

Unlocking the symmetric transfer of irrelevant information: gene–environment interplay and enhanced interhemispheric cross-talk

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Hemispheric specialization influences stimulus processing and behavioural control, affecting responses to relevant stimuli. However, most sensory input is irrelevant and must be filtered out to prevent interference with task-relevant behaviour, a process known as habituation. Despite habituation's vital role, little is known about hemispheric specialization for this brain function. We conducted an experiment with domestic chicks, an elite animal model to study lateralization. They were exposed to distracting visual stimuli while feeding when using binocular or monocular vision. Switching the viewing eye after habituation, we examined if habituation was confined to the stimulated hemisphere or shared across hemispheres. We found that both hemispheres learned equally to ignore distracting stimuli. However, embryonic light stimulation, influencing hemispheric specialization, revealed an asymmetry in interhemispheric transfer of the irrelevant information discarded via habituation. Unstimulated chicks exhibited a directional bias, with the right hemisphere failing to transfer distracting stimulus information to the left hemisphere, while transfer from left to right was possible. Nevertheless, embryonic light stimulation counteracted this asymmetry, enhancing communication from the right to the left hemisphere and reducing the pre-existing imbalance. This sharing extends beyond hemisphere-specific functions and encompasses a broader representation of irrelevant events.

1. Introduction

Hemispheric specializations can influence how stimuli are processed and behaviour is controlled, which in turn affects how an animal responds to relevant stimuli in its environment [1,2]. In a prototypical example, a toad will flee at the sudden appearance of a predator in the left visual field, whereas it will capture prey entering the right visual field [3]. It is also known that the left hemisphere shares information with the right one, whereas the opposite happens to a lesser degree [4,5]. Such extreme hemispheric-spatial specialization is seen in responses to relevant stimuli, but animals spend much of their lifetime dealing with irrelevant events. Given their irrelevance, these stimuli must be filtered out to prevent them from interfering with any ongoing task-relevant behaviour, a capacity manifested by the phenomenon of habituation [6]. Despite the importance of habituation, which is widespread among animal species, there is no evidence about the influence of hemispheric specialization on habituation [7–9]. Therefore, we took advantage of our knowledge of habituation [10] and lateralization [11] in young domestic chickens (*Gallus gallus*) and exposed them repeatedly to a visual distracting stimulus while they were feeding binocularly or using one eye only. We explored whether

habituation depends preferentially on one hemisphere or is implemented bilaterally in the brain. In addition, by switching the viewing eye after habituation had occurred monocularly, we investigated whether habituation was confined to the originally stimulated hemisphere or whether it was evident also in the other hemisphere, which would entail an interhemispheric transfer of distracting information. In particular, we addressed this issue by manipulating an epigenetic factor, light stimulation applied during embryonic development, which is known to interact with genetically determined asymmetries in hemispheric crosstalk [12,13].

2. Material and methods

(a) Participants

We incubated 188 eggs (ROSS-308 *Gallus gallus*) from day 0 to hatching either in complete darkness (dark, $N=93$) or exposed to 60 W LED-light stimulation from day 18 to hatching (light, $N=95$). Chicks were raised in same-sex pairs of comparable weight to ensure each chick had a social companion while avoiding any imbalances in behaviors. Food and water were available ad libitum, and food deprivation was introduced on the night of day 2 post-hatching.

(b) Stimuli, apparatus and procedure

On day 3, a single chick entered a confining box and foraged for crumbles through a frontal circular head-only window for 30 s (figure 1a) either binocularly (BIN; figure 1b) or with a monocular eye-patch (LE, RE; figure 1b). When foraging, the distractor appeared: a train of three flashes (of 200 ms each with an inter-flash interval at 150 ms, for a total of 900 ms) of blue LED-light (1 W), centred on the circular window and 15 cm from the confining box, controlled by a computer via an Arduino Uno circuit. The distractor appeared 16 times: from Trial 1 to 15 every 15 s (habituation); Trial 16 (recovery) occurred after 45 s during which the chick was removed from the confining box and (1) if BIN, an eye-patch was briefly applied to one or the other eye and immediately removed to control for possible arousing effects of the manipulation; (2) if LE or RE, the eye-patch was shifted to cover the eye previously in use (LE-RE; RE-LE). While foraging again, chicks received the last distractor. The duration of the orienting reflex triggered by each distractor was measured in milliseconds, starting from when the chick lifted its head off the food tray in response to the distractor until it resumed pecking. If the chick was not pecking at the food during the scheduled distractor presentation, the distractor was delayed for a maximum of 1 min. Trials where the chick had just lifted its head before the distractor was delivered were excluded from the analysis. Chicks' performance was automatically scored from video recordings using Boris [14].

