Neural Basis of Novel Odour Recognition in Domestic Chicks (Gallus gallus)

Bachelor Thesis

Author: Johannes Oberrauch (Student ID: 7411765)

Supervisor: Univ.-Prof. Dr. Uwe Mayer

Co-Supervisor: Univ.-Prof. Dr. Tatiana Korotkova

Department of Neuroscience

Faculty of Medicine

University of Cologne

February 20, 2026

Eidesstattliche Versicherung

Hiermit versichere ich an Eides statt, dass ich die vorliegende Arbeit "Neural Basis of Novel Odour Recognition in Domestic Chicks (Gallus gallus)" selbstständig und ohne die Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Alle Quellen, die wörtlich oder sinngemäß aus veröffentlichten und nicht veröffentlichten fremden Schriften entnommen wurden, einschließlich mittels KI-Tools generierter Inhalte, sind als solche kenntlich gemacht. Die Arbeit ist in gleicher oder ähnlicher Form im Rahmen einer anderen Prüfung noch nicht vorgelegt worden.
Ich versichere, dass die eingereichte Druckfassung der eingereichten elektronischen Fassung vollständig entspricht.

Johannes Oberrauch

Köln, den 20.02.2026

ABSTRACT

Until the 1960s, it was questioned whether birds, including the domestic chick, are capable of olfaction. Despite significant progress since then, many neural mechanisms regarding olfaction in birds are still not understood, among them the processing of novel olfactory stimuli. The avian hippocampus has been shown to be involved in processing various visual novelties. In this study, we investigated whether the hippocampus also responds to olfactory novelty. We exposed 3-day old chicks to either a familiar or unfamiliar smelling imprinting object. Chicks of both groups displayed similar behaviour, suggesting that the novel odour did not induce neophobia. Latency to approach the object however was significantly less variable in unfamiliar chicks. To quantify neural activity, c-Fos immunohistochemistry was used to analyse the hippocampus, olfactory bulb, nucleus taeniae of the amygdala, septum, and intermediate medial mesopallium. We found significantly less variance in the right ventral and right dorsomedial hippocampus of unfamiliar chicks, suggesting that these areas respond to olfactory novelty in a more synchronized fashion. No significant differences were present in any of the other brain areas. Our results support the idea of a right-hemisphere dominant hippocampus that is involved in general novelty processing beyond vision.

Contents

List of Figures

List of Tables

1 INTRODUCTION

1.1 Olfaction in birds

Research regarding the olfactory system of birds has progressed significantly in the past decades. While for centuries ornithologists believed birds were anosmic, this narrative started to shift by the mid-20th century [1]. The presence of an anatomically complete olfactory system warranted further research, proving birds to be at least microsmatic.

Further, several seminal studies have shown that birds are able to detect odours proficiently and make use of odour cues in a multitude of circumstances. Homing pigeons navigate using an olfactory map based on atmospheric odours and many bird species depend on olfactory cues during their food foraging [2–4]. Most notably, turkey vultures can locate carrion from miles away purely by smell [3]. Relative olfactory bulb (OB) size of birds varies across species and corresponds to life-style needs, as it does in mammals [5].

The olfactory bulb of domestic chicks is averagely sized in terms of relative olfactory bulb size among birds. Furthermore, behavioural evidence shows that domestic chicks detect olfactory cues in various situations (for a review on olfaction in the domestic chick see [1]). For example, chicks exposed to an open field showed a reduced neophobic response if a familiar, reassuring odour was present [6]. Additionally, when presented with a Y-maze where one choice contained their own soiled substrate, and the other choice substrate of an unfamiliar conspecific, chicks reliably chose their own substrate [6]. Moreover, chicks that were previously fed unscented food were reluctant to approach familiar looking food when an orange scented paper was placed below [7, 8].

Orange oil is among the most commonly tested odours and has been shown to be detectable but not fear or appetite inducing [1].

1.2 Domestic chick as an animal model

In avian neuroscience, the domestic chick is the most prominent model next to pigeons, zebra finches and corvids. This is due to several factors, most importantly their abundant availability and their low demands for housing and parental care [9]. As a precocial species, domestic chicks display many perceptual, social and cognitive abilities from

the moment of hatching, allowing for research in just a couple of days old chicks [10]. Hence, domestic chicks have been used in many developmental, psychological and neurobiological studies [11]. Famously, they are the dominant model in research on 'lateralisation' due to significant hemispheric specialization towards specific tasks [12].

Additionally, they are an adequate model for studies investigating novelty detection or neophobia [13, 14]. Neophobia is an instinctual wariness towards novelty in animals. It presents itself through behaviours that suggest a state of heightened alertness, such as an initial safety distance that is often followed by increased interaction with the novelty [7, 13]. For example, young chicks that are confronted with a novel object are slower to approach it than a familiar object [14]. Neophobia also extends to social stimuli. When confronted with either a familiar or unfamiliar conspecific, young chicks engage in more frequent social pecking towards the unfamiliar conspecific [13]. However, while novelty detection and neophobia overlap in their behavioural manifestations, they are distinct in their cognitive processes [14]. Neophobia is associated with the nucleus taeniae of the amygdala (TnA), while the central region of novelty processing appears to be the hippocampus (Hp) [15–17].

The neural bases of olfactory neophobia and novel odour processing in domestic chicks are unknown, as research is limited to behavioural studies.

1.3 Brain areas

1.3.1 Hippocampus

The expansion of the neocortex in humans led to the displacement of more ancestral regions in the brain, such as the hippocampus. In mammals, it lies deep within the inferior temporal lobe, while in birds it is located on the surface of the dorsomedial telencephalon. Additionally, the avian hippocampus shows no clear trilaminar organisation and no clear distinction between dentate gyrus or CA1-CA4 is possible, as it appears as a dense, heterogenous cluster of neurons [17]. Recently however, through immunohistochemical analysis, researchers showed that laminar layers are also present in the avian hippocampus [18]. Yet, continued discussion remains around the topic of potential subdivisions in the hippocampus. In this study, we assume a ventral (HpV), dorsomedial (HpDM) and dorsolateral (HpDL) subdivision, which have become popular

choices [17].

Due to these differences it was long debated whether the avian and mammalian hippocampus are homologue [17, 19]. In the present, they are commonly accepted as such, supported by a strong overlap in spatial cognition, as well as matching interconnectivity and development from similar embryonic fields [17, 19].

The hippocampus in birds has been primarily investigated for its involvement in spatial cognition. Domestic chicks have been shown to navigate using egocentric information, local beacon cues and view-matching strategies [20–22]. A series of immunohistochemical studies by Mayer et al. confirmed the central role of the hippocampus in these tasks [17]. Beyond just spatial learning, the hippocampus of birds seems to serve as a temporary cache during learning and memory formation, as it does in mammals [23]. Indeed, an upregulation of hippocampal activity can be seen in chicks exposed to a novel environment, conspecific and object [13, 14, 24].

The present study tests whether a different sensory modality, through the use of a novel odour, elicits the same reaction in the hippocampus.

