

Long-term Behavioral Effects of Exposure to Imprinting Stimuli in Chicks (*Gallus gallus domesticus*)

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Abstract

Filial imprinting is a type of early developmental learning in which certain species build strong and mostly irreversible connections to objects or individuals. These connections could be shaped by the contribution of several stimuli including the fragments of auditory and visual components. This study aims to describe the duration of the imprinting process as well as estimate long-term behavioural changes in chicks. In this experiment, a total of 78 chicks were used out of which 39 were exposed to imprinting stimuli and the rest served as control. We applied three different tests and replicated them after the imprinting procedure. These tests are Imprinting Preference Test, Social Preference Test and Social isolation Test. Imprinted chicks had a constantly shorter latency to approach the imprinting stimuli for both those two experiments with or without novel objects, whereas no preferences spent time in imprinting stimuli. However, introducing a novel object affected imprinting preferences more in terms of spending a longer time around the hen zone. During social isolation, the control group showed a relatively higher rate of distress calls even though our results don't bear on the significant effect of filial imprinting on changes in distress calling. Overall, this study suggests the presence of long-lasting filial imprinting that is more triggered by external situations.

Keywords: Auditory Stimuli, Domestic Chicks, Filial imprinting, Imprinting Procedure, Learning, Novel Object, Social Isolation

1. Introduction

Filial imprinting is one of the learning processes which plays a significant role in an individual's development stages. This phenomenon was first pointed out by Konrad Lorenz (Lorenz, 1937). Since then, numerous investigations have shown that the early stage of an individual's life shapes their lives in crucial ways, and is affected by the type of exposure. Therefore, filial imprinting is a process where young animals learn and copy the behaviours of their parents, and takes place during sensitive periods which is typically in the perinatal period (sensitive period right after hatching) and constructed behavioural pattern where it is copied from parents' stimuli to survive by young animals, as in chicks (*Gallus gallus domesticus*) (Bolhuis, 1998; Honey, 1998). Filial imprinting comes with a specific stimulus that is consequently merged with animals' characteristics and social preferences later in life. Previous studies have shown that imprinted chicks have preferences for patterns of motion and arrangements of features that are similar to those found in parents or animals they were imprinted from, such as biological motion or comb-like colours (Sluckin, 1972; Lemaire, 2020). Also, they tend to approach appropriately exposed stimulus than non-experienced stimulus. Those repeated approaches of newly hatched chicks led to a preference for the imprinting stimuli (Bateson 1964a, 1966b).

Early studies have shown that the most sensitive period of the imprinting frame is between 14 hours and 24 hours post-hatch (Jaynes, 1957) in domesticated chicks. Also, previous research has stated that the effect of critical variables which serve the process itself, explaining that not only the presence of stimuli but also individuals' predisposition (no learning involved) such as auditory component and the segments of visual stimuli has an integral part of imprinting (Ramsay, 1951). Even though it has been proposed that predispositions could control a chick's preference depending on the type of stimuli that the animal most benefits from (Johnson, 1985; Miura, 2020). In the imprinting process, predisposed preferences can overlap with those already belonging to animals such as biological motions (a live hen), and the specific colour of body parts (colour preferences to the red zone on the comb). Therefore, regarding the type of imprinting stimuli, predisposition could affect characterizing and shaping the memory and learning process of imprinting with different inputs such as mother presence including visual (shape, colour, static or moving) and auditory stimulus (Bateson, 1966; Bolhuis, 1998) as well as making the stimuli more distinguishable. However, some studies suggest that during developmental stages, animals become familiar with the object that has been approximately the first 14-40 hours, after hatching (Bolhuis, 1997; Horn, 1997;

