Individual differences in habituation: Innate covariation between habituation, exploration, and body size in naïve chicks (*Gallus gallus*)

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ABSTRACT

Habituation to novel stimuli has been associated with behavioural differences among individuals in numerous animal species. Because the habituation mechanisms depend on previous experiences with a stimulus, one would expect individuals to develop their habituation capacity based on the life experiences that also shape their behavioural traits. And indeed, in adult lizards, exploratory behaviour and body size correlates with habituation. However, here we show that the same factors correlate with habituation of domestic chicks reared under controlled laboratory conditions and tested in the first 3 days after hatching. This result indicates that the covariation between habituation, exploration, and body size does not necessarily depend on experience. Rather, it represents an innate association between exploratory behaviour and risk assessment, which may provide an immediate survival advantage to new-borns of this precocial avian species.

1. Introduction

Habituation consists in a response decrement to the repetition of an irrelevant stimulus (Thompson and Spencer, 1966). The study of the biological and cognitive mechanisms of habituation have been fascinating researchers since centuries (Thompson, 2009), but many aspects of habituation are still unknown. For example, despite the mechanisms of habituation are phylogenetically very old and their functioning is almost identical in most animal species (Rankin et al., 2009), the rate at which different individuals of the same species habituate to the repetition of the same stimulus varies significantly. Inter-individual differences in habituation have been found in a wide range of species from humans (Blanch et al., 2014; LaRowe et al., 2006; Mangan and O'Gorman, 1969; O'Gorman, 1977) and other primates (Allan et al., 2020) to birds (e.g., Roth et al., 2010), rats (e.g., Glowa and Hansen, 1994), and lizards (Rodríguez-Prieto et al., 2011, 2010). The origin of these differences is unknown. Researchers - so far - have proposed that inter-individual differences in habituation result from either the life history of an individual or from its genetic endowment. However, neither of the two proposals has received full support.

Because habituation mechanisms rely on prior experience with stimuli similar to the one under habituation, life history is the most straightforward explanation for the origin of inter-individual differences in habituation. This idea has been supported, for example, by studies showing that animals living in urban centers are more habituated to human disturbance than individuals of the same species living in the wild (Allan et al., 2020; Blumstein, 2016; Pellitteri-Rosa et al., 2017; Rodríguez-Prieto and Fernández-Juricic, 2005; Samia et al., 2015; Thompson et al., 2018; Vincze et al., 2016). However, life-history is not the only reason for inter-individual differences in habituation. Differences in habituation can be inherited as demonstrated, for example, by Glowa and Hansen (1994); but see also Roth et al. (2010). The authors found that the habituation to a loud sound in three strains of rats (*Ratticus norvegicus*) selected based on the amplitude of their startle response (high-, intermediate-, and low-amplitude) differ in two aspects: the habituation rate (i.e., response decrement relative to the initial startle response) and the number of trials necessary to reach an asymptotic level of response, and complete habituation. Rats in the high-amplitude strain decreased the amplitude startle response over the course of the stimulus repetition to a greater extent than rats in the other two groups; however, at the end of the nine experimental trials, the high-amplitude group had not completed habituation, in contrast with the low- and intermediate-amplitude group. These results suggest the existence of robust phenotypic differences in habituation of the startle response among rat strains.

While the study by Glowa and Hansen (1994) supports the notion that inter-individual differences in habituation are inherited, their results must be interpreted with caution as a genetic cause for

inter-individual differences independent of experience cannot be completely ruled out. Indeed, even when it is possible to trace the genetic relatability between individuals of a species, altricial animals like rats (and the same holds true for the study that we cited by Roth et al., 2010 on black-capped chickadees, *Poecile atricapillus*) spend some amount of time with their parents after birth. Parental effects or experiences within the nest prior to the habituation test can have exerted considerable influence in shaping individuals' behaviour. Neither the explanation of inter-individual differences in habituation based entirely on life-history, nor the explanation based on genetics have received full support, there is no surprise, that yet, no study has systematically investigated the effect of the interaction between prior experience and genetics on this topic. Instead, an increasing corpus of literature shows that inter-individual differences in habituation can be functionally linked to inter-individual differences in other behaviors, like exploratory activity, boldness, and sociability.

Research on inter-individual differences in habituation has focused on the link between habituation rate and the proactivity of an individual (e.g., Carere and Locurto, 2011; Mazza et al., 2018; Verbeek et al., 1994). Proactive individuals which are bolder, more exploratory, and aggressive than reactive individuals are usually the first to approach a novel object. They are expected to complete habituation within less stimulus repetition than reactive individuals. In two studies with lizards, Rodríguez-Prieto et al. (2010, 2011) found that variations in body size and exploratory activity were more strongly associated to inter-individual differences in habituation, than boldness and sociability. In particular, smaller (Rodríguez-Prieto et al., 2010) and more exploratory (Rodríguez-Prieto et al., 2011) lizards decreased the flight-initiation distance over the course of repetition of a dummy predator to a greater extent than larger or less exploratory individuals. Considering the link between habituation and exploratory activity, Rodríguez-Prieto and colleagues concluded that risk assessment, instead of the proactivity–reactivity gradient can affect inter-individual differences in habituation. Animals' reactions to new environments are ambivalent because the tendency to explore or show novelty preference is often accompanied by neophobia. Therefore, habituation may allow an organism to reduce the initial phase of hesitation, and begin the exploratory activity (Dingemanse et al., 2012; Honey et al., 1992; Martin and Réale, 2008). But the role of body size has remained unclear, so far, mostly because the influence of body size and exploratory activity on habituation has been analyzed separately, leaving a gap in our knowledge about their interaction.