1.3.2 Olfactory bulb

As previously discussed, research investigating the role of olfaction in avian species has significantly increased, yet especially neuroanatomical studies remain scarce. The olfactory bulb is located at the anterior end of the brain in birds, as it is in rodents [25, 26]. Avian species that evolved more recently show a trend of smaller relative OB size, perhaps caused by a shift towards vision and audition as primary senses [5, 25]. In birds as in mammals, the OB is not the region of olfactory processing and integration but it rather acts as a ‘relay centre’ [26].

To our knowledge, only one other study used the neural activity marker c-Fos to investigate the olfactory bulb in birds [27].

1.3.3 Septum

Belonging to the limbic system, the septal nuclei are an ancestral part of the vertebrate brain that has been shown to be developmentally and functionally homologue in birds and mammals [28]. A partition into a dorsal (SD), ventrolateral (SVL) and ventromedial

(SVM) subdivision is present in both mammals and birds [29]. It is part of the 'social decision-making network' and is implicated in social cognition of mammals and avian species alike. Social behaviours such as mating, pair-bonding, forming of hierarchies and recognition of familiar conspecifics have all been linked to the septal nuclei [13, 30].

In domestic chicks, septal activity was elevated among individuals that were exposed to an unfamiliar conspecific [13]. Importantly, the medial septal region receives projections from the olfactory bulb, as well as the hippocampal formation [14, 31].

1.3.4 Nucleus taeniae of the amygdala

The nucleus taeniae is part of the amygdala and also part of the 'social decision-making network'. It has been shown to be highly functional on the first day after hatching [13]. Like the septum, it receives direct input from the olfactory bulb [31].

Studies suggest that the TnA plays a vital role in sociosexual behaviour [32]. Male zebra finches with lesioned TnA were never chosen as sexual partners over non-lesioned males [32]. Recently, the TnA has been investigated for its connection to neophobia, as it was observed to respond to a novel environment, object and food [15, 24].

1.3.5 Intermediate medial mesopallium

The intermediate medial mesopallium (IMM) in birds is known for its role in processing visual properties of imprinted objects [33]. In addition to playing a role in visual imprinting, chicks with this region lesioned showed difficulty retaining and forming memories of an imprinting stimulus [34].

No study has investigated the role of the IMM in olfactory novelty yet.

1.4 Immediate early gene *c-fos*

The term immediate early gene (IEG) refers to genes which immediately activate in response to a recently experienced stimulus, without the need of de-novo protein synthesis. A popular IEG is *c-fos*, a gene that encodes c(yclic)-Fos protein, which activates other genes that play a role in cell differentiation, cognition, learning and motor control. Peak levels of c-Fos are reached around 60-90 min post activation. Thus,

sacrificing an animal during this period creates a momentary snapshot of the brain activity during stimulus presentation. By then staining c-Fos and counting activated cells, one can quantify the neural response to a stimulus [35].

In chicks, c-Fos staining has been successfully used in studies investigating spatial, social and olfactory cognition [13, 27, 36]. One downside is its weakness towards confounding factors [35]. If experimental conditions are not well controlled, an unplanned stimulus can invoke c-Fos production, making it indistinguishable during analysis later.

2 OBJECTIVES

The primary goal of this study was to investigate the role of the ventral hippocampus in general novelty processing.

Accordingly, we selected four regions (Hp, IMM, septum and TnA) which have been shown to respond to novelty and process neophobia [13, 14, 24]. In addition to the established regions, we examined the olfactory bulb, an area that has never been analysed in the domestic chick using c-Fos staining.

Previous studies found higher activities in the ventral and dorsomedial subregions of the right hippocampus when chicks were presented a social or visual novelty [13, 14]. Thus, we also expected to find elevated activity in these subdivisions of chicks that were unfamiliar with the odour.

In some cases, the TnA was found to respond to novel objects and to a novel environment in a similar fashion to the hippocampus, albeit likely due to neophobia and not novelty [15, 24]. Research is still unclear about the role the TnA plays in novelty processing. Our results might provide further insight into this question.

Furthermore, we chose to investigate the septum, a region that is part of the social behaviour network [37]. It has been shown to respond to a novel social stimulus [13]. As an imprinting object represents a social stimulus, we hypothesized to see a change of activation pattern in the septum.

The IMM was included in our analysis due to its role in visual imprinting and as a control region [33, 38]. We expected to see no difference of c-Fos activation between groups.

Regarding the behavioural parameters, we expected chicks to experience neophobia towards the novel odour, which would be reflected in behaviours such as higher latency to approach [14].

3 METHODS

3.1 Subjects

We used 24 domestic chicks (*Gallus gallus domesticus*) of the Aviagen ROSS 308 strain. Based on previous studies showing a sex difference, only male chicks were selected [14]. Fertilized eggs were obtained from a commercial hatchery (CRESCENTI Societ‘a Agricola S.r.l. –Allevamento Trepola– cod. Allevamento127BS105/2). They were incubated and hatched in complete darkness at a constant temperature of 37.7° C and humidity of 60%.

The experiment was carried out in accordance with ethical guidelines current to European and Italian laws. The experimental procedures were licensed by the Ministero della Salute, Dipartimento Alimenti, Nutrizione e Sanita‘ Pubblica Veterinaria (permit number: 60/2020-PR).

3.2 Habituation and odour familiarisation

After hatching, chicks were divided into two groups of 12 chicks (Familiar ‘control’ and Unfamiliar ‘experimental’) and individually housed in metal home cages (28 x 32 x 40 cm; W x H x L; Fig. 1a) together with an imprinting object containing 12 holes (red cylinder; 7.5 x 2 cm; H x D; Fig. 1b). A piece of dustless paper (Kimtech) was placed inside the imprinting object and infused with either 1 ml of canola oil for the Unfamiliar group or 1 ml of 5% orange oil (in canola oil) for the Familiar group. Orange oil was chosen as odour, as it is detectable but not aversive to chicks [1]. The chicks were housed for three days with food and water available ad libitum, at a constant temperature of 30-32° C and controlled light conditions of 14 h light and 10 h dark.

Habituation sessions that resemble testing conditions were performed four times a day (two morning and two afternoon sessions). They consisted of five removals of the imprinting object per session that lasted one minute each. Additionally, the odour was refreshed once before morning and once before afternoon sessions. The chicks were handled with identical lab gear (white lab coat and blue gloves) and limited visual exposure to the experimenter.

On the day prior to testing, the chicks were moved to the experimental room and two

final habituation sessions were performed. The chicks were left in the testing room overnight to habituate to the unfamiliar environment.

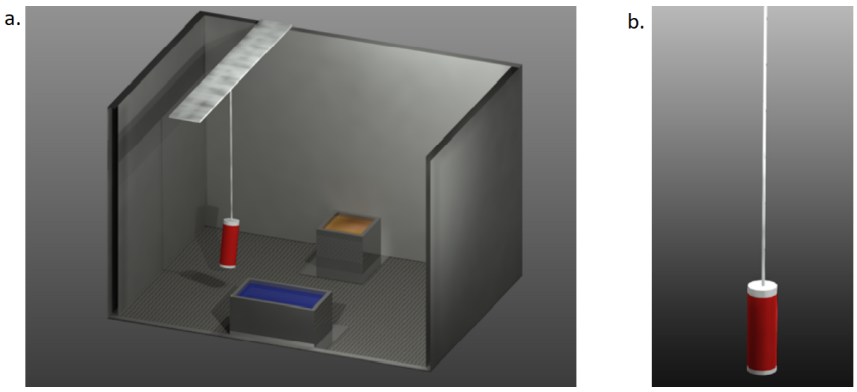

Figure 1: Housing and Imprinting Object. Illustration *a* shows the housing conditions; while dimensions of the cage differed slightly between testing and habituation, the orientation of imprinting object, water and food remained identical. *b* is a model of the imprinting object which additionally had 12 holes to let odour pass through.

