioxide.

Apparatus

I used an I-shaped maze (9 cm × 70 cm) equipped with a 50-cm-long treadmill consisting of a rubber belt at the centre, and an LCD monitor at each end. During imprinting and pre-treatment (Figure II-1A), an infrared sensor and a transparent Plexiglass partition were placed at a point 10 cm from the monitor, and the other monitor was occluded by an opaque partition. When chicks ran and hit the sensor, the rubber belt of the treadmill moved for a short period of 0.3 sec, drawing the chick

backward by *ca.* 30 cm at a time. The treadmill motion was digitally counted, and the number of approaches was recorded for each of the trials, which lasted for 1 hour if not stated otherwise.

In the binary choice test (Figure II-1B), the partitions were removed and the treadmill was turned off. The subject chick was enclosed in a start box placed at the centre for *ca.* 10 sec, and was then allowed to freely go out and choose between the two arms. I recorded the total stay time near each monitor for a period of 5 min, starting from the point when the chick walked out of the starting box. The choice test was repeated twice with the side changed at intervals of 0.5 hours (in Experiment 1 and 2) or ~1–2 min (Experiment 3-1 and 3-2). The behaviour of the subject chick was monitored through a CCD camera (250 kilo pixels) placed on the ceiling, and the videos were stored for offline analysis in a video camera (DCR-SR60, Sony, Japan). The apparatus was placed in a sound-proof wooden box, and the inside of the box was illuminated by infrared LED lamps.

Video clips and point-light animations

For imprinting and pre-treatment, I used a full-screen colour illumination (**plate**), two realistic video clips (**LEGO block** and **real chick**), and seven point-light animations (**LEGO point**, **walking hen**, **stationary dots**, **walking chick**, **scrambled**, **linear motion**, and **stationary chick**). The colour preference was tested using a binary choice between two **plates** with different colours (yellow and red). The BM preference was tested using a binary choice between a pair of point-light animations (**walking hen** and **rotating hen**). For samples of these materials, see Supplemental Information.

The point-light animations were composed of 13 light points and displayed at a

speed of 30 frames/sec. I edited video clips and animations using Adobe Premiere (Elements 7) and the colour was set either to red (R: 255, G: 0, B: 0), yellow (R: 255, G: 255, B: 0) or white (R: 255, G: 255, B: 255). These stimuli were displayed on the LCD monitors (size 10.4", 800 × 600 pixels, Logitec LCM-T102AS, Japan; flash rate: 56–75 Hz, brightness: 230 cd/m², pitch size: 0.264 × 0.264 mm) using free viewer software (A-player, version 6.0) on Windows PC. The width of the presentation was set at 9 cm on the monitor.

Statistical analysis

I used R (version 2.12.0) for non-parametric tests (Kruskal–Wallis test, Steel–Dwass's multiple comparisons, Spearman rank-order correlations, Wilcoxon rank sum test). I also constructed generalised linear models (GLMs) in R, and evaluated them using the Akaike information criteria (AICs). The recorded colour preference score (difference of stay time in binary choice test, sec) was given as a weighted sum of several explanatory variables. As the link, I adopted the logistic function. For details, see our previous report (Miura and Matsushima, 2012) and Supplemental Information.

3. RESULTS & DISCUSSIONS

Experiment 1: Effects of stimulus motion on imprinting

As the first step, I prepared five types of visual stimulus (Figure II-2); full screen colour (**plate**), video clip of a rotating **LEGO block**, point-light animation made from the block (**LEGO point**), BM animation depicting a **walking hen**, and a still pattern of randomly arranged light points (**stationary dots**). I recorded two behaviours, one during imprinting (number of approaches during the 2 hour) and the other during

the test (colour preference score) (Figure II-1).

Among the five groups, significant differences occurred in the number of approaches (Figure II-3; Kruskal–Wallis test, $\chi^2 = 37.05$, $df = 4$, $p = 1.759 \times 10^{-7}$). *Post hoc* Steel–Dwass multiple comparisons (performed in all of the 10 possible pairs) revealed significant differences in the pairs indicated by asterisks. The motionless **plate** resulted in an approach number comparable to that of the **LEGO block**. Conversely, the **LEGO point** group was significantly lower than the **LEGO block** ($t = 3.873$, $p = 1.018 \times 10^{-3}$), even though the **point** depicted the motion of the **block**. Among the three groups trained by point-light animations, the **walking hen** group was higher than the other two (vs. **LEGO point**: $t = 3.816$, $p = 1.279 \times 10^{-3}$; vs. **stationary dots**: $t = 4.073$, $p = 4.454 \times 10^{-4}$), but it was comparable to the **LEGO block** and the **plate**. Furthermore, the **stationary dots** was significantly lower than the **plate** ($t = 3.434$, $p = 5.376 \times 10^{-3}$), which was also motionless. I conclude that motionless colour plates are also attractive, if they are given in full-screen size (9 cm wide). When the colour is presented as moving light points, on the other hand, the BM feature is critical.

A similar pattern of intergroup differences occurred in the colour preference score (Figure II-4) (Kruskal–Wallis, $\chi^2 = 25.01$, $df = 4$, $p = 5.013 \times 10^{-5}$). No significant difference appeared between the **plate** and the **LEGO block**, whereas the **LEGO point** was significantly lower (Steel–Dwass; vs. **LEGO block**: $t = 3.415$, $p = 5.749 \times 10^{-3}$). Among the three point-light animations, the **walking hen** was higher than the other groups (significantly vs. **stationary dots**: $t = 3.092$, $p = 1.700 \times 10^{-2}$; not significantly vs. **LEGO point**: $t = 2.342$, $p = 0.1317$). Clearly, chicks learn the motionless full-screen colour. However, the BM features of the point-light animations facilitate imprinting. In repeated testing on the following day (test 2 after test 1), chicks of each group showed a

highly reproducible colour preference (Supplemental Figure II-S1).

At the individual level, however, no significant correlations appeared between the number of approaches and the colour preference score (Figure II-5).

Spearman rank-order correlation analysis failed to detect significant positive correlation, but a significant negative correlation ($\rho < 0$) occurred in two groups (**LEGO point** and **walking hen**; see the figure legend). This indicates that the number of approaches during imprinting is not assumed to be a reliable predictor of the learned colour preference during testing.

To obtain reliable estimates of the contributing factors, GLM analysis was applied to the colour preference score. I merged all five groups of chicks and constructed a total of 32 GLMs for all possible combinations for five variables: *colour* (red or yellow), *motion* (motionless or motion picture), *point_light* (point-light animation or otherwise), *sex* (female or male), and *approach* (number of approaches in imprinting); see Supplemental Table II-S1 for details. The best and the second-best model in terms of AIC included *point_light* as the most effective variable with a negative coefficient ($\beta_3 < 0$ at confidence level $p = 4.190 \times 10^{-5}$ for the best model, and 3.960×10^{-5} for the second-best model). The less effective variables included *motion* ($\beta_2 > 0$ at $p = 2.020 \times 10^{-3}$ and 1.998×10^{-3}) and *colour* ($\beta_1 > 0$ at $p = 2.700 \times 10^{-3}$ and 2.431×10^{-3}). However, the confidence levels of the estimated coefficients of *sex* (β_4) and *approach* (β_5) were negligible. Here again, the number of approaches failed to predict the learned colour preference.

BM facilitated imprinting, but point-light animations were generally weaker at the population level. At the individual level, however, the chick's approaches to the visual stimulus (during imprinting) and the consequent colour preference (during

testing) were not linked. Critical features of the moving pictures, yet to be characterised, should independently regulate these two aspects of imprinting.

Experiment 2: Effects of BM animation on imprinting

A variety of objects have been used in previous imprinting studies. In its early history, Fabricius and colleagues (Fabricius and Boyd, 1954) systematically investigated these objects and reported that ducklings are imprintable to a wide range of artifacts (such as boxes, balloons, and toy railway trucks) and biological models (human, duck, duckling, stuffed animals, and model ducks). They thus concluded that object features like size, colour and shape do not limit the imprinting. Hess (Hess, 1959) also reported that stuffed brown Leghorn chicken was the least efficient model, and artifacts such as a plain ball or a super-structured ball with attachments (wing, tail and head on it) resulted in a higher score. Imprinting objects should not necessarily look natural.

Object movement is also not critical. Gray (Gray, 1969) showed that chicks are imprinted after exposure to stationary geometrical figures for 24 hours. In quail chicks, preference to colours has a genetic basis (Kovach, 1980), and the chicks changed their preference after exposure to a motionless colour plate (Kabai and Kovach, 1993). However, most studies of imprinting have empirically used moving objects such as a decoy duck moving around a round-shaped runway (Hess, 1959), a cylinder or a box (Horn, 2004), and a LEGO block (Izawa et al., 2001, Yamaguchi et al., 2012) rotating along its vertical axis. The use of artifacts moving in a non-biological manner has been the norm in the imprinting studies, and the role of motion has not been given attention.

In the present study (Experiment 1, Figure II-4, 5), exposure to the colour **plates** proved to be effective similarly to the quail chick study cited above. The **LEGO**

block video on monitor was also as effective as the real LEGO objects (Izawa et al., 2001), suggesting that chicks do not so much care about the depth of moving object. On the other hand, the **LEGO point** animation that depicted the motion of **LEGO block** failed. The **walking hen** animation was strikingly effective despite the point-lights, and I thus assume that the BM feature facilitated the imprinting. In fact, Fabricius stated *“movement of some parts of the body in relation to other parts, as when a duck waddled or a man walked, was an important factor in evoking following in ducklings”* ((Fabricius, 1951) as cited by Sluckin (Sluckin, 1972)). An assembly of objects that maintain relative distance and/or synchronised movements with one another may facilitate imprinting. In the following Experiment 2, I examined whether this idea applied to our case of point-light animations.

To characterise the critical features of the BM picture, I prepared three point-light animations from a video clip of **real chick** (Figure II-6). **Walking chick** was composed of 13 light points, each representing a part of a chick’s body such as the head, neck, body, limbs and tail. **Scrambled** was made from **walking chick** by randomising the spatio-temporal relationship of the light points, thus it had only the movements of points as a common feature with the **walking chick**. Conversely, **linear motion** maintained the global configuration of **walking chick**, but lacked the local movement of points. In this and the following experiments, to avoid possible ceiling effects on imprinting when using yellow, I used red animations.

In number of approaches (Figure II-7), the Kruskal–Wallis test revealed a statistically significant difference among the four groups ($\chi^2 = 19.91$, $p = 1.773 \times 10^{-4}$). I made *post hoc* comparisons in the 3 pairs by assigning the **walking chick** group as the reference control. The **real chick** group was comparable to the **walking chick** (Steel’s

multiple comparison test: $t = 6.427 \times 10^{-1}$, $p = 8.574 \times 10^{-1}$) and so was the **scrambled** ($t = 2.117$, $p = 8.803 \times 10^{-2}$), but the **linear motion** was significantly lower ($t = 3.705$, $p = 6.053 \times 10^{-4}$). Movements of individual points are thus apparently critical.

Colour preference score showed a similar pattern of differences (Kruskal–Wallis; $\chi^2 = 15.68$, $p = 1.320 \times 10^{-3}$). In *post hoc* Steel's multiple comparisons, the **real chick** group and the **scrambled** were comparable to the **walking chick** ($t = 7.559 \times 10^{-1}$, $p = 7.906 \times 10^{-1}$; statistical results were coincidentally equal), whereas the **linear motion** group was lower ($t = 3.402$, $p = 1.861 \times 10^{-3}$). Within each group, repeated tests yielded highly reproducible preference scores (Supplemental Figure II-S2). Between the approach number and the colour preference, I found a significant positive correlation only in the **real chick**, but not in the other three groups (Figure II-S3 for test 1). In the repeated test on the following day (test 2), no significant correlation appeared for any of the four groups (Figure II-S4).

