Behavioural Brain Research 310 (2016) 93–102

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Social predisposition dependent neuronal activity in the intermediate medial mesopallium of domestic chicks (*Gallus gallus domesticus*)

Uwe Mayer*, Orsola Rosa-Salva, Elena Lorenzi, Giorgio Vallortigara

Center for Mind/Brain Sciences (CIMeC), University of Trento, Piazza Manifattura 1, I-38068 Rovereto, TN, Italy

HIGHLIGHTS

- Visually naïve, newly hatched chicks show a preference to approach predisposed stimuli.
- Intermediate medial mesopallium (IMM) responds differently to predisposed and non predisposed stimuli.
- Higher activity in IMM is associated with approach to a non predisposed stimulus, reflecting the need for increased plasticity.
- Activation of IMM is lateralised between the two hemispheres.

ARTICLE INFO

Article history:
Received 17 February 2016
Received in revised form 2 May 2016
Accepted 6 May 2016
Available online 9 May 2016

Keywords:
Intermediate medial mesopallium
Domestic chicks
Immediate early genes
c-Fos
Neuronal basis for social predispositions
Imprinting

ABSTRACT

Species from phylogenetically distant animal groups, such as birds and primates including humans, share early experience-independent social predispositions that cause offspring, soon after birth, to attend to and learn about conspecifics. One example of this phenomenon is provided by the behaviour of newly-hatched visually-naïve domestic chicks that preferentially approach a stimulus resembling a conspecific (a stuffed fowl) rather than a less naturalistic object (a scrambled version of the stuffed fowl). However, the neuronal mechanisms underlying this behaviour are mostly unknown. Here we analysed chicks' brain activity with immunohistochemical detection of the transcription factor c-Fos. In a spontaneous choice test we confirmed a significant preference for approaching the stuffed fowl over a texture fowl (a fowl that was cut in small pieces attached to the sides of a box in scrambled order). Comparison of brain activation of a subgroup of chicks that approached either one or the other stimulus revealed differential activation in an area relevant for imprinting (IMM, intermediate medial mesopallium), suggesting that a different level of plasticity is associated with approach to naturalistic and artificial stimuli. c-Fos immunoreactive neurons were present also in the intermediate layers of the optic tectum (a plausible candidate for processing early social predispositions) showing a trend similar to the results for the IMM.

1. Introduction

The ability to identify animate creatures rapidly and from very early in life is of biological relevance for species as phylogenetically distant as birds and primates [1–3]. This is particularly true for domestic chicks, that are subject to filial imprinting, a learning phenomenon that restricts subsequent social behaviour to an object experienced shortly after hatching [4,5]. In controlled laboratory settings, imprinting can be obtained for a variety of artificial objects. Nevertheless, imprinting is not completely unconstrained in its object. Of particular interest, domestic chicks are facilitated to imprint on naturalistic objects, such as a mother hen or a stuffed red jungle fowl (*Gallus gallus spadiceus*, the wild ancestor of domestic chicks, [6]), over artificial objects [7–10]. This suggested an interaction of two independent mechanisms: a learning process of imprinting and a pre-wired predisposition to approach certain kinds of visual objects that emerges in the first days of life. A series of seminal studies conducted by Gabriel Horn and his collaborators described a preference to approach a stuffed red jungle fowl hen with respect to highly salient artificial stimuli, such as an illuminated red box, in visually naïve chicks (e.g. [11–20], see Ref. [2] for a review). These authors also demonstrated a crucial role for the configuration of features contained in the head and neck of a hen [2,18]. Notably, in one of these studies the canonical fowl was preferred over a so-called "texture fowl" (a jungle fowl that was cut in small pieces attached to the sides of a box in scrambled order). Studies conducted by our group further refined the behavioural characterisation of chicks' approach preferences. In line with what

* Corresponding author.
E-mail address: uwe.mayer@unitn.it (U. Mayer).

http://dx.doi.org/10.1016/j.bbr.2016.05.019
0166-4328/

has been observed in newborns of human and non-human primates [1,3], naïve chicks have a preference for face-like schematic stimuli and photographed faces over control stimuli matched for low-level properties [21–23]. Moreover, chicks are also spontaneously attracted by cinematic patterns typically associated with the motion of animate creatures ([24–27]). However, despite the amount of work done on the behavioural characterisation of the social predispositions displayed by this model organism, very little is known about the neural mechanisms that are subtended by it.

The only attempt to investigate the neuronal basis for this social predisposition of naïve chicks [28] focused on the neural correlates of non-specific stimulating experiences that cause the emergence of the predisposition [11–15,19,20,28]. Results may indicate an involvement of the medial part of the caudal nidopallium (neostriatum according to the early nomenclature, see Ref. [29] for nomenclature change), an area involved in the recognition of species-specific communication [30–33]. Despite its interesting approach, the study of Egorova and Anokhin [28] has severe limitations, such as the small number of subjects used to investigate brain activity (3–5 per condition). Moreover, the activity in the caudal medial nidopallium could simply represent an effect of the increased number of calls emitted by stimulated chicks, compared to the unstimulated controls which were kept in the dark. This makes it difficult to draw firm conclusions from their results, calling for further investigation.

To shed light on these issues, we performed an experiment on the neuronal basis of domestic chicks' predisposition to approach hen-like stimuli, using the immediate early gene product c-Fos as a neuronal activity marker. Immediate early genes play an important role in neuronal plasticity related to learning [34–38] and their products have been successfully used to detect neuronal activity in mammals and birds [39–45].

Social predispositions are bound to interact with imprinting by directing chicks' attention towards appropriate social objects and imprinting is indeed facilitated for naturalistic objects compared to artificial ones [7–10]. Thus, in the present study we focused on the intermediate medial mesopallium, IMM (IMHV, Intermediate medial hyperstriatum ventrale according to the old nomenclature) an area crucially involved in filial imprinting [17,31,46–49]. Although it is known that the preference for hen-like objects is not suppressed by bilateral IMM lesions [31], it has never been investigated whether neuronal mechanisms related to imprinting respond differently to naturalistic and artificial stimuli. We hypothesised that activity within the IMM would differ between chicks that spontaneously approached a stuffed hen or a texture fowl. As regards the direction of the effect, we expected higher activation for the individuals that approach the stuffed hen. The second brain region of interest in this study was the optic tectum (TeO), which represents the avian homologue of the mammalian superior colliculus. In a recent review we have summarised the existing literature identifying candidate brain areas relevant for social predispositions [2], with particular regard to some subpallial (homologs of subcortical) structures. These structures include the optic tectum, which was hypothesised by some scholars to be crucial for early orienting toward social stimuli in both chicks and human newborns (e.g., Johnson [1] hypothesised that the amygdala, pulvinar and superior colliculus were involved in preferential social orienting to faces in human newborns [1]). As for IMM, we thus expected differential activation of TeO in chicks that approached the two stimuli. As a last region of interest we selected the hyperpallium apicale (HA, Hyperstriatum accessorium, old nomenclature), a part of the visual Wulst which is homologue to the visual cortex in mammals [50,51]. We expected to find no difference in the activation of this area, since all chicks were exposed to the same visual environment and the stimuli were well balanced for the low-level perceptual properties.

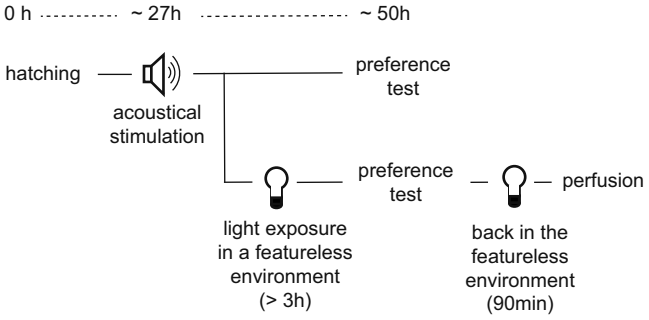


Fig 1. Sequence of experimental procedures. All chicks were acoustically stimulated ~27 h after hatching. The upper line represents the first procedure, in which chicks were kept in the darkness after the acoustical stimulation, until the preference test at ~50 h after hatching. The lower line represents the second procedure, in which chicks were exposed to a featureless environment after the acoustical stimulation and prior to the preference test (again ~50 h post hatch). Chicks that were used for the brain studies were positioned back in the featureless environment after the preference test, where they were kept until perfusion (90 min after the beginning of the preference test).

2. Material and methods

2.1. Subjects

Seventy-six laboratory-hatched, domestic chicks (*Gallus gallus domesticus*), of the "Hybro strain" (a local variety derived from the white leghorn breed), were used. Fertilised eggs were obtained from a local commercial hatchery (Agricola Berica, Montegalda (VI), Italy) and were hatched in individual compartments (12 × 8 cm) separated by thin plexiglass walls, inside dark incubators (Marans P140TU-P210TU). Hatching took place at a temperature of 37.7 °C, with 60% humidity. Approximately 24 h after hatching the temperature was set to 33 °C. To estimate individual hatching time points, each incubator was equipped with an infrared LED lamp and a camera (CCD Board camera 8.47 mm, 1/3"). Photos were captured digitally every 20 min with a time-lapse software (Super Viewer, Somagic Inc) starting at least 24 h before the expected hatching time. Immediately after the end of the test, the chicks used only for the behavioural observations were housed in groups in standard home cages, with food and water available ad libitum and a natural day-night cycle. Soon after, they were donated to local farmers.

