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Biological motion facilitates filial imprinting

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To study the functional role of the predisposed preference for Johansson's biological motion (BM) at an early stage of life, newly hatched domestic chicks, *Gallus gallus domesticus*, were exposed to a variety of motion pictures composed of light points (in red or yellow), and then tested for their learned colour preference. Point-light animations depicting the BM of a walking hen successfully facilitated both the approach activity during imprinting and the learned preference in the test, although significant positive correlations did not appear between these at the individual level. Furthermore, scrambling the light points did not significantly reduce the effects, whereas linear motion of a hen-shaped set of points had no effect. If pretreated with the linear motion, those chicks primed with a high BM preference score showed a high learning score in subsequent imprinting. We conclude that the local movement feature of the BM animation is critical in making chicks approach and learn the associated colour. We propose a scenario wherein naïve chicks have an innate preference for BM, which arises prior to imprinting through nonspecific visual experience in the early posthatch period. The induced BM preference then allows chicks to form a learned colour preference for the associated colour more effectively, leading to the development of tighter social attachment.

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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, Moore, & Morrison, 1988), but also infer various features associated with the animation such as gender (Kozlowski & Cutting, 1977), age (Pavlova, Krageloh-Mann, Birbaumer, & Sikolov, 2002) and emotion (Dittrich, Troscianko, Lea, & Morgan, 1996), and can even identify the individual (Cutting & Kozlowski, 1977). The cognitive capability for BM perception has been thought to be limited only to human adults with mature visual-processing mechanisms, as it develops gradually and slowly in juveniles (Blake, Turner, Smoski, Pozdol, & Stone, 2003; Freire, Lewis, Maurer, & Blake, 2006; Pavlova, Krageloh-Mann, Sokolov, & Birbaumer, 2001).

Recent studies have shown that BM perception might occur in taxonomically remote animals. Cats, *Felis catus*, were trained to discriminate BM animations from non-BM counterparts in an operant conditioning study (Blake, 1993). Marmosets, *Callithrix jacchus* (although only females) inspected a BM animation of a walking hen more than the other patterns (Brown, Kaplan, Rogers, & Vallortigara, 2010). Further pioneering work reported that newly hatched domestic chicks, *Gallus gallus domesticus*, show a preference for the point-light animation depicting a walking hen (BM) over non-BM alternatives (Regolin, Tommasi, & Vallortigara, 2000; Vallortigara & Regolin, 2006; Vallortigara, Regolin, & Marconato, 2005). More recently, we confirmed these findings, as chicks showed a clear BM preference when they had been pretreated by any sort of point-light animation (Miura & Matsushima, 2012). The animation did not necessarily have to be a BM picture, and even an assembly of randomly moving light points was effective. We therefore agree with the idea that BM preference is predisposed (Rosa Salva, Mayer, & Vallortigara, 2015). Chicks may not learn BM, but the BM preference is induced through nonspecific visual experiences. Also, in Vallortigara et al.'s (2005) study, chicks ran in a running wheel in the darkness before the BM preference tests. Besides the visual experiences, chicks may thus have to execute motor activities in response to the stimuli. The critical factors required for the BM preference induction are yet to be specified.

We must stress that the BM preference arises at a very early stage of chick life. This early emergence is also found in humans, as 2-day-old babies prefer the walking hen animation in preference looking tests (Simion, Regolin, & Bulf, 2008). Although it is unclear whether chicks or newborn human babies have processes similar to

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those employed by human adults, the BM preference in the early postnatal (posthatch) period may be based on a common mechanism shared by birds and humans. In this respect, it is interesting that chicks have a similar pattern of functional lateralization of BM preference to that found in humans (Rugani, Rosa Salva, Roglin, & Vallortigara, 2015).

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

In this study, we investigated the possible contribution of BM preference to imprinting. We did not address the possibility that the BM preference is strengthened after imprinting. Specifically, we asked (1) whether chicks would selectively learn the colour associated with BM animations and (2) if pretreated with nonspecific visual stimuli to show a BM preference, whether chicks would score higher in learned colour preference by imprinting. To do that, we 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.

GENERAL METHODS

Subjects and Ethical Note

Domestic white leghorn chicks (Julia strain) were used at 24–48 h posthatch. Fertilized 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 posthatch visual experiences, hatchlings were individually housed and kept in another incubator in complete darkness. After the experiments, chicks were sexed and euthanized by carbon dioxide. A total of 221 chicks (111 males and 110 females) were used. We did not perform any potentially harmful manipulations, invasive sampling of blood or tissues or tagging. 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).