To estimate the critical factors, I merged the colour preference score of the four groups, and constructed GLMs by adopting five explanatory variables: *shape* (real chick image or point light animation), *local_movement*, *global_configuration*, *sex* (female or male) and *approach* (number of approaches in imprinting). The results showed that the best and the second best models included *local_movement* as an effective variable ($\beta_2 > 0$; confidence level $p = 2.480 \times 10^{-5}$ and 8.600×10^{-4} respectively; the test 1 data), and no other variables contributed significantly (Supplemental Table II-S2). Similar results were found for the test 2 data. Taken together, I conclude that local movements of the point-light animations are critical, which separately regulate the attractiveness and the colour learning, yielding a considerable interindividual variance in both aspects of imprinting.

BM and local movements of individual light points

In Experiment 2, I constructed three types of point-light animations from a video clip of **real chick**, and two of these (**walking chick** and **scrambled**) had local movements of points in common. In both imprinting (number of approaches) and tests (colour preference scores), **walking chick** and **scrambled** gave rise to comparable results, whereas **linear motion** was ineffective (Figure II-7, 8). The local movements of individual points thus play a critical role, whereas the geometric relationships among points do not contribute.

The credit for the scrambled animations should be ascribed to Vallortigara and his colleagues (Vallortigara et al., 2005), who were the first to construct a motion picture by randomising the relative locations of light points. All nearby points were thus de-synchronised. In our study, in addition to the location, the phase relationships were also randomised, so that all pairs of points were de-synchronised. In other words, the global configuration (or the geometric associations among points) was preserved in **linear motion**, but it was completely removed in **scrambled** used in this study. It should be noted, however, that **scrambled** gave rise to a slightly lower average than **walking chick** in both behavioural scores (Figure II-7, 8), although this difference was not statistically significant. The partial contribution of the global configuration may be considered.

Experiments 1 and 2 also revealed a high degree of interindividual variance among chicks despite the same visual experiences (see Supplemental Figures II-S3 and II-S4). Variance in the colour preference score may be because of the different sensitivity to the BM pictures. I examined this in the following set of experiments.

Experiment 3-1: Induction of BM preference by pre-treatment

As a first step, I examined if BM preference could be induced by the **linear motion** that proved to be ineffective in imprinting in Experiment 2. Two groups of chicks received pre-treatments, one using **linear motion** and the other **stationary chick**, both composed of white light points (Figure II-9). These chicks were then tested using a binary choice between simultaneously presented **walking hen** and **rotating hen**.

In the number of approaches in pre-treatment (Figure II-10), the **linear motion** group was higher than that of the **stationary chick** in both 1 (Wilcoxon rank sum test: $W = 39.5, p = 4.418 \times 10^{-2}$) (size effect was estimated by Grissom's probability of priority $\hat{p}_s = 0.6172$; (Grissom, 1994)) and 2 ($W = 51.5, p = 4.103 \times 10^{-2}$; $\hat{p}_s = 0.8516$), but the significance level was marginal. In test 1 and test 2 (Figure II-11), similarly, the **linear motion** was higher than the **stationary chick** in their preference of **walking hen** over **rotating hen**, and the difference was significant in test 1 ($W = 52, p = 3.497 \times 10^{-2}$; $\hat{p}_s = 0.8125$) but not in test 2 ($W = 43.5, p = 0.2401$; $\hat{p}_s = 0.6484$). Pre-treatment using **linear motion** thus induced BM preference at the population level with a considerable interindividual variance. The induced BM preference was consistent in the two tests (Figure II-S5), but it lacked a significant correlation with the number of approaches in pre-treatment (Figure II-S6).

Experiment 3-2: Induced BM preference and learned colour preference

For the second step, I examined if a higher BM preference could lead to a higher imprinting score. Two groups of chicks were similarly pre-treated by **linear motion** or **stationary chick** (both in white), tested for their BM preference (**walking hen** over **rotating hen**; both in white), further imprinted using **real chick** (in red), and

then tested for a learned colour preference of red over yellow (Figure II-12). Expecting to detect a larger interindividual variance, I intentionally adopted a weak imprinting paradigm for 15 min.

In the approach number in pre-treatment (Figure II-13), the **linear motion** group was higher than the **stationary chick**, but a significant difference did not appear (Wilcoxon rank-sum test: $W = 152$, $p = 2.099 \times 10^{-1}$; $\hat{p}_s = 0.6354$). In the BM preference score (Figure II-13), however, a significant difference occurred between them ($W = 178$, $p = 2.073 \times 10^{-2}$; $\hat{p}_s = 0.7417$). In the subsequent imprinting (Figure II-14), similarly, the difference was not significant ($W = 152$, $p = 2.109 \times 10^{-1}$; $\hat{p}_s = 0.6333$). In the final colour preference tests (Figure II-14), no significant difference appeared ($W = 155$, $p = 1.710 \times 10^{-1}$; $\hat{p}_s = 0.6458$). At the level of population means, therefore, the induced BM preference seemed not to significantly facilitate imprinting. At the individual level (Figure II-15), however, a significant correlation appeared between the two preference scores in the **linear motion** group (Spearman rank-order correlation: $\rho = 0.7129$, $p = 1.936 \times 10^{-3}$), but not in the **stationary chick** ($\rho = 0.04297$, $p = 8.791 \times 10^{-1}$).

I merged the colour preference scores of two groups and constructed GLMs by adopting five explanatory variables: *BM_preference* (score in the BM preference test), *pre-treatment* (number of approaches in pre-treatment), *imprinting* (number of approaches in imprinting), *type* (animation used in pre-treatment), and *sex* (female or male) (Supplemental Table II-S3). The results showed that *BM_preference* proved to be the most effective variable (β_1 ; confidence level $p = 1.200 \times 10^{-2}$ for the best model, and 1.640×10^{-4} for the second best), whereas all other variables (including *type*) failed to contribute. The induced BM preference thus proved to be a critical determinant of the individual imprintability.

BM preference may precede imprinting

In Experiments 3-1 and 3-2, I pre-treated chicks by exposing them to **linear motion** or **stationary chick** in white. In our previous study (Miura and Matsushima, 2012), exposure to a variety of point-light animations (either BM or otherwise; rotating hen, pendulum, or random motion, but not stationary image of points) made chicks show a BM preference in choices between **walking hen** and **rotating hen**. Here, I confirmed this phenomenon by using **linear motion** (Figure II-10, 11) that was ineffective in imprinting (Figure II-7, 8).

In Experiment 3-2, I failed to detect a statistically significant difference between these two groups of chicks in the mean colour preference score (Figure II-14). However, the score was correlated with the induced BM preference at the individual level (Figure II-15). I therefore assume that the BM preference develops earlier in nature, and the induced BM preference confines the chick's visual learning to specific objects with BM features, most likely the mother hen that moves nearby the chicks. Chicks would then learn the colour of the hen and form a lasting social bond with it.

Those chicks with a high BM preference score tended to approach more in the subsequent imprinting (Experiment 3-2, Supplemental Figure II-S7), but the correlation was not statistically significant ($p = 9.158 \times 10^{-2} > 0.05$) and the effect size ($r = 0.4357$) was not high. Facilitated imprinting cannot be ascribed to the higher approaches, which might have made chicks stay around the monitor for a longer period of time. Rather, higher BM preference may directly cause a higher colour preference, despite how active the chick was during the imprinting. As a future project, I will examine whether the IMM (intermediate medial mesopallium) network for imprinting (Horn, 2004, Yamaguchi et al., 2012) might also be involved in BM preference.

If BM preference really precedes imprinting, this is supposed to be distinct from other forms of innate predisposition reported so far (Johnson and Bolhuis, 1985, Salva et al., 2008, 2010, Rosa Salva et al., 2015). Instead of re-orienting the filial behaviour after imprinting, chicks may be predisposed to learn from specific objects by way of the induced BM preference.

Approaches and preference, two distinct parameters of the filial behaviour

The lack of correlations between the number of approaches in imprinting and the colour preference score in tests need a careful consideration. Hess (Hess, 1959) reported that the strength of imprinting was a function of effort paid by the subject ducklings, and coined the term “law of effort.” In our study (Miura and Matsushima, 2012), a similar correlation appeared between the locomotor activity and the induced BM preference. However, the “law of effort” has been criticised (Moltz et al., 1960), and a consensus has not yet been reached. In terms of the neural events underlying the imprinting, for example, the number of Fos-immunopositive neurons in IMM (the brain area involved for imprinting memory (Horn, 2004)) proved to be linearly related to the learned colour preference at test (McCabe and Horn, 1994). However, more recent bioluminescence quantification of the *c-fos* gene expression in IMM (Yamaguchi et al., 2010) revealed a high link with locomotor activity during imprinting. However, the functional links among locomotion, memory and the neural activities yet remain elusive.

Instead, the number of approaches and the preference score might represent two distinct processes of filial behaviour. The approach number recorded in this study may represent a sort of “vigour” of the subject chicks. Conversely, the colour preference score may represent not only the chick’s preference but also the tendency to leave the

imprinting object, as chicks often shuttled between the two monitors in tests. In a behavioural study mimicking the optimal patch use-behaviour, I found that the patch-use time at a gradually depleting food patch followed a Poisson distribution, suggesting that the decision to leave is a stochastically determined behaviour (Matsusnami et al., 2012). In the present choice test, similarly, the stay time at an option may be reduced if the subject has a higher tendency to leave and explore the alternative, so that a proactive and neophilic chick may have a lower score given an identical preference. Distinct processes might cause distinct interindividual variations between these two parameters. Independent tests for personality (or behavioural syndromes (Sih and Giudice, 2012)) must be done in future to determine its possible link to the preference score.

FIGURES

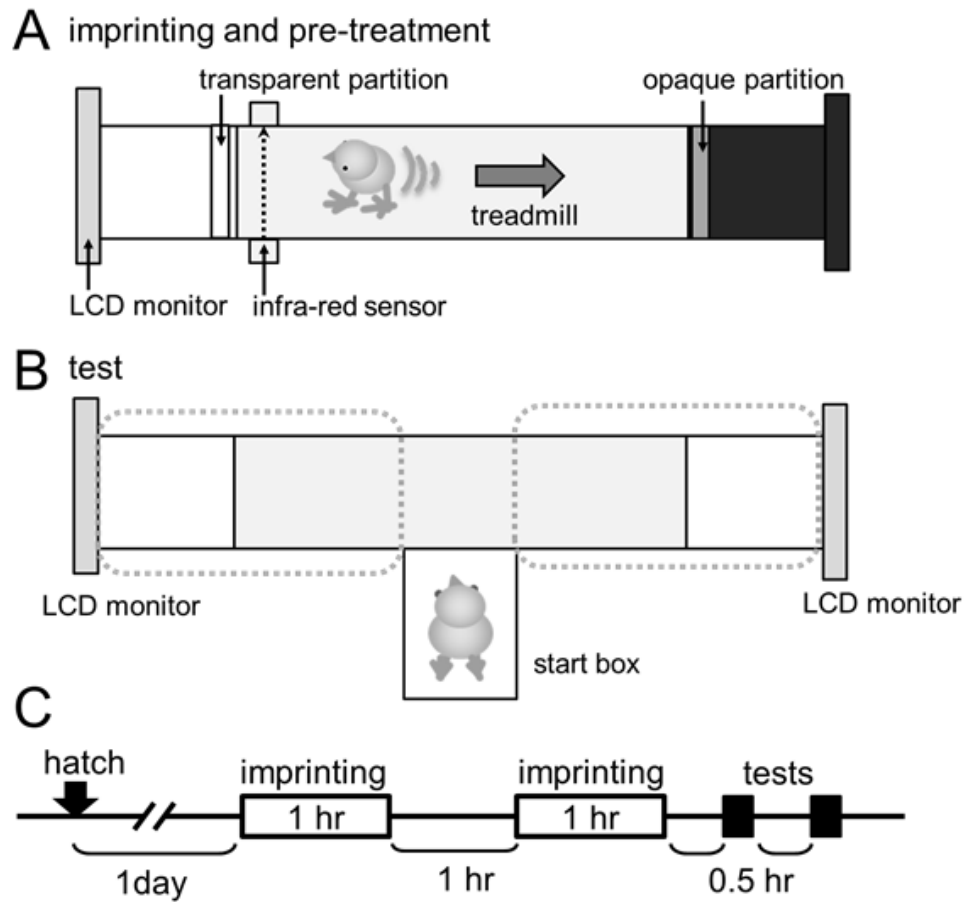


Figure II-1. Apparatus used for imprinting and pre-treatment (A) and testing (B).