Chicks were treated according to two fundamental procedures (Fig. 1), which will be detailedly described below. The main difference between the two was that in the first procedure (upper line of Fig. 1) chicks did not receive any visual experience prior to the moment of the preference test, whereas in the second procedure (lower line of Fig. 1) chicks were habituated to light for some hours before undergoing the preference test. A total of 30 chicks underwent the first procedure. These chicks were used only for behavioural observations to confirm the presence of a predisposition to approach a stuffed fowl, one of the two stimuli later used to study brain activity (stuffed fowl and texture fowl, see below).

A total of 38 chicks underwent the second procedure. All these chicks underwent a preference test between the same two stimuli tested in the first procedure, and their behavioural data were analysed as for the first procedure. Of these 38 chicks, 23 animals were selected for the study of brain activity. Only these individuals were sacrificed (the remaining 15 were donated as described above). The 23 animals used for the study of brain activity were selected because at the preference test they expressed an absolute preference for one of the two stimuli (i.e. they approached only one of the two, spending all their choice time near that stimulus, without alternating between the two). Fourteen of these chicks approached the stuffed fowl and 9 the texture fowl. One brain from the stuffed fowl preference group was damaged during processing

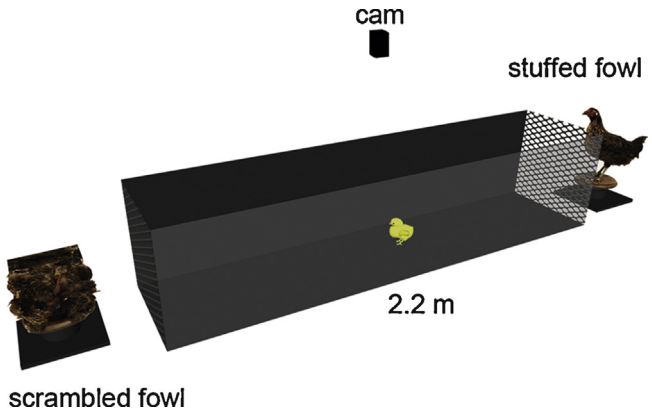


Fig. 2. Experimental setup: the chick was placed in the middle of choice corridor and was free to approach either the texture fowl or the stuffed fowl stimulus, visible through a grid at the two ends of the runway. The chick's behaviour was video-recorded from above.

and was excluded from further analysis, bringing the final sample to 22 (13 chicks that approached the stuffed fowl, 9 the texture fowl).

Finally, an additional group of 8 chicks was used to create a baseline condition for the brain activity study. These subjects were treated according to the second procedure, with the only difference that they were never exposed to the two stimuli. These chicks were also sacrificed at the end of the procedure to investigate brain activation. No behavioural data were collected from them.

2.2. Ethics statement

The experiments reported here comply with the current Italian and European Community laws for the ethical treatment of animals and the experimental procedures were licensed by the *Ministero della Salute, Dipartimento Alimenti, Nutrizione e Sanità Pubblica Veterinaria (permit number 20269/A*).

2.3. Testing apparatus

The testing arena consisted of a 220 cm-long and 44 cm-wide corridor, with lateral walls of 45 cm of height (Fig. 2). The walls and the floor of the corridor were covered with black non-reflective material. The two ends of the corridor were delimited by a black metal grid, behind which the experimental stimuli were visible against a black background. The stimuli were placed on two rotating platforms (5 rpm). On one side of the corridor chicks could see a stuffed fowl hen ("stuffed fowl"), whereas on the other side there was a "texture fowl" (a 9 × 18 cm box, with all side facing surfaces covered with small pieces cut from the pelt of a second identical fowl, attached in scrambled fashion; see Fig. 3 and Ref. [18]). The stuffed fowl hen was acquired from a local taxidermist and was selected to resemble as closely as possible the jungle fowl hen used in previous studies on chicks' social predispositions [18]. These two stimuli were balanced for low-level visual properties such as luminance, colour, visual texture and movement, but they differed in the spatial configuration of the local features that characterise the stuffed fowl (Fig. 3). The left-right placement of the stimuli in the corridor were counterbalanced between subjects. The stimuli were illuminated from above (120 cm). Light (45 W, warm light) was diffused by placing a semi-translucent white plastic sheet under the bulb. Lamp placement was such that most of the corridor was only dimly illuminated, except for the two end-portions of the corridor that were directly adjacent to the stimuli. These two illuminated areas (each 30 cm long) represented the "choice sectors" used to score chicks' approach behaviour toward the stimuli (see below).

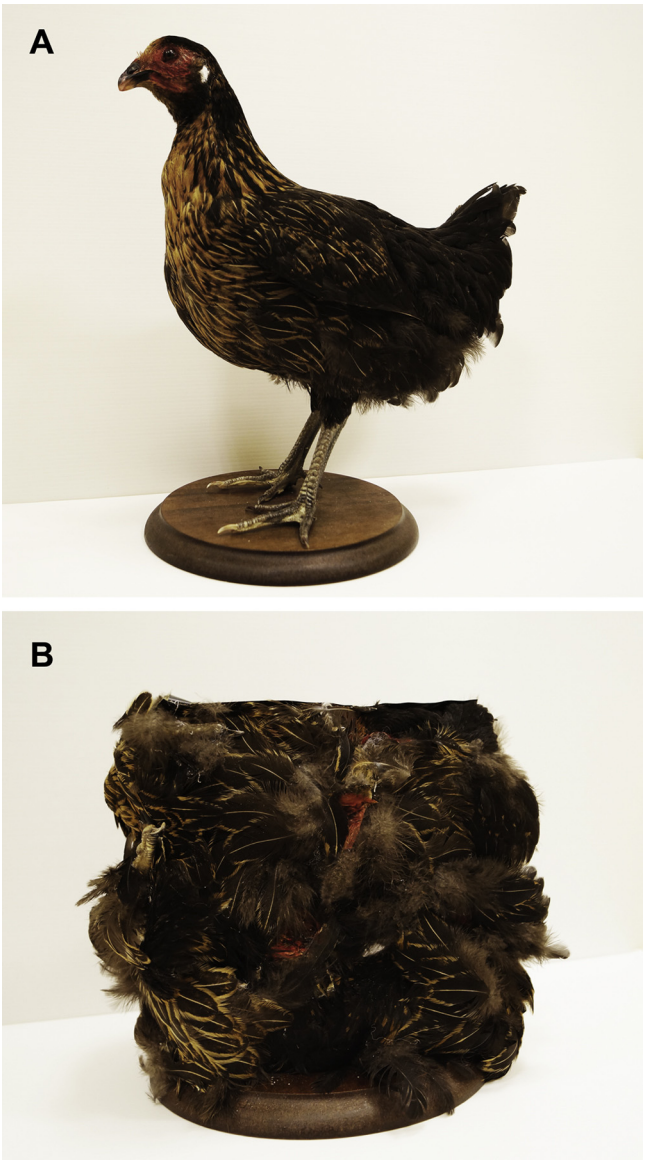


Fig. 3. Photographs of the visual stimuli used. (a) Stuffed fowl. (b) Texture fowl (a similar fowl that was cut in small pieces and attached to the sides of a box in scrambled order).

Behaviour was recorded through a digital camera suspended centrally above the arena and then analysed off line.

2.4. Acoustical stimulation procedure

In order to elicit the subsequent expression of the predisposition, approximately 24 h after hatching (mean age = 27 h, s.e.m. = 0.49), chicks were subject to acoustical stimulation (see Ref. [28]). They were positioned in individual cardboard compartments (10 × 10 cm), inside an additional dark incubator (33 °C) equipped with a loudspeaker. All handling and transportation of the chicks occurred in the dark. Non-species-specific sound stimulation was provided using a digitally constructed audio file composed of non-repeating rhythmic segments of music. For this purpose 15 stereo tracks containing music from different genres were layered and played at the same time. The 3 h audio file was divided into fragments and rendered to a single mono file. The frequency of sound oscillations in the fragments varied from 100 to 12000 Hz and the loudness of the sound varied from 50 to

98 dB. The duration of individual musical fragments varied from 10 to 60 s and the duration of intervals between them varied from 30 to 90 s. Stimulation lasted a total of 180 min (four 45-min sessions with 15-min intervals between sessions). After the end of the stimulation, chicks participating to the first procedure were maintained in individual compartments within a dark incubator until the test. For chicks participating to the second procedure, in order to reduce brain activity caused by the first exposure to light, the chicks were allowed to habituate to light before the test. For this purpose, each chick was exposed for at least 3 h to a featureless environment (a rectangular cage of 28 × 40 × 32 cm, with non-reflecting white walls and floor) that was illuminated from above with a light source (led 240 lx, colour temperature 3000 K) (see Fig. 1).

2.5. Test of the behavioural preference for the stuffed fowl

Chicks of the first procedure were individually tested for their spontaneous preference between the two stimuli (mean age at the time of test = 50.1 h, s.e.m. = 0.89 h). Each subject was placed in the centre of the choice corridor (facing one of the two long walls). Chicks were free to move in the corridor for the duration of the test (8 min), while their behaviour was video recorded. Whenever a chick entered one of the two "choice sectors", this was considered as an approach toward the adjacent stimulus. The chick's starting position with respect to the two long walls, as well as the left-right position of the two stimuli within the apparatus, were counterbalanced across animals. This test procedure was adapted from the works of Gabriel Horn and his collaborators [5,11–20].