(c) Statistical analysis

Data were analysed using linear mixed effects models with subject-specific slope and intercept, as described by [15]. These models were also used to perform an initial outlier analysis, where responses deviating beyond ± 2.5 standard deviations from the predicted values were identified and subsequently discarded from further analysis. The linear models used to analyse the distribution of responses during habituation included the chicks' incubation condition (dark- and light-incubated chicks), the eye in use (BIN, LE-RE and RE-LE), the number of trials (Trial 1 to 15), and their interactions in both fixed and random effect structures. For the analysis of recovery, only

Trials 15 and 16 were considered, together with the incubation condition, the eye in use, and their interaction.

Post-hoc analyses of interactions were performed using Welch's *t*-tests with Tukey correction. All statistical analyses were conducted in R v. 3.5.1 [16]. We used the `lmer()` function from the `lmerTest` package [17] to fit the linear models and the `testInteraction()` function from the `phia` package [18] for the *post-hoc* analyses.

As inferential statistical measures, we provide *p*-values for the estimates of the fixed effects of the linear mixed effects model, with Satterthwaite approximation used to adjust the denominator degrees of freedom in the *F*-tests. Additionally, we present the η_p^2 values as estimates of significant ($p < 0.050$) effect size of *F*-tests and Cohen's *d* values as estimates of significant effect size of *post-hoc t*-tests.

We conducted a sensitivity analysis with G*Power 3.1 [19] on our sample size with α err. prob. = 0.05, power ($1-\beta$ err. prob.) = 0.95 to establish the minimal detectable effects for our experimental design. The results of the sensitivity analysis indicated that the minimal detectable effects were in the small-to-medium range for both the model used to analyse habituation (with a critical $F = 1.28$ and $\eta_p^2 = 0.01$) and the model used to analyse recovery (with a critical $F = 2.26$ and $\eta_p^2 = 0.01$).

3. Results

We analysed a total of 2552 responses, discarding 76 outliers from the initial pool (less than the 3% of the overall responses). The linear mixed effects model used to assess habituation revealed a main effect of the trial ($F_{1, 173.24} = 56.85$, $p < 0.001$, $\eta_p^2 = 0.25$). Specifically, the duration of the chicks' orienting response decreased from Trial 1 to Trial 15 at a negative linear rate of $\beta = -73.58$ ms ($t_{31.41} = -3.18$, $p = 0.003$), in line with habituation. No other main effect or interaction was significant (all $p > 0.050$). The results suggest that all groups of chicks habituated equally to the distractor.

By contrast, chicks' recovery of response changed across conditions. The model used to assess recovery revealed a main effect of trial ($F_{1, 160.92} = 10.28$, $p = 0.002$, $\eta_p^2 = 0.06$), a two-way interaction between trial and eye in use ($F_{2, 160.79} = 4.5420$, $p = 0.012$, $\eta_p^2 = 0.05$), a two-way interaction between trial and incubation condition ($F_{2, 164.88} = 4.49$, $p = 0.013$, $\eta_p^2 = 0.05$), and a three-way interaction between trial, eye in use, and incubation condition ($F_{2, 160.79} = 3.4926$, $p = 0.032$, $\eta_p^2 = 0.04$). Specifically, the orienting reflex recovered in dark-incubated chicks habituated with the left eye when the distractor was presented to the right eye (LE-RE group), showing no transfer of the distractor information from the right to the left hemisphere (figure 1d, difference from Trial 15 to 16, $t_{155} = -5.32$, $p < 0.001$, Cohen's $d = -0.20$). The duration of the orienting reflex elicited via the right eye was significantly longer than that previously elicited via the left eye (figure 1d, difference from Trial 1 to 16 for LE-RE chicks $t_{144} = 2.61$, $p = 0.020$), and comparable to the response elicited via the right eye on the first trial in the other group of dark-incubated chicks (difference from Trial 1 for RE-LE chicks to Trial 16 for LE-RE chicks $t_{295} = 0.07$, $p = 0.944$).