Procedure is schematically shown in (C) for Experiments 1 and 2. Chicks were imprinted twice at a 1-hour interval, and subsequently tested twice at 0.5-hour intervals. During the first post-hatch day and the intervals between the sessions, chicks were individually housed in a dark incubator at 37–38 °C. For experiments 3-1 and 3-2, see Figures 4A and 5A, respectively.

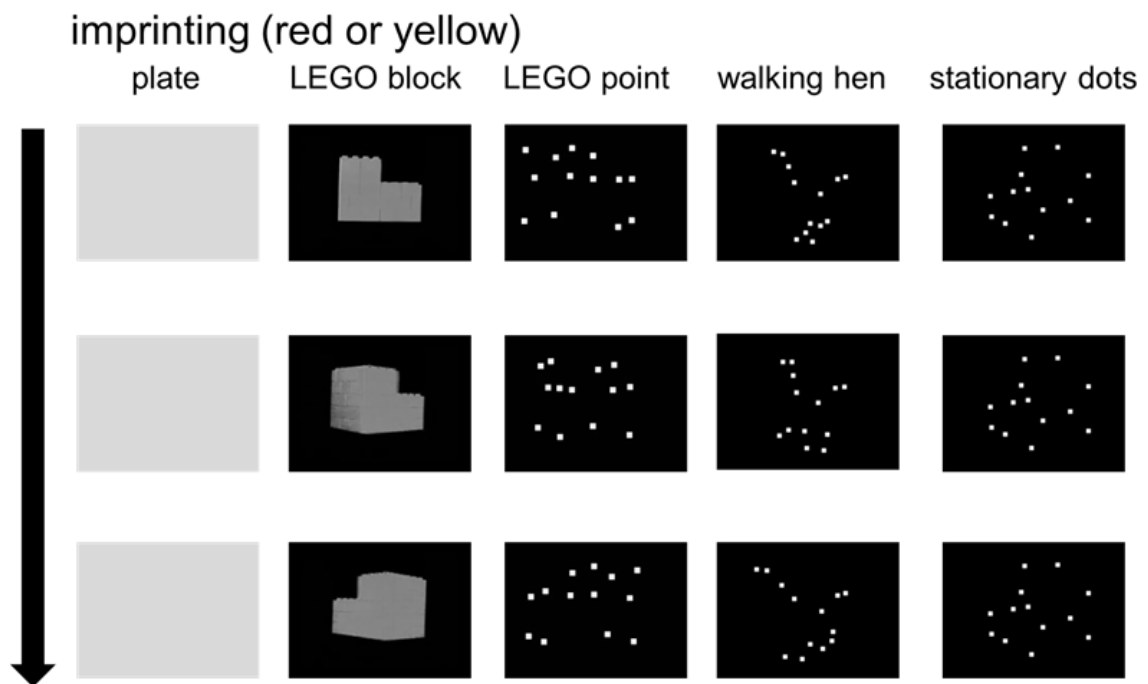


Figure II-2. Effects of motion pictures on the learned colour preference (Experiment 1).
Video clips and point-light animations used for imprinting; for samples, see
Supplemental Information. Pictures were in red or yellow in a balanced manner.

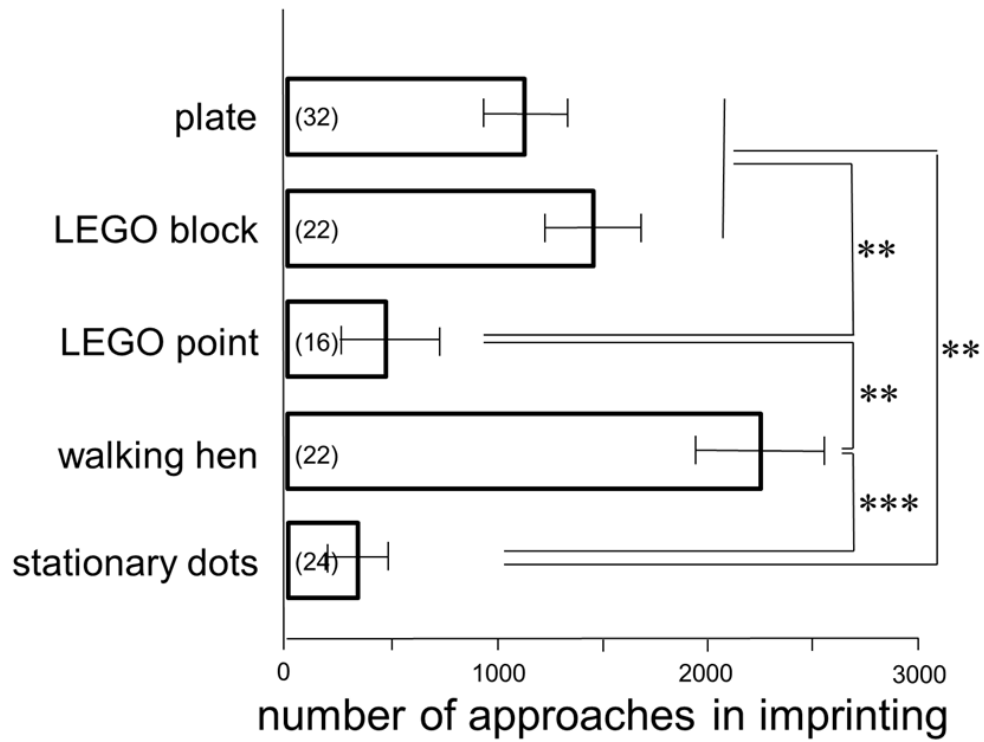


Figure II-3. Effects of motion pictures on the learned colour preference (Experiment 1). Sum of the number of approaches counted during the two imprinting sessions. Columns and bars denote the mean $\pm$ s.e.m. in each group, and numbers in parentheses denote the group size. Asterisks indicate the level of significance after Steel–Dwass multiple comparisons; *: $p < 0.05$; **: $p < 0.01$, ***: $p < 0.001$.

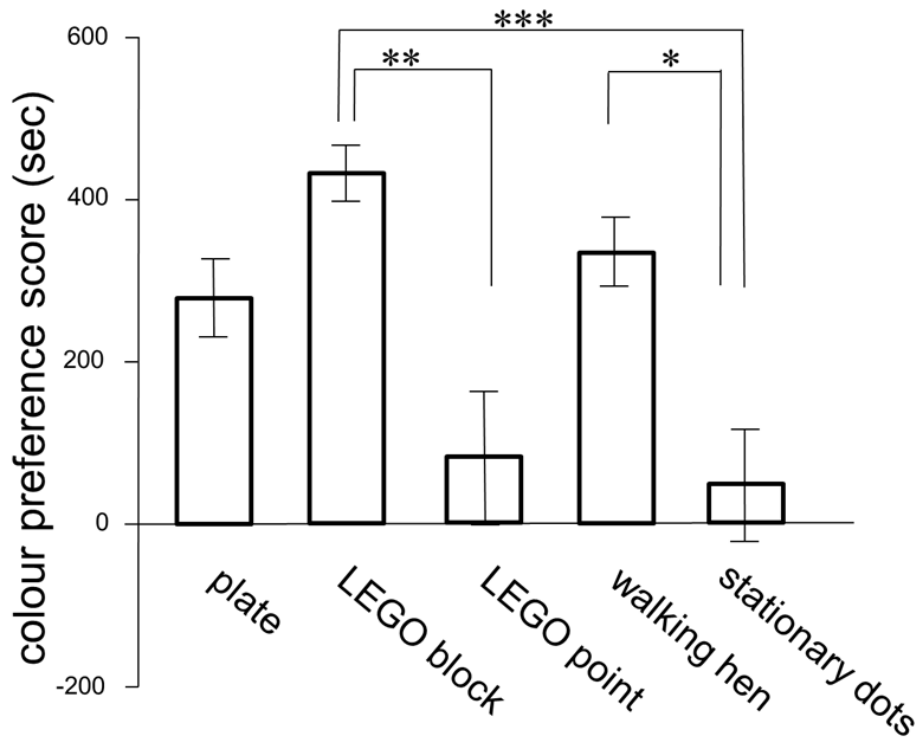


Figure II-4. Effects of motion pictures on the learned colour preference (Experiment 1). Colour preference score measured at tests. Sum of the difference in stay time (imprinting colour minus unfamiliar colour). Columns and bars denote the mean $\pm$ s.e.m. in each group, and numbers in parentheses denote the group size. Asterisks indicate the level of significance after Steel–Dwass multiple comparisons; *: $p < 0.05$; **: $p < 0.01$, ***: $p < 0.001$.

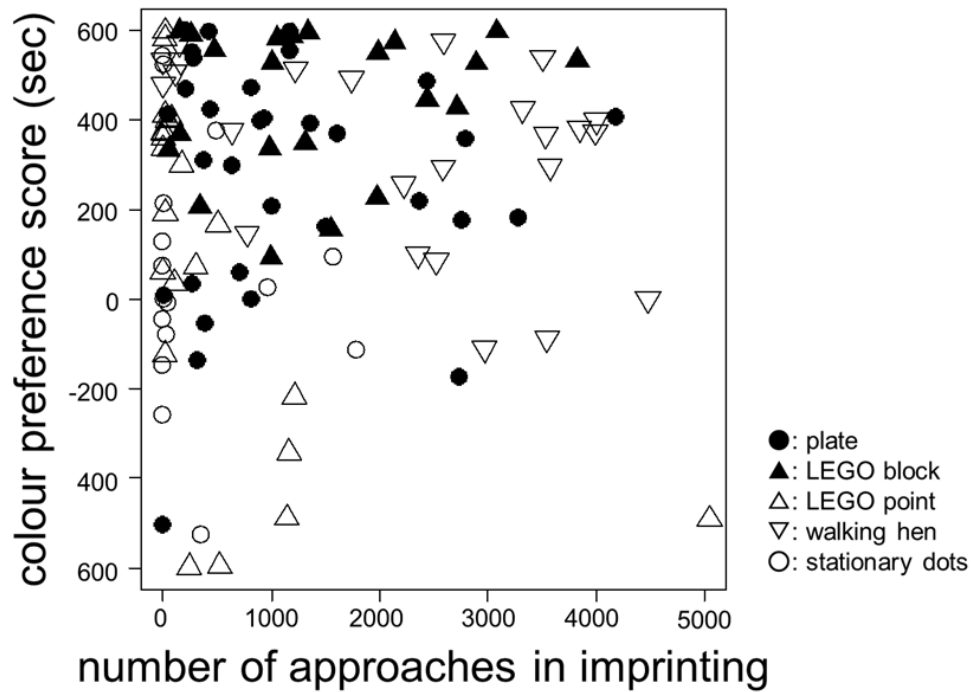


Figure II-5. Effects of motion pictures on the learned colour preference (Experiment 1). Colour preference score was plotted against the number of approaches. Different groups are indicated by different symbols (see inset). At the individual level, no significant positive correlation appeared in any group. Spearman rank-order correlation analysis was repeatedly applied to each group;