2.6. Test session for c-Fos labelling

Also for the second procedure, after at least 3 h the preference test was conducted as described above (mean age at test = 52.9 h, s.e.m. = 0.86). The chicks to be included in the sample for brain activity measurements were selected because they expressed an absolute preference for either stimulus (approaching one stimulus and spending the totality of their choice time near that stimulus, without alternating between the two). Immediately after the end of the test, those chicks were placed back in their familiar 'featureless' cages, where they remained until the time of perfusion. According to our experimental design, chicks were divided in two "choice-groups" based on the stimulus they chose to approach during the spontaneous preference test (either the stuffed fowl, or the texture fowl). We also performed an additional post-hoc baseline condition, requested by an anonymous Reviewer. Chicks in the baseline condition were treated exactly according to the same procedure as the other animals used for brain measurements. The only difference was that, instead of undergoing the preference test between the two stimuli, baseline chicks were placed for an identical amount of time (8 min) in the empty apparatus (no stimuli present). The rationale behind this condition was to measure the baseline activity caused in IMM by aspects of the procedure other than the exposure to the two stimuli, such as light exposure in the featureless environment and exposure to the test set-up. In these baseline brains, in addition to IMM we also measured the activity in the HA as a test of region-specificity.

2.7. Immunohistochemistry

Ninety minutes after the start of the test session, subjects used for brain activity measurements were overdosed with an intramuscular injection of 0.05 ml Ketamine/Xylazine Solution (1:1 Ketamine 10 mg/ml + Xylazine 2 mg/ml) per 10 g of body weight. After 5 min, when the animals became unresponsive (tested by gently pulling the feet and wings), they were immobilised on a plate, the thorax was opened and the heart was exposed. The chicks were perfused transcardially via the left ventricle with cold phosphate-buffered saline (PBS; 0.1 mol, pH = 7.4, 0.9% sodium chloride, 4 °C) for 10 min and then fixed with 4% paraformaldehyde (PFA) in PBS for 10 min. The head was then severed from the body, the skin and the eyes were removed, and the skull was transferred to 4% PFA where it was post-fixed overnight. On the following day, the skull was secured in a stereotaxic head holder (Stoelting, using a Kopf Instruments pigeon head holder). The caudal part of the skull was opened and the brain was exposed. A coronal-plane cut was made with a scalpel blade attached to a micromanipulator, at an orientation of 45°, to ensure that the subsequent sections had the same orientation as in the chick brain atlas of Kuenzel and Masson [52]. The brain was then removed from the skull, post-fixed for approximately 48 h in 4% PFA/PBS containing 20% sucrose at 4 °C, and then transferred to 30% Sucrose/0.4%PFA/PBS for 48–72 h until it sunk. The left and the right hemispheres were separated and processed independently. The hemispheres were frozen at −50 °C in plastic moulds covered with O.C.T (Tissue-Tek freezing medium).

For free-floating staining, six series of 40 μm coronal sections were cut on a Cryostat (Leica CM1850 UV) at −20 °C and collected in PBS. The sections of the first series were used for processing and labelling. The sections of the other series were kept in PBS at 4 °C as backup or for testing antibody specificity (processing without the primary antibody). Endogenous peroxidase activity was depleted by incubation in 0.3% H_2O_2 in PBS for 20 min. After washing in PBS (3 × 5 min), the sections were treated with 3% normal goat serum (S-1000, Vector Laboratories, Burlingame, CA, USA) in PBS for 30 min. The sections were then transferred to the first antibody solution (c-Fos antibody made in rabbit, 1:2000; K-25, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and incubated overnight at 4 °C on a rotator. After several washes in PBS, the secondary antibody reaction was carried out using a biotinylated anti-rabbit solution (1:200, BA-1000, Vector Laboratories) in PBS for 75 min at room temperature. The ABC method was used for signal amplification (Vectastain Elite ABC Kit, PK 6100, Vector Laboratories). Neurons with concentrated c-Fos protein were visualised with the VIP substrate kit for peroxidase (SK-4600, Vector Laboratories). This produced a purple reaction product confined to the cell nuclei of activated neurons. Sections were then transferred to distilled water and serially mounted on gelatine-coated slides. They were dried at 50 °C on a heating plate and counterstained with methyl green (H-3402, Vector Laboratories). After gradual dehydration in ethanol (70%, 80%, 90% and 99% EtOH for 3 min each, and then placed in Xylene) the mounted sections were cover slipped with Eukitt (FLUKA).

2.8. Brain anatomy

Brain sections were examined with a Zeiss microscope at a magnification of 200× and a digital camera (Zeiss AxioCam MRc5). The ZEN Imaging software (Zeiss) was used for the manual counting of immunoreactive (-ir) neurons on a computer screen. Counting was performed blind to the experimental conditions. For counting, a rectangular "enclosure", 150 × 250 μm, was positioned over the different sample areas. Contrast and exposure time of the camera were adjusted so that the image on the screen matched the view under the microscope. Successful immunostaining produces dark purple-black stained nuclei and minimal background staining. Thus, the nuclei of c-Fos-ir neurons were easily discerned from background and non-activated neurons, which were stained light green (see Fig. 5). Every activated c-Fos-ir neuron within the sample rectangular areas was marked on the screen with the "event marker" of the ZEN software, which automatically computed the total number of c-Fos-ir neurons.

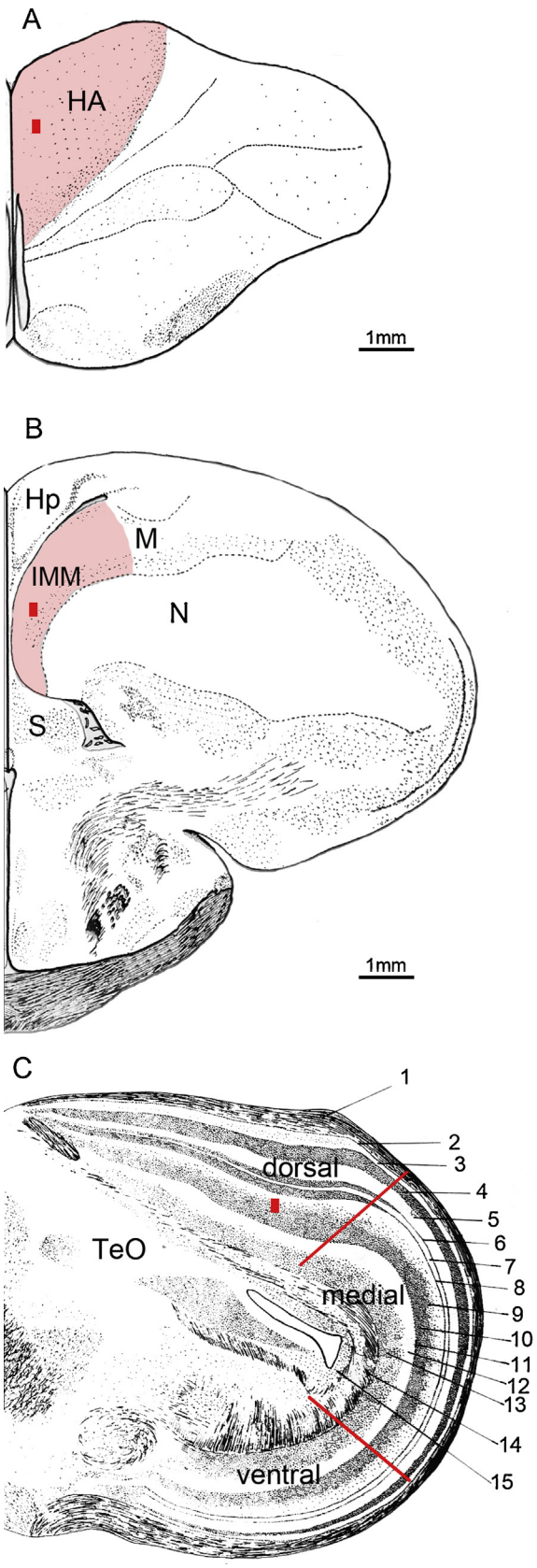


Fig. 4. Typical placement of cell count zones (red rectangles). (a) Schematic view of a coronal section showing a typical placement of the cell count zone within the HA (pale red colour). (b) Schematic view of a coronal section showing a typical placement of the cell count zone within IMM (pale red). (c) Schematic view of a coronal section showing the 15 layers of TeO and its partitioning into dorsal, medial, ventral subdivisions (red lines). Drawings were adapted from the atlas of Kuenzel and Masson [52]. HA—hyperpallium apicale, IMM—intermediate medial mesopallium, N—nidopallium, Hp—hippocampus, S—septum, TeO—tectum opticum. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

Several brain structures showed c-Fos immunoreactivity, including areas of the hyperpallium, mesopallium, nidopallium, hippocampus, septum, olfactory bulb and striatum, together with several mesencephalic and diencephalic structures. In order not to lose statistical power we measured the density of c-Fos-ir neurons only in the three areas of interest: the intermediate medial mesopallium (IMM), the optic tectum (TeO) and the hyperpallium apicale (HA) (Fig. 4).