3.3 Experimental procedure

The cages used on experiment day were different only in dimension (28 x 32 x 36 cm; W x H x L) to the previous cages. The food and water source, as well as the imprinting object were kept in the same orientation. Food and water were available ad libitum during the whole testing procedure and a constant room temperature of 28-30° C was maintained. A camera (Microsoft LifeCam Cinema for Business) was placed 20 cm above each cage to record chick's behaviour. The cages were homogenously illuminated by lamps (25 W warm light) placed 36 cm above the cages, while the rest of the testing room was dark.

The recordings for behavioural evaluation started ten minutes prior to removal of the imprinting object. During the one-minute removal period, the old paper was replaced with a new orange-scented paper for both groups. While for the Familiar group this procedure was identical to their habituation, for the Unfamiliar group, the scent of their imprinting object changed. Recordings continued for another ten minutes after reintroduction of the imprinting object. Exactly one hour after introduction of the stimulus, at c-Fos peak, all 24 chicks were sacrificed and perfused.

3.4 Behavioural measurements

We chose to combine human scoring and automated tracking in our behavioural measurements. While automated tracking excels at handling large data with high temporal resolution, it struggles with complex behaviours, such as head shaking that displays disgust. All behavioural data was analysed and evaluated blind to the experimental conditions.

3.4.1 Manual evaluation

In our manual evaluation we measured three parameters:

1. *Pecks difference:* The number of pecks directed at the imprinting object during the first ten minutes subtracted from the number of pecks during the ten minutes after stimulus.

2. *Approach latency (frames):* The time it took for a chick to touch the imprinting object after stimulus introduction, measured in frames. Video frames were chosen as a time unit to attain the highest possible temporal resolution. Videos were recorded at 30 frames per second. A reference line placed at 40% of the cage from the imprinting object was used as a common starting point (see Fig. 2a).

3. *Disgust reaction:* A binary measurement of whether a chick displayed a disgust reaction after stimulus introduction. A disgust reaction was classified as strong headshaking after close proximity of the beak to the imprinting object.

3.4.2 Automated evaluation

For automated tracking we used DeepLabCut, a markerless pose-estimation software based on deep learning [39].

A single animal approach was used, and ten body parts were defined to create a skeleton (see Fig. 2b). Additionally, the imprinting object was tracked to calculate the distance the chick maintained. For training data, 40 frames from each video were uniformly selected with the DeepLabCut software, totalling 960 frames, far above the recommended amount of 200 [39]. The final model achieved a mean average recall of 0.99 and a mean average precision of 0.97.

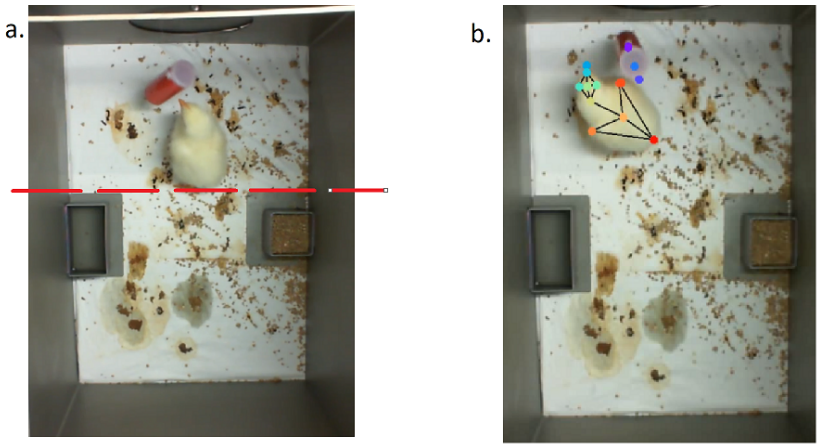

Figure 2: Behavioural measurements. Picture *a* shows where the reference line was placed that was used as starting point for measuring latency. Picture *b* shows an example output of the automated tracking software DeepLabCut; both the chick and the object were tracked.

Using DeepLabCut, we extracted the distance travelled during the first 10 minutes of the test and subtracted it from the distance travelled during the 10 minutes after introduction of the stimulus.

3.5 Immunohistochemistry

Exactly 60 min after the stimulus was introduced, all chicks were overdosed with an intramuscular injection of 0.4 ml of 1:1 ketamine (10 mg/ml) + xylazine (2 mg/ml) solution. According to standardized immunohistochemical procedure, brain activity was quantified by staining the immediate early gene (IEG) product c-Fos [13, 14]. Four series of 40 μm coronal sections were cut at -19° C. From the front of the brain, 15 cuts were collected, containing the olfactory bulb. Then, 40 sections were discarded until the posterior areas were reached. Only the first series of sections was used for c-Fos labelling, others were stored as backup.

3.6 Brain anatomy

All the photos were taken with a Zeiss Axio Imager2 microscope at a magnification of 200 x (eyepiece: 10 x, objective: 20 x with a numerical aperture of 0.5), and a digital camera (Zeiss AxioCam MRc5). Constant conditions were maintained for all photos, while contrast was set to 'best fit' and the gamma value to 0.2 for an optimal visual appearance. The c-Fos-immunoreactive (-ir) cell nuclei appear deep blue while

non-activated nuclei are coloured light green.

For counting cells within the regions of interest, the spot with the highest density of c-Fos-ir cells was selected. Then, a counting area (400 x 400 μm, or 175 x 300 μm in the OB) was placed over this spot and a snapshot was taken (see Fig. 3).

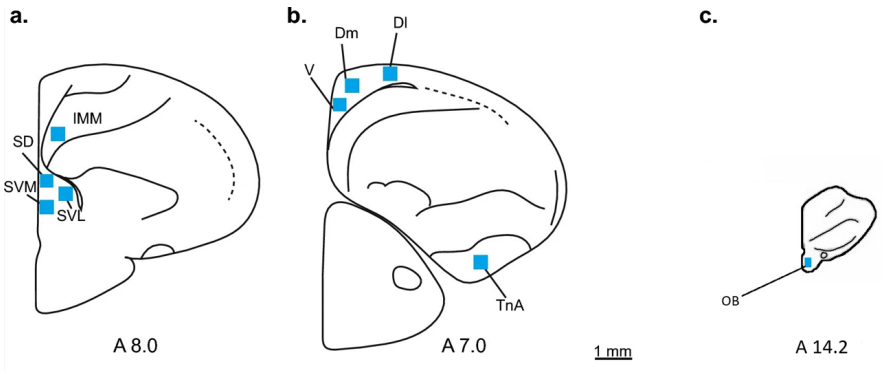

Figure 3: Regions of interest. Illustrations a, b and c show the brain regions analysed and where counting areas where approximately placed.