plate: $\rho = -0.0273, p = 0.8820$

LEGO block: $\rho = 0.1248, p = 0.5787$

LEGO point: $\rho = -0.5994, p = 3.194 \times 10^{-3}$

walking hen: $\rho = -0.4130, p = 4.594 \times 10^{-2}$

stationary dots: $\rho = -0.005904, p = 0.9827$

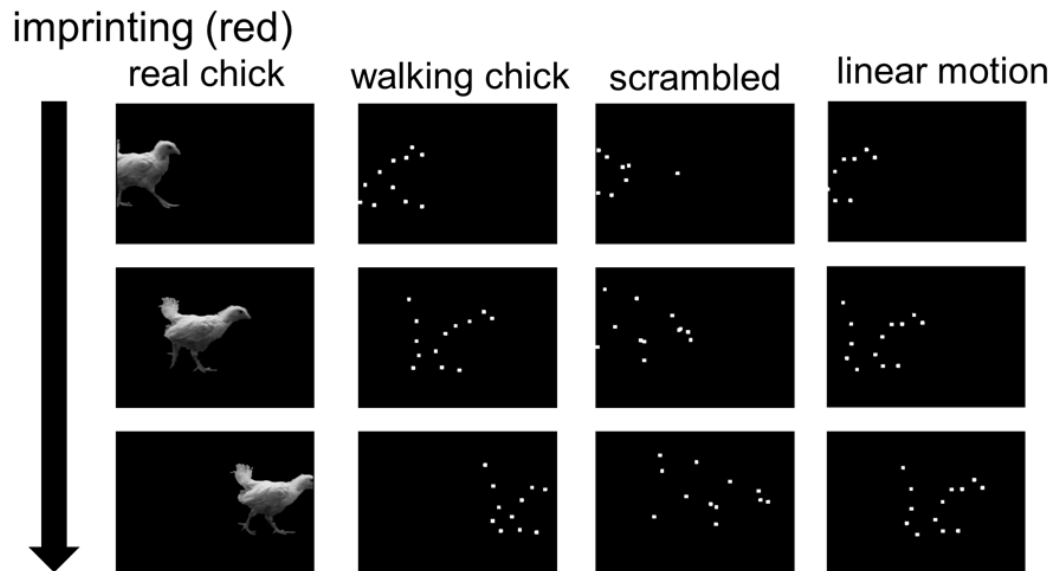


Figure II-6. Effects of BM features on the learned colour preference (Experiment 2).

Video clip and animations. **Walking chick** was made from the **real chick** video.

Scrambled and **linear motion** were made from **walking chick**, each depicting the local movements and the global configuration, respectively.

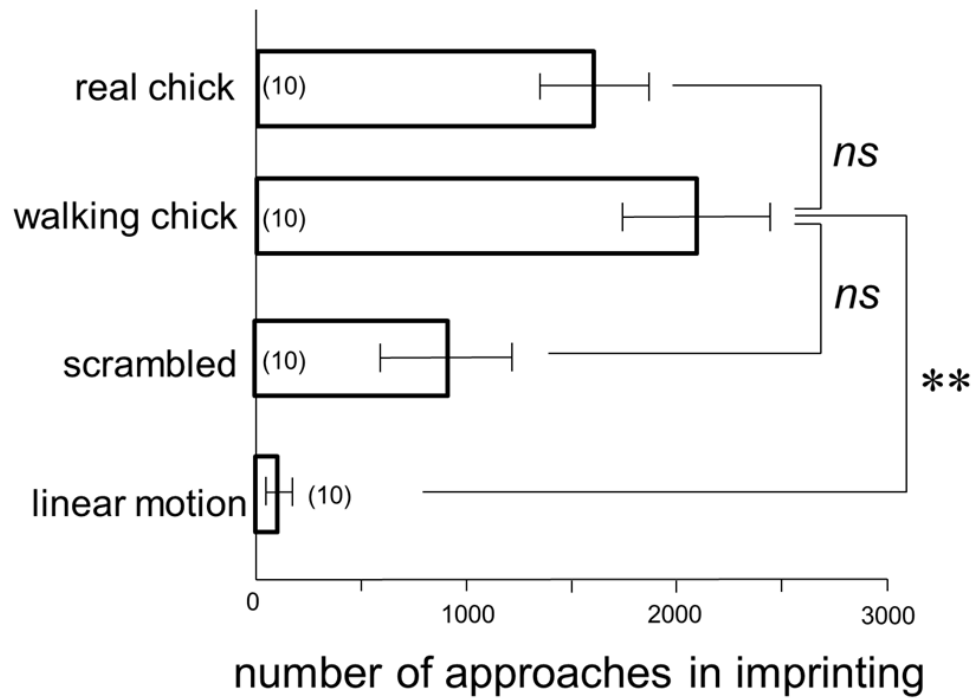


Figure II-7. Effects of BM features on the learned colour preference (Experiment 2).

Sum of the number of approaches. Pairwise multiple comparisons were made by assuming **walking chick** as the reference control group; *: $p < 0.05$; ns: $p > 0.05$ (not significant). Significantly lower scores occurred only in the **linear motion** group.

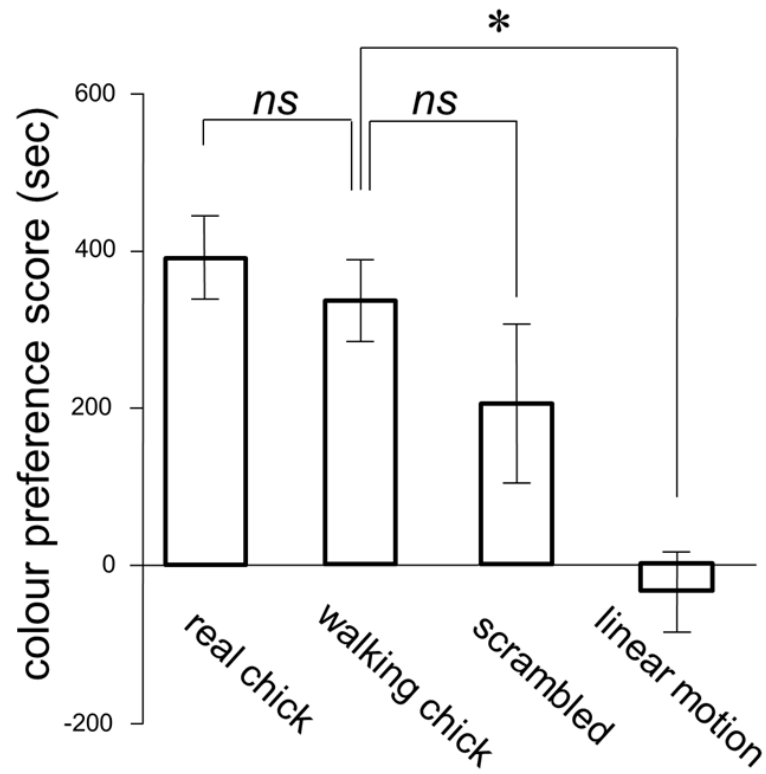


Figure II-8. Effects of BM features on the learned colour preference (Experiment 2).

Colour preferences score. Pairwise multiple comparisons were made by assuming **walking chick** as the reference control group; *: $p < 0.05$; ns: $p > 0.05$ (not significant).

Significantly lower scores occurred only in the **linear motion** group.

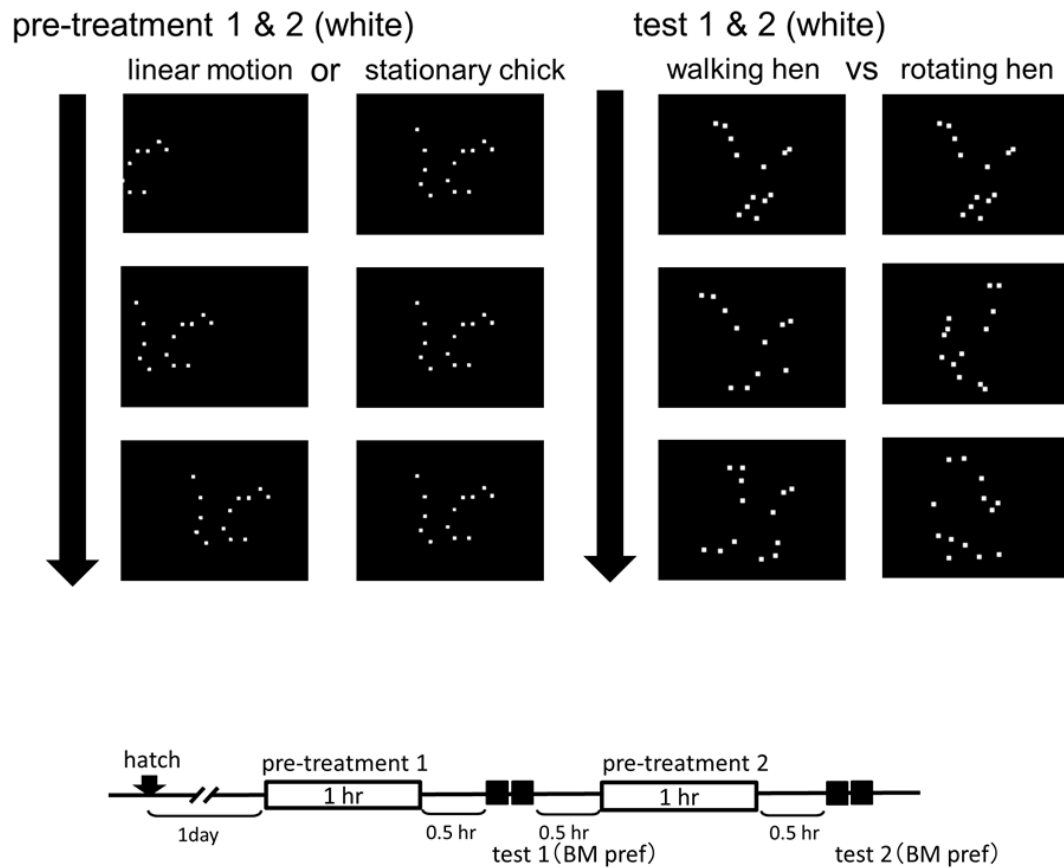


Figure II-9. Effects of motion picture on the BM preference (Experiment 3-1).

Two groups of chicks were repeatedly pre-treated using **linear motion** or **stationary chick** (both in white). At 0.5 hours after each session, each chick was tested twice using binary choice.

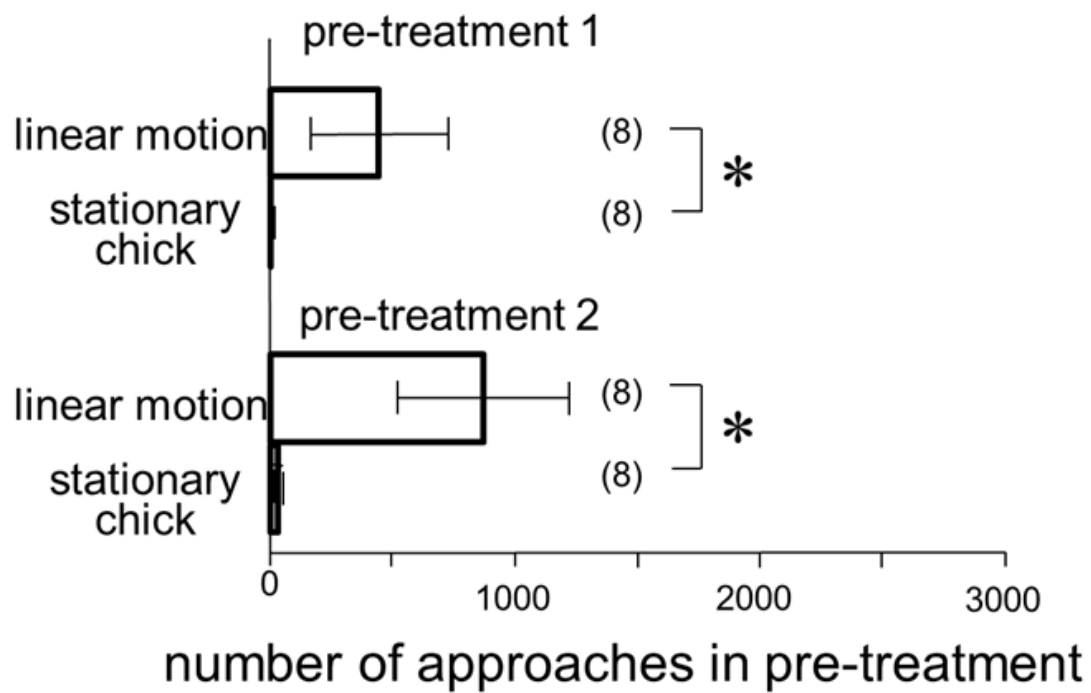


Figure II-10. Effects of motion picture on the BM preference (Experiment 3-1).