To estimate labelled cell density in the IMM we relied anatomically as much as possible on the previous descriptions of this region, known as IMHV (Intermediate medial hyperstriatum ventrale) under the old nomenclature [53–55]. According to the work of McCabe and Horn [54] the IMM would be at the level of the anterior coordinate A7.6 of the Kuenzel and Masson atlas [52]. However, it is worth mentioning that in the atlas [52] coordinates were estimated based on two-week-old broiler chicks, with an average body weight of 300–325 g. In contrast, 1–2 day old chicks used in imprinting studies usually weight around 30 g and would therefore have a different anterior coordinate. For the analysis 5 brain slices were selected from a region where the shape of IMM was corresponding to what is depicted in plate A7.6 of the Kuenzel and Masson atlas [52]. The rectangular enclosure was positioned inside the IMM according to the drawings of Ambalavanar et al. [55], see also Fig. 4B. Labelled cells in the HA were counted from 5 sections of each hemisphere, which were selected in accordance to the region extending from A13.0 to A11.0 of the Kuenzel and Masson atlas [52]. One counting rectangle was positioned within the dorso-medial HA of each section (Fig. 4A). Counting within the TeO of each hemisphere was performed within the dorsal, medial and ventral parts separately (Fig. 4C). In each of these subdivisions we further distinguished between outer layers 1–9, intermediate layers 10–12 and inner layer 13–15 (for the definition of these layers, see the supplementary plate A4.6 in the atlas of Kuenzel and Masson [52]). The counting rectangle was positioned in each of the 9 subdivisions of each hemisphere.

After completing the cell counts, for each animal mean values from the five sections were calculated per hemisphere and cell densities were standardised to 1 mm^2. Thus the calculated neuronal activity of an individual in the IMM and HA was based on 5 counted areas for each hemisphere. Cell counts pooled from the different subdivisions of TeO (dorsal, medial, ventral) were further averaged to estimate overall activity in the TeO. Initially, this was done separately for the outer, intermediate and inner layers. However, because no significant differences were found between these subdivisions, the measured values from all 9 subdivisions on 5 slices of each hemisphere were pooled for further analysis. Thus, the overall estimate of TeO activity in one hemisphere of an individual bird was based on an average from 45 counted areas. The resulting means for individual birds were considered overall indicators for the number of c-Fos-ir neurons and were employed for further statistical analysis.

2.9. Statistical analysis

In order to assess chicks' behavioural preferences, the total time spent in each choice sector was recorded and used to compute a preference index according to the formula: *time near the fowl/(time near the fowl + time near the texture-fowl).* Significant departures from chance level (0.5), which indicated a preference for the pre-

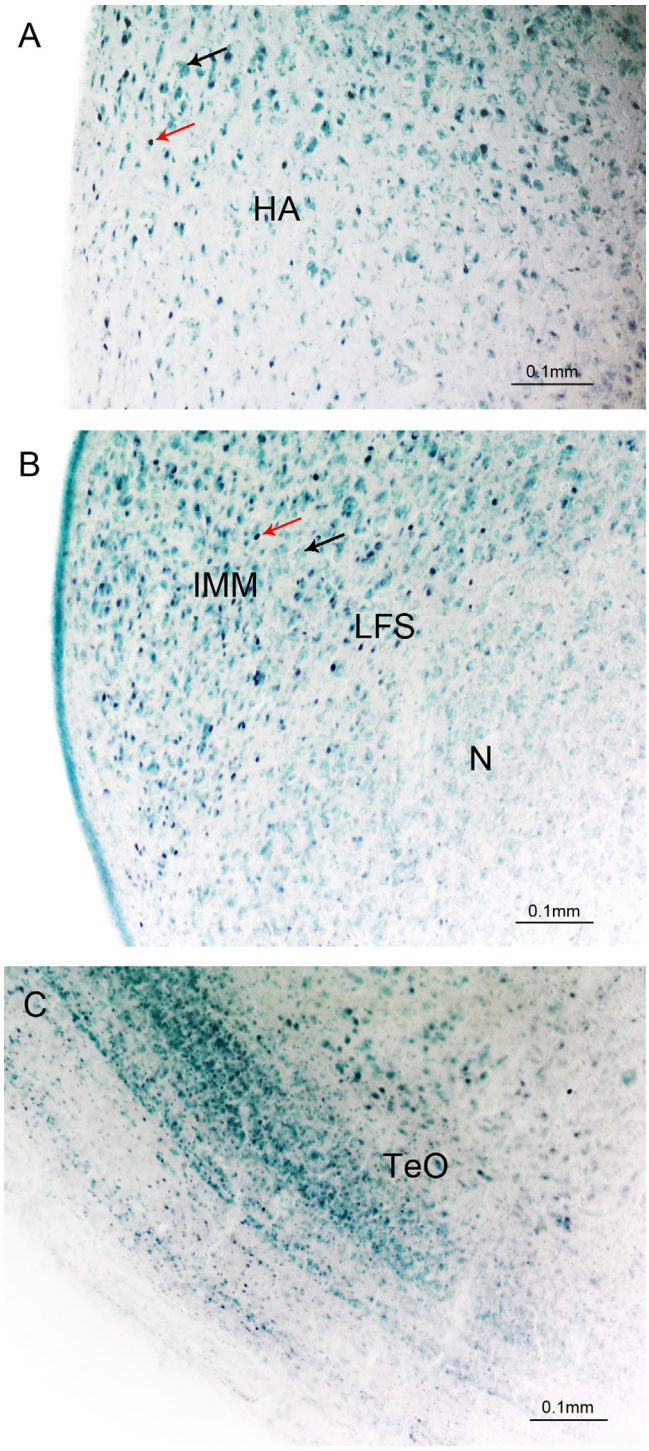


Fig. 5. Labelled neuronal nuclei in the sampled areas of a chick that approached the texture-fowl stimulus (right hemisphere, magnification: 200×). Immunoreactive neurons are stained black (red arrows) and can easily be distinguished from the c-Fos-negative, green-stained neuronal nuclei (black arrow). (a) High number of labelled c-Fos-ir nuclei within HA. (b) High number of labelled nuclei within IMM. (c) Labelled nuclei within different layers of the TeO. HA—hyperpallium apicale, IMM—intermediate medial mesopallium, N—nidopallium, LFS—lamina frontalis superior, TeO—tectum opticum. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

disposed (>0.5) or the control stimulus (<0.5), were estimated by one-sample two-tailed *t*-test.

The presence of difference in the density of IEG-expressing neurons was tested by a mixed-design ANOVA, with a between subject factor "*choice group*" and a within subject factor "*area*" as repeated measure with 6 measured brain regions per brain (3 for each hemisphere). To correct for violation of sphericity a Greenhouse-Geissler correction was applied. For post-hoc analyses, *t*-tests (two tailed) were carried out for each area. To correct for multiple measurements the Bonferroni correction for 12 comparisons was applied. All statistical analyses were performed with the software IBM SPSS Statistic for Windows (Version 22.0).

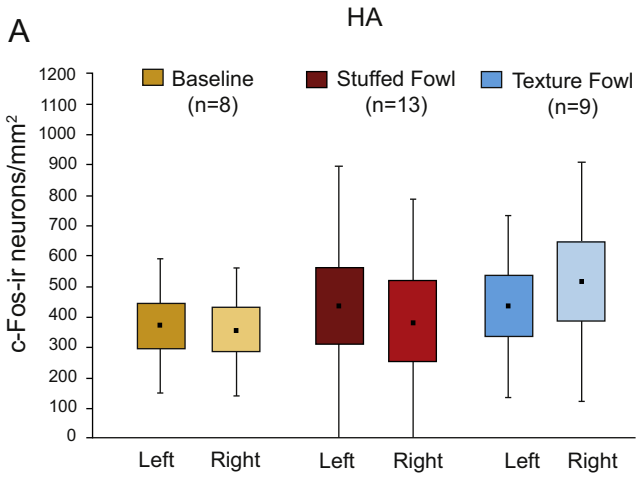
3. Results

3.1. Behavioural results

The choice behaviour of the subjects from the first procedure revealed a clear preference for approaching the stuffed fowl with respect to the texture fowl. In fact, the proportion of time spent near the stuffed fowl during the test was significantly different from chance level ($t_{29} = 2.846$; $p = 0.008$), with an average preference score of 0.73 (s.e.m. = 0.08). This means that on average chicks spent 73% of their total choice time in the sector adjacent to the stuffed fowl. A similar, although non-significant, trend was observed in the chicks treated according to the second procedure, from which we obtained the subjects employed for the neuroanatomical investigations. The average preference score of 0.59 (s.e.m. = 0.07), was not significantly different from chance level ($t_{37} = 1.252$; $p = 0.219$). However, a higher number of chicks approached the stuffed fowl rather than the texture fowl, resulting in an imbalance between the sample sizes of the two groups in the brain study. The chicks to be included in the sample for brain activity measurements were selected because they expressed an absolute preference for either stimulus (having a preference score of either 0, absolute preference for the texture fowl, or 1, absolute preference for the stuffed fowl). These birds were divided into two groups according to the choice they made: chicks that approached the stuffed fowl and chicks that approached the texture fowl.