For the analysis of the hippocampus, eight sections of both hemispheres and each subdivision (HpV, HpDM and HpDL) were selected from an area corresponding to plate A7.8 to A4.6 in the chick atlas [40]. In the septum, five sections of both hemispheres and each subdivision (SD, SVM and SVL) were selected corresponding to A8.8 to A7.6 [40]. All hippocampal and septal subdivisions were independently measured for each subdivision.

In the IMM, TnA and OB, five snapshots of both hemispheres were taken from the area corresponding to plate A9.8 to A8.6, plate A8.8 to A6.4 and plate A14.6 to A14.2 respectively [40].

QuPath (v0.6.0), an open-source software for bioimage analysis, was used for automated counting of c-Fos-ir cells [41, 42]. Nuclei were automatically detected using the built-in watershed-based cell detection algorithm applied to the optical density sum image (Methyl Green as counterstain). Then, a supervised object classifier was trained on manually labelled training images. This was done for each brain region separately. Finally, these classifiers were applied to all images to quantify the density and proportion of c-Fos–ir cells (see Fig. 4).

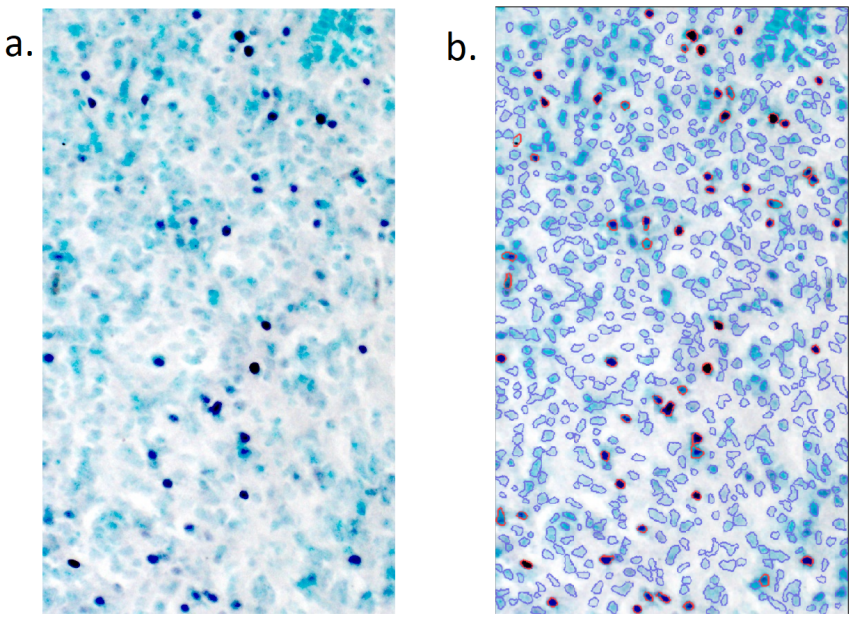

Figure 4: c-Fos staining and counting. Image *a* shows an example image taken from the olfactory bulb (175 x 300 μm) where dark-blue stained c-Fos-ir nuclei can be clearly distinguished from the background; in *b*, the QuPath output of that image is seen, active cells are circled in red.

3.7 Statistical analysis

3.7.1 Behavioural analysis

Behavioural analyses were performed at the level of individual subjects. The primary behavioural measure, latency to first contact with the imprinting object (in frames), was selected a priori as an index of approach behaviour. Exploratory analyses were performed on the other behavioural measures (distance travelled, pecking difference, and disgust reaction). Group differences between conditions (Familiar, Unfamiliar) were assessed using non-parametric tests due to small sample size and non-normal distributions. Wilcoxon rank-sum tests were performed to investigate differences in central tendency, while differences in behavioural variability between conditions were evaluated using Fligner–Killeen tests, a robust non-parametric test for homogeneity of variances. The binary disgust reaction variable was analysed using Fisher's exact test. RStudio was used for all analyses.

3.7.2 Brain analysis

Counts of c-Fos–ir cells and total cells were aggregated for each subject, brain region, and hemisphere across sections. From these merged counts, c-Fos cell density ($cells/mm^2$; see Equation 1) was calculated and used as the primary dependent variable in all statistical analyses. All analyses had three levels (subject × hemisphere × brain region).

$$\text{Cell Density (cells/mm}^2) = \text{Mean count} \times \frac{1}{0.4 \times 0.4} \tag{1}$$

First, we tested whether the novel odour induced an overall higher mean c-Fos activity. Binomial generalized linear models (GLMs) were fitted using the number of c-Fos–ir cells relative to the total number of cells as the response variable. Condition (Familiar vs Unfamiliar), hemisphere (Left vs Right), and their interaction were used as predictors. Repeated measures within subjects were accounted for using cluster-robust standard errors.

Secondly, due to the absence of robust mean differences, further analyses focused on inter-individual variability in c-Fos density. Differences in dispersion between conditions were evaluated separately for each brain region and hemisphere using Fligner–Killeen tests. Among these, hippocampal subdivisions were treated as planned comparisons based on previous studies showing their role in novelty processing. All other regions were analysed exploratorily, applying false discovery rate (FDR) correction to control for multiple comparisons.

Finally, Spearman rank correlation coefficients were calculated to check for relationships between hippocampal c-Fos densities and latency to approach, our significant results. This was treated as an exploratory analysis and used to assess whether individual differences in hippocampal activity covaried with behavioural values.

4 RESULTS

4.1 Behavioural results

Approach latency, disgust reaction, pecking difference and difference in distance travelled were analysed across all 24 chicks (n = 12 per group).

4.1.1 Approach latency

To investigate approach latency (time to first contact, in frames), a Wilcoxon rank sum test was performed, as the data was not normally distributed, but no significant difference between groups was found ($p = 0.17$). Further, a Fligner-Killeen test was applied to check for homogeneity of variances between groups. A significant decrease of variability in latency to approach was found in 'experimental' chicks ($p = 0.024$). The chicks unfamiliar with the odour showed smaller variance in their approach latency (sd = 343.54 frames) than chicks who were already familiar to the odour (sd = 2965.95 frames).

4.1.2 Disgust reaction

Fisher's exact test was used to assess the groups based on whether they displayed a disgust reaction (characterized by head shaking). Of 12 chicks familiarised with the odour since hatching, 3 displayed at least one disgust reaction. In contrast, 8 out of 12 chicks in the Unfamiliar group reacted with disgust at least once. No significant difference was found ($p = 0.099$).

4.1.3 Pecking frequency

The number of pecks directed at the imprinting object during the first 10min was subtracted from the number of pecks in the 10min after reintroduction to obtain a difference value (Δ pecks). No significant difference between groups was observed in the mean peck count (Wilcoxon rank sum test; $p = 0.603$) or in the variability (Fligner-Killeen test; $p = 0.760$).