Number of approaches in pre-treatment 1 and 2. Note that significant differences appeared even after pre-treatment 1.

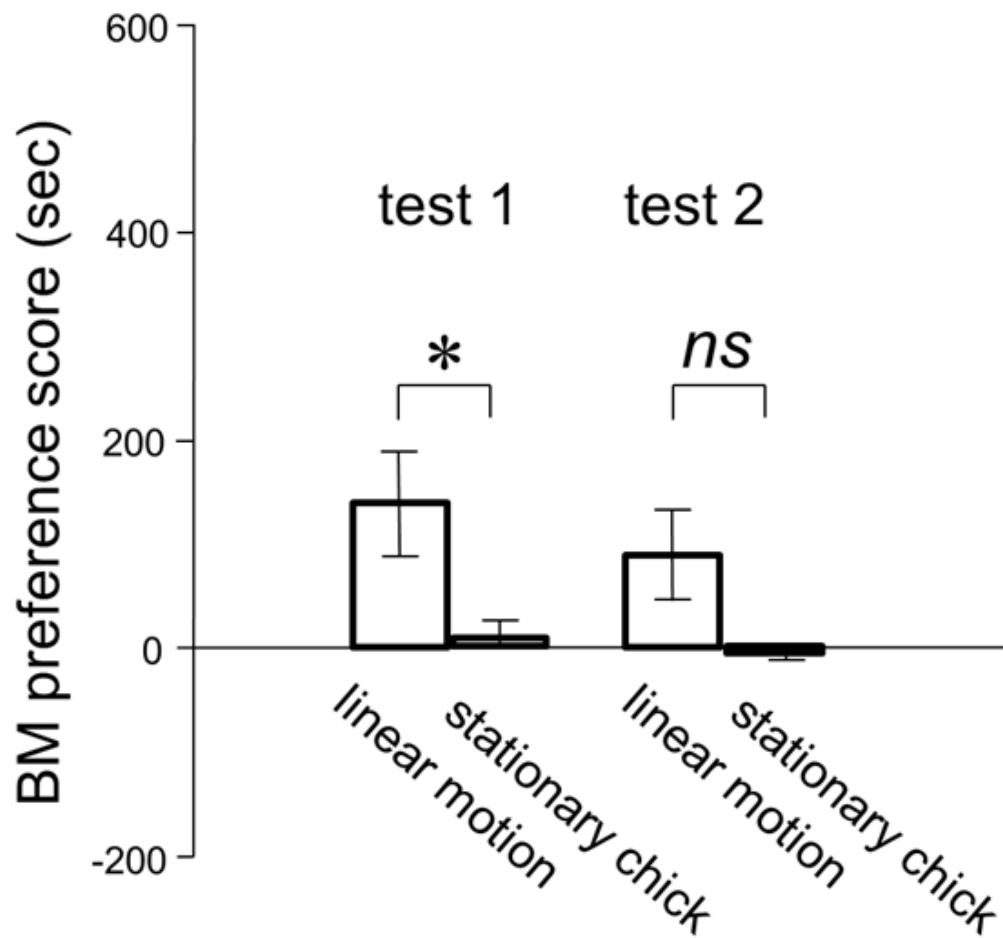


Figure II-11. Effects of motion picture on the BM preference (Experiment 3-1).

BM preference scores (**walking hen** minus **rotating hen**) measured at test 1 and 2. Note that significant differences appeared even after pre-treatment 1.

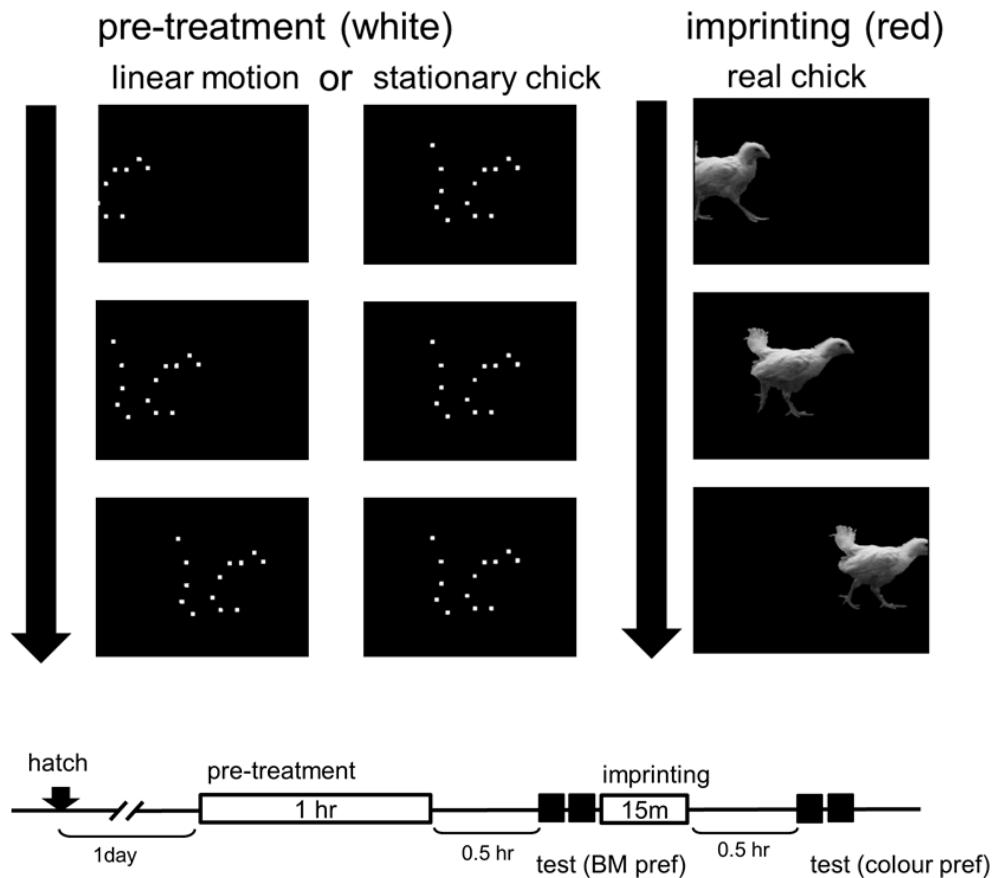


Figure II-12. Effects of the induced BM preference on the learned colour preference (experiment 3-2). Two groups of chicks were pre-treated once by **linear motion** or **stationary chick** (both in white), tested for the BM preference, and then imprinted briefly for 15 min by **real chick** (in red) before the colour preference test (red over yellow).

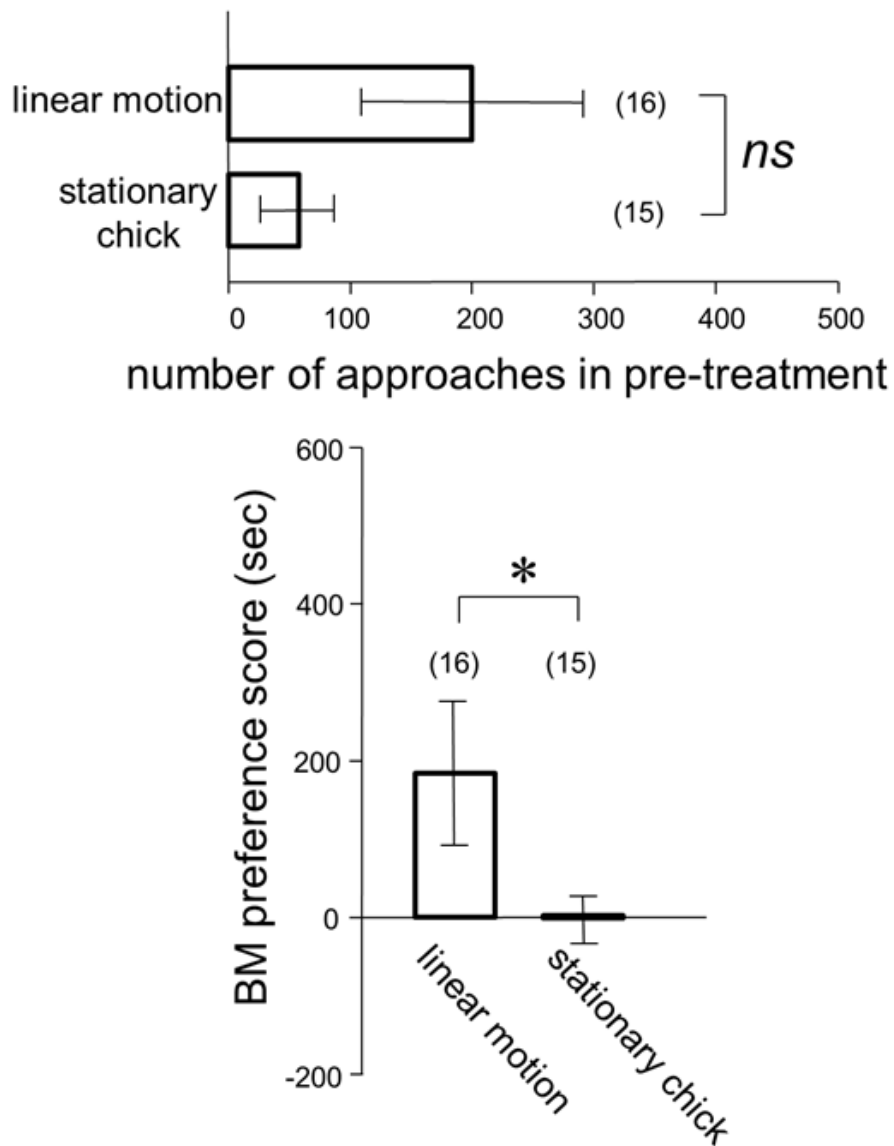


Figure II-13. Effects of the induced BM preference on the learned colour preference (experiment 3-2). Results of pre-treatment. Between the two groups, significant differences appeared in the BM preference.

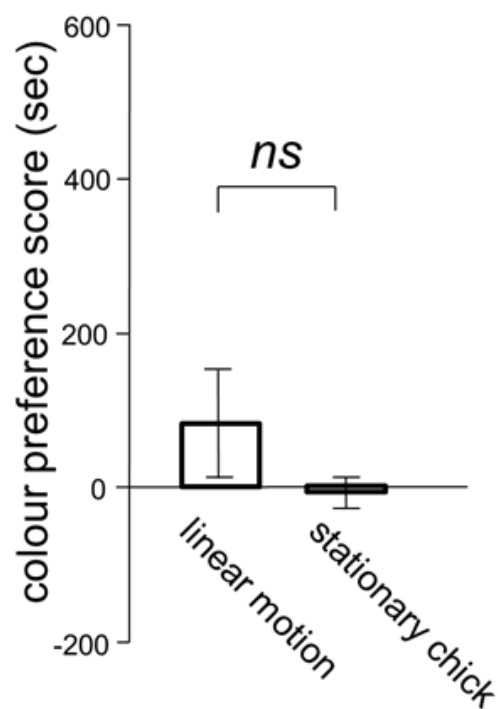
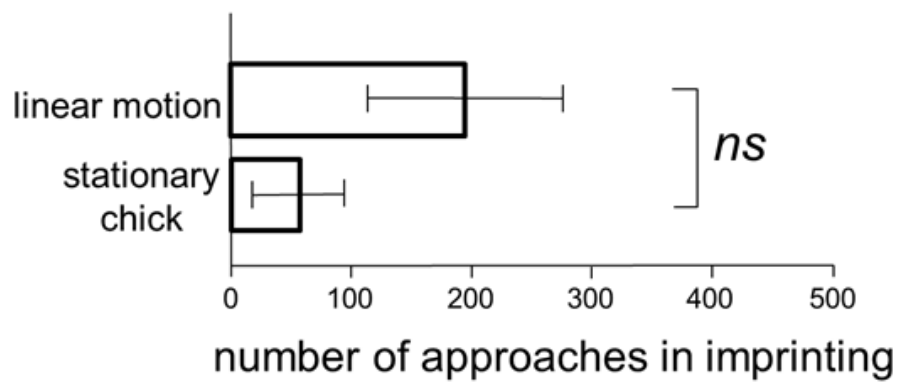


Figure II-14. Effects of the induced BM preference on the learned colour preference (experiment 3-2). Results of imprinting. Between the two groups, significant differences did not appear in the colour preference.