Johnson, 1989), the difference in approach between the novel and familiar objects does not always produce absolute results (Van Kampen, 1991). Bateson et al.'s (Jackson, 1974) investigations showed that in some situations, chicks tend to approach novel objects rather than familiar(imprinted) objects (Salzen, 1968; Meyer, 1968). The research underlined that long-term exposure to novel objects in different imprinting phases changes the chicks' motivation of approaching novel objects. Unexpectedly, chicks presented closer interactions with the novel object the first 30 minutes after imprinting started whereas not after 60 minutes. Versace et al. assessed visual experience-based preferences for approaching a stuffed hen versus a scramble hen. Following stages of the study, they observed that chicks tended to discover the environment more and other stimuli (Versace, 2017). However, a predisposed preference for naïve chicks hatching in the dark with a lack of prior visual experience showed that the preference for approaching stuffed jungle fowl hen is higher compared to artificial objects, or even a distorted version of the same stimulus (Johnson, 1988). In addition to chicks' approaches to face-like configurations, predisposed preferences to moving objects have also been observed. The studies focused on animacy cues underlined that self-moving and accelerating objects are more attractive than static (Mascalzoni, 2013) and linear objects (Rosa-Salva, 2016). This finding suggests that naïve chicks actively seek out stimulation for conspicuous objects (Bateson, 1968), visual and auditory stimuli as they would do in natural environments (Bateson 1966a, 1968b). Regarding that, naïve chicks actively seek out stimulation for either conspicuous or naturalistic objects which highlights the early approach preferences may have an adaptive function of promoting attachment towards social companions rather than inanimate objects.

In birds, Filial imprinting is largely mediated by different biological factors which contribute to memory and learning processes. It has been suggested by previous studies that the neural process forms an integral part of imprinting and the filial imprinting is also under the control of specific brain regions and neural pathways (Horn, 1998; Maekawa et al., 2006; Nakamori et al., 2010; Yamaguchi et al., 2012). After hatching, a specific region of the brain called Wulst (VW) located in the telencephalon is in charge processing of detailed visual information in chicks (Nakamori, 2017). The histological studies showed that Wulst conjugates to the mammalian visual cortex and then, visual information is transmitted to the region called medial mesopallium (IMM) where the imprinting memory is both retained (Reiner et al. 2004) and neurons are the most responsive to the several imprinting stimuli (McCabe, 1999; Nicol, 1999).

In light of these various factors, filial imprinting is likely to be induced in several ways. The current uncertainties about the way of development of filial imprinting and its impact on chicken welfare in production brought more questions worth focusing on. Thus for a better understanding of these phases, in this study, we will focus on the fact that imprinting can be a practical way of improving chicken welfare. Therefore, we will present the role of the filial imprinting phenomenon on a chick's long-term social behavioural response and its interplay with the presence of imprinting stimuli and novel objects. We first aimed at testing the duration of the imprinting process to see how long imprinting last. Following this, we expect that the more we replicate the test, imprinted chicks will approach the imprinting stimuli in a shorter time than the unimprinted chicks. Secondly, we observed the effect of the presence of imprinting stimuli on chicks' preferences towards imprinting stimuli and novel objects. Regarding that, we hypothesize that introducing a novel object would make imprinting stimuli more distinguishable and affect the approaching time towards imprinting stimuli. Lastly, we examined long-term stress toleration based on assessing distress calling. We expect to see a reduction in distress calling by individuals who were attached to the imprinting stimuli since they would cope with a stressful situation more than control chicks when we separated them from a social group and imprinting stimuli.

The experiments were approved by the local ethical committee for animal experimentation in Linköping, license NO 14916-2018.

2. Materials and Methods

2.1. Animals and Housing

The experiment was conducted on domesticated chickens White Leghorn (*Gallus gallus domesticus*). One hundred eggs from the commercial hatchery, Lohmann Sverige AB, were transported by car to Linköping University and placed into the fridge until the day of incubation(September 7th in 2022). Eggs were placed in an incubator of 'Kruijt' hatchery at Linköping University. All eggs were incubated in darkness at 37.8 Celcius degrees and 55% RH (Relative Humidity) for 21 days. When hatching started, they were transferred into a hatcher at 37.5 Celcius degrees and 65% RH. The eggs were checked 2 times per day in the dark. A red headlamp was used during the hatching. A total of 78 chicks hatched: 77 of them hatched between 12 and 48 hours and 1 in 72 hours. Imprinting took place during hatching until the chicks were placed in their home pen. To avoid imprinting towards other stimuli, in

