Effect of embryonic light exposure on laterality and sociality in quail chicks (Coturnix coturnix japonica)

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ABSTRACT

Lateralization is the ascendency of one side of the body or the brain to control and perform motor or perceptual functions. Light exposure during prenatal life can modulate the expression of visual lateralization (strength and direction) in a variety of species, including birds. Individual lateralization provides cognitive advantages, but the function of lateralization at the population level is less obvious. Recent studies support the hypothesis that intra-specific population-level lateralization may favour coordination between asymmetrical individuals during synergistic (cooperative) social interactions. Since cohesion requires a good coordination, one can therefore imagine that a higher alignment of lateralization within a group, by making coordination between individuals easier, may promote social cohesion. Our study investigated the effect of light on laterality and social behaviour in Japanese quail chicks (*Coturnix coturnix japonica*). We compared chicks coming from eggs exposed to light during incubation to chicks coming from eggs maintained in complete darkness. We expected light-exposed (LE) chicks to be more aligned than dark-incubated (DI) ones and we wanted to test whether the most aligned chicks were also the most coordinated. We assessed laterality, social motivation, synchronization and spatial cohesion within groups of LE and DI chicks. The prenatal light conditions did not affect either laterality or chicks' social behaviour, and there was no turning bias at the group level. An absolute laterality index characterizing the alignment of chicks living together showed that the groups with the highest indices were not necessarily the groups with the greatest synchronization and spatial cohesion. This suggests that light does not consistently induce laterality in Japanese quail chicks and that the alignment of the chicks' turning bias does not influence their social cohesion. Quails are widely used in farm industry and scientific research and better knowledge of the consequences of incubation conditions on their behavioural asymmetries and sociality could help improve their handling and welfare.

Keywords: turning bias; visuo-motor lateralisation; prenatal experience; lateralisation level; behavioural synchronization; spatial cohesion

1 INTRODUCTION

Laterality is when one side of the body takes advantage over the other one to perform motor or sensory activities (Glick, 1985). It exists in humans and most animal species, including invertebrates (e.g. see Güntürkün et al., 2020; Rogers et al., 2013; Versace and Vallortigara, 2015 for recent reviews). Among behavioural asymmetries, visual lateralization has been widely studied in birds because their eyes are placed laterally and the optic nerves decussate almost completely (Ehrlich and Mark, 1984; Remy and Güntürkün, 1991; Takatsuji et al., 1983; Weidner et al., 1985). This makes them natural "split-brain" models whose eyes and each contralateral hemisphere can be tested independently, which may help in understanding the brain asymmetries that govern lateralization.

Light is known to modulate visual lateralization and hence visually guided behaviours, particularly in birds (e.g. domestic chicks: Casey and Karpinski, 1999; Rogers, 1990, 1982; bobwhite quail: Casey and Lickliter, 1998; pigeons: Güntürkün, 1993; for a review see Chiandetti, 2017). Asymmetrical stimulation of the right eye due to the position of the embryo in the egg (which causes the left eye to be occluded because it is oriented towards the body) is decisive for several forms of visual lateralization (Chiandetti, 2017). In the domestic chick, over-exposure of the right eye to light can reinforce the population bias whereas exposure of the left eye can induce a reversal of this bias. Incubation in the dark can suppress the development of some forms of lateralization at the population level (Rogers, 1990).

Individual lateralization (when each individual favours one side over the other) can give a cognitive advantage, but the function of lateralization at the population level (when most individuals favour the same side) is less obvious (see Vallortigara and Rogers, 2020 for a review of costs and benefits of these two degrees of lateralization). According to some authors, the alignment of individuals' biases may have evolved as an evolutionary stable strategy in which asymmetrical individuals must coordinate their behaviour with that of other asymmetrical individuals (Ghirlanda et al., 2009; Ghirlanda and Vallortigara, 2004; Vallortigara, 2006; Vallortigara and Rogers, 2020, 2005). For

65 Ghirlanda et al. (2009), population-level lateralization could be advantageous in intra-specific social
66 interactions by making coordination easier. Thus, synergistic activities could favour individuals with
67 the same lateralization because they can, for instance, have an easier time coordinating physical
68 activities (see also Frasnelli and Vallortigara, 2018). Here we wanted to check whether groups made
69 of individuals with the same lateralization would indeed have an easier time synchronizing their
70 activities and staying cohesive.

71 Although the domestic chick and the pigeon have been the two most popular models since
72 researchers began studying visual lateralization in birds, more recently Japanese quails have also
73 been studied more extensively (e.g. Casey and Sleigh, 2014; Valenti et al., 2003; Zucca and Sovrano,
74 2008). Japanese quails have been domesticated for centuries (Cheng et al., 2010). Because of their
75 relatively small size and fast development, they quickly became popular in both farm industry and
76 scientific research. Although quails are difficult to observe in the wild due to their small size and their
77 secretive habits, several studies have shown that, during the breeding season, they live in small
78 family groups or single pairs, in areas where they hardly tolerate other quails (Farris, 1964; Nichols,
79 1991; Wetherbee, 1961). Japanese quail chicks do not naturally exhibit turning bias and footedness
80 (Casey, 2005; Casey and Sleigh, 2001). However, the chicks, but not the adults, are lateralized in the
81 "pebble floor" task (which consists in distinguishing edible grains from pebbles stuck to the ground)
82 (Valenti et al., 2003). Adult quails, on the other hand, appear to be lateralized in a detour task using a
83 social target (Zucca and Sovrano, 2008) and when they are trained to run across either a left- or a
84 right-turning runway to obtain sexual access to a conspecific of the opposite sex (Gülbetekin et al.,
85 2009, 2007). Recently, by manipulating prenatal visual stimulation after having removed the shell
86 and inner-shell membrane of the egg, Casey and Sleigh (2014) managed to induce individual- and
87 population-level motor lateralization in Japanese quail chicks. Stimulated chicks showed a turning
88 bias that unstimulated chicks did not show. Although embryonic exposure to light is likely to be
89 reduced in wild quails, stimulating embryos with light could be useful to manipulate lateralization, at

the individual as well as at the population level, and to better understand the link between lateralization and social behaviour.