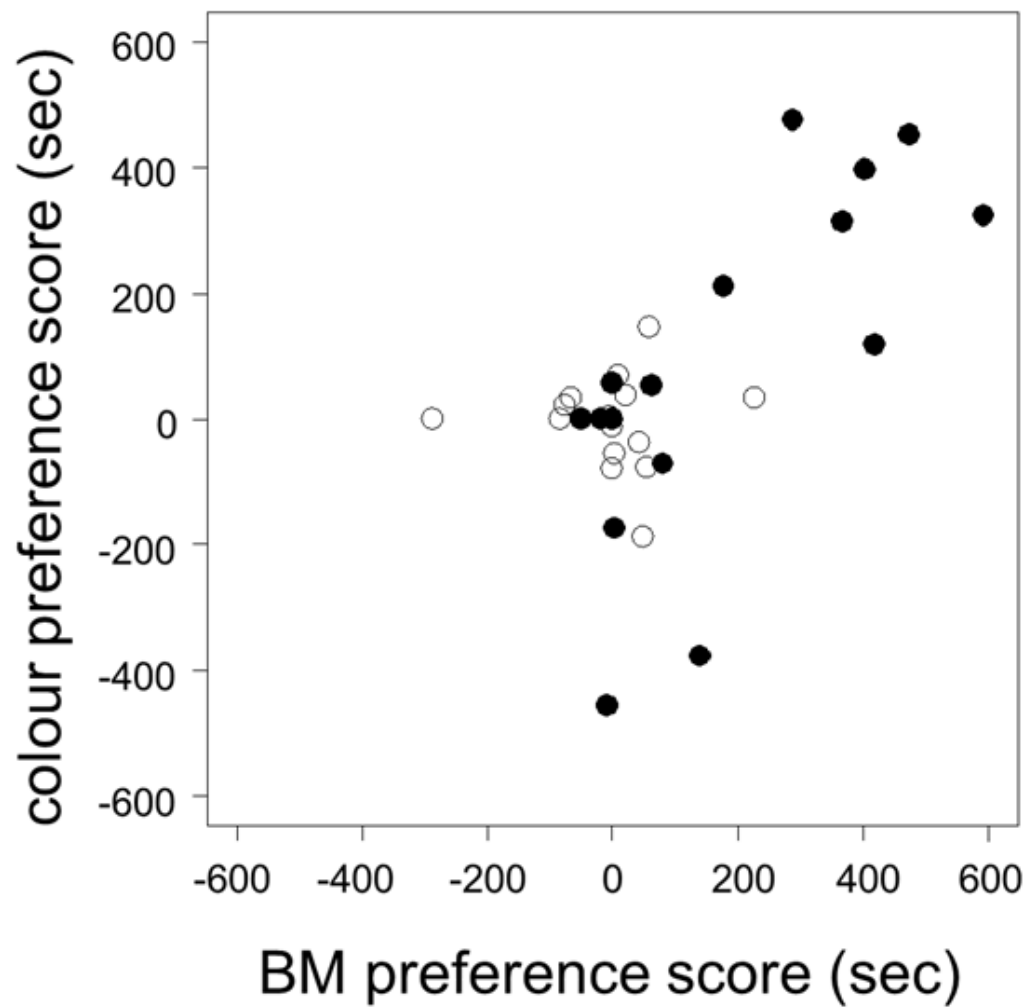


Figure II-15. Effects of the induced BM preference on the learned colour preference (experiment 3-2). Distinct patterns of distribution appeared, suggesting a link between the two scores.

SUPPLEMENTAL DATA

Inventory of the supplemental information

Experiment 1: Effects of stimulus motion on imprinting

Figure II-S1: High reproducibility of the learned colour preference in the repeated test sessions

Table II-S1: Generalized linear models (GLMs) revealed a significant contribution of the stimulus motion to the learned colour preference

Experiment 2: Effects of BM (biological motion) animation on imprinting

Figure II-S2: High reproducibility of the learned colour preference in the repeated test sessions

Figure II-S3, II-S4: No individual-level correlations between the number of approaches in imprinting vs. the colour preference score in tests (test 1, 2)

Table II-S2: GLMs revealed a significant contribution of the factor of local movements of the stimuli to the learned colour preference

Experiment 3-1: Induction of BM preference by pre-treatment using motion picture

Figure II-S5: High reproducibility of the induced BM preference in the repeated test sessions (test 1 and test 2)

Figure II-S6: No individual-level correlations between the number of approaches in pre-treatment vs. the BM preference score in tests (test 1 and test 2)

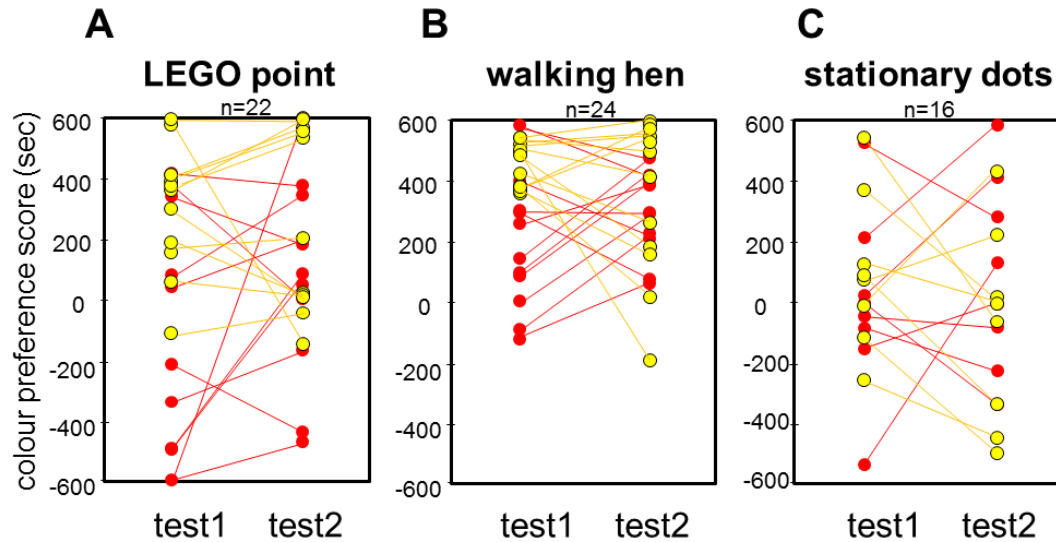
Experiment 3-2: Induced BM preference and learned colour preference by imprinting

Figure II-S7: Factors that potentially contribute to the individual-level variance in the BM preference score, the number of approaches in imprinting, and the learned colour preference

Table II-S3: GLMs revealed a significant contribution of the induced BM preference to the learned colour preference

Figure II-S1: *Experiment 1*

High reproducibility of the learned colour preference in the repeated test sessions



In Experiment 1, three groups of chicks imprinted by point-light animations (**LEGO point**, **walking hen** and **stationary dots**) were tested twice, one immediately after the imprinting (test1), and another on the next day (test2). Colour preference score (difference in the stay time near the imprinted colour minus un-imprinted colour) is plotted in each individual. Red and yellow indicate the imprinted colour. Wilcoxon signed rank tests detected no significant difference between test1 and test2.

LEGO point (red): $V = 19$, $p = 0.2402$, **LEGO point** (yellow): $V = 32.5$, $p = 0.9834$
walking hen (red): $V = 20$, $p = 0.1514$, **walking hen** (yellow): $V = 49.5$, $p = 0.1543$
stationary dots (red): $V = 12$, $p = 0.4609$, **stationary dots** (yellow): $V = 27$, $p = 0.2500$

Table S1: *Experiment 1*

Generalized linear models (GLMs) revealed a significant contribution of the stimulus motion to the learned colour preference

Models	AIC	β_0 (Intercept)	β_1 (Colour)	β_2 (Motion)	β_3 (Point_light)	β_4 (Sex)	β_5 (Approach)
Null	1654.2	<u>252.1</u>	—	—	—	—	—
1	1631.6	<u>187.1</u>	<u>153.1</u>	<u>170.1</u>	<u>-226.5</u>	16.86	—
2	1633.4	<u>195.3</u>	<u>154.0</u>	<u>169.5</u>	<u>-226.3</u>	—	—
Full	1635.3	<u>193.5</u>	<u>151.7</u>	<u>174.0</u>	<u>-228.6</u>	16.94	-0.01

GLMs were constructed for the colour preference score in test1. Data obtained from five groups of chicks were merged. The two best models in terms of AIC were shown.

The full model was defined as:

$$X = \beta_0 + \beta_1 * colour + \beta_2 * motion + \beta_3 * point_light + \beta_4 * sex + \beta_5 * approach + r_i$$

where X represents the colour preference score with logistic link function. As explanatory variables, I configured 5 factors without interactions;

colour (categorical variable = 0 for red, 1 for yellow) in imprinting

motion (categorical = 0 for **plate** and **stationary dots**, 1 for the others)

point_light (categorical = 0 for **plate** and **LEGO block**, 1 for the other pictures)

sex (categorical = 0 for females, 1 for males)

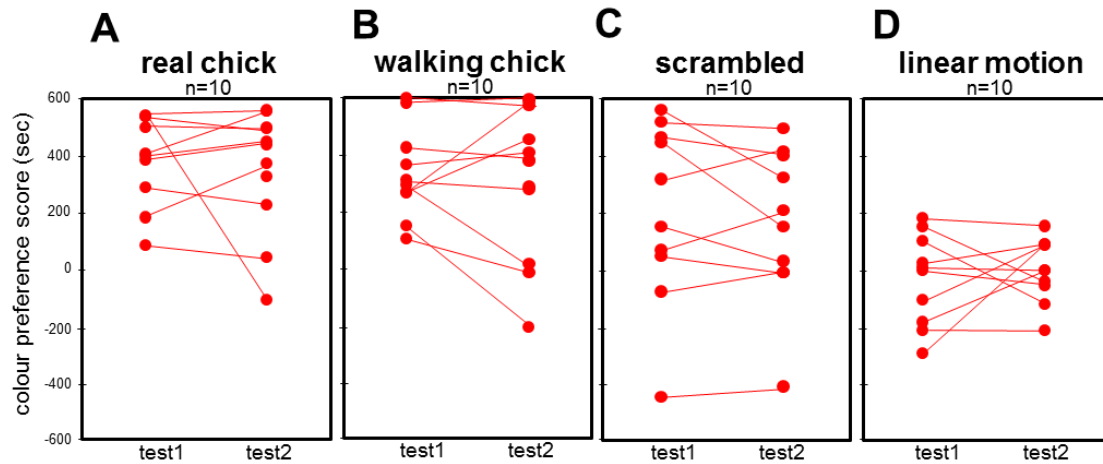
approach (integer = number of approaches in imprinting)

r_i denotes residual individual variance.