Apparatus

We 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 pretreatment (Fig. 1a), an infrared sensor and a transparent Plexiglas 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 0.3 s, drawing the chick backwards by about 30 cm at a time. The

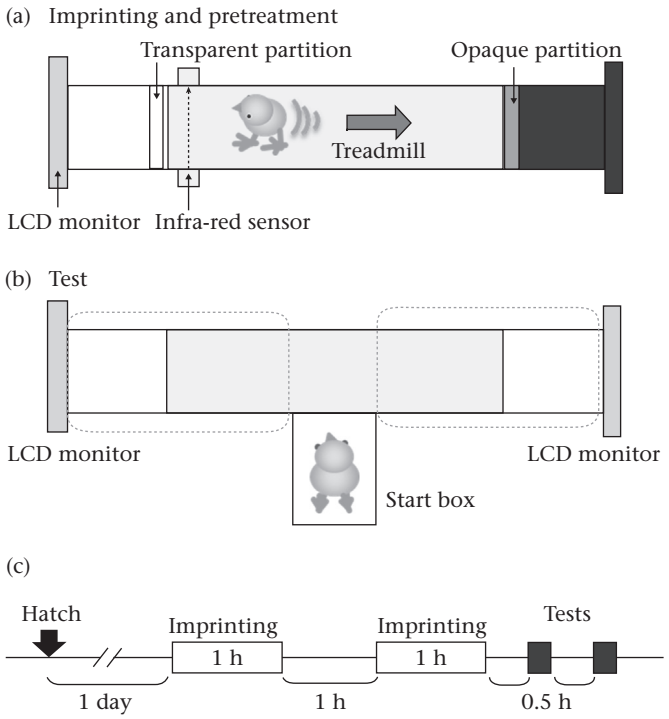

Figure 1. Apparatus used for (a) imprinting and pretreatment and (b) testing. (c) The procedure is shown schematically for experiments 1 and 2. Chicks were imprinted twice at a 1 h interval, and subsequently tested twice at 0.5 h intervals. During the first posthatch day and the intervals between the sessions, chicks were individually housed in a dark incubator at 37–38 °C. For experiments 3-1 and 3-2, see Figs 4a and 5a, respectively.

treadmill motion was digitally counted, and the number of approaches was recorded for each of the trials, which lasted for 1 h if not stated otherwise.

In the binary choice test (Fig. 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 about 10 s, and was then allowed to freely go out and choose between the two arms. We 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 start box. The choice test was repeated twice with the side changed after 0.5 h (in experiments 1 and 2) or about 1–2 min (experiments 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 recorder (DCR-SR60, Sony, Japan). The apparatus was placed in a soundproof wooden box, which was illuminated by infrared LED lamps.

Video Clips and Point-light Animations

For imprinting and pretreatment, we 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). These are available in the Supplementary Material. The videos/animations were accompanied by sounds, except those used in the BM preference test (Walking hen and Rotating hen) and the colour preference test (test (red) and test (yellow)). The same sound source

accompanied different videos in each experiment: repetition of 'clicks' synchronized with the LEGO block motion (experiment 1) and repetition of distress 'peep' calls (experiments 2 and 3). See the Supplementary Material for detailed accounts of the sound.

The point-light animations were composed of 13 light points and displayed at a speed of 30 frames/s. We 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/m^2; 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

We used R (version 2.12.0, R, The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org) for nonparametric tests (Kruskal–Wallis test, Steel–Dwass multiple comparisons, Spearman rank-order correlations, Wilcoxon rank sum test). We also constructed generalized linear models (GLMs) in R, and evaluated them using the Akaike information criterion (AIC). The recorded colour preference score (difference of stay time in binary choice test, s) was given as a weighted sum of several explanatory variables. As the link, we adopted the logistic function. For details, see our previous report (Miura & Matsushima, 2012) and the Supplementary Material.

EXPERIMENT 1: EFFECTS OF STIMULUS MOTION

Methods

As the first step, we prepared five types of visual stimulus (Fig. 2a): full screen colour (Plate), video clip of a rotating LEGO block, point-light animation made from the block (LEGO point; see Fig. S8 of the Supplementary Material), BM animation depicting a Walking hen and a still pattern of randomly arranged light points (Stationary dots). We recorded two behaviours (Fig. 1c), one during imprinting (number of approaches during the 2 h of observation) and the other during the test (colour preference score).

Results

Among the five groups, significant differences occurred in the number of approaches (Fig. 2b; Kruskal–Wallis test: $\chi^2_4 = 37.05$, $P < 0.001$). Post hoc Steel–Dwass multiple comparisons (performed in all of the 10 possible pairs) also revealed significant differences (Fig. 2b). 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 group ($t = 3.873$, $P = 0.001$), even though the LEGO point depicted the motion of the LEGO block. Among the three groups trained by point-light animations, the Walking hen group was higher than the other two (versus LEGO point: $t = 3.816$, $P = 0.001$; versus Stationary dots: $t = 4.073$, $P < 0.001$), but it was comparable to the LEGO block and the Plate groups. The Stationary dots group was significantly lower than the Plate group ($t = 3.434$, $P = 0.005$), which was also motionless. We 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, however, the BM feature is critical.

A similar pattern of intergroup differences occurred in the colour preference score (Fig. 2c; Kruskal–Wallis: $\chi^2_4 = 25.01$, $P < 0.001$). No significant difference appeared between the Plate and the LEGO block groups, whereas the LEGO point group was significantly lower (Steel–Dwass: versus LEGO block: $t = 3.415$, $P = 0.006$). Among the three point-light animations, the Walking hen group was higher than the others (significantly versus Stationary dots: $t = 3.092$, $P = 0.017$; not significantly versus LEGO point: $t = 2.342$, $P = 0.132$). Clearly, chicks learned the motionless full-screen colour. However, the BM features of the point-light animations facilitated imprinting. In repeated testing on the following day (Test 2 after Test 1), chicks of each group showed a highly reproducible colour preference (Supplementary Fig. S1).