3.2. Immunohistochemistry

Sample photomicrographs of c-Fos-ir neurons in the brain areas examined can be found in Fig. 5A–C. The ANOVA (between subject factor "*choice-group*" with 2 levels and within subject factor "*area*" with 6 levels) revealed significant differences in the number of c-Fos-ir neurons between the two groups in a region-dependent fashion (interaction of *area*choice-group:* $F_{(2.645,52.895)} = 5.236$; $p = 0.004$). Importantly, IMM counting showed significant differences in the density of c-Fos-ir neurons between the two experimental groups (Fig. 6B). The density of labelled neurons in the right IMM of birds that approached the texture fowl (mean ± s.e.m: 1259.3 ± 131.7 c-Fos-ir neurons/mm2) was two-fold higher compared to the right IMM of the stuffed fowl preference chicks (mean ± s.e.m: 576.4 ± 136.7 c-Fos-ir neurons/mm2). Post hoc *t*-test comparison between the right IMM of birds that approached the texture fowl with those that approached the stuffed fowl: $t_{20} = -3.452$; $p = 0.003$ (after a Bonferroni correction for 12 measurements: $p = 0.036$). Such group difference was not present in the left IMM: $t_{20} = -1.191$; $p = 0.247$. The IMM activity in birds that approached the texture fowl showed also a significant lateralisation (Fig. 6B). Post hoc *t*-test: Right IMM (texture fowl) vs. Left IMM (texture fowl): $t_8 = -3.316$; $p = 0.011$ (Bonferroni correction for 12 measurements: $p = 0.132$). The difference within IMM was region specific and not due to overall activity of the brains, since the activation in HA (visual Wulst) was nearly identical in both groups (Fig. 6A). A non-significant trend for enhanced activity was also present within the TeO of the right hemisphere (Fig. 7), in the group of chicks that approached the texture fowl (mean ± s.e.m: 403.15 ± 136.6 c-Fos-ir neurons/mm2) compared to chicks that



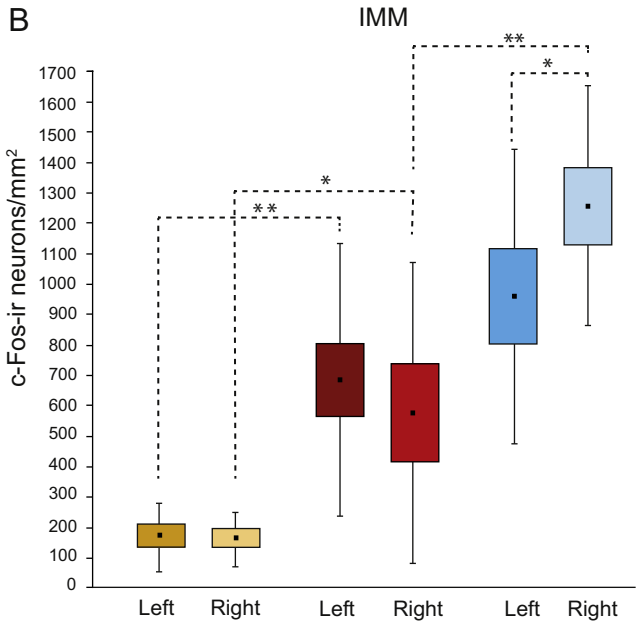


Fig. 6. Measured c-Fos-ir densities in the left and right HA and IMM of three groups of chicks. (a) A high number of c-Fos-ir neurons is present in the left and right HA of all groups. (b) Only a very low number of c-Fos-ir neurons is present in the IMM of the baseline control group, it is significantly higher in both hemispheres of the stuffed fowl group and it is at the highest level in the texture fowl group. Significantly higher number of c-Fos-ir neurons is present in the right IMM of chicks that approached the texture fowl compared to the stuffed fowl group. Moreover only this group shows also significant lateralisation. Graph-plot: mean (black square), s.e.m. (box) and s.d (whisker). (* indicates $p < 0.05$; ** indicates $p < 0.01$). Densities of c-Fos-ir neurons per mm^2 are represented on the Y-axis. Choice group and hemispheres are on the X-axis: results are represented for the baseline control group by the two yellow boxes on the left-hand side, for the stuffed fowl choice group by the two red boxes in the middle, and for the texture-fowl choice group by the two blue boxes on the right-hand side. In all groups the left hemisphere is the darker shaded box. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

approached the stuffed fowl (mean ± s.e.m: 245.0 ± 88.8 c-Fos-ir neurons/mm2).

As suggested by an anonymous Reviewer we performed an additional post hoc baseline activity control experiment. Chicks of this group underwent the same procedure as others that were used for brain activity measurements (lower line, Fig. 1), with the only difference that, instead of expressing a choice between the two stimuli, they were exposed to an empty corridor. The IMM of these baseline control chicks contained only a very low number of c-Fos-ir neurons, which was significantly lower than in the other two groups (Fig. 6B). The measured c-Fos-ir density in the left IMM of the baseline group (mean ± s.e.m: 172.0 ± 41.13 c-Fos-ir neurons/mm2) was three times lower than in the left IMM of the stuffed fowl choice group (mean ± s.e.m: 685.54 ± 124.52 c-Fos-ir neurons/mm2), showing a highly significant difference $t_{19} = 3.142$; $p = 0.005$. Also the difference to the left hemisphere of the texture fowl group (mean ± s.e.m: 960.00 ± 161.64 c-Fos-ir neurons/mm2) was highly significant $t_{15} = 4.468$; $p < 0.001$. Likewise, the activity in the right IMM of the baseline control group (mean ± s.e.m: 164.67 ± 33.39 c-Fos-ir neurons/mm2) was more than three times lower than in the right IMM of the stuffed fowl group (mean ± s.e.m: 576.4 ± 136.7 c-Fos-ir neurons/mm2) and revealed a significance level of $t_{19} = 2.290$; $p = 0.03$. The activity was more than six times lower compared to the right IMM of the texture fowl group (mean ± s.e.m: 1259.3 ± 131.7 c-Fos-ir neurons/mm2), also this difference was highly significant $t_{15} = 7.612$; $p < 0.001$. The densities of c-Fos-ir neurons were almost at the same level in the HA of all groups and both hemispheres (Fig. 6A), showing that differences that were present in the IMM were specific to this brain region.

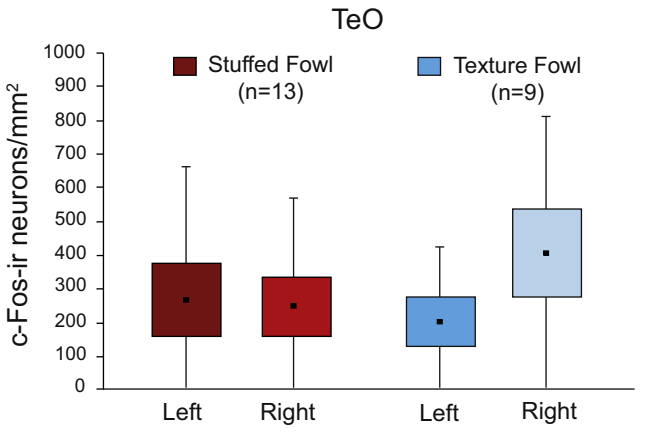


Fig. 7. Measured c-Fos-ir densities in the TeO. Although this trend is not significant, the right tectum shows higher number of c-Fos-ir neurons in the texture fowl group.

4. Discussion

The present study demonstrates different activation of the IMM in chicks that approached a naturalistic visual stimulus (stuffed fowl) compared to those that approached a texture fowl. In a spontaneous choice test with visually naïve chicks (first procedure) we were able to obtain a significant preference for the stuffed fowl over a texture fowl, thus successfully replicating the results of Johnson and Horn [18]. In chicks that were exposed to light prior to testing (second procedure), the strength of the preference decreased. In line with our initial hypothesis comparison of brain activity in these subjects after their choice revealed a differential activation in the IMM. Contrary to our expectations a significantly higher number of c-Fos-ir neurons was present in the IMM of chicks that approached the texture fowl, if compared to chicks that approached the stuffed fowl. However, neuronal activity in IMM of both groups was higher than the baseline condition, where it was nearly absent. This indicates that IMM activity is up-regulated by processing of either of the two stimuli. As expected, such differences were not present in the HA, suggesting that the differential activity in IMM was region specific. Contrary to what we predicted, the number of c-Fos-ir neurons was also not significantly different in the TeO, although a slightly higher activity level was present in the right TeO of birds that approached the texture fowl.

The IMM in chicks is important for learning and retention of visual properties of an imprinting object [49,56,57] and it contains neurons that respond to visual features of the imprinting object after training [58,59] (see Refs. [60–64] for other areas involved in imprinting). At the same time, the IMM does not contribute to the emergence of predisposed preferences in chicks [31]. In chicks, lesions to this area abolish learned object preferences, but chicks can still develop predisposition for the stuffed fowl [31]. These results suggested that information about a complex object, which resembled the chicks' own species, is stored in a different manner than information derived from a relatively simple, but very salient, artificial object. In line with this evidence, IMM activity in our experiment differed depending on whether the chicks approached the stuffed fowl or the texture fowl. This indicates that the spatial arrangement of the otherwise identical local visual features in the two stimuli provides input to IMM, which is different enough to influence its neuronal activity.

Given the very low c-Fos expression in the baseline condition, the higher activity found in the IMM of chicks that approached the texture fowl or the stuffed fowl is likely to reflect increased plasticity in these two groups. Indeed, c-Fos as an IEG product is rapidly activated after an increase of neuronal activity and plays an important role in neuronal plasticity related to learning [34–38]. The higher c-Fos expression in the texture fowl group thus indicates increased plasticity in this group. This can be ascribed to the fact that this group was exposed to a visual object that does not fit to the innate template representing the appearance of the preferred naturalistic objects. Encoding the properties of this object can require the storing of additional information for the aspects that do not fit the pre-wired template. A similar phenomenon has been found in sexual-imprinting in zebra finches [65]. In this species sexual imprinting on conspecifics is facilitated, but cross-species rearing can originate also sexual preferences for non-conspecifics. Rearing by non-conspecifics and subsequent preference for them is associated with higher IEGs expression than preference for conspecifics, in brain areas related to sexual imprinting [65]. In a situation closer to the present study, domestic chicks that show better recognition of their non-naturalistic imprinting object (a red box) have also higher number of Fos-like immunoreactive neurons in the IMM, compared to chicks that have poor recognition performance [54]. Thus, chicks that develop a better representation of the artificial imprinting object, as revealed by their superior recognition performance, have increased expression of plasticity markers in IMM. Also, electrophysiological recordings of spontaneous activity of IMM neurons in chicks imprinted on a red box or on the stuffed fowl revealed a significant correlation with approach counts during training, but only for individuals imprinted on the red box [66]. This evidence reflects increased spontaneous IMM activity after learning of a stimulus to which chicks do not show a predisposition. Finally, it should be noted that the concentration of noradrenaline in the IMM correlates with the strength of filial imprinting for artificial stimuli, but not with the predisposition for hen-like objects [14,67]. Noradrenaline has a crucial role in memory consolidation (see also Ref. [16]), in line with our argument that there is a greater need for learning, and thus plasticity, in chicks exposed to artificial stimuli that do not fit the preferred template.