4.1.4 Difference in distance travelled

Accurate distance travelled by each chick was calculated from DeepLabCut tracking data. The difference from before to after stimulus introduction was calculated to represent a change in behaviour (Δ distance travelled). A Wilcoxon rank sum test ($p = 0.520$) and a Fligner-Killeen test ($p = 0.546$) with FDR correction showed no significant difference of the mean or variance between groups.

4.2 Immunohistochemical results

All 24 brains were successfully extracted, however, only 19 of those, 10 from the 'experimental' group and 9 of the 'control' group, could be analysed due to a technical dysfunction of the cryostat. Additionally, some areas were damaged during staining. A detailed subject count for each area is contained in Table 1.

A repeated measures ANOVA was performed to investigate differences in the measured brain activity across the different regions among hemispheres and group. However, no significant interactions were found.

4.2.1 Planned analysis

For the planned analysis, the Hippocampus region was divided into three subdivisions (HpDL, HpDM and HpV).

First, we checked for differences in c-Fos activation to test our hypothesis of novel odour inducing an increase in ventral hippocampus activity. However, no significant difference in mean c-Fos density was observed between conditions in any hippocampal subdivision or hemisphere (all $p > 0.1$). Further, we found no significant interactions between hemisphere and treatment when testing mean activation levels (all $p > 0.1$).

Given these results, we focused on analysing inter-individual c-Fos expression variability. A significant reduction in variance of c-Fos activation between hemisphere and treatment was found in the right ventral (sd-ratio $= 0.339$, $p = 0.029$) and right dorsomedial (sd-ratio $= 0.387$, $p = 0.016$) subdivisions of chicks that were exposed to a novel odour. This effect was not present in the left hemisphere or in the dorsolateral subdivision .

4.2.2 Exploratory analysis

In addition to the planned analyses, we performed exploratory analyses of inter-individual variability in c-Fos activation in the other brain regions (OB, septal subdivisions, IMM, and TnA). Differences in variance were evaluated using Fligner–Killeen tests applied separately for each region and hemisphere (see Table 1 for full values).

Trends in the variability of c-Fos activation between groups were found in the right ventrolateral septum (sd-ratio = 0.475, $p = 0.075$) and the left olfactory bulb (sd-ratio = 2.373, $p = 0.090$) but did not survive FDR correction for multiple comparisons (see Table 1).

Table 1: Variance analysis of c-Fos activation across brain regions using Fligner-Killeen test. FDR correction was only calculated for exploratory analyses.

Area	Hemisphere	n (Familiar)	n (Unfamiliar)	sd-ratio	p (raw)	Analysis type	p (FDR corrected)
HpDM	Right	8	8	0.388	0.017	planned	-
HpV	Right	8	8	0.339	0.029	planned	-
HpDL	Left	9	10	1.193	0.136	planned	-
HpDL	Right	8	8	0.616	0.211	planned	-
HpDM	Left	9	10	0.545	0.355	planned	-
HpV	Left	9	10	0.845	0.618	planned	-
SVL	Right	9	9	0.476	0.075	exploratory	0.545
OB	Left	8	9	2.373	0.091	exploratory	0.545
SVM	Left	9	9	0.765	0.182	exploratory	0.682
SD	Right	9	9	0.607	0.227	exploratory	0.682
SD	Left	9	9	1.266	0.378	exploratory	0.773
IMM	Left	9	10	1.461	0.387	exploratory	0.773
TnA	Right	9	8	1.277	0.554	exploratory	0.849
SVL	Left	9	9	0.888	0.566	exploratory	0.849
SVM	Right	9	9	0.922	0.816	exploratory	0.938
OB	Right	9	10	1.067	0.881	exploratory	0.938
IMM	Right	9	8	0.790	0.902	exploratory	0.938
TnA	Left	9	10	0.872	0.938	exploratory	0.938

4.3 Correlations between behavioural and brain parameters

Spearman rank correlation analyses were conducted to check for correlations between the significant behavioural parameter, latency to approach, and the hippocampal subregions analysed. They revealed no significant correlation between latency to approach and hippocampal c-Fos activation of either hemisphere, indicating that the neural activity was not induced by the approach behaviour.

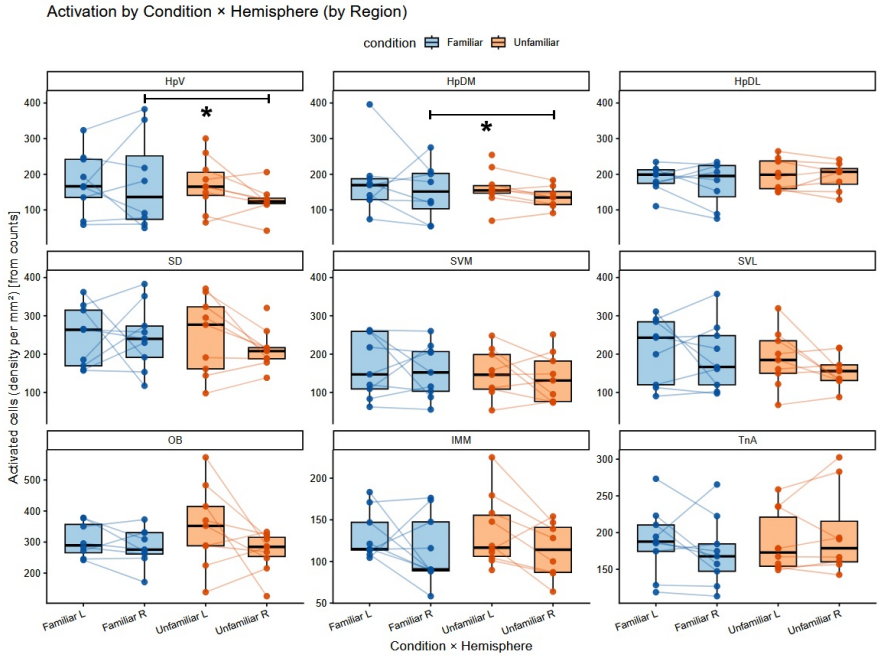

Figure 5: Activated Cell Count. Complete figure of c-Fos-ir cell count in all analysed areas. Comparing the significant areas to Fig. 6 shows that the differences in variance of HpV-R and HpDM-R are caused by activated cells and not by total cell count (* for $p < 0.05$).