Here, to test the effect of light on population-level laterality and social behaviour, and to determine whether the alignment of behavioural asymmetries can facilitate social interactions between individuals, we compared the turning bias, temporal synchronisation, spatial cohesion and social motivation of groups of quail chicks coming from eggs that had been either incubated in the dark or temporarily exposed to light before the end of incubation. We expected that exposure to light would create differences in visuo-motor lateralization between exposed and unexposed chicks, and we wanted to see if the most lateralized chicks were also the most coordinated and socially motivated.

2 MATERIAL AND METHODS

All the experiments were carried out in accordance with the European Communities Council Directive of 22th September 2010 (2010/63/EU) and were approved by the regional ethics committee (French "Comité Rennais d'Ethique en matière d'Expérimentation Animale / n°7", permit number: APAFIS#17476-2018110716226608 v2).

2.1 Incubation

We placed commercial fertilized Japanese quail eggs ("Les cailles de Chanteloup", Corps-Nuds, France) in two incubators (Ova-Easy Advance 380, Brinsea). From day 1 of incubation to day 15, incubation parameters were the same for all eggs: 37.7 °C, 45% humidity and a rotation of 45 degrees every 30 minutes, and both incubators were in the dark. After 15 days of incubation, to induce hatching, we changed the parameters of both incubators to 37.7°C, 65% humidity and no rotation. At the same time, we interchanged half of the eggs of each incubator in order to avoid any incubator bias. From that moment, the eggs of one of the incubators were lit continuously for 41 hours by a "daylight" led (6400°K, 600 lumens) placed in front of the incubator while the eggs of the second incubator stayed in the dark. After the 41 hours of exposition to light, we again placed all the

eggs in the dark until hatching on day 17 to 18, that is within 24 hours after the end of the stimulation. Since light intensity within the incubator was 20-30 Lux, luminous exposure was $2.9x10^6$ to $4.4x10^6$ lx.s (for comparison, luminous exposure was $0.7x10^6$ to $3.6x10^6$ in Rogers, 1982). Japanese quail's eggs have brown spots that may reduce the amount of light that reaches the embryo. We therefore decided to stimulate the eggs longer, and thus started the stimulation earlier, than in the study of Casey and Sleigh (2014) on quails. However, the end of the stimulation was similar to theirs.

2.2 Maintenance of the chicks

The day of hatching (day 1, D1), we weighed the chicks, ringed them with one plastic ring on each leg, and placed them in heated plastic cages (L90xl40xH30cm) with a plastic net and wood shavings on the floor. We provided food and water ad libitum. Dark-incubated (DI) and light-exposed (LE) chicks were in the same room but not in the same cages. At post-hatching day 3 (D3), we separated 120 of the chicks that had hatched in 2x10 groups of six chicks (n=60 DI and 60 LE chicks). At this age chicks cannot be sexed so we have constituted the groups without knowing their sex ratio. After 3 weeks, we sexed the birds and found that the sex ratio did not differ between DI and LE birds (DI: females = 36, males = 23; LE: females = 31, males = 27; Fisher's exact test, p=0.46, https://biostatgv.sentiweb.fr/?module=tests/fisher).

On the day of hatching, when we took all the chicks that had hatched, DI chicks (n=106) were heavier than LE chicks (n=104) (mean±SD: DI chicks' weight = 10.51±0.93 g, LE chick's weight = 10.26±0.79 g; Student t=2.03, ddl=208, p-value=0.04), but the effect was small (Hedges' g = (10264 - 10507)／862.06 = 0.28; https://www.socscistatistics.com/effectsize/default3.aspx). Anyway, by mixing chicks of different weights within each experimental group, we constituted groups of DI and LE chicks whose average weights were not significantly different (n=60 chicks in each group, DI chicks' weight = 10.43±0.68 g, LE chicks' weight = 10.28±0.66 g; Student t=1.17, ddl=118, p=0.24). We placed all the six-chick groups in the same room in separate metal cages (L100xl70xH65cm) equipped with a heating lamp, a feeder and a drinking trough (Figure 1). The different groups could hear but not see

each other. Room temperature was 20 to 25°C and the artificial light followed a L:D rhythm of 12:12. During the first week of life, we provided green light during the night to enable the chicks to see the feeder and drinking trough if they need to eat or drink. We counterbalanced the positions of the DI and LE chicks' groups in the room in order to avoid a bias due to the position of the cage. We also counterbalanced the positions of the feeder and the drinking trough within the cages. We turned off heating lamps after 15 days. Under natural conditions, quail chicks are able to regulate their body temperature and they become independent by 11 days after hatching, (Mills et al., 1997; Orcutt, Jr. and Orcutt, 1976).