This experiment aims at addressing two unresolved issues in the literature of inter-individual differences in habituation. First, we tested whether inter-individual differences in habituation are innate, rather than the by-product of life-history. Second, we tested whether inter-individual differences in habituation can be better explained by an interaction between individuals' body size and exploratory activity, rather than the separate effect of these two variables.

To this aim, we capitalized on inexperienced 3-day old chicks of the domestic fowl (*Gallus gallus*). Because chicks can be observed immediately after hatching under complete experimental control over pre- and post-natal experiences (Chiandetti et al., 2015; Turatto et al., 2019; Vallortigara, 2012), this animal is an optimal model to study the innateness of inter-individual differences. Furthermore, we focused on a set of variables that have been previously studied in other species – namely, body size, exploratory activity, boldness, social reattachment. We expected that if the covariation between habituation and these variables is innate, we would find that habituation, exploratory activity, and body size would also covary in inexperienced young chicks.

2. Methods and materials

2.1. Subjects

We tested 104 domestic chicks (females = 51) of the Ross 308 strain (Aviagen). Chicks hatched in our laboratory at a temperature of 37.7 °C and humidity of 50–60 %. On hatching day, they were housed in individual cages (22 × 30 × 40 cm, width, height, depth) with a red cylinder hanging from above (6 × 7.5 cm, diameter, height) as imprinting object. Throughout the experimental days, chicks had full access to food and water from within their cages. The illumination of the room followed a 12:12 dark:light cycle and the temperature was kept at 31.5 °C.

2.2. Behavioural assays (Day 1)

Chicks were observed when 2 days old. The arena was a white cube (40 × 40 x 40 cm, width, height, depth) divided in 2 chambers by a transversal plastic wall (*polionda ®)* communicating through a sliding door that was manually controlled by the experimenter. A set of 16 black cones was placed on the floor of each chamber. The whole experiment was recorded by two cameras located 30 cm above the chicks' head. The chicks' movements were tracked using a custom made opencv-python script using the HSV colour space to estimate of the middle point in the chick's back. The script was based on the cv2.inRange(), cv2.minEnclosingCircle() and cv2.moments() functions of the cv2 and imutils packages.

We focused on three main behavioural domains: exploration, boldness, and social reattachment. The set up changed throughout the behavioural assays to test all the three domains in a single trial.

2.2.1. Assay I: Exploratory activity

Chicks were gently moved in one of the 2 chambers of the arena using a cylindric box. Then, they were left free to explore the new environment. To encourage the chicks to explore the chamber, half of the cones on the floor hid food and chicks could feed from the cones as they explored the chamber (Chiandetti et al., 2005). The presence of food also mimicked the ecological situation in which a chick peck on the floor to search for new resources. We measured the latency (s) to move the first step in the chamber, the latency (s) to peck the first cone, the time (s) spent freezing, the time (s) spent pecking at food, the proportion of cones that chicks uncovered and so obtained food from and the amount of the area explored (%). The amount of the area explored was calculated by dividing the floor of the chamber into 9 sections and counting the number of sections occupied by the chicks during the exploration. This first assay lasted 5 min.

2.2.2. Assay II: Boldness

By the end of the first assay, the first chamber of the arena was covered with an opaque plastic roof. After a minute break, the sliding door separating the two chambers was removed, letting the chicks move to the unfamiliar chamber (for a similar procedure, see Bryan Jones and Mills, 1983). We measured the latency (s) for the focal chick's head to cross the threshold of the sliding door and the latency (s) to leave the familiar chamber with the whole body. This second phase lasted for a maximum of 10 min. Chicks who did not enter the new chamber were given a ceiling latency of 600 s on both the variables.

2.2.3. Assay III: Social reattachment

At the beginning of this assay, the chick was restrained in an opaque cylinder and moved to one corner of the arena in a counterbalanced fashion across individuals. Then, both the roof of the first chamber and the plastic wall separating the two chambers were removed. An imprinting object was hung from above on the opposite corner of the chick, at which point the latency (s) to reattach with the familiar object (touching it with the body) was measured after the chick was released (for the rationale of this test, see Vallortigara et al., 1990). A ceiling was set at 5 min for this task. Thereafter, the chick was restrained again with the opaque cylinder and placed on a different corner of the arena. The imprinting object was then moved on one of the two corners perpendicular to that of the chick and an unfamiliar blue rectangular object (8 × 12 × 3 cm, width, height, depth) was hung on the opposite corner.

