Observations of the Development of Inferior and Olfactory Conchae and Temporary Nasal blockages in Chicken Embryos

Abstract

The development of the nasal conchae in chick embryos at embryonic days (ED) 8, ED12 (H&H stage 38), and ED14 (H&H stage 39) was examined. In addition, the presence of epithelial tissue blocking the nasal passageways in the chick embryo was confirmed. This experiment was conducted as a preliminary step to exploring when in development embryonic chicks are able to display olfactory learning. The olfactory conchae were present with a mostly completed morphology in day 8 embryos and grew larger by day 12. The inferior conchae were in a primitive state of development at day 8. By day 12, the main structure of the inferior conchae was evident, and by day 14 they seemed to be in their final form. This and other evidence from the literature suggests that it may be possible for chicks to imprint to odors beginning at about day 13, although there is not yet conclusive proof of this.

Introduction

Until the 1960’s, it was widely assumed that birds do not have a very powerful olfactory system. However, more recent studies have found that olfactory cues can in fact influence the behavior of birds in important ways. Furthermore, it has been shown that exposure to odorants in the embryo can affect behavior after hatching. The earliest study to examine this response (Tolhurst and Vince 1976) exposed chick embryos that had just pierced through the inner shell membrane of the egg to air containing one of four specific odors. The responses to these odor stimuli were observed, and it was found that exposure to odorants significantly affected rates of behaviors such as beak-clapping or headshakes and also increased heart rate. This indicated that at least by embryonic day 21 (ED21), which is just prior to hatching, chicks can in fact sense odors in the environment. More recent studies have taken this further, finding that chick embryos can show behavioral responses after hatching to odors that they were exposed to while in the egg. In this study (Sneddon et al. 1998), chick embryos were continuously exposed to strawberry flavoring from ED15 to ED20. The flavoring was either circulated in the air around the egg, applied to the shell, or injected directly into the egg. All of these methods of treatment led to a specific preference for strawberry odors, indicating that the
chicks are able to detect and imprint to it at some point between day 15 and day 20. This result is particularly interesting because it exposed the chicks to odor stimuli in ways that would mimic natural means of exposure, which seems to provide further evidence that olfactory imprinting can occur in a natural environment between ED15 and ED20. However, Sneddon et al. did not find exactly when in development the ability of chick embryos to imprint to odors begins.

Another study (Lalloué et al. 2003) found that an olfactory response is possible beginning at ED13 and not before. However, it is important to note that responses were only examined using electrophysiological measurements in this study, so it is not known whether the observed electrophysiological response to stimulation can actually cause a change in behavior. In addition, odor stimuli were applied by dissecting the nasal area to reveal the olfactory conchae and directly injecting air with dissolved odorants onto the exposed conchae. While this type of technique is useful for examining whether olfactory receptor neurons at a particular developmental stage have the basic capability to respond to odors, it is very different from the ways that chicks would naturally be exposed to odors from their environment. Therefore, it is far from certain that chicks exposed to odor stimuli in a more natural way would show similar neuronal responses. Finally, it is important to note that the response at day 13 was a relatively small one; the amplitude of this response increased substantially at day 14, and increased further as the embryo developed further (Figure 1). So, while there is some neuronal activity at day 13 in response to odorant stimulation, it

![Figure 1: Mean amplitudes (with standard error) of electrophysiological responses to four different odors applied to chick embryos at different stages in development. Notice that as the embryo becomes more developed, the physiological response to odor stimulation becomes stronger.](image)
is much less than the response in older embryos. Still, it is interesting that there is some possibility of a response to odor stimulation as early as ED13. In addition, while there was no electrophysiological response to odors in embryos younger than 13 days, Lalloué et al. speculate that some olfactory response that could not be picked up by their recording technique is still possible before day 13. This is because ciliated neurons have been found in the olfactory epithelium as early as day 11, and the presence of cilia is one way to define functional maturity for olfactory receptor neurons. Then, it seems like it at least could be possible for chicks to respond to odor stimuli much earlier than has been shown in behavioral studies.