At the individual level, however, significant positive correlations did not appear between the number of approaches and the colour preference score (Fig. 2d; Spearman rank-order correlation analysis: Plate: $r_S = -0.027$, $P = 0.882$; LEGO block: $r_S = 0.125$, $P = 0.579$; LEGO point: $r_S = -0.599$, $P = 0.003$; Walking hen: $r_S = -0.413$, $P = 0.046$; Stationary dots: $r_S = -0.006$, $P = 0.983$). This indicates that the number of approaches during imprinting is not 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. We 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 Supplementary Table 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 $P < 0.001$ for the best model). The less effective variables included Motion ($\beta 2 > 0$ at $P = 0.002$) and Colour ($\beta 1 > 0$ at $P = 0.003$). However, the confidence levels of the estimated coefficients of Sex (β4) and Approach (β5) 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 characterized, should thus independently regulate these two aspects of imprinting.

EXPERIMENT 2: EFFECTS OF BM ANIMATION

Methods

To characterize the critical features of the BM picture, we prepared three point-light animations from a video clip of Real chick (Fig. 3a). 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 randomizing the spatiotemporal relationship of the light points (see Fig. S9 of the Supplementary Material); thus it had only the local 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, we used red animations.

Results

In number of approaches (Fig. 3b), the Kruskal–Wallis test revealed a statistically significant difference between the four groups ($\chi^2 = 19.91$, $P < 0.001$). We made post hoc comparisons in the three pairs by assigning the Walking chick group as the reference control. The Real chick group was comparable to the Walking