Therefore, the chick was equidistant from both the imprinting object and the unfamiliar object. Once the chick was released, we measured the latency (s) to reach the imprinting object, the latency (s) to reach the unfamiliar object and a proximity index computed as $I = \frac{dU - dI}{dU + dI}$, were dU is the distance from the unfamiliar object and dI is the distance from the imprinting object. This index ranges from -1 to $+1$ and positive values indicate a greater distance from the unfamiliar object, i.e., greater levels of social reattachment. The ceiling was set at 5 min.

2.2.4. Habituation test (Day 2)

The habituation test took place on the following day. Chicks were tested in the same order used for the behavioural assay the day before. They were placed within a running wheel (30 cm in diameter) located on one of the short sides of a black rectangular arena (45 × 50 × 160 cm, width, height, depth) (Dissegna et al., 2018). Chicks were motivated to run within the wheel in attempt to reach their imprinting object. Only chicks that run for a minimum distance of 10 m within 5 min in the wheel were tested (for a similar procedure see, Chiandetti and Turatto, 2017). Two loudspeakers played the habituation stimuli at 30 cm above the chick's head.

Chicks were randomized in two habituation conditions before the test. In one condition the habituation stimulus consisted of a hen cluck (730 ms, 90 dB SPL); in the other condition the habituation stimulus was a rooster alarm call (730 ms, 90 dB SPL). These two stimuli were chosen to test chicks' habituation to two unfamiliar calls when they are part of the vocal repertoire of the same or different species. In both conditions, the habituation stimulus was repeated 10 times (Trials 1–10) at pseudo-random intervals ranging from 30 s to 60 s. The stimulation was manually controlled by the experimenter, so that if the chick was not running at the scheduled interval, the stimulation was delayed up to a maximum of 60 s. The experiment terminated for chicks that stopped for a longer time. The time, distance and direction of the chicks' run were displayed by an *Arduino* circuit. We measured the number of times each chick froze in response to the stimulus and the freezing duration (s). The whole experiment was videorecorded.

2.3. Data analysis

First, we inspected the density distributions of variables obtained from each assay. The variables displaying a bimodal distribution were transformed into categorical variables. A median split on the variable score was used to divide chicks into two groups, namely "high" and "low" identifiers (i.e., the latency to peck the first cone and the proportion of area explored (Assay I)), the latency to protrude the head and the body (Assay II) and the proximity index (Assay III). The remaining latencies were log transformed because positively skewed (i.e., the latency to move the first step (Assay I), the latencies to reach the imprinting object and the unfamiliar one (Assay III)).

A principal component analysis (PCA) was conducted on a mixed correlation matrix (i.e., Pearson correlations for the continuous variables (r), tetrachorics (r_{tet}) for the dichotomous items, and the biserial (r_{pb}) correlations for the various mixed variables) between the 11 variables resulting from the behavioural assays. The resulting components were rotated according to a promax procedure, which allowed the factors to correlate with each other. Factor scores were assigned to each chick using the regression method to obtain reliable empirical estimates of the individual differences across the expected broader behavioural traits (i.e., Exploratory Activity, Boldness, and Social Reattachment). Again, we replaced factor scores with a bimodal distribution with categorical variables. We analysed the correlation between chicks' factor scores, sex, and weight (g), using robust correlation coefficients and t-test on maximum-likelihood estimator differences (Wilcox, 2011). In order to analyse whether behavioural differences had affected the likelihood of a chick reaching the criterion to begin the habituation test (2 categories: Not Run; Run) and to complete the test (2 categories: Not

Tested; Tested) we used a proportion z-test, with s.e. $= \sqrt{\frac{\pi_{H0}*(1-\pi_{H0})}{n}}$, where π_{H0} represents the expected proportion of cases in each category.

We estimated the latent intercept and habituation rate for each individual chick that completed the habituation test (n = 46) using latent curve model (LCM). We fitted two models: a liner model with the 10 Trials as observed indicators, and an intercept and a linear slope as latent growth factors; and a quadratic model which also included a quadratic slope as an additional latent factor. The intercept represented the initial duration of the freezing response of chicks; the linear slope quantified the monotonical (linear) habituation rate; and the quadratic slope accounted for the change of the monotonical rate over Trials, hereafter the quadratic habituation rate (Lane et al., 2013). The raw linear habituation rate is a negative number, but in all our analysis we expressed this rate as a positive value by multiplying individual habituation rate by -1 for ease of interpretability. (i.e., the largest this value, the highest the rate of habituation, and the greater the response decrement relative to the initial response. The linear habituation rate accounts for the steepness of the line tangent to the habituation curve. Larger values of linear habituation rate indicate steeper habituation in the initial trials. Whereas smaller values indicate more gradual habituation. The quadratic habituation rate accounts for how the habituation curve slows over time. Larger values of the quadratic habituation rate indicate more abrupt flattening of the habituation curve, as the animal approaches the asymptotic level of response. Whereas smaller values of the quadratic habituation rate indicate more gradual flattening of the habituation curve and approach of the asymptotic level of response. The model with the quadratic habituation rate fitted our data significantly better than the model with a linear habituation rate alone (χ^2_{diff} [4] = 48.82, $p < .001$)). Therefore, we focused our analysis on this model. We also merged the two stimuli conditions (hen cluck vs rooster alarm call) because there was no significant difference in the habituation rate and intercept of the resulting habituation curves (for the intercept: difference = 1.12, $t(36.97) = 0.30$, $p = 0.769$; Cohen's $d = 0.09$; for the linear habituation rate: difference = 0.99, $t(30.73) = 0.95$, $p = 0.350$; Cohen's $d = 0.29$; for the quadratic habituation rate: difference $= -0.14$, $t(24.50) = -1.91$, $p = 0.068$; Cohen's $d = -0.59$). We tested if differences in chicks' body size (weight) and behaviour (factor scores) predicted their initial duration of freezing and habituation rate, in terms of both the individuals' linear and quadratic habituation rate obtained from the LCM model. In particular, based on the previous research (Rodríguez-Prieto et al., 2011, 2010), we expected to find a positive relationship between habituation rate, body size (weight) and Exploratory Activity. Because the interplay between these factor has never been studied before, we decided to test and analyse the effects on habituation of individual differences in Boldness and Social reattachment, despite the previous research by Rodríguez-Prieto et al. (2010, 2011) showed the these behaviours had no effect on habituation rate (Rodríguez-Prieto et al., 2011, 2010). In particular, based on the previous research, we expected to find a positive relationship between habituation rate, body size (weight) and Exploratory Activity only as in Rodriguez-Prieto et al. (2011).