As is apparent from the method used by Lalloué et al. to expose the embryos to odorants, the conchae, and in particular, the superior (olfactory) conchae, are important in

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**Figure 2:** Series of coronal sections of the nasal cavity of a fully developed chick showing the appearance of the nasal conchae at different locations. See Appendix for a simpler 3-dimensional figure showing the relative locations of the nasal conchae.

Key to labels: 1, nasal septum; 2, vestibular concha; 3, duct of lateral nasal gland; 4, nasal bone; 5, maxillary bone; 6, inferior concha; 7, nasal cavity; 8, inferior recess of nasal cavity; 9, palatine sinus 10, intermaxillary bone; 11, lateral nasal gland; 12, superior (olfactory) concha; 13, olfactory region of nasal cavity; 14, respiratory region of nasal cavity; 15, lateral palatine fissure; 16, median palatine fissure; 17, eye; 18, orbital sinus; 19, palate; 20, maxillary sinus; 21, aperture of maxillary sinus

Source: Adapted from Romanoff 1960, as redrawn from Mihalkovics 1898
detection of odors. Each side of the chick nose contains 3 conchae: the superior (olfactory) concha, the inferior (also respiratory or middle) concha, and the vestibular concha (Figure 2, Appendix). The olfactory conchae are where most of the olfactory epithelium, and therefore most of the olfactory receptor cells, are located. The inferior conchae have a distinctive, scrolled shape (Figure 2, Appendix). They serve a respiratory function, warming and moistening the air before it enters the lungs. At certain points in development, there may be some olfactory epithelium on the inferior conchae, but this is only temporary (Romanoff 1960). The vestibular conchae are also involved in respiration as well as in creating blockages in the nasal passageways, as will be discussed later. The vestibular conchae will not be examined in depth here, however.

The conchae begin to form relatively early in development. The inferior conchae first appear in the 4 day chick and the superior conchae appear soon after, at ED 5 (Romanoff 1960). Still, the amount of surface area increases as the conchae develop further. This is important because it has been shown in rats that increased surface area allows for more the presence of more olfactory epithelium. In particular, one study has shown that over a period of time where the odor sensitivity in young rats greatly increases, the surface area of the olfactory receptor sheet increases 8-fold and the number of olfactory neurons increases 12-fold. Then, an increase in surface area of the conchae and other structures that support olfactory epithelium in rats seems to correlate with an increase in odor sensitivity (Meisami 1989). Therefore, it seems possible that increases in the surface area of the olfactory conchae during their development are critical for the development of odor sensitivity in birds.

To begin to understand the impact that nasal anatomy has on the development of the ability to respond to olfactory stimuli, it is first necessary to examine embryonic development of the nose on a more general level. For instance, it would be useful to know at what point in development the surface area of the olfactory conchae increases most and whether that increase in surface area is correlated with increased odor responses. However, before it is possible to even examine that, it is necessary to compare the size of the olfactory conchae in a series of embryos at different ages to determine where the surface area appears to be increasing the most. That comparison is the primary
goal of this experiment. In addition, development of the inferior conchae was examined to be able to compare their development with that of the olfactory conchae.

**Materials and Methods**

Chicken embryos were obtained (CBT Farms, Chestertown, MD) and were harvested at a series of ages from ED 5 to ED 20. Each embryo was staged using the Hamburger and Hamilton (1951) staging series and the heads were saved. The specimens from days 8 and 11 were not part of this group and so they were not staged rigorously when harvested. The day 12 embryo was stage 38; the day 14 embryo was stage 39. It should be noted that the day 12 embryo was harvested at about 1 AM on the night of day 12 while the day 14 embryo was harvested at about 3 PM on day 14, so these specimens were actually only about 1.5 days apart in development.

The specimens used in this experiment were preserved in different ways. Heads from 8 and 11 day embryos were harvested as part of a separate experiment and saved in 70% ethanol for about 3 months before they were prepared for freezing. The 14 day head used here was stored in 2% paraformaldehyde for 8 days before it was prepared for freezing. The 12 day head was stored in 4% paraformaldehyde 1 day before it was prepared for freezing.