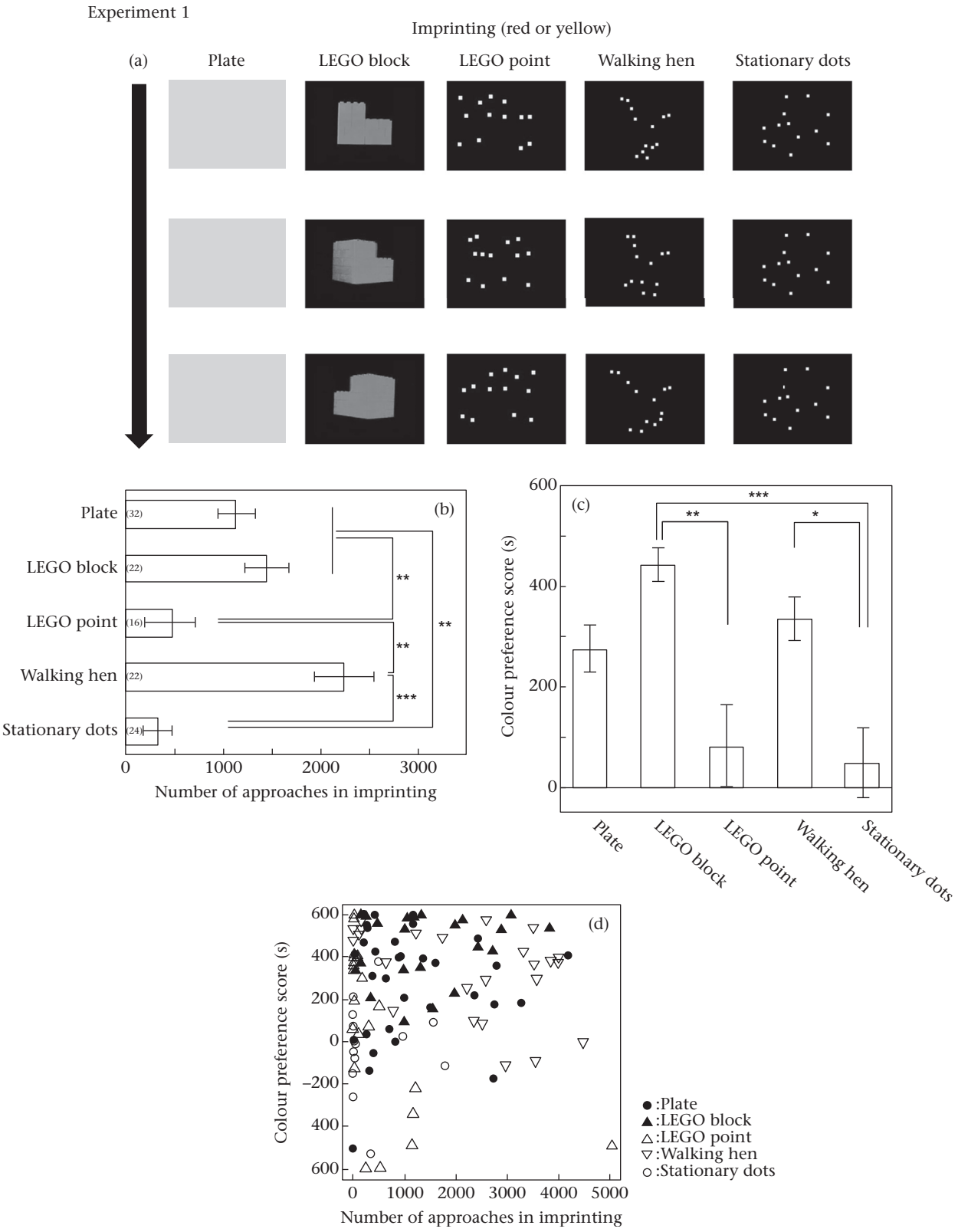

Figure 2. Effects of motion pictures on the learned colour preference (experiment 1). (a) Video clips and point-light animations used for imprinting; for samples, see Supplementary Material. Pictures were in red or yellow in a balanced manner. (b) Sum of the number of approaches counted during the two imprinting sessions. (c) Colour preference score measured at tests. Sum of the difference in stay time (imprinting colour minus unfamiliar colour). Columns and bars denote the mean ± SEM in each group, and numbers in parentheses denote the group size. Asterisks indicate the level of significance after Steel–Dwass multiple comparisons: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. (d) Colour preference score plotted against the number of approaches. Different groups are indicated by different symbols.

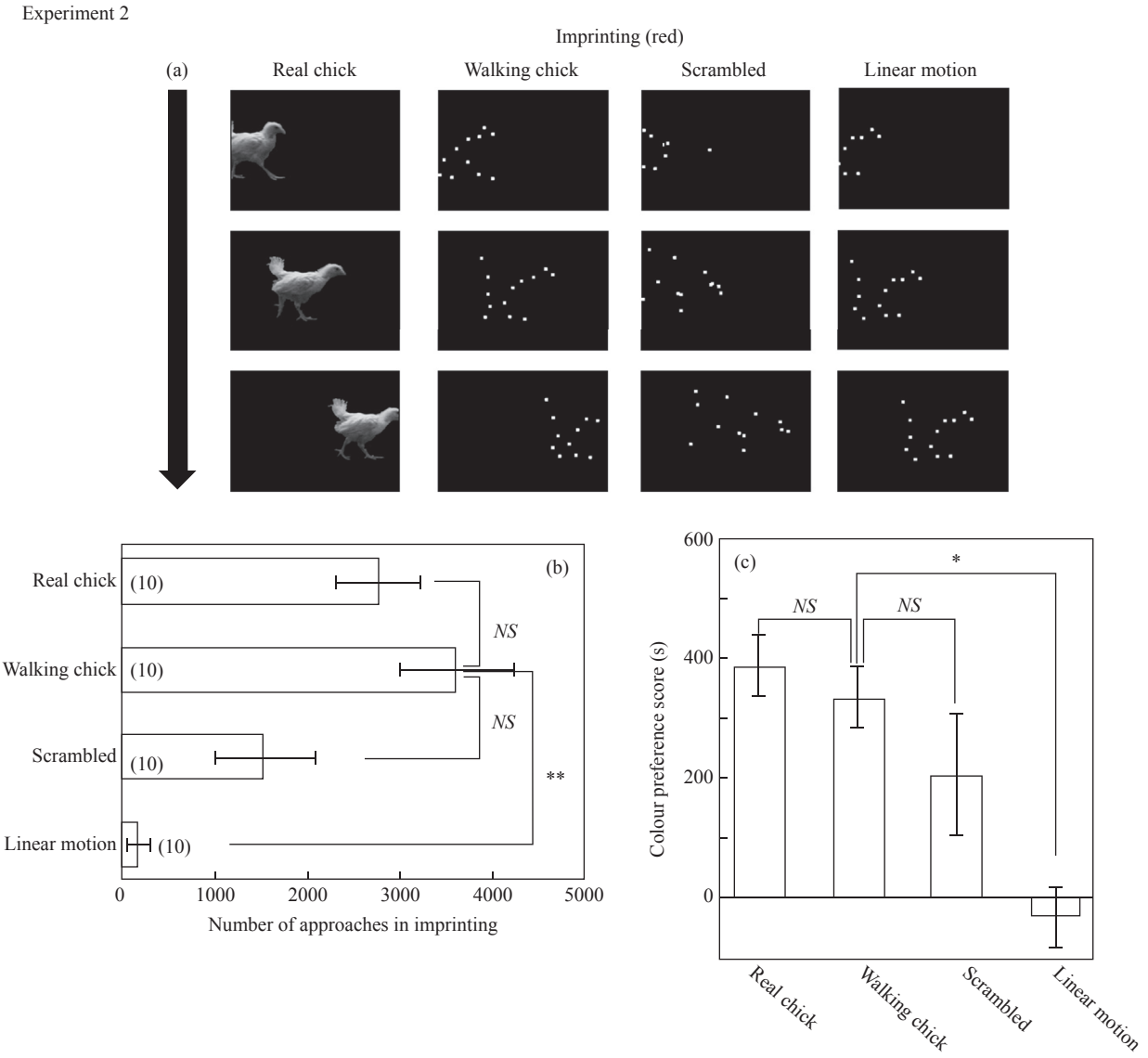

Figure 3. Effects of BM features on the learned colour preference (experiment 2). (a) Video clip and animations. Walking chick was made from the Real chick video. Scrambled and Linear motion were made from Walking chick, each depicting the local movements and the global configuration, respectively. (b) Sum of the number of approaches. (c) Colour preferences score. Columns and bars denote the mean ± SEM in each group, and numbers in parentheses denote the group size. Asterisks indicate the level of significance after Steel–Dwass multiple comparisons made by taking Walking chick as the reference control group: $^{*}P < 0.05$; $^{**}P < 0.01$.

chick group (Steel–Dwass multiple comparison test: $t = 0.6427$, $P = 0.857$) and so was the Scrambled group ($t = 2.117$, $P = 0.088$), but the Linear motion group was significantly lower ($t = 3.705$, $P < 0.001$). Movements of individual points are thus apparently critical.