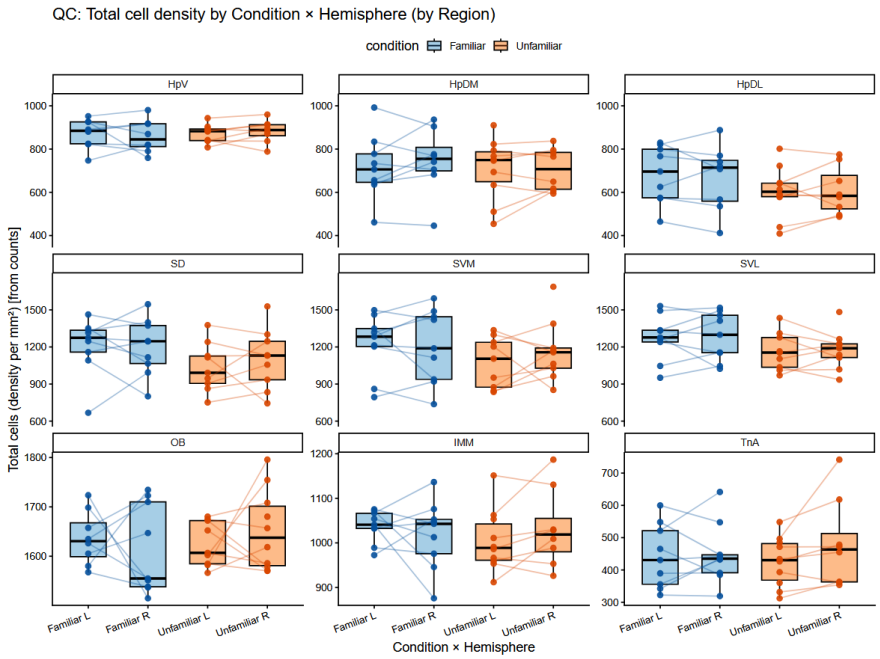

Figure 6: Total Cell Count. Complete figure showing total cell count obtained from QuPath.

5 DISCUSSION

The present study had two main goals. First, to gain insight into the processing of novel odours in the chick brain, specifically the ventral subdivision of the right hippocampus which is thought to play a role in general novelty processing [14, 17]. Secondly, it served as an exploratory study, as research investigating the neural basis of novel odour processing is severely underrepresented in avian research.

5.1 Behavioural results

In the analysis of our behavioural results, we found only one significant difference. Chicks that were unfamiliar with the orange odour displayed less variability in their latency to approach the object . None of the other parameters (disgust reaction, pecking frequency, distance travelled) were significantly different between groups.

While the role of olfaction in birds has been historically underestimated, it still remains complementary to vision or audition. When chicks were presented with food that was either novel or familiar in appearance and either scented or unscented, a significant increase in latency to eat the scented food was observed. However, this effect was only present, if the food was also novel in appearance [43]. As a result, it was hypothesized that olfactory novelty only leads to neophobia in case of additional visual novelty [1]. Contrary to this, one study did see a neophobic reaction when chicks were presented food that was only novel in smell [7].

It is important to note, that in our experiment, the novel odour was introduced through an imprinting object. This stimulus was not only familiar in appearance, but it also acted as a naturally calming social stimulus. It is likely that the combination of visual and social familiarity in our experiment offered strong reassurance to the chicks, negating any neophobia that the odour might have caused. Additionally, orange oil as odour has not been shown to elicit fear or avoidance in chicks, unlike blood or cat odour [1].

This could also explain why chicks unfamiliar with the odour approached the imprinting object in a significantly less variable fashion. Young male chicks specifically have been shown to be attracted by novelty [44]. Considering that our stimulus did not induce neophobia, it was likely seen as an interesting novelty, giving reason for an eventual approach. Chicks in the Familiar group on the other hand were already familiar

with the odour. Thus, their approach time might be more variable due to some chicks approaching instantly, while others show no interest at all.

Finally, social intersubject pecking has been reliably shown to be elevated towards conspecifics [13, 45]. However, pecking frequency seems to only increase towards social novelty. For example, a study investigating the behaviour of chicks towards a novel visual object found no increase in pecking frequency between groups [14]. We saw no increase in pecking frequency towards an inanimate imprinting object of novel odour, suggesting that social pecking might be constrained to conspecifics. Further research that targets this question specifically is required.

5.2 Role of the hippocampus

Previously, it has been shown that the ventral and dorsomedial subdivisions of the hippocampus seem to play a significant role in processing novel social and visual information in chicks [13, 14, 17]. While we did not find an increase of c-Fos-ir cells in these subdivisions, we did observe a significant difference in variability of c-Fos activation between the two groups. This effect was present in the right ventral and right dorsomedial hippocampus. Unfamiliar chicks displayed significantly decreased variability of c-Fos-ir cells in comparison to the Familiar group. We propose that the novel odour was detected by 'experimental' chicks and caused a shared experience that led to a more synchronised neural activity. Among 'control' chicks however, some might have focused on the renewed odour, while others may have paid less attention to it and focused on things such as feeding. These results align with our significant behavioural parameter, where Unfamiliar chicks approached the object in a less variable fashion.

This difference in variance appeared only in the hippocampus of the right hemisphere. Lateralisation of the domestic chick brain has been extensively researched [12]. Domestic chicks imprinted on a scented object were only able to discriminate between an unscented and a familiar scented object when they were allowed to use their right nostril [46]. This suggests a right hemisphere dominance in odour discrimination tasks. Moreover, the right hippocampus seems to be dominant in visual and social learning [13, 14]. Our research reinforces the idea of a right hippocampus dominance in novelty detection.

5.3 Role of the olfactory bulb

To our knowledge, there is only one other study that investigated the olfactory bulb in birds using c-Fos staining. Homing pigeons were exposed to a series of artificial odours to investigate the claim of an olfactory map for navigation located in the dorsolateral hippocampus. They found that OB c-Fos activity was only influenced by the absence of odour (filtered air) and not by novelty of odour [27]. In the present study, we found no difference in c-Fos activity when chicks experienced a novel or familiar, but identical odour, confirming their findings of OB activity.

While research about the olfactory bulb in birds is limited, mammalian olfactory bulbs have seen extensive research [47, 48]. Our results indicate that the OB is not a region of odour processing or learning, but an interface to secondary areas such as the piriform cortex [26]. This reinforces the idea that the avian olfactory system is not just structurally, but also functionally homologue to the mammalian counterpart.

5.4 Role of the nucleus taeniae of the amygdala

In our analysis, we did not find any significant interactions in the nucleus taeniae of the amygdala. As previously stated, it is likely that the chicks did not experience neophobia due to reassurance by the imprinting object.

Several studies have investigated the TnA of domestic chicks in various novel experiences. However, not as an area of general novelty processing, but as an area of novelty processing in connection to neophobia [15, 16, 24]. Yet, some studies that observed a neophobic reaction in the behaviour of chicks, did not find a corresponding response in the TnA [13, 14]. For example, chicks exposed to an unfamiliar conspecific did not show heightened activity of the TnA [13]. Interestingly, a recent study observed a response in the TnA not just from a novel object, but also from novel foods, possibly suggesting a role in olfactory novelty [15]. Importantly however, the study presented chicks with a novel food that was also novel in appearance, thus it cannot be ruled out that they responded to visual novelty.

Further research is required to explain these contrasting findings of the TnA in novelty processing.

5.5 Role of the septum

We included the septum in our analysis, because of its known involvement in various social behaviours [29]. Most recently, elevated levels of c-Fos-ir cells were observed in the dorsal and dorsomedial subdivisions of the septum [13]. Furthermore, the olfactory bulb of pigeons has been shown to project to parts of the medial telencephalic wall, which is where the medial septum lies [31]. As imprinting objects represent a social stimulus, we expected to see a difference between groups in septal c-Fos activation.

However, no significant differences between groups were observed in any of the three septal subdivisions. As previously discussed, chicks might have disregarded olfactory novelty given the visual familiarity of the imprinting object.