Preparation for freezing on the 12 and 14 day embryos involved first dissecting the head to remove the eyes, the lower beak, and the back of the head. This was not done for the day 8 embryo. The eyes were removed from the day 11 embryo, but in the process, all nasal tissue was accidentally cut away as well. Therefore, there was no usable data from the 11 day embryo, and it is not included in the results. After initial preparation, embryos were placed in a series of 10%, 20%, and 30% phosphate-buffered sucrose solutions to reverse the shrinkage that occurs after preservation with paraformaldehyde. The 12 day embryo was placed immediately into a 30% sucrose solution. After a specimen had absorbed enough water that it would sink in the 30% sucrose solution, which takes at least 3-5 days after it is first placed in sucrose solution, it was frozen in freezing matrix using dry ice. Blocks were stored at –80°C and sections were cut using a cryostat. It should be noted that while sections were approximately coronal, they were not perfectly coronal. Therefore, the sections were cut at a small angle, and one side of a section was usually further dorsal than the opposite side.
Some sections were stained using alcian blue and nuclear fast red. Alcian blue stains acid mucosubstances blue, including cartilage in particular, while nuclear fast red is a red nuclear stain. Slides were then examined under a microscope and observations were recorded. Pictures were taken on a digital camera and were enhanced using Adobe Photoshop 7. All images were rotated so that the orientations used to describe sides of the nose as left or right would correspond to the appearance of the image on the page.

Results

Observations of Conchae

8 day embryo

The youngest embryo examined here is at day 8 of development. Here, it is clear that the conchae have begun to form. Both the olfactory and inferior conchae are in a relatively primitive stage of development at this point, but the olfactory conchae seem to be further developed than the inferior conchae (Figure 3B). One thing that is immediately apparent is that none of the scrolling characteristic of the inferior conchae is evident at this stage. On the other hand, the olfactory conchae at this stage are in a form that resembles their final shape (Figure 3). In fact, it even appears that the cartilage structure that supports the conchae is partially formed in the olfactory conchae of the 8 day embryo, while the corresponding structural components of the inferior conchae are not apparent at ED 8 (Figure 3).

In addition, it seems that the olfactory conchae have reached a greater percentage of their eventual size than the inferior conchae have by day 8. The inferior conchae do begin ventral to the olfactory conchae at this age, as the right nasal concha is visible 680 \( \mu m \) after the right nare opens while the first appearance of the right olfactory concha is 830 \( \mu m \) after the right nare opens. So, at the beginning of the nose there are areas that only have inferior conchae (Figure 4). Then, when the olfactory conchae do begin to form, the most ventral portions seem to be tucked in behind the inferior conchae (Figure 5). This does not happen in older embryos, as the inferior concha has already broken its attachment to the superior end of the nasal cavity by the time the olfactory cavity begins to form. The fact that the inferior conchae has not done that yet in the 8 day embryo provides further evidence that the olfactory conchae are better developed at this stage. Soon, though, the inferior concha moves away from the superior end as it approaches the
stages shown in Figure 3. Moving further dorsally, it becomes apparent that unlike in later embryos, the inferior conchae end before the olfactory conchae. This is evident as the more dorsal areas of the nasal fossae have olfactory conchae even where the nasal conchae are no longer present (Figure 6). The observation that the olfactory conchae seem more developed is also supported by comparing the overall depth of the respiratory concha with that of the corresponding olfactory concha. The depth of the right inferior concha from the ventral end to the dorsal end is 960 µm, while the depth of the right olfactory concha is about 1000 µm, although it was difficult to determine the exact depth of the olfactory concha because of technical problems with some of the slides. Then, the olfactory and inferior conchae have similar ventral-to-dorsal measurements in the day 8 embryo. By comparison, in later stages, the inferior conchae are substantially larger, as will be described.
Figure 3: (A) Approximately coronal section of 8 day embryo taken 1290 µm dorsal to the opening of the right nare and showing inferior conchae on both sides as well as one olfactory concha (100x magnification). The section is stained with Alcian blue and nuclear fast red, which stains cartilage and other acid mucosubstances blue. It is apparent that while there is some staining, there is no organized cartilage structure in the inferior conchae. Note that the reason why this section and all others are not symmetrical is that the sections are not exactly coronal. For this specimen, the right side of each section is further dorsal than the left side. (B) Section of 8 day embryo from an area 1570 µm dorsal to the opening of the right nare and 280 µm dorsal to that shown in A. Here, it is evident that the cartilage structure is beginning to develop in the olfactory conchae.
Figure 4: Approximately coronal section of 8 day embryo taken from an area 730 µm dorsal to the opening of the right nare stained with Alcian blue and nuclear fast red (100x magnification). The initial development of the right inferior concha is visible here. It is also apparent from this picture that the inferior concha is first visible ventral to where the olfactory concha first appears.
Figure 5: Approximately coronal section of 8 day embryo taken from an area 1000 µm dorsal to the opening of the right nare. (100x magnification) The ventral end of the right olfactory concha is visible here, and it is apparent that the inferior concha is covering the olfactory concha, which does not happen at this point in older embryos.
Figure 6: Approximately coronal section of 8 day embryo taken from an area 1560 µm dorsal to the opening of the right nare stained with Alcian blue and nuclear fast red. (40x magnification with additional zoom on camera). The olfactory conchae are visible here, while the inferior conchae are no longer present. In later stages, the inferior conchae extend further dorsally than the olfactory conchae, but at this stage they do not.
12 day embryo