To test the Exploratory Activity × Weight interaction, we performed two separate linear regressions, the first, including only the main effects of chicks' body size (weight) and behaviour (factor scores); the second, including the additional Exploratory Activity × Weight interaction term. We looked at the change in R^2 as a test of model improvement following the inclusion of the Exploratory Activity × Weight interaction and tested whether this change was statistically significant. All the analyses were carried out in R 3.6.2 (R Core Team, 2021). The PCA was carried out using the command *principal()* on a mixed correlation matrix obtained using the *mixedCor()* function (both part of the "*psych*" package (Revelle, 2020)); for robust two-samples tests and correlation coefficients we used the "*WRS2*" package (Mair and Wilcox, 2020); for LCM we used the "*lavaan*" package (Rosseel, 2012).

3. Results

A PCA was used to assess the performance of the chicks in the Behavioural assays. This statistical analysis allowed us to condense the variables used to assess behavioral differences between chicks into a smaller set of factors. A factor score was assigned to individual chicks, which expresses their relative standing on each factor. Variability of factor scores reflects behavioural differences across chicks.

Based on Kaiser's criterion, 3 components were retained from the PCA which in combination explained 70 % of the variance of the observed variables. The variables clustered accordingly to the three assays suggest that component 1 represents chicks' level of Exploratory Activity, component 2 their level of Boldness, and component 3 their level of Social Reattachment. Details of the PCA are reported in the Supplementary Material.

3.1. Smaller chicks are more exploratory and bolder than larger ones

Individuals' factor scores (behavioural differences) were correlated with body size and sex using the most appropriate correlation index as specified in the Data Analysis section. There was a positive correlation between factor scores of Exploratory Activity and Boldness ($r_{tet} = 0.31$) and between scores of Exploratory Activity and Social Reattachment ($r_{pb} = 0.32$), but not between Boldness and Social Reattachment ($r_{pb} = 0.10$). Chicks with high Exploratory Activity or Boldness score were globally smaller (for Exploratory Activity: $t(101.85) = 3.09$, $p = .036$; *Cohen's d* = .46); for Boldness: ($t(95.80) = 2.74$, $p = .030$; *Cohen's d* = .40) than chicks with low scores. Chicks' level of Social Reattachment was independent from their body size. There were no significant differences in behaviour of males and females.

3.2. Bolder chicks are more likely to complete a running-wheel test

A proportion test was used to evaluate how many chicks scored high in Boldness, ran, and completed the running wheel task. Chicks with higher level of boldness were more likely to run in the wheel for the minimum distance to start the habituation test (n. chicks that ran in the wheel = 62, prop. of chicks with high level of boldness running in the wheel: $\hat{\pi} = 38/62 = 0.61$, $z = 2.06$, $p = .019$). Bolder chicks were also more likely to complete the 10 trials of the habituation test (n. chicks that concluded the habituation test = 43, prop. of chicks with high level of boldness that completed the habituation test: $\hat{\pi} = 26/43 = .60$, $z = 1.72$, $p = .042$). The other behaviours and chicks' body size did not affect their probability to run in the wheel.