Conversely, dark-incubated animals that learned to ignore the distractor via the right eye showed no recovery of the orienting response after the eye switch (figure 1e, dark-incubated RE-LE chicks, $t_{153} = -1.02$, $p = 0.308$), showing transfer of distractor representation from the left to the right hemisphere. The same holds true for both right- and left-viewing light-incubated groups (figure 1d, light-incubated LE-RE

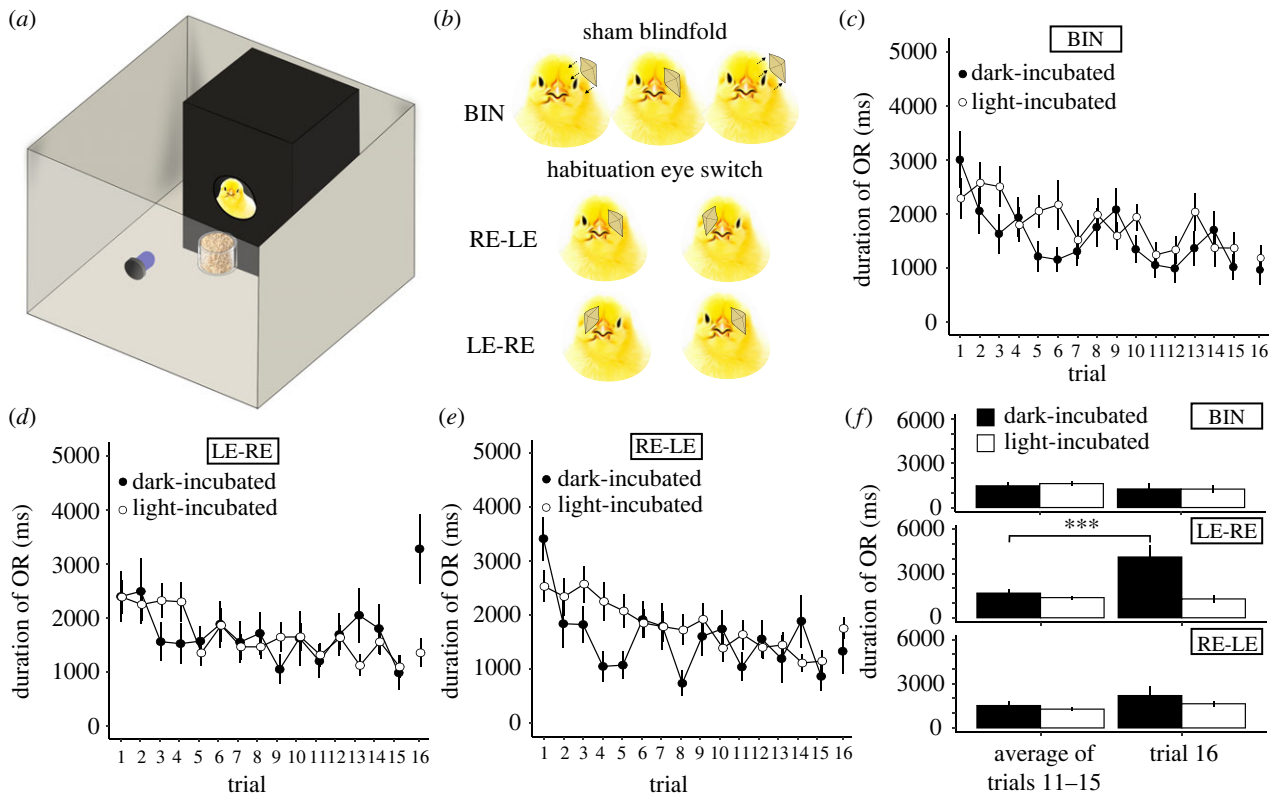


Figure 1. (a) Layout of the apparatus. (b) Depiction of the eye patch applied to obtain sham blindfold for BIN chicks and monocular occlusion in RE and LE chicks. (c) BIN ($N = 63$; females = 34) chicks' performance in response to the light flashes. (d) Performance of dark- ($N = 30$; females = 14) and light-incubated chicks ($N = 32$; females = 15) trained with LE in use and then tested for transfer of filtering with the RE. (e) Performance of dark- ($N = 31$; females = 15) and light-incubated chicks ($N = 32$; females = 16) trained with RE in use and then tested for transfer of information with the LE. (f) Contrast of duration of the OR in all groups from training to transfer trial. BIN, binocular; LE, left eye in use; RE, right eye in use; OR, orienting reflex; *** $p < 0.001$.

chicks $t_{175} = -0.58$, $p = 0.562$; figure 1e, light-incubated RE-LE chicks, $t_{163} = -1.32$, $p = 0.185$). Simple arousing induced by removal and re-application of the eye patch on the new eye is unlikely to elicit a similar response recovery, as binocular chicks still effectively filter out the distractor even after a comparable manipulation (figure 1c, dark-incubated BIN chicks, $t_{130} = 0.02$, $p = 0.985$; light-incubated BIN chicks, $t_{163} = 0.25$, $p = 0.800$). The results were confirmed also when the average of the last three habituation trials, as a measure of the asymptote of the habituation curve [20], were contrasted with trial 16 (figure 1f, difference in the mean orienting reflex from Trials 11–15 to 16 for LE-RE dark-incubated chicks, $t_{175.94} = 5.35$, $p < 0.001$, Cohen's $d = -0.30$, for all other conditions $p > 0.050$).

No sex difference was observed (for all effects involving chicks' sex, $p > 0.050$).

4. Discussion

The ability to habituate to irrelevant or harmless stimuli that appear on either side of the body is crucial for an animal's survival. Advantageously, both hemispheres exhibit comparable efficiency in ignoring the irrelevant distractor either under monocular or binocular view. Although embryonic light stimulation does not affect the overall habituation capacity of each hemisphere [21], crucially it significantly impacts interhemispheric transfer. Specifically, a directional bias in cross-talk transmission of distractor information is present in dark-incubated chicks, meaning that the right hemisphere is unable to transfer the to-be-filtered information