5.6 Role of the intermediate medial mesopallium

Our results suggest no involvement of the intermediate medial mesopallium in novel odour processing. While the IMM is known as the region for visual imprinting, no studies report higher activation of the IMM in the context of neophobia or olfaction [38, 49]. Our findings add to this, showing that the IMM is not involved in novel olfactory processing.

6 CONCLUSION

A series of recent studies showed that the hippocampus of domestic chicks responds to novel stimuli with a heightened neural activity [13, 14, 17]. Together, these findings suggest that the hippocampus plays a central role in learning beyond the spatial domain, and is possibly responsible for novelty processing *per se*.

While we found no such increase in c-Fos activity, we did observe significantly lower variability of c-Fos activity in the right ventral and right dorsomedial hippocampus of experimental chicks. This proves that the chicks were able to detect the novel odour, despite olfaction playing a lesser role to vision or audition in birds. We propose that olfactory novelty influenced the hippocampus to a different degree than visual novelty, acting as a subtle stimulus that evoked a more synchronised response of the hippocampus. Further, our results confirm the right hippocampus dominance in novelty related processing.

This study introduced the novel odour through an imprinting object, which offers significant reassurance to young chicks in otherwise stress-inducing situations. Future experiments should focus on introducing the novel odour via air, thereby disconnecting the odour from a physical object. In addition to an 'unfamiliar' and 'familiar' group, a 'control' group exposed to filtered air should be included.

A Appendix

A.1 List of resources used during writing

I declare that the entirety of this thesis was written by me, unless otherwise marked (through citation marks).

Large language models were never used as a writing tool, besides as a writing assistance the way a supervisor would act. For example, during the formatting of LaTeX code or proof-reading.

References

[1] R.Bryan Jones and Timothy J Roper. "Olfaction in the Domestic FowlA Critical Review". In: *Physiology & Behavior* 62.5 (1997), pp. 1009–1018. DOI: 10.1016/S0031-9384(97)00207-2.

[2] Anna Gagliardo. "Forty years of olfactory navigation in birds". In: *Journal of Experimental Biology* 216.12 (2013), pp. 2165–2171. DOI: 10.1242/jeb.070250.

[3] David C. Houston. "Scavenging Efficiency of Turkey Vultures in Tropical Forest". In: *The Condor* 88.3 (1986), pp. 318–323. DOI: 10.2307/1368878.

[4] Anna C. Gsell, Julie C. Hagelin, and Dianne H. Brunton. "Olfactory sensitivity in Kea and Kaka". In: *Emu - Austral Ornithology* 112.1 (2012), pp. 60–66. DOI: 10.1071/MU11052.

[5] Jeremy R. Corfield et al. "Diversity in olfactory bulb size in birds reflects allometry, ecology, and phylogeny". In: *Frontiers in Neuroanatomy* 9 (2015). DOI: 10.3389/fnana.2015.00102.

[6] R.Bryan Jones and Michael J. Gentle. "Olfaction and behavioral modification in domestic chicks (Gallus domesticus)". In: *Physiology & Behavior* 34.6 (1985), pp. 917–924. DOI: 10.1016/0031-9384(85)90014-9.

[7] R. Bryan Jones. "Food Neophobia and Olfaction in Domestic Chicks". In: *Bird Behavior* 7.2 (1987), pp. 78–81. DOI: 10.3727/015613887791918051.

[8] I. Turro, R.H. Porter, and M. Picard. "Olfactory cues mediate food selection by young chicks". In: *Physiology & Behavior* 55.4 (1994), pp. 761–767. DOI: 10.1016/0031-9384(94)90057-4.

[9] Rafael Freire and Susan J. Hazel. "Are chicken minds special?" In: *Animal Sentience* 2.17 (2017). DOI: 10.51291/2377-7478.1223.

[10] Lori Marino. "Thinking chickens: a review of cognition, emotion, and behavior in the domestic chicken". In: *Animal Cognition* 20.2 (2017), pp. 127–147. DOI: 10.1007/s10071-016-1064-4.

[11] V. H. B. Ferreira, V. Guesdon, and L. Calandreau. "How can the research on chicken cognition improve chicken welfare: a perspective review". In: *World's Poultry Science Journal* 77.3 (2021), pp. 679–698. DOI: 10.1080/00439339.2021.1924920.

[12] Lesley Rogers and Giorgio Vallortigara. "When and Why Did Brains Break Symmetry?" In: *Symmetry* 7.4 (2015), pp. 2181–2194. DOI: 10.3390/sym7042181.

[13] Carlos Daniel Corrales Parada et al. "Neural basis of unfamiliar conspecific recognition in domestic chicks (Gallus Gallus domesticus)". In: *Behavioural Brain Research* 397 (2021), p. 112927. DOI: 10.1016/j.bbr.2020.112927.

[14] Anastasia Morandi-Raikova, Alba Cumplido-Mayoral, and Uwe Mayer. "Novel object recognition in the dorsomedial and ventral hippocampus of young domestic chicks (Gallus gallus)". In: *Brain Structure and Function* 231.2 (2026), p. 21. DOI: 10.1007/s00429-026-03078-9.

[15] Emilie C. Perez et al. "Object and food novelty induce distinct patterns of c-fos immunoreactivity in amygdala and striatum in domestic male chicks (Gallus gallus domesticus)". In: *Behavioural Brain Research* 381 (2020), p. 112453. DOI: 10.1016/j.bbr.2019.112453.

[16] Melanie G. Kimball et al. "Novel objects alter immediate early gene expression globally for ZENK and regionally for c-Fos in neophobic and non-neophobic house sparrows". In: *Behavioural Brain Research* 428 (2022), p. 113863. DOI: 10.1016/j.bbr.2022.113863.

[17] Anastasia Morandi-Raikova and Uwe Mayer. "Spatial cognition and the avian hippocampus: Research in domestic chicks". In: *Frontiers in Psychology* 13 (2022), p. 1005726. DOI: 10.3389/fpsyg.2022.1005726.

[18] Antonio Abellán, Ester Desfilis, and Loreta Medina. "Combinatorial expression of Lef1, Lhx2, Lhx5, Lhx9, Lmo3, Lmo4, and Prox1 helps to identify comparable subdivisions in the developing hippocampal formation of mouse and chicken". In: *Frontiers in Neuroanatomy* 8 (2014). DOI: 10.3389/fnana.2014.00059.

[19] Georg F. Striedter. "Evolution of the hippocampus in reptiles and birds". In: *Journal of Comparative Neurology* 524.3 (2016), pp. 496–517. DOI: 10.1002/cne.23803.

[20] Giorgio Vallortigara. "Learning of colour and position cues in domestic chicks: Males are better at position, females at colour". In: *Behavioural Processes* 36.3 (1996), pp. 289–296. DOI: 10.1016/0376-6357(95)00063-1.

[21] Lucia Regolin, Giorgio Vallortigara, and Mario Zanforlin. "Object and spatial representations in detour problems by chicks". In: *Animal Behaviour* 49.1 (1995), pp. 195–199. DOI: 10.1016/0003-3472(95)80167-7.