3.3. Exploratory activity and body size effects on chicks' habituation

LCM was used to analyze the duration of the freezing over the 10 stimulus repetitions. The LCM returned the estimated habituation curve model coefficients (intercept, linear, and quadratic slope) used to predict the duration of freezing on each trial for the entire sample. It also computed the same coefficients for individual chicks. The LCM model including both the linear and quadratic habituation rate explained 60 % of the variance of individuals' duration of the freezing over Trials. Overall, habituation was attested by a significant decrement of the duration of the chicks' freezing from Trial 1–10 ($a = 19.29$, $se = 2.345$, $p < .001$; $b_{lin} = -4.57$, $se = 0.683$, $p < .001$; $b_{quad} = 0.32$, $se = 0.053$, $p < .001$) (see Fig. 1, panel a). This result suggests that the mean freezing response of the chicks to the first trial was 19.29 s, with a monotonic decrement of − 4.573 s every Trial. The positive quadratic habituation rate of 0.32 suggests that the decrement became flatter at each Trial, namely that animals' learning curve was approaching an asymptotic level. There was a positive correlation between the duration of the initial freezing and both the linear habituation rate ($r = .67$; $p < .001$, Fig. 1, panel b) and the quadratic habituation rate ($r = .78$; $p < .001$). There was a strong positive correlation between the linear
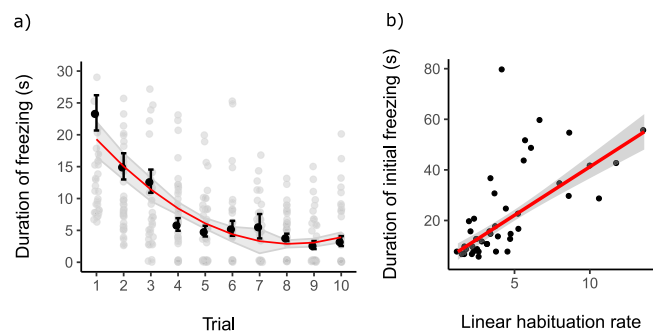

Fig. 1. Panel a) shows the overall habituation curve of chicks. Black dots represent the observed average freezing duration (y-axis) per Trial (x-axis). Gray dots represent individuals' freezing duration in each trial. The red line represents the estimated latent growth curve. Error bars express the S.E.M. Panel b) depicts the relationship between the duration of the initial freezing of chicks and their linear habituation rate obtained from the LCM. The linear habituation rate was transformed into a positive number for ease of interpretability. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and quadratic habituation rate ($r = .98$; $p < .001$). Chicks that froze for longer on the first Trial showed a steeper initial habituation and reached the asymptotic level of response more quickly. The variance around both the intercept and the linear habituation rate was also significant ($\sigma^2_{int} = 177.189$, $p < .001$; $\sigma^2_{lin} = 9.865$, $p = .029$) indicating individual differences in the initial response to the stimulus and the habituation rate. Whereas the variance around the quadratic habituation rate was not significant ($\sigma^2_{quad} = 0.030$, $p = .274$). This revealed that all chicks approached their asymptotic level approximately at the same pace.

3.4. Body size is positively correlated with chicks' initial freezing

Two linear regression models were used to test the effect of individuals' factor scores (behavioural differences) and body size on initial freezing duration. One model included the main effects of body size and exploration activity, separately; the other, the interaction between them. We compared the goodness of fit (in terms of R^2 change) of these two models to determine which one was the best to account for individual differences in the initial chicks' freezing. Table 1 summarizes the multiple regression model coefficients of the relationship between chicks' behavioural differences, body size (formalized by their weight), and the duration of the initial freezing response to the habituation stimulus. The model with Weight, Exploration Activity, Boldness, Social Reattachment, and the interaction Weight × Exploration Activity did not fitted our data significantly better than the model including only main effects (for the main effect model: $R^2 = 0.28$, $F(4, 38) = 3.77$, $p = 0.011$; for the interaction model: $R^2 = 0.31$, $F(5, 37) = 3.42$, $p = 0.012$; R^2 change = 0.03, $F(1, 37) = 1.75$, $p = 0.194$). Thus, we focused our analysis on the most parsimonious model including only the following predictors: Weight, Exploration Activity, Boldness, and Social Reattachment. The analysis revealed a significant main effect of Body size ($F(1, 38) = 12.51$, $p = 0.001$, $\eta^2_p = 0.25$). The duration of the initial freezing increased from smaller to larger chicks with a rate of $\beta = 0.88 \pm 0.25$, $t(38) = 3.54$, $p = 0.001$. No other main effect was significant ($p > .054$).

3.5. Chicks' habituation rate depends on the interaction between exploratory activity and body size

Two linear regression models were used to test the effect of individuals' factor scores (behavioural differences) and body size on individuals' habituation rates (linear and quadratic). As in the previous analysis, one model included the main effects of body size and exploration activity, separately; the other, the interaction between them. We

Table 1
Unstandardized coefficients of the two robust regression models for the Initial freezing, the linear habituation rate and the quadratic habituation rate extracted from the quadratic model of the habituation curve.