The colour preference score showed a similar pattern of differences (Kruskal–Wallis: $\chi^2 = 15.68$, $P = 0.001$). In post hoc Steel–Dwass multiple comparisons, the Real chick and Scrambled groups were comparable to the Walking chick group ($t = 0.7559$, $P = 0.791$; statistical results were coincidentally equal), whereas the Linear motion group was lower ($t = 3.402$, $P = 0.002$). Within each group, repeated tests yielded highly reproducible preference scores (Supplementary Fig. S2). Between the approach number and the colour preference, we found a significant positive correlation only in the Real chick, but not in the other three groups (Fig. S3 for Test 1). In the repeated test on the following day (Test 2), no significant correlation appeared for any of the four groups (Fig. S4).

To estimate the critical factors, we 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 $P < 0.001$; the Test 1 data), and no other variables contributed significantly (Supplementary Table S2). Similar results were found for the Test 2 data. Taken together, we 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.

EXPERIMENT 3-1: PRETREATMENT INDUCTION OF BM PREFERENCE

Methods

As a first step, we examined whether BM preference could be induced by the Linear motion that proved to be ineffective in imprinting in experiment 2. Two groups of chicks received pretreatments, one using Linear motion and the other Stationary chick, both composed of white light points (Fig. 4a). These chicks were then tested using a binary choice between simultaneously presented Walking hen and Rotating hen.

In the number of approaches in the pretreatment (Fig. 4b), the Linear motion group was higher than that of the Stationary chick group in both pretreatment 1 (Wilcoxon rank sum test: $W = 39.5$, $N_1 = 8$, $N_2 = 8$, $P = 0.044$; size effect was estimated by Grissom's probability of priority $\hat{p}_s = 0.6172$; Grissom, 1994) and pretreatment 2 ($W = 51.5$, $P = 0.041$; $\hat{p}_s = 0.8516$), but the significance level was marginal. In Test 1 and Test 2 (Fig. 4c), similarly, the Linear motion group was higher than the Stationary chick group in their preference of Walking hen over Rotating hen, and the difference was significant in Test 1 (Wilcoxon rank sum test: $W = 52$, $N_1 = 8$, $N_2 = 8$, $P = 0.035$; $\hat{p}_s = 0.8125$) but not in Test 2 ($W = 43.5$, $P = 0.240$; $\hat{p}_s = 0.6484$). Pretreatment using Linear motion thus

