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Research report

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

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## 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.

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## 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

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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.

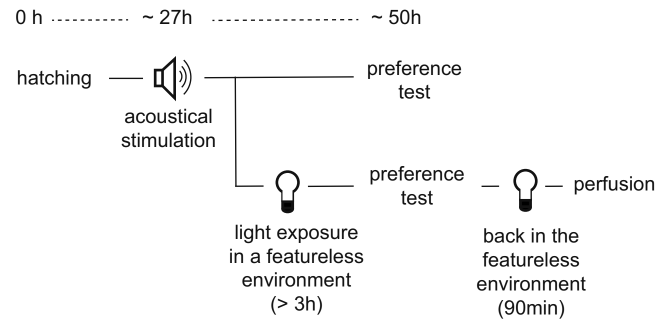


**Fig 1.** Sequence of experimental procedures. All chicks were acoustically stimulated ~27 h after hatching. The upper line represents the first procedure, in which chicks were kept in the darkness after the acoustical stimulation, until the preference test at ~50 h after hatching. The lower line represents 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