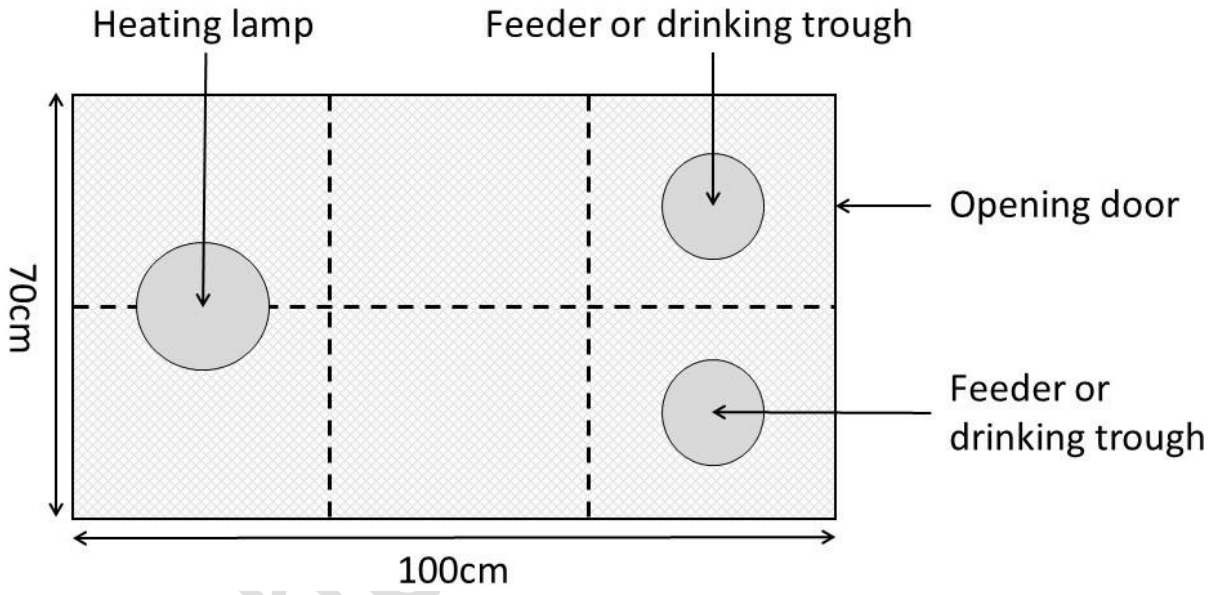


Figure 1: Schematic representation of a home cage. The dotted lines show the virtual boundaries we used to locate the chicks during behavioural observations.

2.3 Laterality tests

On D3, once the six-chick groups were formed, we tested the turning bias of the 120 chicks by placing each of them once in a T-maze (Figure 2). The T-maze was in a soundproof room homogeneously lighted. We regularly changed the orientation of the maze in the room (90° turn each time) so that a quarter of the chicks (n=15 DI and 15 LE chicks) passed the test in each of the four positions. At the beginning of each test, the experimenter placed a chick in the longest, stem branch of the T-maze,

facing the wall, and stood still behind this branch. The chick was free to move in the maze during 3 minutes. The test stopped as soon as the chick turned in one of the distal branches (i.e. both legs were in the branch; see Figure 2) or after 3 minutes, and the experimenter noted which side (left or right) the chick chose. We also tested all the chicks in the same way on D10-11 and D22-23. In order to adapt to their growth, we used a larger maze (Figure 2). We also had to conduct the tests over two days because they moved much less at these ages (Figure 3).

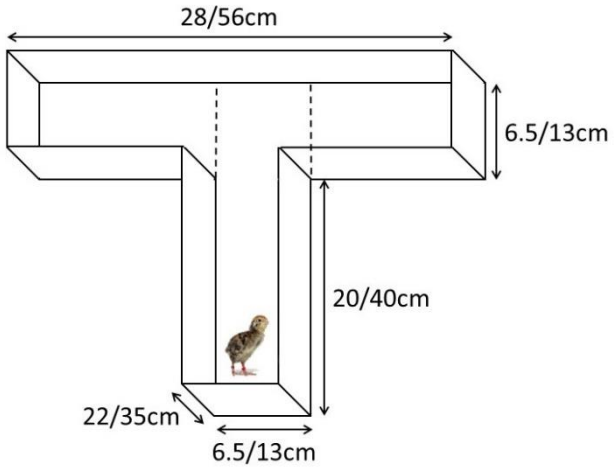


Figure 2: T-maze that we used to test the laterality of the chicks. The smallest dimensions correspond to the size of the maze that we used on D3, and the largest dimensions to the size of the maze that we used on D10-11 and D22-23. We considered that the chick had chosen one branch of the maze when both legs crossed one of the two virtual lines represented here as dotted lines.

2.4 Behavioural observations

In order to evaluate the spatial cohesion and the temporal coordination of each group of six chicks, we observed the chicks directly in their home cage with the instantaneous scan-sampling method. Each group was observed 60 times during 12s in two 135-min sessions (one from 8:00 h to 10:15 h and one from 15:30 h to 17:45 h), with an interval of 4min30s between two consecutive scans of the same group. We noted the behaviour (resting, moving, exploring, eating, drinking, observing, vocalising, preening and touching another chick) and position of all the chicks in each group. To position the chicks, we virtually divided the cage into six 34x34cm squares (Figure 1). Behavioural

observations were made on D4, D7, D9, D11 and D15, amounting to 300 scans for each group of 6 chicks.

2.5 Social motivation test

On D16 and D17, the chicks were isolated one by one in their home cage to test their social motivation. We removed all the chicks from the same home cage and then put them back one by one in this cage. There was only one chick at a time in the home cage and the other chicks were kept together in a room far enough away so that the isolated chick could not hear them. When they lose visual contact with peers, quail chicks usually try to re-establish contact by emitting distress calls that are calls of high social value (Guyomarc'h and Guyomarc'h, 1996). These calls are loud calls that are easily recognisable and they are the only loud calls that are produced when quail chicks are isolated. We noted the latency of the first distress call and the total number of calls during 3 minutes. After these 3 minutes, the isolated chick was removed, placed back with the others and another chick was placed alone in the cage for 3 minutes, until all six chicks had been tested.