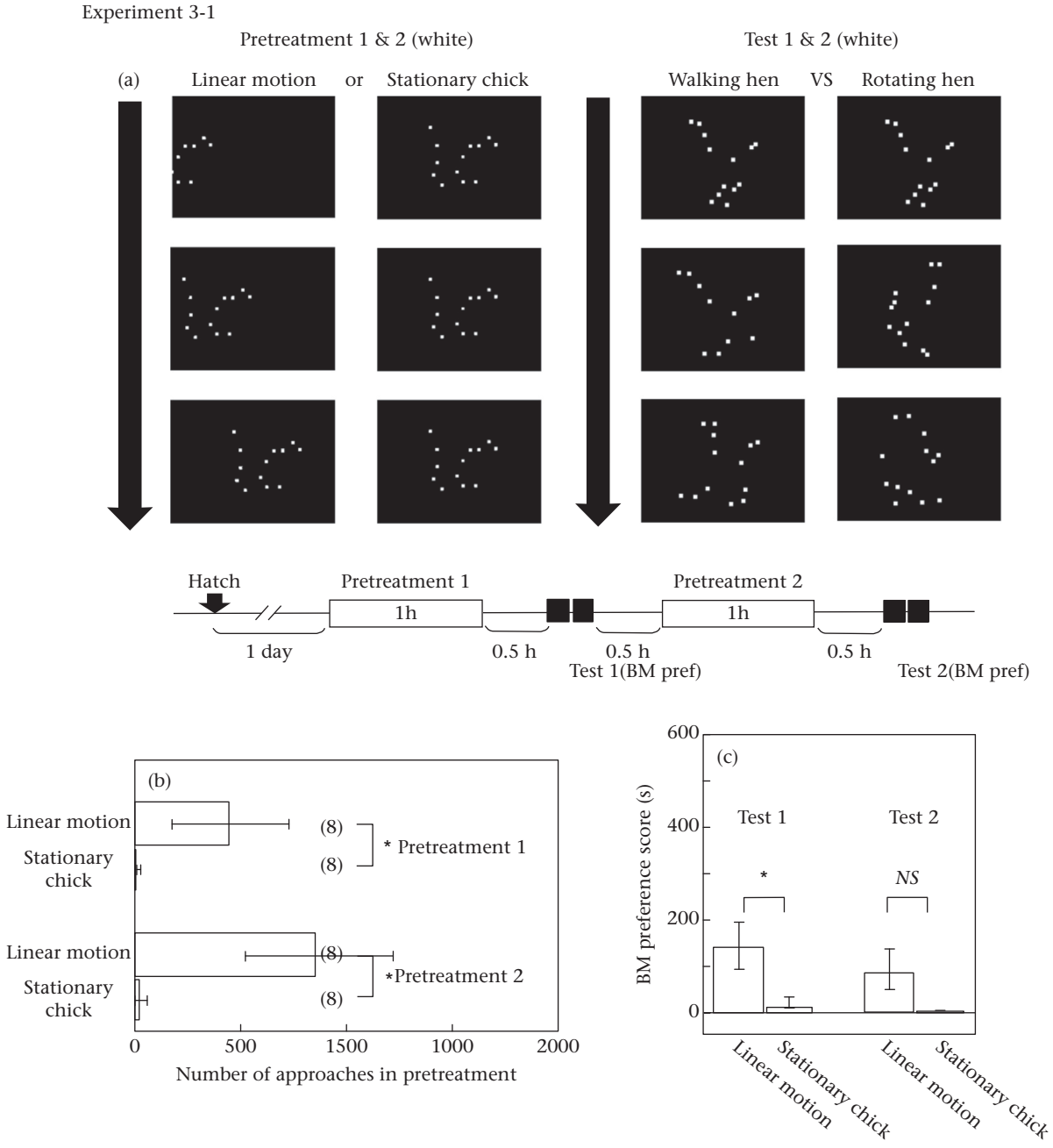

Figure 4. Effects of motion pictures on the BM preference (experiment 3-1). (a) Two groups of chicks were repeatedly pretreated using Linear motion or Stationary chick (both in white). At 0.5 h after each session, each chick was tested twice using binary choice. (b) Number of approaches in pretreatment 1 and 2. (c) BM preference scores (Walking hen minus Rotating hen) measured at Tests 1 and 2. Columns and bars denote the mean ± SEM in each group, and numbers in parentheses denote the group size. Asterisks indicate the level of significance after Steel–Dwass multiple comparisons: $^*P < 0.05$.

induced BM preference at the population level with a considerable interindividual variance. The induced BM preference was consistent in the two tests (Fig. S5), but it lacked a significant correlation with the number of approaches in the pretreatment (Fig. S6).

EXPERIMENT 3-2: INDUCED BM AND LEARNED COLOUR PREFERENCES

Methods

For the second step, we examined whether a higher BM preference could lead to a higher imprinting score. Two groups of chicks were similarly pretreated 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 (Fig. 5a). Expecting to detect a larger interindividual variance, we intentionally adopted a weak imprinting paradigm for 15 min.

Results

In the approach number in the pretreatment (Fig. 5b), the Linear motion group was higher than the Stationary chick group, but not significantly so (Wilcoxon rank sum test: $N_1 = 16, N_2 = 15$; $W = 152$, $P = 0.210$; $\widehat{p}_s = 0.6354$). In the BM preference score (Fig. 5c), however, a significant difference occurred between them ($W = 178$, $P = 0.021$; $\widehat{p}_s = 0.7417$). In the subsequent imprinting (Fig. 5d), similarly, the difference was not significant ($W = 152$, $N_1 = 16$, $N_2 = 15$, $P = 0.211$; $\widehat{p}_s = 0.6333$). In the final colour preference tests (Fig. 5e), no significant difference appeared ($W = 155$, $P = 0.171$; $\widehat{p}_s = 0.6458$). At the level of population means, therefore, the induced BM preference seemed not to significantly facilitate imprinting. At the individual level (Fig. 5f), however, a significant correlation appeared between the two preference scores in the Linear motion group (Spearman rank-order correlation: $r_S = 0.713$, $P = 0.002$), but not in the Stationary chick group ($r_S = 0.043$, $P = 0.880$).

We merged the colour preference scores of two groups and constructed GLMs by adopting five explanatory variables: BM_preference (score in the BM preference test), Pretreatment (number of approaches in pretreatment), Imprinting (number of approaches in imprinting), Type (animation used in pretreatment) and Sex (female or male) (Supplementary Table S3). The results showed that BM_preference was the most effective variable ($\beta 1$; confidence level $P = 0.012$ for the best model and $P < 0.001$ 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.

DISCUSSION

Visual Features for Imprinting

A variety of objects have been used in previous imprinting studies. In its early history, Fabricius and Boyd (1954) systematically investigated these objects and reported that mallard ducklings, *Anas platyrhynchos*, imprint on a wide range of artefacts (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 such as size, colour and shape do not limit the imprinting. Hess (1958) also reported that a stuffed brown leghorn chicken was the least efficient model, and artefacts 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 thus do not necessarily need to look natural.

Object movement is also not critical. Gray (1960) showed that chicks are imprinted after exposure to stationary geometrical figures for 24 h. In quail chicks, *Coturnix japonica*, preference for colours has a genetic basis (Kovach, 1980), and the chicks changed their preference after exposure to a motionless colour plate (Kabai & Kovach, 1993). However, most studies of imprinting have empirically used moving objects such as a decoy duck moving along a round runway (Hess, 1958), a cylinder or a box (Bolhuis, 1991; Horn, 2004) and a LEGO block (Izawa et al., 2001; Yamaguchi et al., 2012) rotating along its vertical axis. The use of artefacts moving in a nonbiological manner has been the norm in imprinting studies, and the role of motion has not been given attention.