	Initial freezing			Linear habituation rate			Quadratic habituation rate		
	B	C.I.	p	B	C.I.	p	B	C.I.	p
Exploratory Activity	11.21	-0.19–22.60	.054	9.12	-0.21 –18.45	.055	-0.51	-1.05–0.04	0.068
Boldness	1.91	-9.41–13.23	.709	-0.69	-2.44 – 1.06	.429	0.04	-0.06–0.14	0.439
Social Reattachment	-4.13	-10.13–1.87	.171	0.21	-0.72 – 1.14	.654	-0.01	-0.07–0.04	0.658
Weight	0.88	0.37–1.38	**.001**	-0.03	-0.15 – 0.08	.564	0.01	-0.01–0.01	0.522
Exploratory Activity × Weight				0.19	0.35 – 0.03	**.019**	0.01	0.01 – 0.02	**0.026**

compared the goodness of fit (in terms of R^2 change) of these two models to determine which one was the best to account for individual differences in the initial chicks' freezing. Table 1 summarizes the coefficients of the multiple regression model used to determine whether behavioural differences and body size explained chicks' habituation rate expressed in terms of individuals' linear habituation rate. Fig. 2 shows the relationship between Body size (weight), Exploratory Activity and chicks' habituation rate. As can be seen, there is a positive relation between the linear habituation rate and Body size (weight) only among chicks with a high level of Exploratory Activity. This observation was confirmed by multiple regression analysis. The model with the Weight × Exploration Activity interaction term fitted our data significantly better than the model with only main effects (for the main effect model: $R^2 = 0.27$, $F(4, 38) = 3.58$, $p = 0.014$; for the interaction model: $R^2 = 0.38$, $F(5, 37) = 4.46$, $p = 0.003$; R^2 change $= 0.11$, $F(1, 37) = 6.04$, $p = 0.018$). The chicks' Body Size differently predicted the habituation rate depending on the Exploratory Activity, as indicated by a significant Weight × Exploration Activity interaction. Post hoc analysis of the interaction revealed that the quadratic rate of habituation increased from small to large chicks in the most exploratory group with a rate of $\beta = 0.21 \pm 0.053$, $t(18) = 3.99$, $p < 0.001$; $R^2 = 0.44$, $F(1,18) = 15.98$, $p < 0.001$. The same rate remained stable along chicks' body size in the least exploratory group ($\beta = 0.029 \pm 0.049$, $t(21) = 0.595$, $p = 0.558$; $R^2 = 0.02$, $F(1, 21) = 0.35$, $p = 0.557$).

We performed the same analysis on the quadratic habituation rate (see, Table 1). The results replicated those obtained on the linear habituation rate. The interaction model with the Weight × Exploration Activity as an additional coefficient over Boldness and Social Reattachment fitted our data significantly better than the model including only main effects (for the main effect model: $R^2 = 0.26$, $F(4, 38) = 3.42$, $p = 0.017$; for the interaction model: $R^2 = 0.36$, $F(5, 37) = 4.13$, $p = 0.004$; R^2 change $= 0.10$, $F(1, 37) = 5.38$, $p = 0.025$). Post hoc analysis of the interaction revealed that the quadratic rate of habituation increased from small to large chicks in the most exploratory group with a rate of $\beta = 0.01 \pm 0.003$, $t(18) = 3.78$, $p = 0.002$; $R^2 = 0.44$, $F(1,18) = 14.28$, $p = 0.001$. The same rate remained stable along chicks' body size in the least exploratory group ($\beta = 0.002 \pm 0.002$, $t(21) = 0.695$, $p = 0.495$; $R^2 = 0.02$, $F(1, 21) = 0.48$, $p = 0.494$).

3.6. Smaller chicks in the high exploratory activity group complete habituation earlier than larger chicks

Previous analyses show that habituation rate (both linear and quadratic) is positively associated with chicks' body size in the High Exploratory Activity group. We further analyzed this relationship to determine whether body size is also associated with the number of trials necessary to complete habituation in this group of chicks.

For each chick, we determined the trial at which the estimated habituation curve approached the asymptotic level of response, defined as the trial t (Trial of complete habituation) at which the predicted
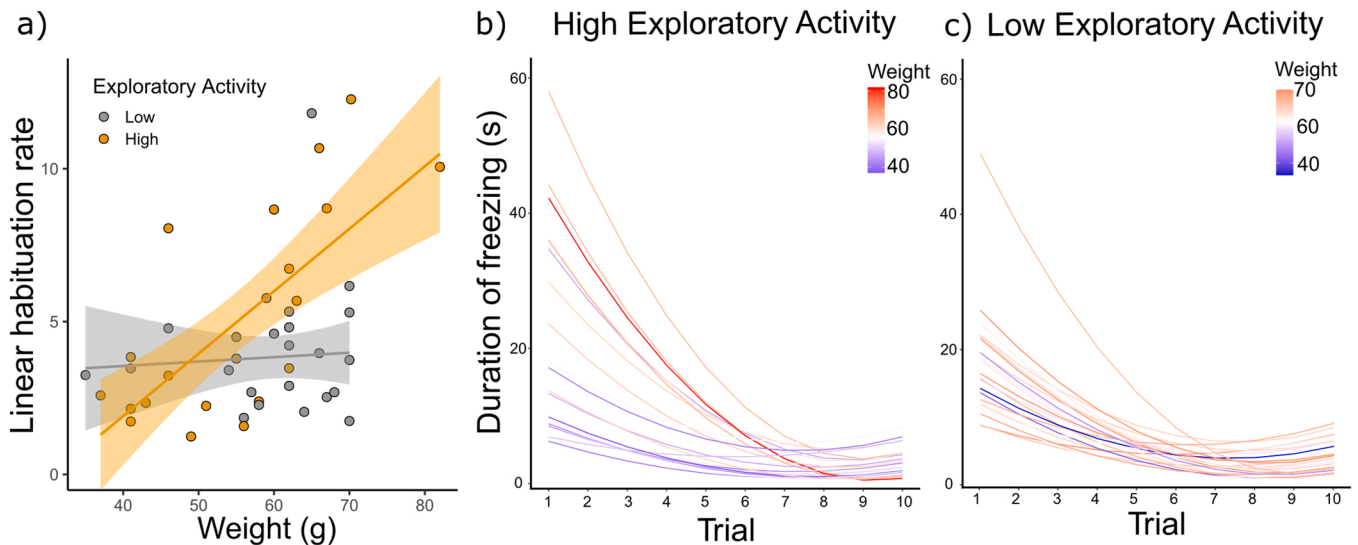