This test measures both social motivation and emotivity. However, by leaving the tested chick in the home cage, that is in a familiar environment, we tried to make it feel as safe as possible. This allowed us to avoid an emotional or fear response due to novelty and a supposedly dangerous situation and to thus preferentially measure the social dimension of the chicks' response.

2.6 Statistical analyses

We compared the number of DI and LE chicks having turned (left or right) or not in the T-maze, and the number of chicks turning left and right, using Chi-squared tests within (goodness of fit) and between (homogeneity) the experimental conditions, using Microsoft Excel 2019.

To evaluate social behaviour, we calculated two indices from the scans (Lumineau et al., 2001). The first index was a temporal synchronisation (TS) index. To calculate this index, we distinguished two types of behaviours: high-energy behaviours (eating, drinking, moving, exploring and vocalising) and

low-energy behaviours (resting, observing, preening and pecking another chick). If, for one day of observation, A is the number of chicks of the same group having high-energy behaviours in one scan "i", R is the number of chicks having low-energy behaviours in the same scan and N the total number of chicks per group (6) multiplied by the total number of scans per day (60), then: TS=$\sum_{i=1}^{60} \frac{|Ai-Ri|}{N}$. TS was 1 when all the chicks of the group were having the same type of behaviour (either high- or low-energy) in all the scans and 0 when half of the chicks were having one type of behaviour while the other half was having the other one. We obtained one TS value per group of 6 chicks and per day of observation. The second index was a spatial cohesion (SC) index. In each scan, there was n_1 chicks in the virtual square 1, n_2 in the virtual square 2… and n_6 in the virtual square 6. Using these values, we calculated the ratio between the variance and the mean number of chicks per virtual square per scan. This ratio was 6 when all the chicks were in the same square, and 0 when there was one chick in each square. We then averaged the values of the 60 scans to obtain one SC value per group of six chicks and per day of observation. We compared DI and LE chicks' TS and SC indices obtained for all days with permutation tests using Rd_kheradPajouh_renaud to handle nuisance variables and 50000 permutations (R v4.0.0 with permuco package; Frossard and Renaud, 2019; R Core Team, 2020).

To compare the social motivation of the two experimental groups, we performed Mann-Whitney tests with the latency of the first distress call and the total number of calls as variables (R v4.0.0; R Core Team, 2020).

Finally, in order to see if there was a correlation between the alignment of laterality and the social cohesion within the groups of 6 chicks, we calculated for every group and for each test day in the T-maze an absolute laterality index (AbsLI) whose formula was: |L-R|/L+R where L was the number of chicks who turned left and R the number of chicks who turned right. This index was 1 when all the chicks turned on the same side and 0 when half of the chicks turned on one side and the other half on the other side. We kept only the groups in which at least three of the six chicks chose to turn into

223 one of the two arms during the test for further analyses. We used AbsLI values to make linear
224 regressions and calculate correlation coefficients with TS and SC indices.

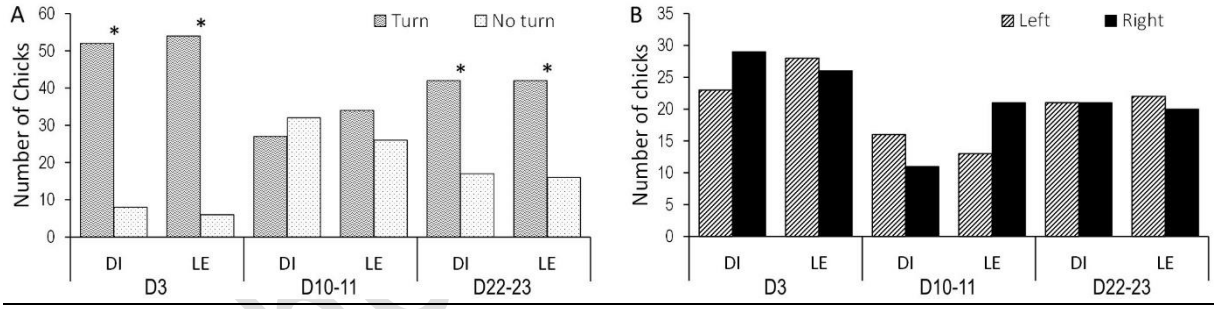
225 3 RESULTS

226 3.1 Effect of light exposure

227 3.1.1 Laterality (T-maze) tests

228 At D3 and D22-23, there were significantly more chicks that turned left or right than chicks that did
229 not turn (Figure 3A; see Table 1 for statistical results). At D10-11, since many chicks did not turn (54%
230 of the DI chicks and 43% of the LE ones), this difference was not significant (Figure 3A).

231 Whatever the day, there was no within- or between-group difference in the number of chicks turning
232 left and those turning right (Table 1; Figure 3B).



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234 Figure 3: Summed results of the laterality tests at D3, D10-11 and D22-23. (A) Number of chicks that
235 turn or did not turn in the T-maze; (B) Number of chicks that turned left and right. DI: Dark-Incubated
236 chicks; LE: Light-Exposed chicks. * Goodness of fit Chi-squared test, p<0.001.

237 Table 1: Comparisons within and between the experimental conditions (DI and LE) of the number of
238 chicks turning or not turning, and turning left or right in the T-maze. Results of goodness of fit (within
239 experimental conditions) and homogeneity (between experimental conditions) Chi-squared tests.