As in the 8 day embryo, the inferior conchae begin ventral to where the olfactory conchae begin. The left inferior concha is first visible here 1520 µm from the initial opening of the nares. At its most ventral end, the inferior concha appears as a small structure entering the nasal cavity from the superior edge supported by a cartilage support structure emerging from the top of the septum (Figure 7). This appears to be the same support structure that was visible with Alcian blue staining on the olfactory conchae of the day 8 embryo, but it is substantially more developed by day 12. In addition, unlike in the day 8 embryo, it is found in the inferior conchae as well as the olfactory conchae here. Moving further dorsally, both the soft tissue and the cartilage structure gradually begin to curl. The next important milestone in development of the inferior concha is where it begins to enter from the side of the nasal cavity wall instead of from the superior edge (Figure 8, 9). This is important because the olfactory conchae form superior to the inferior conchae, so this switch is necessary to provide room for the olfactory conchae in the nasal cavity. This change also increases the surface area of the inferior conchae where it happens, which is important for their respiratory function.

It is interesting to note that when the inferior concha reaches its full size in the day 12 embryo, it is actually fused with the inferior wall of the nasal cavity (Figure 9). This is not the case in the 14 day embryo or in the adult chick. In addition, the scrolls themselves are wound tightly together in the inferior concha, leaving no surface area between them (Figure 9). This is also not the case in the 14 day embryo, as described later, or when development is complete (Figure 2). Then, it seems that some important aspects of development in the inferior conchae are not complete by day 12.

Just dorsal to the point where the inferior conchae seem to reach their maximum size, the ventral edge of the olfactory conchae becomes visible. The left olfactory concha is first visible 2700 µm dorsal to the opening of the nares, which is 1280 µm dorsal to the ventral edge of the left inferior concha. It is interesting that at the most ventral edge of the olfactory conchae, the cartilage structure is not visible yet (Figure 10). In fact, on the left olfactory concha, it only becomes visible about 280 µm dorsal to the ventral edge of the concha. This suggests that there is a layer of epithelium approximately 280 µm thick on the ventral end of the concha. Once the cartilage structure is visible in the olfactory...
conchae, it begins to grow larger in the superior to inferior direction, increasing the size of the conchae (Figure 11). This growth continues until the dorsal end of the olfactory concha is neared. This end is very different from the ventral end of the olfactory conchae; moving dorsally, a cartilage projection begins to fill the lateral edge of the concha as it continues to grow longer in the superior to inferior direction (Figure 12). The edge of the concha begins to move closer to the lateral wall, and as this happens, the layer of structural cartilage inside of it becomes thicker. Then, this cartilage begins to shrink and the epithelial tissue around it also shrinks somewhat, moving closer to the lateral wall of the nasal cavity. However, the epithelial tissue does not appear to shrink in the superior to inferior direction, so it persists near the lateral wall until the supporting cartilage has nearly completely ended (Figure 13). At this point, the olfactory epithelium also ends. The depth of the left olfactory concha from the ventral end to the dorsal end is 1640 µm in the 12 day embryo.