Fig. 2. Panel a) depicts the relationship between chicks' Body Size (operationalized by their weight), their level of Exploratory Activity and their linear habituation rate. The categories (Low vs High Exploratory Activity) refer to chicks' factor scores compared to the sample average. The habituation rate was transformed into positive values. The shaded areas represent S.E.M. The Body Size was positively associated with chicks' habituation rate only in more exploratory chicks. Panel b-c) illustrate the latent growth habituation curve in High Exploratory Activity group (panel b) and in the Low Exploratory Activity group (panel c) as a function of the chicks' body size (curve color). In the High Exploratory Activity group, the steepness of the habituation curve increases from small (blue color) to large (red color) chicks. Furthermore, shorter durations of initial freezing (Trial 1) are related to flatter curves and an earlier asymptote than longer duration of initial freezing. The same relationship is not present in the Low Exploratory Activity group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

duration of the freezing response is lower than the predicted duration of the freezing response at trial $t + 1$. On average, chicks completed habituation in 7.79 trials (sd = 0.94), with no significant difference between chicks in the High Exploratory Activity and Low Exploratory Activity group (mean High Exploratory Activity = 7.90, sd = 0.90; mean Low Exploratory Activity = 7.69, sd = 0.94, $t(39.61) = -0.70$, $p = 485$). To test the effect of body size on the number of trials to complete habituation, we performed two separate linear regressions, including the main effects of chicks' body size (weight) and behaviour (factor scores) for the High and Low Exploratory Activity groups. In the High Exploratory Activity group, the number of trials to complete habituation increased from small to large chicks ($\beta = 0.048 \pm 0.019$, $t(16) = 2.48$, $p = 0.014$). The same relationship was not significant in the Low Exploratory Activity group ($\beta = -0.002 \pm 0.022$, $t(19) = -0.17$, $p = 0.908$). This result reveals that smaller chicks in the High Exploratory Activity completed habituation earlier than larger chicks with the same Exploratory Activity.

4. Discussion

In a world full of information, animals have evolved habituation mechanisms to ignore repetitive irrelevant stimuli and selectively focus on new ones (Cowan, 1988; Ramaswami, 2014; Sokolov, 1963). Because habituation depends on prior stimulus repetition, one would expect that inter-individual differences in habituation entirely rely on previous interactions between an organism and a stimulus or items that resemble that stimulus. Instead, our results show that individual differences in habituation, behaviour, and morphological factors are functionally linked and co-vary from early chicks' life. Because a laboratory-reared new-born chick, whose experiences are intentionally reduced to a minimum, might sample from a limited number of memories established in the first few days of life and is naïve to the stimuli presented at test, the possibility that experience produced the observed individual differences is unlikely. Rather, our results suggest the link between habituation, exploratory activity and body size of chicks is innate.

Our work overcomes the limitation of the previous studies that attempted to demonstrate that individual differences in habituation are innate (Glowa and Hansen, 1994; Roth et al., 2010) and could not completely rule out the possibility that early maternal experiences might have influenced their results. By testing a precocial bird like the chick of domestic fowl, which is independent from parental cares, we provided strong support to the hypothesis of the innateness of inter-individual differences in habituation. Still, it is important to disclose that the existence of innate differences in habituation does not undermine the role that life-history exerts on this form of learning, as demonstrated by several studies (Allan et al., 2020; Blumstein, 2016; Pellitteri-Rosa et al., 2017; Rodríguez-Prieto and Fernández-Juricic, 2005; Samia et al., 2015; Thompson et al., 2018; Vincze et al., 2016). Rather, our discovery paves the way to new research on habituation. Because innate individual differences in habituation may affect precocial experiences of organisms in a significant way, and given the high relevance of habituation mechanisms for a normal neurodevelopment (McDiarmid et al., 2017), future research, for example, may exploit our battery of tests to investigate the genetic bases of the association between exploratory activity, body size, and habituation, an aspect that we did not address in the present study.