[22] Tommaso Pecchia, Anna Gagliardo, and Giorgio Vallortigara. "Stable Panoramic Views Facilitate Snap-Shot Like Memories for Spatial Reorientation in Homing Pigeons". In: *PLoS ONE* 6.7 (2011). Ed. by Wulfila Gronenberg, e22657. DOI: 10.1371/journal.pone.0022657.

[23] Uwe Mayer, Shigeru Watanabe, and Hans-Joachim Bischof. "Spatial memory and the avian hippocampus: Research in zebra finches". In: *Journal of Physiology-Paris* 107.1 (2013), pp. 2–12. DOI: 10.1016/j.jphysparis.2012.05.002.

[24] Anastasia Morandi-Raikova and Uwe Mayer. "The effect of monocular occlusion on hippocampal c-Fos expression in domestic chicks (Gallus gallus)". In: *Scientific Reports* 10.1 (2020), p. 7205. DOI: 10.1038/s41598-020-64224-9.

[25] Stanley Cobb. "The Size of the Olfactory Bulb in 108 Species of Birds". In: *The Auk* 85.1 (1968), pp. 55–61. DOI: 10.2307/4083624.

[26] Rui Alvites et al. "The Olfactory Bulb in Companion Animals—Anatomy, Physiology, and Clinical Importance". In: *Brain Sciences* 13.5 (2023), p. 713. DOI: 10.3390/brainsci13050713.

[27] P. E. Jorge et al. "Odours stimulate neuronal activity in the dorsolateral area of the hippocampal formation during path integration". In: *Proceedings of the Royal Society B: Biological Sciences* 281.1783 (2014), p. 20140025. DOI: 10.1098/rspb.2014.0025.

[28] L. Puelles et al. “Comparison of the Mammalian and Avian Telencephalon from the Perspective of Gene Expression Data”. In: *European Journal of Morphology* 37.2 (1999), pp. 139–150. DOI: 10.1076/ejom.37.2.139.4756.

[29] James L. Goodson, Andrew K. Evans, and Laura Lindberg. “Chemoarchitectonic subdivisions of the songbird septum and a comparative overview of septum chemical anatomy in jawed vertebrates”. In: *Journal of Comparative Neurology* 473.3 (2004), pp. 293–314. DOI: 10.1002/cne.20061.

[30] K. Nishizawa, E.-I. Izawa, and S. Watanabe. “Neural-activity mapping of memory-based dominance in the crow: neural networks integrating individual discrimination and social behaviour control”. In: *Neuroscience* 197 (2011), pp. 307–319. DOI: 10.1016/j.neuroscience.2011.09.001.

[31] Anton Reiner and Harvey J. Karten. “Comparison of Olfactory Bulb Projections in Pigeons and Turtles”. In: *Brain, Behavior and Evolution* 27.1 (1985), pp. 11–27. DOI: 10.1159/000118717.

[32] Maki Ikebuchi, Toshikazu Hasegawa, and Hans-Joachim Bischof. “Amygdala and Socio-Sexual Behavior in Male Zebra Finches”. In: *Brain, Behavior and Evolution* 74.4 (2009), pp. 250–257. DOI: 10.1159/000264660.

[33] Brian J. McCabe. “Visual Imprinting in Birds: Behavior, Models, and Neural Mechanisms”. In: *Frontiers in Physiology* 10 (2019), p. 658. DOI: 10.3389/fphys.2019.00658.

[34] B.J. McCabe, G. Horn, and P.P.G. Bateson. “Effects of restricted lesions of the chick forebrain on the acquisition of filial preferences during imprinting”. In: *Brain Research* 205.1 (1981), pp. 29–37. DOI: 10.1016/0006-8993(81)90717-4.

[35] Sandra Yasbeth Lara Aparicio et al. “Current Opinion on the Use of c-Fos in Neuroscience”. In: *NeuroSci* 3.4 (2022), pp. 687–702. DOI: 10.3390/neurosci3040050.

[36] Uwe Mayer et al. “Hippocampus and medial striatum dissociation during goal navigation by geometry or features in the domestic chick: An immediate early gene study: Environmental Geometry Engages HF of Domestic Chicks”. In: *Hippocampus* 26.1 (2016), pp. 27–40. DOI: 10.1002/hipo.22486.

[37] Lauren A. O'Connell and Hans A. Hofmann. "The Vertebrate mesolimbic reward system and social behavior network: A comparative synthesis". In: *Journal of Comparative Neurology* 519.18 (2011), pp. 3599–3639. DOI: 10.1002/cne.22735.

[38] Tomoharu Nakamori et al. "Neural basis of imprinting behavior in chicks". In: *Development, Growth & Differentiation* 55.1 (2013), pp. 198–206. DOI: 10.1111/dgd.12028.

[39] Alexander Mathis et al. "DeepLabCut: markerless pose estimation of user-defined body parts with deep learning". In: *Nature Neuroscience* 21.9 (2018), pp. 1281–1289. DOI: 10.1038/s41593-018-0209-y.

[40] W. J. Kuenzel and M. Masson. *A stereotaxic atlas of the brain of the chick (gallus domesticus)*. 1st. Arkansas Academy of Science, 1988.

[41] Peter Bankhead et al. "QuPath: Open source software for digital pathology image analysis". In: *Scientific Reports* 7.1 (2017), p. 16878. DOI: 10.1038/s41598-017-17204-5.

[42] Nicholas J. Morriss et al. "Automated Quantification of Immunohistochemical Staining of Large Animal Brain Tissue Using QuPath Software". In: *Neuroscience* 429 (2020), pp. 235–244. DOI: 10.1016/j.neuroscience.2020.01.006.

[43] Nicola M. Marples and Timothy J. Roper. "Effects of novel colour and smell on the response of naive chicks towards food and water". In: *Animal Behaviour* 51.6 (1996), pp. 1417–1424. DOI: 10.1006/anbe.1996.0145.

[44] Giorgio Vallortigara, Lucia Regolin, and Mario Zanforlin. "The development of responses to novel-coloured objects in male and female domestic chicks". In: *Behavioural Processes* 31.2 (1994), pp. 219–229. DOI: 10.1016/0376-6357(94)90008-6.

[45] D. W. Rajecki, Barbara Ivins, and Barbara Rein. "Social discrimination and aggressive pecking in domestic chicks." In: *Journal of Comparative and Physiological Psychology* 90.5 (1976), pp. 442–452. DOI: 10.1037/h0077212.

[46] G. Vallortigara and R.J. Andrew. "Olfactory lateralization in the chick". In: *Neuropsychologia* 32.4 (1994), pp. 417–423. DOI: 10.1016/0028-3932(94)90087-6.

[47] Roberto Tirindelli et al. "From Pheromones to Behavior". In: *Physiological Reviews* 89.3 (2009), pp. 921–956. DOI: 10.1152/physrev.00037.2008.

[48] D G Moulton and L M Beidler. "Structure and function in the peripheral olfactory system." In: *Physiological Reviews* 47.1 (1967), pp. 1–52. DOI: 10.1152/physrev.1967.47.1.1.

[49] G. Horn, B.J. McCabe, and P.P.G. Bateson. "An autoradiographic study of the chick brain after imprinting". In: *Brain Research* 168.2 (1979), pp. 361–373. DOI: 10.1016/0006-8993(79)90176-8.