Turn/ No turn	DI	LE	DI/LE

D3	**X^2=32.27**	**df=1**	**p<0.001**	**X^2=38.40**	**df=1**	**p<0.001**	X^2=0.32	df=1	p>0.05
D10-11	X^2=0.42	df=1	p>0.05	X^2=1.07	df=1	p>0.05	X^2=1.42	df=1	p>0.05
D22-23	**X^2=10.59**	**df=1**	**p<0.001**	**X^2=11.65**	**df=1**	**p<0.001**	X^2=0.02	df=1	p>0.05
Left/Right		DI			LE			DI/LE	
D3	X^2=0.69	df=1	p>0.05	X^2=0.07	df=1	p>0.05	X^2=0.62	df=1	p>0.05
D10-11	X^2=0.93	df=1	p>0.05	X^2=1.88	df=1	p>0.05	X^2=2.67	df=1	p>0.05
D22-23	X^2=0.00	df=1	p>0.05	X^2=0.09	df=1	p>0.05	X^2=0.05	df=1	p>0.05

D: Day; DI: Dark-Incubated chicks; LE: Light-Exposed chicks. Significant results are in bold.

3.1.2 Temporal synchronisation

Temporal synchronisation decreased with time regardless of the experimental condition (DI/LE: F=0.59, p=0.45; Day: F=42.12, p<0.0001; interaction: F=1.06, p=0.38). The older the chicks were, the less synchronized they were (Figure 4). There was a significant decrease after D4 and temporal synchronization at D15 was significantly lower than at D7 and D9 (Pairwise comparisons using permutation t tests; D4 compared to the other days: p=0.02 in all cases; D15 compared to D7 and D9: p=0.04 in both cases). A Bayes factor analysis confirmed a strong effect of time but a negligible effect of the experimental condition and of its interaction with time (supplementary Table 1).

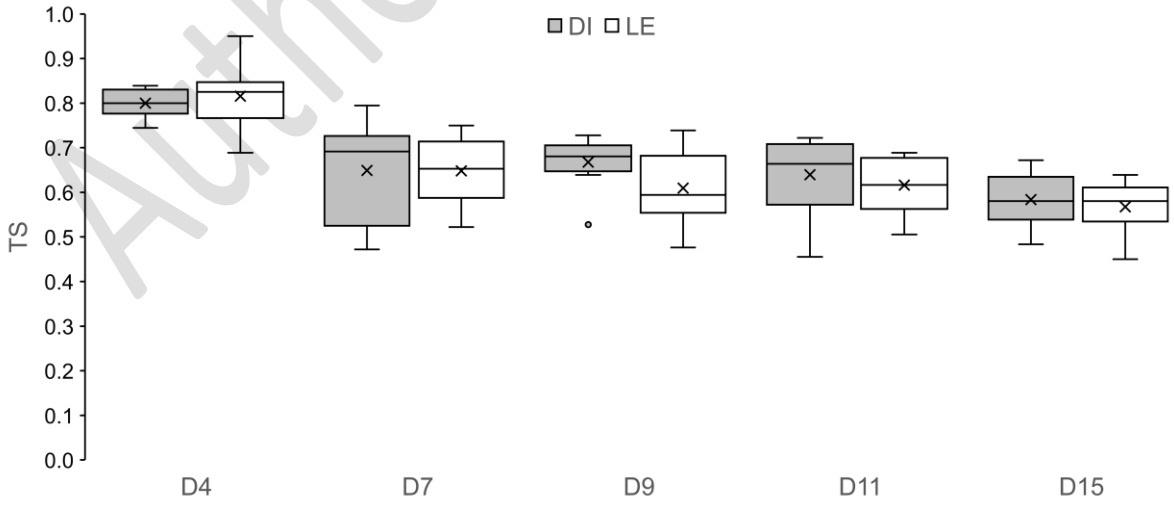

Figure 4: Temporal synchronization index (TS) of dark-incubated (DI, grey boxes) and light-exposed (LE, white boxes) chicks during development (D=post-hatching day). Boxplot: middle line=median,

lower limit of the box=1st quartile, upper limit of the box=3rd quartile, whiskers=1.5 inter-quartile range. Cross: mean.

3.1.3 Spatial cohesion

After a significant decrease between D4 and D7, spatial cohesion remained relatively stable over time, regardless of the experimental condition (DI/LE: F=0.00, p=0.98; Day: F=5.54, p<0.001; interaction: F=0.87, p=0.49; Pairwise comparisons using permutation t tests; D4 significantly different of all other days except D15: p=0.02 for D7, D9 and D11 and 0.30 for D15; no other significant difference; Figure 5). Again, a Bayes factor analysis confirmed an effect of time but a negligible effect of the experimental condition and of its interaction with time (supplementary Table 1).