In the present study (experiment 1, Fig. 2), exposure to the colour Plates proved to be effective similarly to the quail chick study cited above. The LEGO block video on a monitor was also as effective as the real LEGO objects, suggesting that chicks do not care much about the depth of a moving object. On the other hand, the LEGO point animation that depicted the motion of a LEGO block failed. The Walking hen animation was strikingly effective despite the point-lights, and we thus assume that the BM feature facilitated the imprinting. According to Sluckin (1964), Fabricius stated that the movement of some parts of the body in relation to other parts is an important factor in eliciting following in ducklings. An assembly of objects that maintain relative distance and/or synchronized movements with one another may facilitate imprinting. In experiment 2, we examined whether this idea applied to our case of point-light animations.

BM and Local Movements of Individual Light Points

In experiment 2, we 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 (Fig. 3). The local movements of individual points thus play a critical role, whereas the geometric relationships among points do not contribute.

Vallortigara et al. (2005) were the first to construct a motion picture by randomizing the relative locations of light points. Most of the neighbouring points were thus desynchronized. In our study, in addition to the location, the phase relationships were also randomized, so that all pairs of the points were perfectly desynchronized from one another. 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. Note, however, that Scrambled gave rise to a slightly lower average than Walking chick in both behavioural scores (Fig. 3b, c), although this difference was not statistically significant. The global configuration of the animation may thus have only a partial effect.

Experiments 1 and 2 also revealed a high degree of interindividual variance among chicks despite the same visual experiences (see Supplementary Figs S3,S4). Variance in the colour preference score may be because of the different sensitivity to the BM pictures. We examined this in experiment 3.

BM Preference may Precede Imprinting

In experiments 3-1 and 3-2, we pretreated chicks by exposing them to Linear motion or Stationary chick in white. In a previous study (Miura & Matsushima, 2012), exposure to a variety of point-light animations (either BM or otherwise; rotating hen, pendulum