As the olfactory conchae fill more of the nasal cavity, the inferior conchae begin to become smaller (Figure 12, 13). Unlike in the 8 day embryo and like in the fully developed embryo, they continue past the dorsal end of the olfactory conchae. The scrolls continue to shrink further until they gradually taper to an end (Figure 14), with the left inferior concha of this specimen ending 640 µm dorsal to the end of the olfactory concha. The total depth of the left inferior concha in this specimen is 3000 µm.
**Figure 7:** Approximately coronal section of 12 day embryo taken from an area 1880 µm dorsal to the opening of the left nare. (A) 40x magnification; the area marked in red is magnified further in B; (B) 100x magnification. This image shows part of the most ventral portion of the inferior concha.
Figure 8: Approximately coronal section of 12 day embryo taken from an area 2420 µm dorsal to the opening of the left nare (40x magnification). This image shows the inferior conchae after they have begun to curl. It is also apparent that on the left side, the cartilage structure is beginning to shift so that the attachment point is the lateral edge instead of the superior edge, providing space for the olfactory concha.
Figure 9: Approximately coronal section of 12 day embryo taken from an area 2680 µm dorsal to the opening of the left nare; (A) 40x magnification; the area marked in red is shown in B; (B) 100x magnification. By this point, the attachment point of the inferior concha has moved from the superior edge to the inferior wall of the nasal cavity. This is also where the left inferior concha seems to be at its maximum size. It appears to fuse with the inferior wall of the nasal cavity, and the scrolls themselves are also fused together. Therefore, the inferior conchae do not appear to be fully developed by day 12.
Figure 10: Approximately coronal section of 12 day embryo taken from an area 2800 µm dorsal to the opening of the left nare; (A) 40x magnification. Some of the bones and other features of the nasal area are clearly visible in this picture, so those are marked here for reference. The area marked in red is shown in B and C at 100x magnification. (B) The beginning of the olfactory concha is clear in this section. However, this area is ventral to where the cartilage invaginates to form the structure of the olfactory concha, as highlighted in C. (C) Similar image to B with cartilage structure highlighted in blue using Adobe Photoshop. This makes clear that the cartilage structure has not invaginated yet to form the concha, but there is still olfactory epithelium visible at this level.
Figure 11: Series of sections showing the growth of the olfactory conchae moving dorsally in approximately coronal sections of the 12 day chick embryo. (A) 40x magnification of section 3300 µm dorsal to the left nare. Important features are marked, and cartilage structure was also colored blue for emphasis using false color in Adobe Photoshop. The area marked in the area magnified further in E; B, C, and D were taken in similar areas on other sections. (B) 100x magnification of a section 3060 µm dorsal to the nares; (C) 100x magnification of a section 40 µm dorsal to B; (D) 100x magnification of a section 140 µm dorsal to C; (E) 100x magnification of a section 180 µm dorsal to D
Figure 12: Approximately coronal section of 12 day embryo taken from an area 3880 µm dorsal to the opening of the left nare. This region is where the olfactory conchae reach their largest superior-to-inferior size. The cartilage projection on the lateral end of the olfactory concha that joins with the main cartilage structure near the dorsal end of the olfactory concha is visible here; at this point, however, it has not joined with the main cartilage yet. The inferior conchae are visibly smaller by this point as well.
**Figure 13:** Series of sections showing the dorsal end of the left olfactory concha in a 12 day chick (40x magnification); (A) 4080 µm dorsal to the left nare; (B) 160 µm dorsal to A; (C) 80 µm dorsal to B; (D) 40 µm dorsal to C; (E) 80 µm dorsal to D. The gradual ending of the cartilage structure followed by the end of the olfactory epithelium on the outside of the conchae is visible here. It is also apparent that the inferior conchae are becoming smaller here.

**Figure 14:** Section from 12 day chick embryo from 4960 µm dorsal to the left nare. The region near the dorsal end of the inferior conchae is visible here. There is no longer scrolling visible in the inferior conchae at this point.
Day 14 Observations

At day 14, the conchae look relatively similar to their appearance at day 12. The most important change seems to be that there is further development in the inferior conchae. By day 14, some apoptosis seems to have taken place on the tissue in the scrolls because they are no longer touching the edge of the nasal cavity or overlapping within the concha (Figure 15). The olfactory conchae, by contrast, look approximately the same as in the day 12 embryo (Figure 16). The depth of the right olfactory concha in this specimen is 1340 µm and the depth of the left olfactory concha is 1300 µm. The depth of the left inferior concha in this specimen is 3820 µm while the depth of the right inferior concha is 3920 µm.