We also discovered that exploratory activity moderates the relationship between body size and habituation in chicks. Specifically, we found that body size positively correlates with the habituation rate only in the most exploratory chicks. In this group, the habituation rate increases from small to large individuals, with smaller chicks completing habituation earlier than larger chicks. From a theoretical standpoint, this discovery expands the notion that exploratory activity and habituation rate are both expressions of a broader latent trait of risk assessment, as proposed by Rodríguez-Prieto et al., (2010, 2011). The new element that we introduce is that the most exploratory animals have different abilities to habituate based on their body size. This finding may

have interesting implications if one considers that individuals with similar exploratory activity may be more likely to compete for access to new resources. In our experiment, for example, exploratory activity was measured in an assay in which chicks were searching food while exploring the arena. It is probable that the most exploratory chicks resulting from our assay would be in competition one against the other to access to new food resources when in group. One can easily imagine that the high food drive of these chicks may exacerbate the physical rivalry between small and large chicks with similar behavioural characteristics. From such physical rivalry, small chicks may benefit if they complete habituation before large chicks start the exploration of the new environment.

Our study emphasizes the existence of idiosyncrasies in habituation, which have been overlooked by classical habituation theories (Groves and Thompson, 1970; Sokolov, 1963; Wagner, 1981). Due to the scarce theoretical consideration devoted to individual differences in habituation, we are unable to confidently offer a theoretical explanation to reconcile our results with these classic explanations of habituation at this time.

Instead, the ideas offered by the recent Hall and Rodriguez's (2017, 2019, 2020) theory may be more informative on this topic. This theory holds that a new stimulus elicits the expectation that an indeterminate event will occur, namely the expectation that the stimulus has an excitatory link with another event. This expectation increases the stimulus salience, which eventually causes the organism to respond. When a stimulus is shown repeatedly in isolation, an inhibitory learning process (extinction) diminishes this expectation, and consequently the stimulus salience, determining habituation. The stronger the expectation that something will happen when a stimulus is presented, the higher the initial responsiveness and the rate of habituation. Chicks' habituation differences may reflect the strength of this expectation across individuals.

Such indeterminate expectation includes the possibility that a harmful event will follow, as evidenced by the emergence of a defensive response in our chicks. Remarkably, according to this theory, the emergence of this indeterminate expectation increases the uncertainty about the following events. To minimize the uncertainty, animals engage in exploratory behavior aimed at learning the consequences of events. Hence the close connection between the animal's exploratory behaviour and habituation of a defensive responses. Finally, as proposed by the authors, such expectation can be either genetically determined or the product of previous experiences, so that individual differences in habituation are expected to be innate, in line with our results.

This account of individual differences in habituation spurs new research on the topic. Indeed, Hall and Rodriguez's (2017, 2019, 2020) theory makes fascinating predictions about how experience can modulate individual expectations about events and, as a result, differences in habituation across the life span. For example, it suggests that an individual who has learned through personal experience that stimuli are always followed by relevant consequences will exhibit higher responsiveness to new stimulation and greater habituation rate than another individual who experienced the same stimuli without repercussions.

Aside from the results on habituation, we found that bold chicks are more likely to run consistently in the wheel than shy individuals, which suggests that boldness represents a strong sampling bias in the context of the running-wheel paradigm rather than being directly linked to the habituation capacity of chicks as expected based on the classification of individuals along a proactive-reactive continuum (e.g., Carere and Locurto, 2011; Mazza et al., 2018; Verbeek et al., 1994). The fact that bold animals are more likely to be tested is known from the literature (e. g., Carter et al., 2012) but it is worth stressing that, for the first time, we were able to quantify this sampling bias in a paradigm that is widely used to study chicks' early cognition (e.g., Chiandetti and Turatto, 2017; De Tommaso et al., 2019) and imprinting (e.g., Horn, 1998).

5. Conclusions

To conclude, we showed that the presence of inter-individual differences in habituation capacity of chicks is linked with their exploratory activity and body size soon after birth. Obviously, we could not untangle the direction of the relationship between the factors involved. Indeed, it is possible that a different habituation capacity may have affected chicks' exploratory propensity and body size condition, but the opposite may also be true. Whatever the causal role among these factors, we showed that their reciprocal influence shapes the early interaction of chicks with new stimuli in the absence of relevant life experiences. More research is needed to explore whether our findings apply to different species or to strains of chicks raised in different circumstances. Despite our strain's (Ross 308) high genetic homogeneity, we were able to find differences across chicks. This indicates possible predisposed variability in chicks' behaviour and suggests that these differences could occur also in more ancient strains whose behavioural phenotype has been less subjected to human selection. In general, the implications of innate individual differences in habituation for the development of organisms require further investigation.

Authors' contributions

A.D. and C.C. conceived the study; C.C. supervised the experiment; A.D. collected the data; A.D. and M.G. performed the analysis; A.D. drafted the manuscript, C.C. and M.G. revised the manuscript. All authors approved the final version of the manuscript.

Ethics approval

This experiment complies with the current European Community and Italian laws for the ethical treatment of animals and has been approved by the Organismo Preposto al Benessere Animale of the University of Trieste and licensed by the Italian Health Ministry (permit 88/2019-PR).

Data Availability

The datasets supporting this article has been uploaded as part of the electronic supplementary material.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.beproc.2022.104705.

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