For each model, statistically significant coefficients were indicated by different types such as: bold letter: $p < 0.05$, bold letter with an underline: $p < 0.01$, bold/Italic letter with an under: $p < 0.001$. Figures in ordinary letter indicate that the probability of including zero (thus is not significant) is estimated to be higher than 0.05.

Figure II-S2: *Experiment 2*

High reproducibility of the learned colour preference in the repeated test sessions



The learned colour preference (red over yellow) was compared between test1 and test2 at individual level. Wilcoxon signed-rank test revealed no significant difference.

real chick: $V = 24.5$, $p = 0.7910$

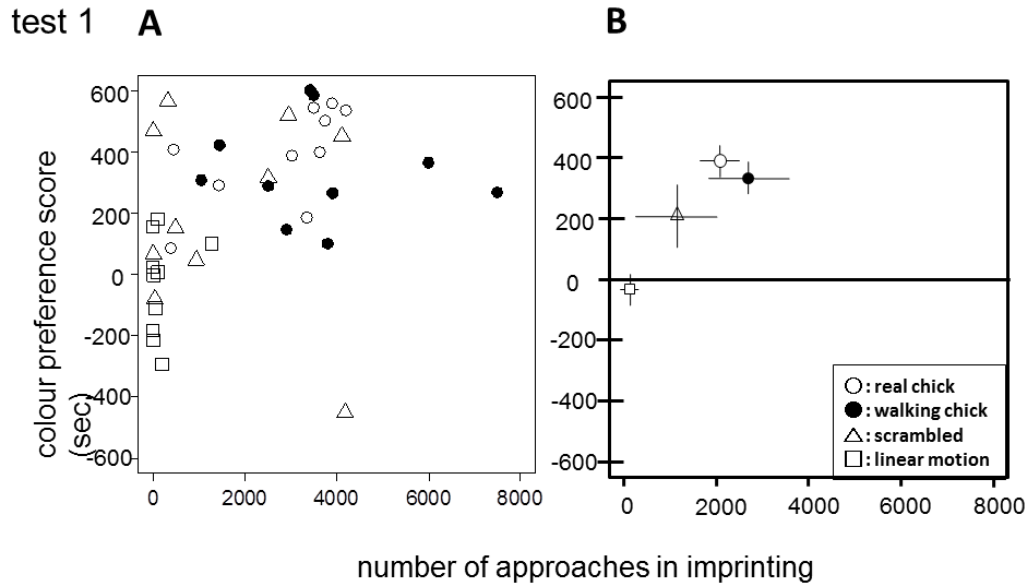
walking chick: $V = 33$, $p = 0.6074$

scrambled: $V = 24$, $p = 0.9102$

linear motion: $V = 25$, $p = 0.8457$

Figure II-S3: *Experiment 2*

No individual-level correlations between the number of approaches in imprinting vs. the colour preference score in test (Results of test 1)



Colour preference score (y-axis: results of test1) was plotted against number of approaches in imprinting (x-axis) at individual (A) and population level (B). In (A), a regression line was derived for each group, except **linear motion** in which the x-axis data (number of approaches) were accumulated around 0. Significant **positive** correlation at $p < 0.05$ appeared only in the group of **real chick** (shown in red).

Real chick: $\rho = 0.7333, P = 0.021$

Walking chick: $\rho = -0.2364, P = 0.514$

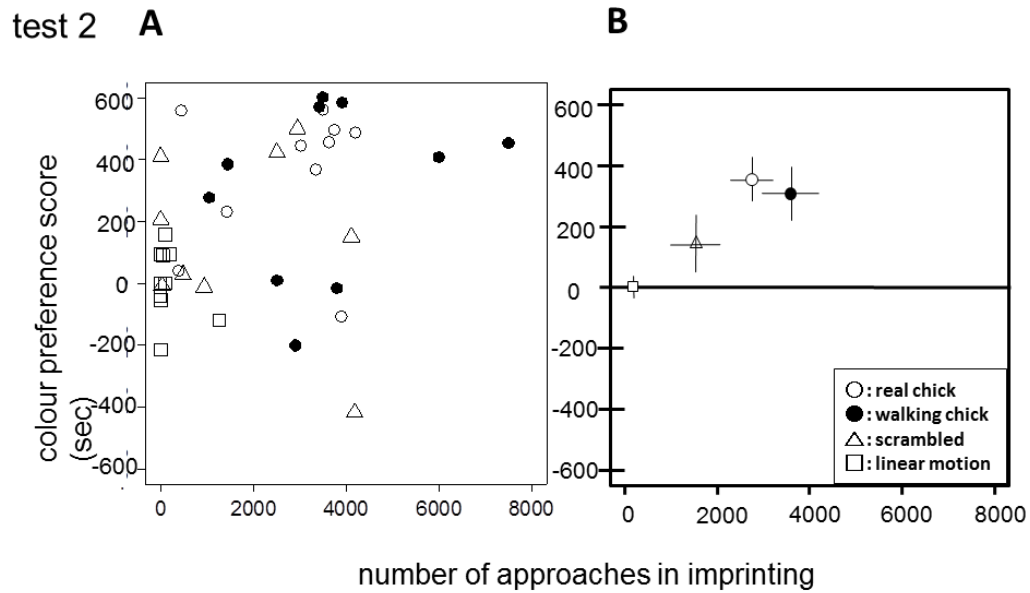
Scramble: $\rho = -0.1033, P = 0.776$

Linear motion: $\rho = 0.0061, P = 0.987$

In (B), mean and s.e.m. were plotted for each group as in Figure S2.

Figure II-S4: *Experiment 2*

No individual-level correlations between the number of approaches in imprinting vs. the colour preference score in test (Results of test 2)



Results of test2 on the next day after imprinting were used for y-axis. No significant correlation appeared at individual level in all of the four groups.

Real chick: $\rho = 0.1394, P = 0.707$

Walking chick: $\rho = 0.4182, P = 0.232$

Scramble: $\rho = -0.1757, P = 0.651$

Linear motion: $\rho = 0.0092, P = 0.980$

Table S2: *Experiment 2*

Generalized linear models (GLMs) revealed a significant contribution of the factor of local movements of the stimuli to the learned colour preference in tests (Test 1 and Test 2)

Models	AIC	β_0 (Intercept)	β_1 (Shape)	β_2 (Local movement)	β_3 (Global configuration)	β_4 (Sex)	β_5 (Approach)
Test 1							
Null	562.2	<u>223.9</u>	—	—	—	—	—
1	546.7	-188.5	—	<u>394.4</u>	155.4	—	—
2	548.3	-33.1	119.25	<u>302.85</u>	—	—	—
Full	552.0	-158	59	<u>338.4</u>	110.8	0.008	33.4
Test 2							
Null	549.8	<u>201.69</u>	—	—	—	—	—
1	542.2	-186.99	—	<u>328.55</u>	187.69	—	—
2	542.9	-213.23	—	<u>325.22</u>	187.32	66.52	—
Full	545.7	167.9	52.8	254.4	138.9	67.9	0.014

GLMs were constructed for the colour preference score in test1 and test2. Data obtained from four groups of chicks were merged. The two best models in terms of AIC were shown. Logistic link function was adopted. The full model was defined as:

$$X = \beta_0 + \beta_1 * shape + \beta_2 * local_movement + \beta_3 * global_configuration + \beta_4 * sex + \beta_5 * approach + r_i$$

The following five factors were included as explanatory variables without interactions:

shape (categorical = 0 for the three point light animations, 1 for **real chick**)

local_movement (categorical = 0 for **linear motion**, 1 for **real chick, walking chick** and **scrambled**)

global_configuration (categorical =0 for **scrambled**, 1 for others)

sex (categorical = 0 for females, 1 for males)

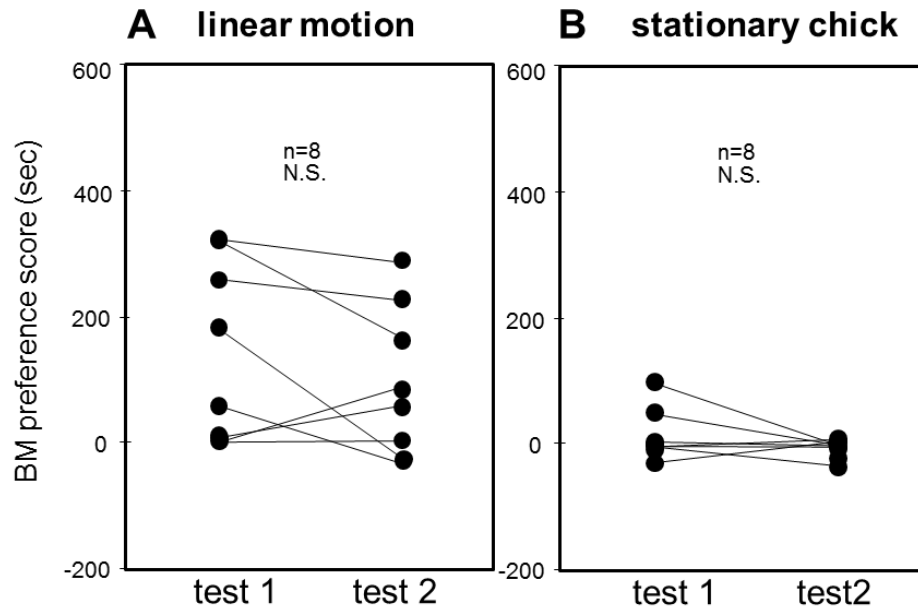
approach (integer = number of approaches in imprinting)

r_i denotes residual individual variance.

See table S1 for the significance of the estimated coefficients.

Figure II-S5: *experiment 3-1*

High reproducibility of the induced BM preference in the repeated test sessions



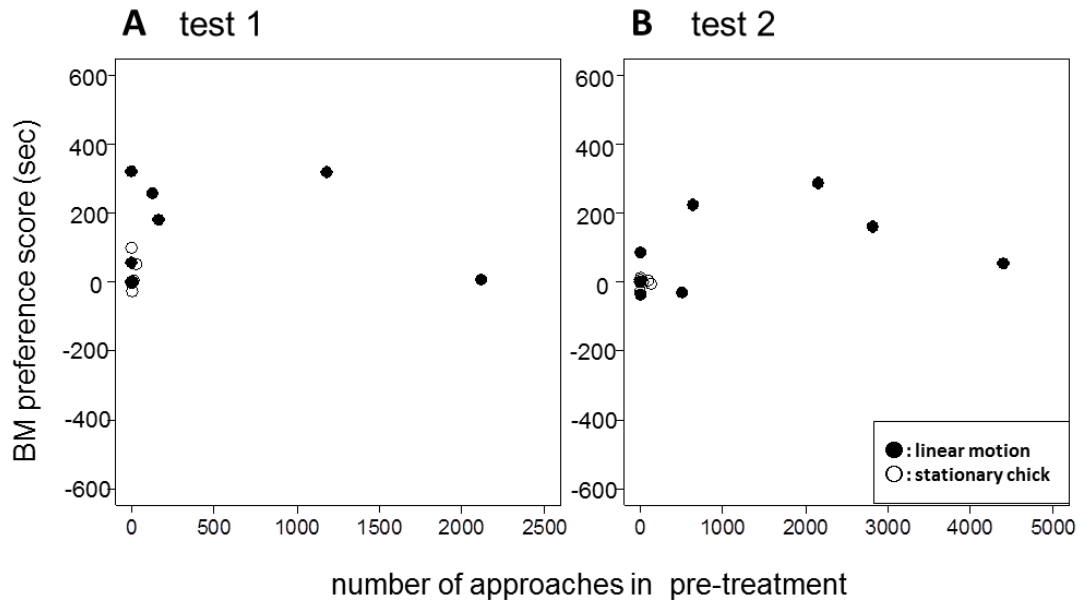
The BM preference was measured by the difference in the stay time near **walking hen** minus **rotating hen**. Wilcoxon signed rank test revealed no significant difference between test1 and test2.

linear motion: $V = 21$, $p = 0.2812$

stationary chick: $V = 19.5$, $p = 0.4062$

Figure II-S6: *experiment 3-1*

No individual-level correlations between the number of approaches in pre-treatment vs. the BM preference score in tests (test 1 and test 2)



BM preference score (y-axis, A for test1 and B for test2) was plotted against the number of approaches in pre-treatment (x-axis) in two groups of chicks. See inlet for the abbreviations. Spearman rank-order correlation analysis revealed no significant correlation in all of these 4 sets of data.