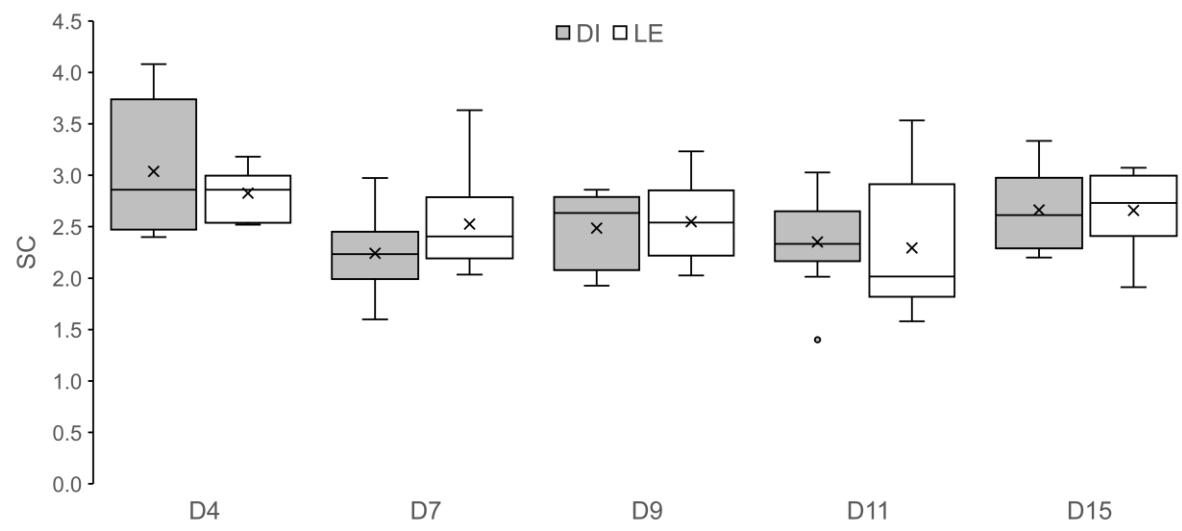

Figure 5: Spatial cohesion index (SC) of dark-incubated (DI, grey boxes) and light-exposed (LE, white boxes) chicks during development (D=post-hatching day). Boxplot: middle line=median, lower limit of the box=1st quartile, upper limit of the box=3rd quartile, whiskers=1.5 inter-quartile range. Cross: mean.

3.1.4 Social motivation

There was no difference in social motivation between the two experimental conditions as measured by the latency before the first distress call (Mann-Whitney W=1757.5, n_{DI}=59, n_{LE}=60, p=0.95) or the total number of calls (Mann-Whitney U=1625.5, n_{DI}=59, n_{LE}=60, p=0.44) (Figure 6).

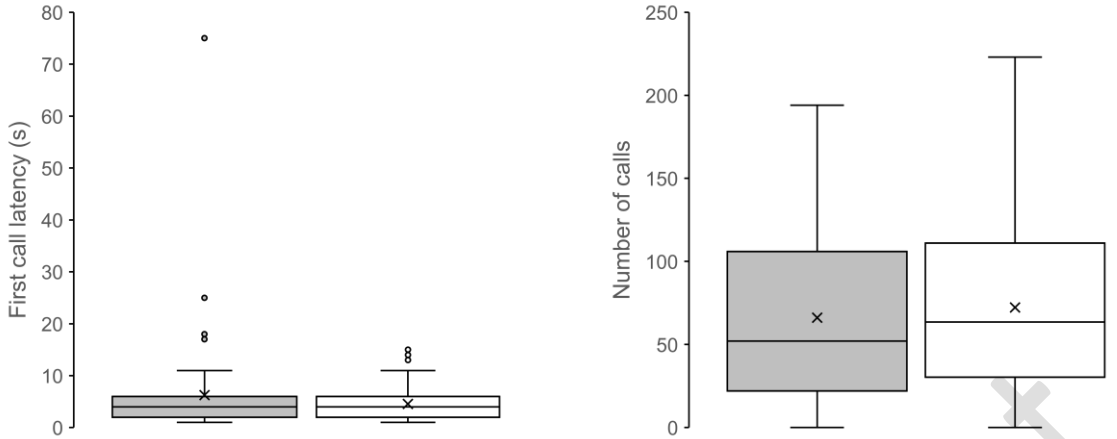

Figure 6: (A) Latency before the first distress call (s) and (B) total number of calls of dark-incubated (DI, grey boxes) and light-exposed (LE, white boxes) chicks during the social motivation test. For better readability, chicks that did not vocalize (n=2 DI and n=2 LE chicks), and therefore had a latency score of 180 s, were excluded from the latency graph. Boxplot: middle line=median, lower limit of the box=1st quartile, upper limit of the box=3rd quartile, whiskers=1.5 inter-quartile range. Crosses: mean.

3.2 Correlation between laterality alignment and social cohesion

The AbsLI index measured the extent to which the chicks living in the same cage turned in the same direction in the T-maze test. Whatever the day, there was no correlation between AbsLI and temporal synchronisation or spatial cohesion (Figure 7). This means that the most aligned chicks were not necessarily the most synchronized and cohesive. Since social motivation was only measured once in each chick, we could not test the correlation between AbsLI and the number and latency of distress calls.