Another interesting finding of the present study is the lateralisation in chicks that approached the texture fowl, with higher activity in the right IMM. Filial imprinting has long been known to be lateralised in the IMM [68]. Admittedly, our results seem to be at odds with the observation that biochemical and structural changes consequent on imprinting (on artificial objects) are more marked in the left hemisphere [16] (but see [69,70] for exceptions to this general pattern). However, the lateralisation pattern is more complex than that, with a crucial interaction between the different types of information to be stored and the time course of memory formation. The left and right IMM have been hypothesised to undergo parallel memory consolidation processes with different temporal profiles (see Ref. [71]). However, in the present study we chose to allow chicks only a very brief exposure to the two stimuli (8 min), consistent with the duration of the behavioural tests typically used for predispositions. This makes it difficult to "stage" our data with regard to the time course of memory formation in classical imprinting studies, where time is counted after the end of a much longer exposure period. Interestingly, with only 20 min of exposure to the imprinting object Bradley et al. [72] found transient structural synaptic changes limited to the right hemisphere. Also, some behavioural, pharmacological and morphological studies, suggest the right hemisphere has a crucial role in the early stages of imprinting recall, in line with what we found in the present study [73].

The right hemisphere's dominance observed in the present study could be also due to functional specialisation of the right hemisphere to encode some features characterising the texture fowl to a greater degree than the stuffed fowl. Indeed, the two hemispheres are hypothesised to encode different aspects of the visual stimuli [71,74–78]. Right hemisphere's functions include the detection of change in a complex stimulus or in its spatial context ([75,76,79,80] see also evidence from electrophysiology studies reviewed in Johnston and Rose [73]). Preferential use of the right hemisphere to monitor the environment has also been considered a default condition that is used when there is no strong reason to involve the left hemisphere. On the contrary, the left hemisphere would be in charge of processing salient cues that identify the category of the object and are used in the guidance of the response [81,82]. It is possible that the scattered placement of the salient local features, scrambled all over the surface of the texture fowl, could make it more difficult to process it in terms of its unnatural configuration of features. This more demanding configuration processing could cause enhanced recruitment of the right hemisphere [74,78,79]. Finally, it should be noted that this adds to the list of lateralisation effects observed in chicks hatched from dark incubated eggs (e.g., [79,83,84]; for the role of light exposure in lateralisation development see Refs. [85–89]).

In contrast to what we observed in the IMM and contrary to our initial expectations, the inner, intermediate and outer layers of the optic tectum (TeO) did not show a significant difference between the two groups, even though we observed a slight, but not significantly higher number of c-Fos-ir neurons in the right hemisphere of chicks that preferred the texture-fowl (Fig. 7). Interestingly, lateralised expression of IEGs has been reported also in TeO of zebra finches after sexual imprinting, however the lateralisation occurred independently from the experimental condition and was in favour of the left hemisphere [90]. The lack of clear differences within the optic tectum might be related to the fact that chicks were exposed to a rich and complex visual scene. Not only both stimuli were visible to the exposed chicks, but they both also contained various types of visual information, like movement and visual configurations of different sizes. Other visual textures where also present in the visual environment during the test phase, like those created by the grids at the end of the corridor and the floor and walls of the corridor itself. In the deeper layers of the optic tectum [91,92], there are different types of neurons that respond to a variety of different visual properties [93,94], see also Ref. [2]. Given this, we can imagine that such a highly complex visual stimulation should activate multiple neuronal populations in both groups. This could have masked any effect of the only aspect in which the visual scene had any variation (i.e. the configuration of the two stimuli).

Despite these partial limitations, our findings represent the first evidence of different neuronal responses to naturalistic and artificial stimuli in naïve chicks. Our results pave the way for further investigations on the neural bases of social predispositions in the

only animal model available for these phenomena. Future investigations could be devoted to confirm and extend these findings to other samples of chicks demonstrating a significant preference for the naturalistic stimulus at the behavioural level. In addition, based on our results we suggest that further studies focusing on tectum as a region of interest could benefit from providing more controlled and specific visual stimulation to the different experimental groups, to activate different types of neurons separately (e.g. by exposing each group of chicks separately to a different stimulus, rather than using a free choice paradigm in which all individuals see both stimuli).

Acknowledgements

This work was funded by an ERC Advanced Grant to GV (PREMESOR ERC-2011-ADG 20110406, Project No: 295517). We are thankful to Dr. Jasmine Loveland for her useful comments and language editing of the final manuscript.