(a) Linear motion: $\rho = 0.0982$, $P = 0.817$

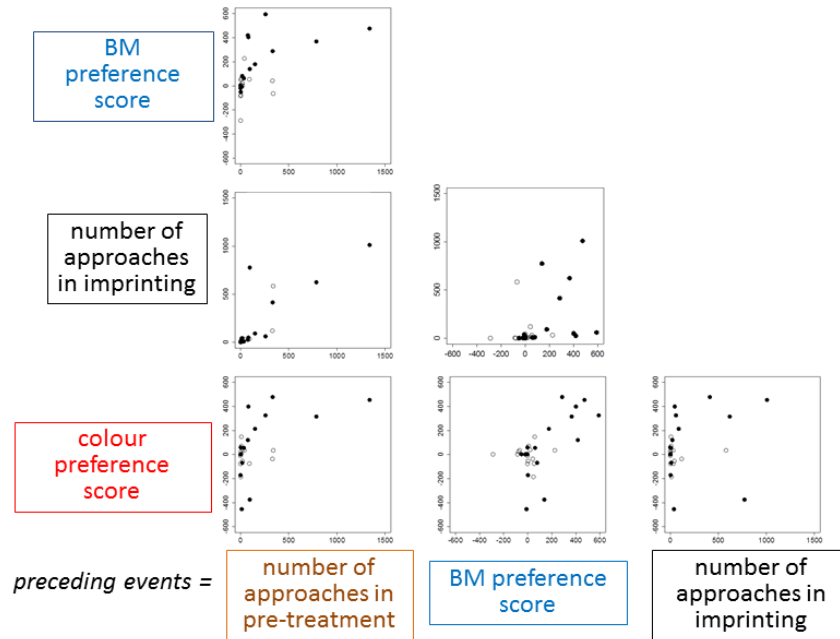
Stationary chick: $\rho = 0.3000$, $P = 0.470$

(b) Linear motion: $\rho = 0.5476$, $P = 0.171$

Stationary chick: $\rho = 0.0123$, $P = 0.977$

Figure II-S7: *experiment 3-2*

Factors that potentially contribute to the individual-level variance in the BM preference score, the number of approaches in imprinting, and the learned colour preference



The measured behavioural parameters (y-axis) were plotted against each of the preceding events (x-axis). As shown in Figure 5F, filled and open circles indicate individuals pre-treated by **linear motion** and **stationary chick**, respectively. Spearman rank-order correlation analysis revealed significant **positive** correlation in four out of these combinations in **linear motion**, and in only one in **stationary chick**. (shown in **red**).

Linear motion ($df = 14$)

Top: BM preference score

against number of approaches in pretreatment: $\rho = 0.8340, P < 0.001$

Middle: number of approaches in imprinting

against number of approaches in pretreatment: $\rho = 0.9083, P < 0.001$

against BM preference score : $\rho = 0.7410, P = 0.001$

Bottom: colour preference score

against number of approaches in pretreatment: $\rho = 0.7041, P = 0.002$

against BM preference score: $\rho = 0.7129, P = 0.002$

against number of approaches in imprinting: $\rho = 0.4467, P = 0.083$

Stationary chick (Sample size (N) =15)

Top: BM preference score

against number of approaches in pretreatment: $\rho = 0.5519, P = 0.329$

Middle: number of approaches in imprinting

against number of approaches in pretreatment: $\rho = 0.8650, P < 0.001$

against BM preference score : $\rho = 0.5202, P = 0.047$

Bottom: colour preference score

against number of approaches in pretreatment: $\rho = 0.2383, P = 0.392$

against BM preference score: $\rho = 0.0430, P = 0.880$

against number of approaches in imprinting: $\rho = -0.0558, P = 0.844$

Table S3: *Experiment 3-2*

Generalized linear models (GLMs) revealed a significant contribution of the induced BM preference to the learned colour preference

Models	AIC	β_0 (Intercept)	β_1 (BM preference)	β_2 (Pretreatment)	β_3 (Imprinting)	β_4 (Type)	β_5 (Sex)
Null	421.3	39.4	—	—	—	—	—
1	406.8	-34.8	0.5	0.2	—	—	—
2	407.9	-23.2	<u>0.7</u>	—	—	—	—
Full	409.7	-28.9	0.5	0.5	-0.4	28.9	-26.9

GLMs were constructed for the colour preference score. Data obtained from two groups of chicks were merged. The full model was defined as:

$$X = \beta_0 + \beta_1 * BM_preference + \beta_2 * pre-treatment + \beta_3 * imprinting + \beta_4 * type + \beta_5 * sex + r_i$$

The following five factors were included as explanatory variables without interactions:

BM_preference (integer = BM preference score (sec))

pre-treatment (integer = number of approaches in pre-treatment)

imprinting (integer = number of approaches in imprinting)

type (type of the animation used in pre-treatment = 0 for stationary, 1 for linear motion)

sex (categorical = 0 for females, 1 for males)

r_i denotes residual individual variance.

See table S1 and S2 for abbreviations.

General Discussion: Scientific Significance of the Present Study

It was Vallortigara and his colleagues who reported the biological motion (BM) preference in newly hatched chicks for the first time (Vallortigara *et al.*, 2005, 2006). In these reports, he argued that the BM feature of the mother hen makes chicks easily imprinted in natural environment. However, some questions have remained unanswered for his argument. First, the reported BM preference is too weak to facilitate imprinting. Second, chicks in his report do not distinguish between hen (conspecific) and cat (predator) displayed in point-light animations. Third, but most importantly, his presumption has not been experimentally tested. In the present thesis, I tried to answer these questions by systematically studying the development and function of the BM preference in chicks.

In chapter 1, I studied the developmental process of the BM preference in the early post-hatch stage. I confirmed their finding that the BM preference is innate. However, in a clear contrast to their original finding, I found that visual experience of any point-light animations is necessary for inducing the BM preference, particularly in male. After the induction, chicks turned out to show a selective preference to hen over cat in point-light animation. In addition, I found a correlation between locomotor activity during the induction process and the induced BM preference, somehow reminiscent to the “law of effort” reported by Hess (1958).

In chapter 2, I studied possible function of the induced BM preference, with an assumption that the preference helps chicks to selectively learn the visual features such as color during the imprinting. It did as expected, and the score of learned color preference was higher when the color was associated with the BM animation mimicking

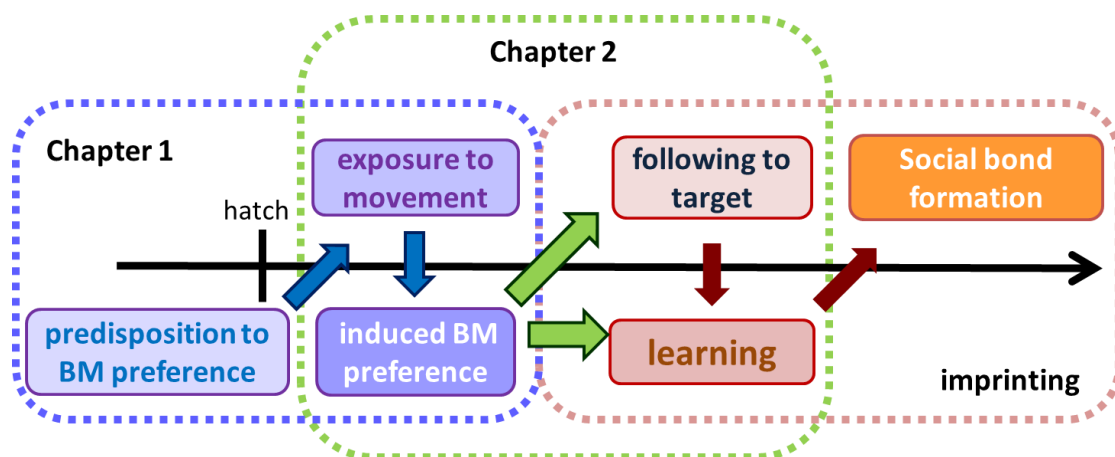
a walking hen or a walking chick. I also found that local movements of light points of BM (rather than the global feature) facilitate imprinting. Furthermore, those chicks which had been pre-treated to show a higher BM preference tended to show a higher color preference score after a brief imprinting using a real chick video clip. The present results therefore support the Vallortigara's idea that the innate BM preference restricts the target of imprinting to their mother.

Based on the series of findings, I propose that a following cascade of developmental events. Initially, newly-hatched chicks have an innate preference to BM, but the preference is very weak and not functional at the beginning. After hatching, chicks are visually exposed to moving objects of any sorts, and the BM preference is induced and becomes functional. The induced and strengthened BM preference then makes chicks selectively learn visual features (such as color) of those targets that have a BM feature. It is to be stressed that chicks do not have to run more in order to get imprinted more. Irrespectively of what the chicks do during the imprinting procedure, those chicks with a higher BM preference score showed a higher color learning score in imprinting. Processes and mechanisms underlying the BM preference may have a functional link to those responsible for the imprinting, particularly for the color memory formation. The hidden link however remains to be elucidated.

Our findings in domestic chicks may give us a hint for understanding the development of social bonds in humans. Two-year-old autistic children fail to show selective attention to BM images (Klin et al. 2009). The authors argue that a genetically predisposed lack of BM preference makes the children's experiences increasingly atypical, leading to severe deficits in social skills throughout development. In this respect, I pay attention to the inter-individual variance in the BM preference found in

this study (Figure II-5F). Those chicks with BM preference score < 200 sec (and thus lacking a learned colour preference after imprinting) may fail to develop normal social behaviours. Besides the possible genetic basis, the developmental consequences of the variation in BM preference should be studied in social behaviours in adults (such as aggressive and sexual behaviours).

Another question remains unanswered on the evolutionary origins of the BM preference in early stages of life found in both chicks and humans. It is unknown whether some of the findings in newly-hatched chicks can be applied to new-born human babies, simply because birds are located systematically very far from humans. The commonality may have stemmed from a common ancestry which had BM preference, or otherwise, the BM preference may have repeatedly evolved independently in different taxa. Further comparative studies are necessary in other amniotic animals including lizards and non-primate mammals.



ACKNOWLEDGEMENTS

First, I would like to express my sincerest gratitude to Prof. Toshiya Matsushima. He has spent so much time to enormous support and has given many insightful comments.

I am very grateful to George Vallorita (Trent University, Italy) and Lucia Regolin (University of Padua) for their valuable cooperation in my experiments.

I am grateful to the colleagues in the sub-department of Behavioural Neurobiology in the Biology Department, Hokkaido University. Critical comments by Dr. Masayo Soma, Dr. Kazuhiro Wada, Prof. Makoto Mizunami were particularly valuable.

Helpful comments on the manuscript by Dr. Masayo Soma and valuable suggestions on the statistical computations by Dr. Ai Kawamori are highly appreciated.

I appreciate Mr. Naohumi Katou (Hokkaido University) to make apparatuses in my study.

This study was supported by grant-in-aid for scientific research to T.M. from the Japanese Society for Promotion of Sciences (#22570070) and from the Japanese Ministry for Education, Culture, Sports, Science and Technology (#22120502) for Innovative Area on the “Study of the neural dynamics for understanding communication in terms of complex hetero systems.”

Finally, I have had the support and encouragement of the members of Matsushima laboratory and other members of laboratories of Animal Behaviour and Intelligence.

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7. ○三浦桃子、松島俊也、

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