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<td>960 µm (right)</td>
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Table 1: Comparison of dorsal-ventral depth of olfactory and inferior conchae in 8 day, 12 day, and 14 day specimens

Figure 15: Coronal section from 14 day embryo showing inferior conchae in a region relatively similar to that shown in Figure 10 (40x magnification), taken 3260 µm dorsal to the right nare. It is clear that the inferior conchae are more developed here as apoptosis seems to have removed excess tissue, allowing for an increase in surface area in the conchae.
Figure 16: Coronal sections from 14 day embryo stained with Alcian blue and nuclear fast red and showing olfactory conchae (A) near the ventral edge of the conchae and (B) once they have already begun to grow in size, both at 40x magnification. They clearly look similar to the olfactory conchae in day 12 embryos. On the other hand, as in Figure 16, the inferior conchae appear more developed here than at 12 days. Sections were taken (A) 3620 µm and (B) 3860 µm dorsal to the opening of the right nare.
Observations of nasal blockages

It is apparent from examining the ventral area of the day 12 embryos that the nares do not directly connect with the nasal passageway (Figure 17). There is an invagination of the nares on the outside, and then near the back of the nares, a separate nasal cavity located inferior to that is visible. For about 50 µm, the two passageways are visible in the same cross-section, but no direct passage between them is visible there or either dorsal or ventral to that area. However, there is a layer of tissue that does stretch directly between the nares and the nasal cavity. This tissue appears to be the Füllgewebe described by Weber (1950), a tissue that blocks the passage from the nares to the nasal cavity. The Füllgewebe surrounds the vestibular concha, a cartilage structure that branches off from the superior end of the septum (Figure 17).

There still appears to be Füllgewebe blocking direct access from the nares to the nasal cavity at day 14 (Figure 18). It seems that part of it may be beginning to disintegrate by day 14 (Figure 18C), but this is not certain from the sections and would seem unlikely based on the pattern of development found in the quail by Weber.

Part of the passageway that is blocked by Füllgewebe extends relatively far beyond the dorsal end of the nares at both day 12 and day 14 (Figure 19). The section shown in 19B also allows us to observe the staining of Füllgewebe with Alcian blue and Nuclear fast red staining. From that section, it appears that the Füllgewebe stains an intense pink color.

Knowledge of this staining pattern enables us to identify Füllgewebe in the day 8 embryo as well. It is not immediately obvious at this stage of development that the tissue labeled in Figure 20 is Füllgewebe. However, Weber’s (1950) description of the blockages in quail would suggest that the day 8 chick should have Füllgewebe in the nose in the orientation apparent in Figure 20. The observation that the labeled tissue also stains intense pink like the Füllgewebe in Figure 19B confirms that this tissue is in fact what Weber describes.
Figure 17: Approximately coronal section of 12 day chick embryo showing an area (A) 1360 µm from the opening of the left nare (40x magnification); (B) 80 µm dorsal to A (40x); (C) 80 µm dorsal to B (40x); (D) same section as C (40x with additional zoom). It is apparent from these sections that there is a direct pathway between the nares and the nasal cavity that is filled by an epithelial tissue blockage (called the Füllgewebe by Weber) at this stage. False color has been added to A-C to highlight the Füllgewebe in each section as well as the cartilaginous vestibular concha, which is a permanent structure, unlike the Füllgewebe. D shows the appearance of these structures without highlighting.
Figure 18: Coronal section of 14 day chick embryo showing the Füllgewebe blocking the path from the nares to the nasal cavity. (A) 40x magnification; the area marked in red is magnified further in B and that marked in purple is magnified further in C. False color was added here to highlight the Füllgewebe and the vestibular concha, as in Figure 17; (B) 100x magnification of the area marked in red on A showing the tissue blocking the nares from the nasal passageway; (C) 100x magnification (with additional zoom) of the area marked in purple in A showing the area where the Füllgewebe may be beginning to degenerate.
**Figure 19:** Approximately coronal section of 14 day embryo taken from an area 2440 µm dorsal to the opening of the right nare stained with Alcian blue and nuclear fast red. Although the nares are already closed, the passageway blocked by the Füllgewebe continues for a considerable distance dorsal to the nares. This is also apparent in the sections of the 12 day embryo shown in Figures 7 and 8. This section also shows that the Füllgewebe stains bright pink with this stain.
**Figure 20**: Approximately coronal section of 8 day embryo taken 390 µm dorsal to the opening of the right nare. By comparing the staining pattern of this and other nearby sections to Figure 19B and to Weber’s description of the Füllgewebe, it seems clear that the tissue in this sample is Füllgewebe.
Discussion