to the left hemisphere. However, the same transfer is possible after light exposure.

In other words, our data showed that the naturally biased visual experience has potentiated communication from the right to the left hemisphere, improving access to contralateral visual information [12,13,21] about distracting events, and annulling pre-established asymmetries [13,22]. It is not the first time that ontogenetic plasticity has been revealed to benefit the unstimulated hemisphere, thus allowing that hemisphere to participate in the control of behaviour [12], but with our contribution we both exemplify how an environmental factor (light) reduces an inherent asymmetry when lateralization is not beneficial and enhances the understanding of what is shared between left and right hemispheres [1,23]. We demonstrate that this sharing extends beyond the information specific to each hemisphere's pivotal functions and includes a more general representation of the irrelevant events. According to one model of habituation [24], we posit that what is transferred, in the very least, from the habituating hemisphere to the other is the neural representation of the stimulus. This transferred representation can then be used by the receiving hemisphere (to predict the upcoming events. Interestingly, considering the lack of response recovery, it could be speculated that the degree of response suppression achieved by the initial hemisphere could also be shared with the other hemisphere. It is indeed established that various commissures within the pigeon brain assume an asymmetrical role in interhemispheric transfer at different levels of visual processing (e.g. anterior commissure: [25]; tectal commissure: [26]; supraoptic commissure: [27]). In the chicken brain, intact tectal and posterior commissures

suppress lateralized responses to non-rewarding beads [28]. In the latter study, environmental stimulation was not controlled, though it is reasonable to assume that light was applied at some point in the commercial hatchery, which aligns with the findings obtained in our present work. This hypothesis paves the way for studying further both the kind of information transferred and the neuroanatomical and physiological basis of the phenomenon we observed.

Ethics. The observations followed the Italian and European Union directives on animal research and license no. 88/2019-PR by the Ministero della Salute.

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

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