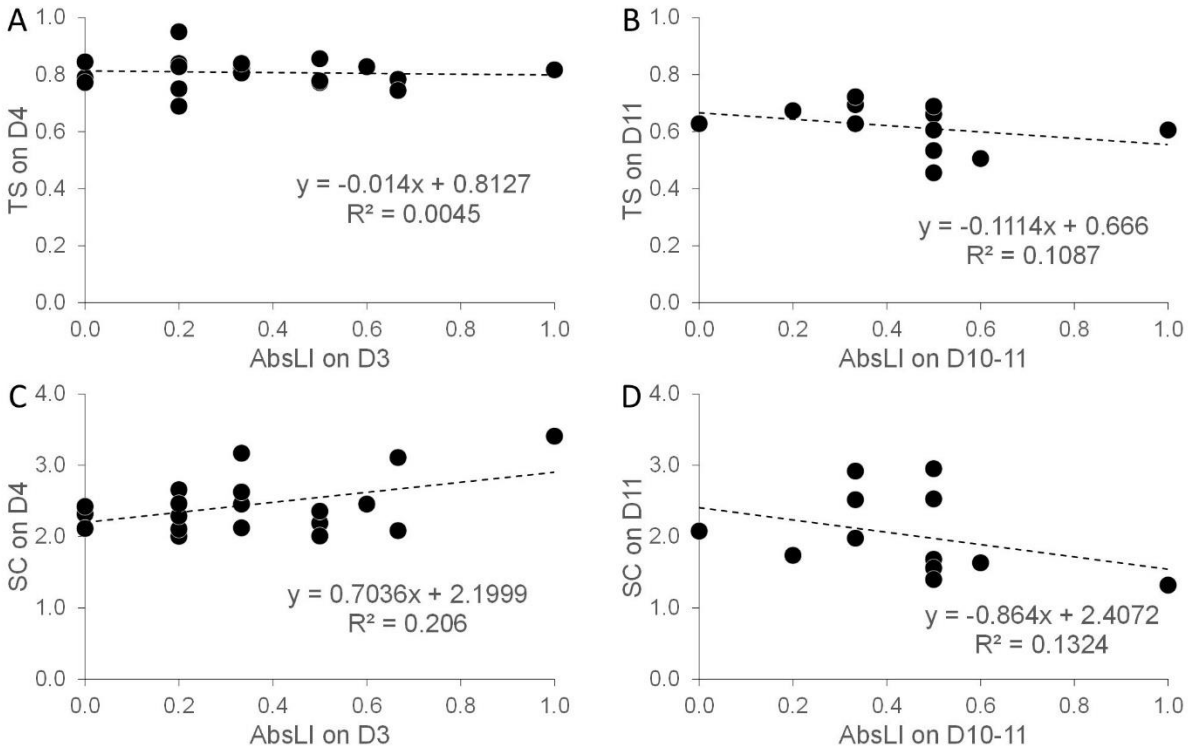

Figure 7: Correlations between AbsLI and (A,B) temporal synchronization (TS) and (C,D) spatial cohesion (SC), at (A,C) D3-4 and (B,D) D10-11. Dotted line: linear regression.

4 DISCUSSION

By comparing laterality (at the group level and as measured by turning bias) and social behaviour of groups of chicks exposed or not to light at the end of incubation, we studied both the effect of light on the development and alignment of lateralization and the link between laterality and sociality. Since the degree, the direction and the alignment of lateralization of some visually-guided behaviours can be modulated by the amount of light received during incubation in a variety of species such as zebra fish, topminnows, pigeons and domestic chicks (see Chiandetti, 2017 for a review), we expected light exposure to influence the alignment of visuo-motor lateralization and to create a difference between our two experimental conditions. However, our protocol of exposure to light during incubation had no effect either on the alignment of turning bias or on spatial cohesion, temporal synchronisation and social motivation of our groups of chicks.

297 Exposure to light during incubation had no significant effect on chicks' visuo-motor laterality in the T-
298 maze test. Yet, in the domestic hen and the bobwhite quail, two species that are closely related to
299 the Japanese quail, exposure to light before hatching can reinforce visual (Rogers, 1982) and motor
300 (Casey and Karpinski, 1999; Casey and Lickliter, 1998) lateralization at the population level. This
301 effect is thought to be due to the position of the embryo in the egg: in the egg, the embryo's body
302 occludes the left eye and only the right eye can receive light. Since the avian visual system crosses
303 almost completely, the left hemisphere is therefore more stimulated than the right one (Rogers,
304 1990). Occluding the right eye and stimulating the left one (therefore the right hemisphere) can
305 reverse the group bias (Casey and Karpinski, 1999; Casey and Lickliter, 1998; Rogers, 1990). Thus,
306 asymmetrical light stimulation in the egg can create a bias. Here, we observed no group turning bias
307 in our chicks, even after exposing them to light during incubation. This suggests that light stimulation
308 through the shell does not modulate the particular visuo-motor lateralization that we measured in
309 the Japanese quail. Casey and Sleigh (2014) recently obtained an effect of light during incubation, but
310 they had to open the eggshell and to take out the head of the chick to directly stimulate one of its
311 eyes. It is therefore possible that our light stimulation was not strong enough to play a role in the
312 development of visuo-motor asymmetries. In the domestic hen, a stimulation of 250 to 350 lux of the
313 egg (which corresponds to values as low as 25 to 35 lux for the embryo) is enough to obtain an effect
314 on visual lateralization (Rogers, 1990). But, while hens' eggshells are white, those of Japanese quail
315 have brown spots that may reduce the amount of light that reaches the embryo. Light stimulation
316 did have an effect though: dark-incubated chicks were significantly heavier than light-exposed chicks
317 on the day of hatching. This weight difference may be because light-exposed chicks began to hatch a
318 bit earlier (no more than 9 hours) than dark-incubated chicks. Previous research has shown that
319 exposing eggs to white light can alter the time of hatching (Narahari et al., 1988). This happens
320 especially when light stimulation is too strong (Fairchild and Christensen, 2000; Siegel et al., 1969).
321 Considering this, it might be possible that in our study an excess of light stimulation has made

hatching earlier in the light-exposed group, which might have somehow disrupted the full development of lateralization.

Another explanation could be that we made our stimulation outside the sensitive period of the Japanese quail. In the domestic chick, the embryos need to be exposed to light during the last two days of incubation to obtain an effect. Although two hours of stimulation are sufficient to create a group bias, stimulation has to last between 2.5 to 6 hours to consolidate this bias, so that it does not change if the other eye is stimulated (Rogers, 1990). More recently, an early sensitive period has been discovered in the domestic chick: light exposure occurring well before the development of a functional visual system also plays a role in the development of visual lateralization (Chiandetti et al., 2013; Chiandetti and Vallortigara, 2019). Since no data about sensitive periods to light (either late or early) are available for the Japanese quail, it remains to be determined whether our stimulation was within or outside a light-sensitive period.