From these observations, it is possible to describe some basic characteristics of development in the chick nose between days 8-12 and days 12-14. It seems that the olfactory conchae are already formed by day 8, but they do grow substantially between then and day 12. The inferior conchae are much less developed at day 8, while by day 12, their basic structural elements are formed. Still, even at day 12, the inferior conchae are not entirely developed. This is apparent because the scrolls of the inferior conchae are very wide in the 12 day embryo, causing the scrolls to overlap with each other and with the walls of the nasal cavity. Considering that the function of the scrolling is to provide increased surface area for the air to touch, it seems clear that there should be more air space in the respiratory conchae when they become functional than we see in the 12 day embryo. Indeed, by day 14, the scrolls are thinner and look more like the scrolls of the fully developed chick as shown in Figure 2. It seems likely that to get to this point, apoptosis must occur in some cells of the inferior conchae. This is reasonable because the inferior conchae begin as solid bulges, as apparent in the day 8 embryo (Figures 4 and 5). Somewhere between day 8 and day 12, the cartilage structure appears to enter the inferior conchae to give them their characteristic shape. Still, the hollowing out of the areas around the scrolls is a separate step of development that seems to occur between days 12 and 14.

While the olfactory conchae do not show these morphological changes, they do seem to be growing throughout this period. The olfactory conchae are clearly longer at day 12 than at day 8 on a superior to inferior measurement. Surprisingly, they are actually smaller at day 14 than at day 12. This indicates that there may be some apoptosis in the olfactory conchae similar to what was observed in the inferior conchae. It could also be because of individual differences between the 12 day and 14 day specimens, which is a possibility because we only examined one of each specimen. Finally, it could be because these embryos were prepared using different techniques, so the conchae could have shrunken or become enlarged in different ways with different techniques. Then, a more rigorous study testing a more complete series of days and where all specimens are prepared the same way would help to examine whether this is a real effect or not.
The observation that the olfactory conchae seem to be mostly developed at day 12 indicates that it could be possible for the olfactory receptor neurons to respond to odors in the environment as soon as the neurons are mature, at around day 13. This is because, as suggested by Meisami’s (1989) work in rats, growth of the olfactory conchae could result in increased sensitivity to odors. Based on the small amount of quantitative data described above, it seems likely that the surface area of the olfactory conchae could reach a peak by day 12, although more quantitative analysis is necessary to examine this further. It is still far from certain that the chicken embryo can actually sense odorant cues in ovo, since, as described in the introduction, the experiments that showed that the neurons are functionally competent at day 13 use an unnatural method of stimulation. Still, these observations of the conchae do not seem to rule it out.