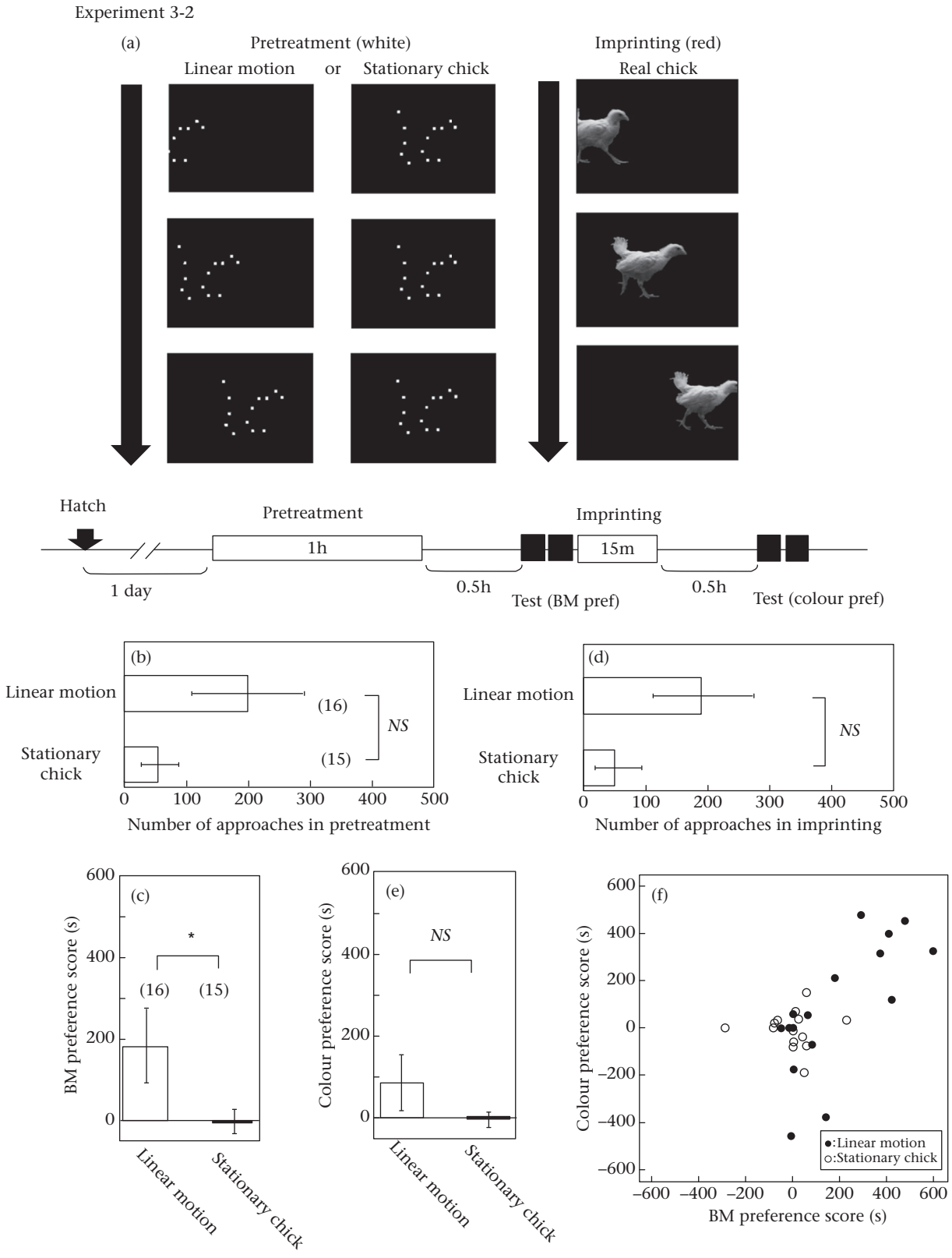

Figure 5. Effects of the induced BM preference on the learned colour preference (experiment 3-2). (a) Two groups of chicks were pretreated once by Linear motion or Stationary chick (both in white), tested for the BM preference, and then imprinted briefly for 15 min by Real chick (in red) before the colour preference test (red over yellow). (b, c) Results of the pretreatment. (d, e) Results of imprinting. (f) Colour preference score plotted against BM preference score. Filled and open circles denote individuals pretreated by Linear motion and Stationary chick, respectively. Columns and bars denote the mean ± SEM in each group, and numbers in parentheses denote the group size. Asterisks indicate the level of significance after Steel–Dwass multiple comparisons: $^{*}P < 0.05$.

or random motion, but not stationary image of points) made chicks show a BM preference in choices between Walking hen and Rotating hen. Here, we confirmed this phenomenon by using Linear motion (Fig. 4) which was ineffective in imprinting (Fig. 3).

In experiment 3-2, we failed to detect a statistically significant difference between these two groups of chicks in the mean colour preference score (Fig. 5e). However, the score was correlated with the induced BM preference at the individual level (Fig. 5f). We 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 which moves near 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 approached more in the subsequent imprinting (experiment 3-2, Supplementary Fig. S7; the number of approaches in imprinting plotted against the BM preference score in the Linear motion chicks). The correlation was statistically significant ($P = 0.001$) and the effect size ($r_S = 0.741$) was high. Facilitated imprinting can therefore be ascribed to the higher number of approaches, which might have made chicks stay around the monitor for a longer time. Otherwise, a higher BM preference may directly cause a higher colour preference, despite how active the chick was during the imprinting. As a future project, we will examine whether the intermediate medial mesopallium network for imprinting (Horn, 2004) might also be involved in BM preference.

The predisposed preference of chicks for BM is analogous to the predisposition for 'fowl' (Bolhuis, Johnson, & Horn, 1985; Johnson et al., 1985) and 'face' (Rosa Salva et al., 2015; Rosa Salva, Regolin, & Vallortigara, 2010). In the former case of 'fowl,' chicks had received nonspecific sensory stimuli and/or motor execution (e.g. running under diffuse illumination or in darkness), and the preference for a stuffed fowl subsequently appeared. In the latter case, chicks had been imprinted to a face-shaped piece of cardboard, before they showed a preference for the 'eyes & mouth' arrangement of blobs. It is unknown, however, whether the 'fowl' and the 'face' predispositions are functionally linked to the memory formation of filial imprinting.

In the present study, the predisposed BM preference appeared immediately after visual exposure to a nonspecific animation (experiment 3-1, Fig. 4). If the BM predisposition emerged prior to imprinting, chicks would readily learn visual features of any nearby objects with a BM feature. It is unknown, however, which of these predisposed processes arises first, how these are ordered in time and how they are causally linked with one another in the course of filial imprinting.

In this respect, we must note a close similarity to the chick's preference for self-propelled causality in the study by Mascalzoni, Regolin, and Vallortigara (2010): naïve chicks acquired a learned preference for the colour that was associated with the self-motion object. The determination process of the predispositions, the induction time course through development and the functional roles played by the predisposed preference, as well as the underlying neural and cognitive mechanisms (either genetic or epigenetic), will be important topics of future research.

Approaches and Preference: Two Parameters of Filial Behaviour

The lack of correlations between the number of approaches in imprinting and the colour preference score in tests needs careful consideration. Hess (1958) reported that the strength of imprinting was a function of the effort made by the subject ducklings, and coined the term 'law of effort'. In our previous study (Miura & Matsushima, 2012), a similar correlation appeared between the locomotor activity and the induced BM preference. However, the 'law of effort' has been criticized (Moltz, Rosenblum, & Stettner, 1960). In a more recent paper, a significant correlation was reported between the approach activity during imprinting and the preference score at test (Bateson & Jaeckel, 1974). That study used a large number of chicks (>100), compared to our present study (8–32 chicks per group). We may thus have failed to detect a significant correlation in these small samples. Regardless, the link between the approach and the preference is not thought to be tight.

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, we 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 (Matsunami 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 & Giudice, 2012) must be done in future to determine its possible link to the preference score.

Relevance to Social Bond Formation in Humans

Our findings in domestic chicks may help us understand the development of social bonds in humans. Two-year-old autistic children fail to show selective attention to BM images (Klin, Lin, Gorrindo, Ramsay, & Jones, 2009). The authors argued 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, we may consider the interindividual variance in the BM preference found in this study (Fig. 5f). Those chicks with BM preference score <200 s (and thus lacking a learned colour preference after imprinting) may have failed 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 (such as aggressive and sexual behaviours) in adults.

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Supplementary Material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.anbehav.2016.03.025.

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