To determine individual lateralization, you have to test each individual several times, and to determine population-level lateralization, you have to test many individuals at least once. Because quail chicks grow very fast, our logistical conditions did not allow us to repeat the laterality tests enough times in a short period of time to determine individual lateralization during the first days of life. We therefore decided to focus on laterality at the population-level by testing more chicks but only once. Even if there is no evidence of a motor population bias in Japanese quail chicks, Casey (2005) showed that about 50% of the individuals show a turning bias (either to the left or to the right, in equal proportion). Although there was no turning bias at the group level, it is therefore possible that the number of individually lateralized chicks differed between the two experimental groups. Moreover, lateralization is task-specific. We therefore cannot exclude that chicks would have shown a group bias in other tasks such as the pebble floor task, for which a transitory visual lateralization has already been evidenced (Valenti et al., 2003).

346 Exposure to light had no effect on social cohesion and motivation either. It has also been shown that
347 exposure of the embryo to light has no effect on dispersal in domestic chicks (Wichman et al., 2009).
348 However, when domestic chicks are exposed to light during incubation, they tend to be more
349 aggressive and to peck more at conspecifics than dark-incubated chicks (Rogers, 1982). Moreover,
350 whereas dark-incubated chicks peck more at unfamiliar than at familiar individuals, light-exposed
351 chicks show no preference (Riedstra and Groothuis, 2004). Since the two eyes do not play the same
352 role in social recognition (Rogers and Andrew, 2002; Vallortigara and Andrew, 1994), this light-
353 induced effect on the distinction between familiar and unfamiliar individuals is likely due to an
354 alteration of the way each hemisphere process visual information. However, no effect of light
355 exposure during incubation was shown on some social behaviours such as individual recognition or
356 choice behaviour between familiar and unfamiliar chicks (Deng and Rogers, 2002). Since these
357 behaviours are more influenced by postnatal light experience (Deng and Rogers, 2002), we might
358 have obtained different results by manipulating exposure to light after hatching.

359 Regardless of prenatal treatment, we observed a decrease in chicks' temporal synchronisation.
360 Chicks are not able to regulate their body temperature before 11 days of life (Orcutt, Jr. and Orcutt,
361 1976). To keep warm, they thus sleep close from each other, which requires them to synchronize
362 their activities (Formanek et al., 2011). Then, they become more and more independent with time
363 and they show less and less social motivation (François et al., 1998). This could explain the decrease
364 that we observed in our study. Spatial cohesion, on the other hand, remained relatively stable over
365 time. This means that chicks stayed relatively close from each other while doing various activities.
366 This might be explained by the fact that they spent a lot of time resting under the heater at the
367 beginning of the experiment, and then spent more time close to the feeder and the drinking trough
368 (without necessarily eating or drinking). In large aviaries (25-30 m^2) reproducing semi-natural
369 conditions, spatial cohesion decreases between the age of 4 and 15 days (Lumineau et al., 2001). Our
370 home cages might therefore have been too small for the chicks to disperse. It could also have
371 something to do with the absence of any visual barriers in the home cage, since opaque barriers

appear to be important for the development of spatial cognition and its lateralization in the domestic chick (Freire and Rogers, 2005). In addition, as they had nowhere to hide, there may have been no incentive for the chicks to move away from other chicks.

Finally, by using an index that could measure the degree of alignment of chicks independently of prenatal treatment, we could check whether there was a correlation between group lateralization and social cohesion (i.e. temporal synchronization and spatial cohesion). The results showed that chicks that were more aligned were not necessarily better synchronized and more cohesive. This suggests that there may be no modulation of social behaviour by group lateralization in the Japanese quail. In a complementary way, Versace et al. (2020) have just shown that social environment can modulate individual- but not population-level lateralization in fruit flies (Drosophila melanogaster). The link between lateralization and social interactions is therefore probably not so straightforward and more studies are definitely needed to better understand it. The Japanese quail could be useful to explore this issue further, and, since it is a domestic species, and although our study is primarily fundamental, such studies could also help to better understand the consequences of domestication and to improve handling and welfare of species used in laboratory and farm industries. Respect for animal welfare is a major issue in farm industries. In animal husbandry, in order to withstand high densities, animals must be more socially tolerant and less emotive. Understanding the mechanisms for the development of sociality and emotivity is therefore an essential prerequisite for the adaptation of animals to farming conditions.

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Supplementary Table 1: Bayes factor analysis of temporal synchronization and spatial dispersion

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Temporal synchronisation

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[1] Experimental condition (DI/LE) + Cage: 0.3211489 ± 0.86%

[2] Time (Day) + Cage: 3.81146e+16 ± 0.63%

[3] Experimental condition (DI/LE) + Time (Day) + Cage: 1.611854e+16 ± 1.12%

[4] Experimental condition (DI/LE) + Time (Day) + Experimental condition (DI/LE):Time (Day) + Cage: 4.681978e+ 15 ±4.07%

Against denominator: Temporal Synchronisation ~ Cage

Spatial cohesion

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[1] Experimental condition (DI/LE) + Cage: 0.279752 ± 0.86%

[2] Time (Day) + Cage: 129.6506 ± 0.45%

[3] Experimental condition (DI/LE) + Time (Day) + Cage: 37.23607 ± 1.27%

[4] Experimental condition (DI/LE) + Time (Day) + Experimental condition (DI/LE):Time (Day) + Cage: 9.711455 ± 6.99%

Against denominator: Spatial Cohesion ~ Cage

Bayes factor type: BFlinearModel, JZS