It should also be noted that there is a reasonable mechanism for odors to reach the olfactory conchae at this stage even though Tolhurst and Vince did not find any response before day 21. This is because they used odors in air, and since the chick is not breathing air until just before hatching, it would be expected that it could not show a significant response to odors in the air around the shell. Still, it is possible for odorants outside the shell to diffuse into the egg through the chorioallantoic membrane (CAM), along with oxygen and other molecules. In fact, the rate of oxygen uptake through the CAM increases substantially at about day 12 (Rahn et al. 1979). This is occurring because the embryo is growing rapidly at around day 12, so it needs increased oxygen. Because the overall rate of diffusion into the egg is increasing at this point, it would be reasonable to expect that the rates of diffusion into the egg for other molecules, including odorants, that would be carried in along with oxygen would also increase. When these odorants do enter the shell through the CAM, they could enter the blood along with oxygen. They could then be transferred to the amniotic fluid, which is bathing the embryo and probably does reach the olfactory conchae. While it has not been shown whether birds can respond to odorant molecules in amniotic fluid, many studies have shown that mammals can respond to odorants dissolved in amniotic fluid in utero (Schaal and Orgeur 1992). Then, there is a reasonable pathway by which odorants could enter the egg from the outside and reach the olfactory conchae in the nose before the chick begins breathing air.
One observation that does seem like it could make early sensing of odors more difficult is the blockage between the nares and the nasal cavity. This blockage, the Füllgewebe, blocks the only direct path between the outside of the embryo and the olfactory conchae. It is present at day 8, day 12, and day 14, meaning that it could prevent odor stimuli from reaching the conchae at these stages. Still, it is possible that odorants could somehow pass through the epithelial tissue of the Füllgewebe. It seems likely that there would be some amniotic fluid bathing the nasal cavity even if it is blocked by tissue, and if amniotic fluid can move through the Füllgewebe, it should be possible for odorants to move through it as well. Still, it would be interesting to examine the tissue further to test what can diffuse through them. Weber (1950) has examined blockages of this type in quail, which are similar to chickens in development (Weber 1950). This study found that the blockages are composed of some type of granular epithelium (Figure 21). Weber also found that the blockage does become more

**Figure 21:** (A) Diagram of nasal blockages in 10 day quail embryo (which should correspond to an 11-12 day chick embryo); (B) Image of Füllgewebe in 10 day quail embryo. (C) Diagram of nasal blockages in 12 day quail embryo (corresponding to about a 13-14 day chick embryo); (D) Image of Füllgewebe in 12 day quail embryo. Legend: Bm = basal membrane, Bk = basal nuclei, Me = Cell walls; Fk = Füllgewebe nuclei; Gr = granula; Vk = vacuole; dFk = degenerating Füllgewebe nuclei. Adapted from: Weber 1950
permeable near the middle of development, as the density of the Füllgewebe decreases to about 50% of its original level by day 11 in quail. The quail has an incubation period of only 18 days, instead of 21 for the chicken, so this should correspond to about day 12-13 in the chick. Then, it seems that the characteristics of this blockage could allow odors to reach the olfactory receptor neurons on the olfactory conchae at day 13.

Then, it seems that there is a convergence of factors that would allow chick embryos to respond to begin responding to odorants at around day 13. However, further studies are necessary to show whether any type of odor learning can actually happen at these early stages. The most basic way to test this would be to look for behavioral responses to exposure to odors beginning at day 13. One possibility for ways to do this would be to expose embryos to odorants for short periods, ie. from day 13-day 14 for some and from day 15-16 for others. Another method could be to begin exposure at different time points and test whether beginning exposure earlier would give a stronger response. While the first method could provide a more specific temporal localization of the beginning of olfactory sensory ability, the second method is closer to what would occur in nature, and therefore it could be more likely to generate a response.

It could also be useful to explore the development of this system in more detail. One obvious way to do that would be to examine a more complete series of embryos to find exactly where important steps in the development of the conchae occur. Another important area for future analysis would be to examine the number of olfactory receptor neurons in the olfactory conchae at different ages. It seems possible that as found in the rat by Meisami (1989), an increased surface area on the olfactory conchae and increased density of olfactory receptor neurons could be correlated to an increase in olfactory sensitivity in embryonic chickens. Another way to test the influence of development of the conchae on the ability of embryos to imprint to odors would be to destroy the conchae using a virus and subsequently test the ability of the chicks to respond to odors. The laryngotracheitis virus has been shown to destroy the conchae in chicks (Bang and Bang 1967). However, since this would be done in chicks post-hatching, a study like this would only test the influence of the conchae in the ability of chicks to respond to odors in general, and would not specifically examine embryonic imprinting.
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Literature Cited


Appendix

Figure A-1: Diagram of the chick nose showing the patterns of airflow through the conchae and the relative positions of the conchae in a side view of the nose. This macroscopic view of the conchae serves as a companion to the more detailed diagrams in Figure 2.
Source: Bang and Bang 1967