References

[1] M.H. Johnson, Subcortical face processing, Nat. Rev. Neurosci. 6 (2005) 766–774, http://dx.doi.org/10.1038/nrn1766.
[2] O. Rosa Salva, U. Mayer, G. Vallortigara, Roots of a social brain: developmental models of emerging animacy-detection mechanisms, Neurosci. Biobehav. Rev. 50 (2015) 150–168, http://dx.doi.org/10.1016/j.neubiorev.2014.12.015.
[3] Y. Sugita, Face perception in monkeys reared with no exposure to faces, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 394–398, http://dx.doi.org/10.1073/pnas.0706079105.
[4] P. Bateson, Thirty years of collaboration with Gabriel Horn, Neurosci. Biobehav. Rev. 50 (2015) 4–11, http://dx.doi.org/10.1016/j.neubiorev.2014.09.019.
[5] B.J. McCabe, Imprinting, Wiley Interdiscip. Rev. Cogn. Sci. 4 (2013) 375–390, http://dx.doi.org/10.1002/wcs.1231.
[6] F.E. Zeuner, A History of Domesticated Animals, Hutchinson & Co., London, 1963.
[7] J.J. Bolhuis, W.J. Trooster, Reversibility revisited: stimulus-dependent stability of filial preference in the chick, Anim. Behav. 36 (1988) 668–674, http://dx.doi.org/10.1016/S0003-3472(88)80149-0.
[8] J.J. Cherfas, A. Scott, Impermanent reversal of fillial imprinting, Anim. Behav. 29 (1981) 301, http://dx.doi.org/10.1016/s0003-3472(81)80180-7.
[9] J.P. Kent, Experiments on the relationship between the hen and chick (*Gallus gallus*): the role of the auditory mode in recognition and the effects of maternal separation, Behaviour 102 (1987) 1–13, http://dx.doi.org/10.1163/156853986X00018.
[10] E.A. Salzen, C.C. Meyer, Reversibility of imprinting, J. Comp. Physiol. Psychol. 66 (1968) 269–275, http://dx.doi.org/10.1037/h0026349.
[11] J.J. Bolhuis, G. Horn, Delayed induction of a filial predisposition in the chick after anaesthesia, Physiol. Behav. 62 (1997) 1235–1239, http://dx.doi.org/10.1016/S0031-9384(97)00231-X.
[12] J.J. Bolhuis, M.H. Johnson, G. Horn, Effects of early experience on the development of filial preferences in the domestic chick, Dev. Psychobiol. 18 (1985) 299–308, http://dx.doi.org/10.1002/dev.420180403.
[13] J.J. Bolhuis, M.H. Johnson, G. Horn, Interacting mechanisms during the formation of filial preferences: the development of a predisposition does not prevent learning, J. Exp. Psychol. Anim. Behav. Process. 15 (1989) 376–382, http://dx.doi.org/10.1037/0097-7403.15.4.376.
[14] D.C. Davies, M.H. Johnson, G. Horn, The effect of the neurotoxin DSP4 on the development of a predisposition in the domestic chick, Dev. Psychobiol. 25 (1992) 251–259, http://dx.doi.org/10.1002/dev.420250403.
[15] N.G. Hampton, J.J. Bolhuis, G. Horn, Induction and development of a filial predisposition in the chick, Behaviour 132 (1995) 451–477, http://dx.doi.org/10.1163/156853995X00667.
[16] G. Horn, Pathways of the past: the imprint of memory, Nat. Rev. Neurosci. 5 (2004) 108–120, http://dx.doi.org/10.1038/nrn1324.
[17] M.H. Johnson, G. Horn, Dissociation of recognition memory and associative learning by a restricted lesion of the chick forebrain, Neuropsychologia 24 (1986) 329–340, http://dx.doi.org/10.1016/0028-3932(86)90018-7.
[18] M.H. Johnson, G. Horn, Development of filial preferences in dark-reared chicks, Anim. Behav. 36 (1988) 675–683, http://dx.doi.org/10.1016/S0003-3472(88)80150-7.
[19] M.H. Johnson, J.J. Bolhuis, G. Horn, Interaction between acquired preferences and developing predispositions during imprinting, Anim. Behav. 33 (1985) 1000–1006, http://dx.doi.org/10.1016/S0003-3472(85)80034-8.
[20] M.H. Johnson, D.C. Davies, G. Horn, A sensitive period for the development of a predisposition in dark-reared chicks, Anim. Behav. 37 (1989) 1044–1046, http://dx.doi.org/10.1016/0003-3472(89)90148-6.
[21] O. Rosa-Salva, L. Regolin, G. Vallortigara, Faces are special for newly hatched chicks: evidence for inborn domain-specific mechanisms underlying spontaneous preferences for face-like stimuli, Dev. Sci. 13 (2010) 565–577, http://dx.doi.org/10.1111/j.1467-7687.2009.00914.x.
[22] O. Rosa Salva, T. Farroni, L. Regolin, G. Vallortigara, M.H. Johnson, The evolution of social orienting: evidence from chicks (*Gallus gallus*) and human newborns, PLoS One 6 (2011) e18802, http://dx.doi.org/10.1371/journal.pone.0018802.
[23] O. Rosa Salva, L. Regolin, G. Vallortigara, Inversion of contrast polarity abolishes spontaneous preferences for face-like stimuli in newborn chicks, Behav. Brain Res. 228 (2012) 133–143, http://dx.doi.org/10.1016/j.bbr.2011.11.025.
[24] G. Vallortigara, L. Regolin, F. Marconato, Visually inexperienced chicks exhibit spontaneous preference for biological motion patterns, PLoS Biol. 3 (2005) e208, http://dx.doi.org/10.1371/journal.pbio.0030208.
[25] G. Vallortigara, L. Regolin, Gravity bias in the interpretation of biological motion by inexperienced chicks, Curr. Biol. 16 (2006) R279–R280, http://dx.doi.org/10.1016/j.cub.2006.03.052.
[26] E. Mascalzoni, L. Regolin, G. Vallortigara, Innate sensitivity for self-propelled causal agency in newly hatched chicks, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 4483–4485, http://dx.doi.org/10.1073/pnas.0908792107.
[27] O. Rosa-Salva, M. Grassi, E. Lorenzi, L. Regolin, G. Vallortigara, Perception of animacy in visually naive domestic chicks (Gallus gallus): spontaneous preference for speed changes, Cognition (2016) (submitted for publication).
[28] O.V. Egorova, K.V. Anokhin, Experimental analysis of the processes of systems genesis: expression of the c-fos gene in the chick brain during treatments inducing the development of the species-specific results-of-action acceptor, Neurosci. Behav. Physiol. 33 (2003) 209–216, http://dx.doi.org/10.1023/A:1022186911789.
[29] E.D. Jarvis, O. Güntürkün, L. Bruce, A. Csillag, H. Karten, W. Kuenzel, et al., Avian brains and a new understanding of vertebrate brain evolution, Nat. Rev. Neurosci. 6 (2005) 151–159, http://dx.doi.org/10.1038/nrn1606.
[30] D.L. Duffy, G.E. Bentley, G.F. Ball, Does sex or photoperiodic condition influence ZENK induction in response to song in European starlings? Brain Res. 844 (1999) 78–82, http://dx.doi.org/10.1016/S0006-8993(99)01915-0.
[31] G. Horn, B.J. McCabe, Predispositions and preferences. Effects on imprinting of lesions to the chick brain, Anim. Behav. 32 (1984) 288–292, http://dx.doi.org/10.1016/S0003-3472(84)80349-8.
[32] S. Ribeiro, G.A. Cecchi, M.O. Magnasco, C.V. Mello, Toward a song code: evidence for a syllabic representation in the canary brain, Neuron 21 (1998) 359–371, http://dx.doi.org/10.1016/S0896-6273(00)80545-0.
[33] R. Stripling, S.F. Volman, D.F. Clayton, Response modulation in the zebra finch neostriatum: relationship to nuclear gene regulation, J. Neurosci. 17 (1997) 3883–3893.
[34] A. Fleischmann, O. Hvalby, V. Jensen, T. Strekalova, C. Zacher, L.E. Layer, A. Kvello, M. Reschke, R. Spanagel, R. Sprengel, et al., Impaired long-term memory and NR2A-type NMDA receptor-dependent synaptic plasticity in mice lacking c-Fos in the CNS, J. Neurosci. 23 (2003) 9116–9122.
[35] J.F. Guzowski, Insights into immediate-early gene function in hippocampal memory consolidation using antisense oligonucleotide and fluorescent imaging approaches, Hippocampus 12 (2002) 86–104, http://dx.doi.org/10.1002/hipo.10010.
[36] M.W. Jones, M.L. Errington, P.J. French, A. Fine, T.V.P. Bliss, S. Garel, et al., A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories, Nat. Neurosci. 4 (2001) 289–296, http://dx.doi.org/10.1038/85138.
[37] S. Kubik, T. Miyashita, J.F. Guzowski, Using immediate-early genes to map hippocampal subregional functions, Learn. Mem. 14 (2007) 758–770, http://dx.doi.org/10.1101/lm.698107.
[38] A. Lanahan, P. Worley, Immediate-early genes and synaptic function, Neurobiol. Learn. Mem. 70 (1998) 37–43, http://dx.doi.org/10.1006/nlme.1998.3836.
[39] A.J. Cole, D.W. Saffen, J.M. Baraban, P.F. Worley, Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation, Nature 340 (1989) 474–476, http://dx.doi.org/10.1038/340474a0.
[40] U. Mayer, H.-J. Bischof, Brain activation pattern depends on the strategy chosen by zebra finches to solve an orientation task, J. Exp. Biol. 215 (2012) 426–434, http://dx.doi.org/10.1242/jeb.063941.
[41] U. Mayer, S. Watanabe, H.-J. Bischof, Hippocampal activation of immediate early genes Zenk and c-Fos in zebra finches (*Taeniopygia guttata*) during learning and recall of a spatial memory task, Neurobiol. Learn. Mem. 93 (2010) 322–329, http://dx.doi.org/10.1016/j.nlm.2009.11.006.
[42] U. Mayer, T. Pecchia, V.P. Bingman, M. Flore, G. Vallortigara, Hippocampus and medial striatum dissociation during goal navigation by geometry or features in the domestic chick: an immediate early gene study, Hippocampus 26 (2016) 27–40, http://dx.doi.org/10.1002/hipo.22486.
[43] C.V. Mello, D.S. Vicario, D.F. Clayton, Song presentation induces gene expression in the songbird forebrain, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 6818–6822.
[44] J. Milbrandt, A nerve growth factor-induced gene encodes a possible transcriptional regulatory factor, Science 238 (1987) 797–799, http://dx.doi.org/10.1126/science.3672127.
[45] T.V. Smulders, T.J. DeVoogd, Expression of immediate early genes in the hippocampal formation of the black-capped chickadee (Poecile atricapillus) during a food-hoarding task, Behav. Brain Res. 114 (2000) 39–49, http://dx.doi.org/10.1016/S0166-4328(00)00189-3.

[46] J.J. Bolhuis, R.C. Honey, Imprinting, learning and development: from behaviour to brain and back, Trends Neurosci. 21 (1998) 306–311, http://dx.doi.org/10.1016/S0166-2236(98)01258-2.
[47] G. Horn, Imprinting, learning, and memory, Behav. Neurosci. 100 (825) (1986), http://dx.doi.org/10.1037/0735-7044.100.6.825.
[48] G. Horn, Neural bases of recognition memory investigated through an analysis of imprinting, Philos. Trans. R. Soc. Lond. B: Biol. Sci. 329 (1990) 133–142, http://dx.doi.org/10.1098/rstb.1990.0158.
[49] B.J. McCabe, G. Horn, P.P.G. Bateson, Effects of restricted lesions of the chick forebrain on the acquisition of filial preferences during imprinting, Brain Res. 205 (1981) 29–37, http://dx.doi.org/10.1016/0006-8993(81)90717-4.
[50] H.J. Karten, W. Hodos, W.J.H. Nauta, A.M. Revzin, Neural connections of the visual wulst of the avian telencephalon. Experimental studies in the pigeon (*Columba livia*) and owl (*Speotyto cunicularia*), J. Comp. Neurol. 150 (1973) 253–277, http://dx.doi.org/10.1002/cne.901500303.
[51] L. Medina, A. Reiner, Do birds possess homologues of mammalian primary visual, somatosensory and motor cortices? Trends Neurosci. 23 (2000) 1–12, http://dx.doi.org/10.1016/S0166-2236(99)01486-1.
[52] W.J. Kuenzel, M. Masson, A Stereotaxic Atlas of the Brain of the Chick (*Gallus Domesticus*), Johns Hopkins University Press, 1988 (accessed 15.2.16) http://agris.fao.org/agris-search/search.do?recordID=US9000051.
[53] G. Horn, B.J. McCabe, P.P.G. Bateson, An autoradiographic study of the chick brain after imprinting, Brain Res. 168 (1979) 361–373, http://dx.doi.org/10.1016/0006-8993(79)90176-8.
[54] B.J. McCabe, G. Horn, Learning-related changes in Fos-like immunoreactivity in the chick forebrain after imprinting, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 11417–11421.
[55] R. Ambalavanar, E.A. Van der Zee, J.J. Bolhuis, B.J. McCabe, G. Horn, Co-expression of Fos immunoreactivity in protein kinase (PKCγ)-positive neurones: quantitative analysis of a brain region involved in learning, Brain Res. 606 (1993) 315–318, http://dx.doi.org/10.1016/0006-8993(93)91000-I.
[56] D.B.J. McCabe, J. Cipolla-Neto, G. Horn, P. Bateson, Amnesic effects of bilateral lesions placed in the hyperstriatum ventrale of the chick after imprinting, Exp. Brain Res. 48 (1982) 13–21, http://dx.doi.org/10.1007/BF00239568.
[57] G. Horn, Visual imprinting and the neural mechanisms of recognition memory, Trends Neurosci. 21 (1998) 300–305, http://dx.doi.org/10.1016/S0166-2236(97)01219-8.
[58] B.J. McCabe, A.U. Nicol, The recognition memory of imprinting: biochemistry and electrophysiology, Behav. Brain Res. 98 (1999) 253–260, http://dx.doi.org/10.1016/S0166-4328(98)00091-6.
[59] G. Horn, A.U. Nicol, M.W. Brown, Tracking memory's trace, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 5282–5287, http://dx.doi.org/10.1073/pnas.091094798.
[60] N. Aoki, S. Yamaguchi, T. Kitajima, A. Takehara, S. Katagiri-Nakagawa, R. Matsui, et al., Critical role of the neural pathway from the intermediate medial mesopallium to the intermediate hyperpallium apicale in filial imprinting of domestic chicks (*Gallus gallus* domesticus), Neuroscience 308 (2015) 115–124, http://dx.doi.org/10.1016/j.neuroscience.2015.09.014.
[61] F. Maekawa, O. Komine, K. Sato, T. Kanamatsu, M. Uchimura, K. Tanaka, et al., Imprinting modulates processing of visual information in the visual wulst of chicks, BMC Neurosci. 7 (2006) 75, http://dx.doi.org/10.1186/1471-2202-7-75.
[62] T. Nakamori, K. Sato, Y. Atoji, T. Kanamatsu, K. Tanaka, H. Ohki-Hamazaki, Demonstration of a neural circuit critical for imprinting behavior in chicks, J. Neurosci. 30 (2010) 4467–4480, http://dx.doi.org/10.1523/JNEUROSCI.3532-09.2010.
[63] S. Yamaguchi, N. Aoki, T. Kitajima, E. Iikubo, S. Katagiri, T. Matsushima, et al., Thyroid hormone determines the start of the sensitive period of imprinting and primes later learning, Nat. Commun. 3 (2012) 1081, http://dx.doi.org/10.1038/ncomms2088.
[64] S. Yamaguchi, N. Aoki, D. Kobayashi, T. Kitajima, E. Iikubo, S. Katagiri, et al., Activation of brain-derived neurotrophic factor/tropomyosin-related kinase B signaling accompanying filial imprinting in domestic chicks (*Gallus gallus* domesticus), Neuroreport 22 (2011) 929–934.
[65] C. Huchzermeyer, P. Husemann, C. Lieshoff, H.-J. Bischof, ZENK expression in a restricted forebrain area correlates negatively with preference for an imprinted stimulus, Behav. Brain Res. 171 (2006) 154–161, http://dx.doi.org/10.1016/j.bbr.2006.03.034.
[66] J.K. Payne, G. Horn, Long-term consequences of exposure to an imprinting stimulus on spontaneous impulse activity in the chick brain, Behav. Brain Res. 13 (1984) 155–162, http://dx.doi.org/10.1016/0166-4328(84)90145-1.
[67] D.C. Davies, G. Horn, B.J. McCabe, Noradrenaline and learning: effects of the noradrenergic neurotoxin DSP4 on imprinting in the domestic chick, Behav. Neurosci. 99 (1985) 652–660, http://dx.doi.org/10.1037//0735-7044.99.4.652.
[68] G. Horn, P. Bradley, B.J. McCabe, Changes in the structure of synapses associated with learning, J. Neurosci. 5 (1985) 3161–3168.
[69] A.N.B. Johnston, L.J. Rogers, Right hemisphere involvement in imprinting memory revealed by glutamate treatment, Pharmacol. Biochem. Behav. 60 (1998) 863–871, http://dx.doi.org/10.1016/S0091-3057(98)00073-2.
[70] G. Horn, S.P.R. Rose, P.P.G. Bateson, Monocular imprinting and regional incorporation of tritiated uracil into the brains of intact and split-brain chicks, Brain Res. 56 (1973) 227–237, http://dx.doi.org/10.1016/0006-8993(73)90337-5.
[71] R.J. Andrew, The differential roles of right and left sides of the brain in memory formation, Behav. Brain Res. 98 (1999) 289–295, http://dx.doi.org/10.1016/S0166-4328(98)00095-3.
[72] P. Bradley, P.G. Horn, P. Bateson, Imprinting, Exp. Brain Res. 41 (1981) 115–120, http://dx.doi.org/10.1007/BF00236600.
[73] A.N.B. Johnston, S.P.R. Rose, Memory and lateralized recall, in: R.J. Andrew, L.J. Rogers (Eds.), Comp. Vertebr. Lateralization, Cambridge University Press, Cambridge, 2002, pp. 533–581.
[74] R.J. Andrew, L.J. Rogers, The nature of lateralization in tetrapods, in: R.J. Andrew, L.J. Rogers (Eds.), Comp. Vertebr Lateralization, Cambridge University Press, Cambridge, 2002, pp. 94–126.
[75] G. Vallortigara, R.J. Andrew, Lateralization of response by chicks to change in a model partner, Anim. Behav. 41 (1991) 187–194, http://dx.doi.org/10.1016/S0003-3472(05)80470-1.
[76] G. Vallortigara, R.J. Andrew, Differential involvement of right and left hemisphere in individual recognition in the domestic chick, Behav. Processes 33 (1994) 41–57, http://dx.doi.org/10.1016/0376-6357(94)90059-0.
[77] G. Vallortigara, Right hemisphere advantage for social recognition in the chick, Neuropsychologia 30 (1992) 761–768, http://dx.doi.org/10.1016/0028-3932(92)90080-6.
[78] L.J. Rogers, G. Vallortigara, R.J. Andrew, Divided Brains, The Biology and Behaviour of Brain Asymmetries, Cambridge University Press, Cambridge, UK, 2013 (accessed 16.2.16) https://books.google.it/books/about/Divided_Brains.html?hl=it&id=lbchAwAAQBAJ.
[79] O. Rosa Salva, L. Regolin, E. Mascalzoni, G. Vallortigara, Cerebral and behavioural asymmetries in animal social recognition, Comp. Cogn. Behav. Rev. 7 (2012) 110–138.
[80] L.J. Rogers, Early learning after hatching, in: L.J. Rogers (Ed.), Dev. Brain Behav. Chick, CAB International, Cambridge, UK, 1995, pp. 72–119.
[81] R.J. Andrew, Memory formation and brain lateralization, in: R.J. Andrew, L.J. Rogers (Eds.), Comp. Vertebr. Lateralization, Cambridge University Press, Cambridge, 2002, pp. 582–633.
[82] G. Vallortigara, Segregation and integration of information among visual modules, Behav. Brain Sci. 22 (1999) 398–399, http://dx.doi.org/10.1017/S0140525X99582022.
[83] C. Deng, L.J. Rogers, Social recognition and approach in the chick: lateralization and effect of visual experience, Anim. Behav. 63 (2002) 697–706, http://dx.doi.org/10.1006/anbe.2001.1942.
[84] A.N. Johnston, L.J. Rogers, P.R. Dodd, [3H]MK-801 binding asymmetry in the IMHV region of dark-reared chicks is reversed by imprinting, Brain Res. Bull. 37 (1995) 5–8, http://dx.doi.org/10.1016/0361-9230(94)00249-5.
[85] L.J. Rogers, S.W. Bolden, Light-dependent development and asymmetry of visual projections, Neurosci. Lett. 121 (1991) 63–67, http://dx.doi.org/10.1016/0304-3940(91)90650-I.
[86] L.J. Rogers, H.S. Sink, Transient asymmetry in the projections of the rostral thalamus to the visual hyperstriatum of the chicken, and reversal of its direction by light exposure, Exp. Brain Res. 70 (1988) 378–384, http://dx.doi.org/10.1007/BF00248362.
[87] L.J. Rogers, Light input and the reversal of functional lateralization in the chicken brain, Behav. Brain Res. 38 (1990) 211–221, http://dx.doi.org/10.1016/0166-4328(90)90176-F.
[88] L.J. Rogers, Early experiential effects on laterality: research on chicks has relevance to other species, Laterality 2 (1997) 199–219, http://dx.doi.org/10.1080/713754277.
[89] L.J. Rogers, Development and function of lateralization in the avian brain, Brain Res. Bull. 76 (2008) 235–244, http://dx.doi.org/10.1016/j.brainresbull.2008.02.001.
[90] C. Lieshoff, J. Große-Ophoff, H.J. Bischof, Sexual imprinting leads to lateralized and non-lateralized expression of the immediate early gene zenk in the zebra finch brain, Behav. Brain Res. 148 (2004) 145–155.
[91] B. Hellmann, O. Güntürkün, M. Manns, Tectal mosaic: organization of the descending tectal projections in comparison to the ascending tectofugal pathway in the pigeon, J. Comp. Neurol. 472 (2004) 395–410, http://dx.doi.org/10.1002/cne.20056.
[92] G. Marín, J.C. Letelier, P. Henny, E. Sentis, G. Farfán, F. Fredes, et al., Spatial organization of the pigeon tectorotundal pathway: an interdigitating topographic arrangement, J. Comp. Neurol. 458 (2003) 361–380, http://dx.doi.org/10.1002/cne.10591.
[93] D. Wylie, C. Gutierrez-Ibanez, J.M. Pakan, A.N. Iwaniuk, The optic tectum of birds: mapping our way to understanding visual processing, Can. J. Exp. Psychol. Rev. Can. Psychol. Exp. 63 (2009) 328–338, http://dx.doi.org/10.1037/a0016826.
[94] J. Verhaal, H. Luksch, Neuronal responses to motion and apparent motion in the optic tectum of chickens, Brain Res. 1635 (2016) 190–200, http://dx.doi.org/10.1016/j.brainres.2016.01.022.