the dark, the chicks were, equally, moved from the incubator to imprinting and control (no experiment) boxes. The lamp (240 V, 40 W) was attached above the imprinting boxes where they could directly see the model hen. The lamp was turned on as soon as chicks were placed into imprinting boxes. During the imprinting procedure, newly hatched chicks were positioned into two boxes where they could see the model hen in the middle. As imprinted stimuli, both rotating model hen and hen sound (tidbitting sound, used by the hen to teach their chicks where food is) were used. The hen sound was played through a speaker and the whole imprinting procedure lasted 90 minutes. At the end of the imprinting, the chicks were moved into four home pens (39 chicks placed into two home pens as the control group - CC; and 39 chicks placed into two as the imprinted group - IC). All home pens contained food, water and a heat roof. As a part of the experiment, we attached model hens on the heat roofs and placed them in the IC pens to observe the duration of imprinting by exposing the stimulus. We extended all home pens when chicks were one week old and built perches inside. All chicks were tagged and tracked according to their leg numbers and leg rings which were switched three times as individuals grew up. The whole imprinting procedure, meanwhile, placing control chicks in the dark box and later to their home pens were performed with another master student Lovisa Åkerstedt who also did her experiment with the same chicks.

2.2. General Procedure and Experiments

The study occurred at 'Kruijt' hatchery at Linköping University and took seven weeks after the chicks hatched (October, 30th to November, 16th in 2022). The study had four steps: Imprinting Procedure, Imprinting Preference Test, Social Preference Test and Social Isolation Test. All tests were done in the darkness and each test was replicated one time when they were 5 weeks old for both Imprinting Preference and Social Isolation tests- except the Imprinting Preference Test which was replicated four times when the chicks were two and four weeks old. We randomly selected individuals from each compartment during the tests. Since the experiment took place in the hatchery where technician visited daily during the experiment, the arenas were covered with a curtain to prevent possible distraction of the chicks from external in case the experimenter walk around the arena, or the technician come inside to the hatchery to feed the other chicks in the meanwhile.

All phases of Imprinting and Social Preference tests were video recorded by GoPro (Hero5 Black or a Hero10 Black). Since we divided arenas into three sections (Figure 5.), we noted

the time spent in each section in seconds during video analysis. For statistical analysis, we used IBM SPSS software 29. and Levene's t-test (p-value <0.05) to assess the statistical significance of the differences between tests, treatments and sex.

All graphs were made by Excel tools (Microsoft 365)

2.2.1. Imprinting Procedure

We designed a set-up for the imprinting procedure, including two imprinting stimuli which are the stuffed hen (Figure 1.) and the tidbitting hen sound (the recording was taken from [www.github.com](https://github.com/zebular13/ChickenLanguageDataset); link: <https://github.com/zebular13/ChickenLanguageDataset>) by attaching them on a rotating plate with a diameter of 45 cm. We aimed to create a natural set-up where chicks can see and hear the hen, shortly after hatching. Thus, we introduced two stimuli including a stuffed hen and the hen sound in the background. Due to the sensitivity of the imprinting frame, the 24-hour-old chicks had the highest preference score for the imprinting stimuli (Bolhuis, 1998; Honey, 1998), we exposed them to the imprinting stimuli within 24 hours after hatching. We, equally, placed newly hatched chicks both inside the control box(chicks which were not exposed to the imprinting procedure) and the imprinting box (where the imprinting procedure occurred). Imprinting training occurred in a dark room for 90 minutes, only using a spotlight and heat lamp over a rotating plate to make the imprinting stimulus visible to chicks.



Figure 1. Imprinting Procedure set-up



Figure 2. Rotating plate with stuffed hen



Figure 3. Imprinting set-up with two stimuli; auditory stimulus comes from the speaker

2.2.2. Experiment 1: Imprinting Preference Test

The test was done in two arenas, small and bigger, in case three-day-old chicks may become less motivated to reach the stimuli in the longer arena. Both arenas were built in corridor shape by using cardboard boxes and covered with shower curtains (Figure 4). The floor of the arena was covered with sawdust to prevent possible sliding while chicks were exploring the arena. The whole experiment was done in the dark.

During the experiment, we used the same imprinting stimuli (both tidbitting sound and stuffed hen) where we already exposed the chicks in the imprinting procedure to see. For the first experiment, we built a small arena with 45x31x31 cm and visually divided it into three parts of 15 cm (Figure 5.). We examined the preference of the chicks toward imprinting stimuli by calculating the total time spent in each section. The main reason for dividing the arenas into three sections was to examine latency and how much time the chicks spent in the middle and the hen zone by making equal measurements for each parameter (latency, middle and hen zone). For the last two replications, we followed the same method but used a bigger arena with 72x31x31 cm and divided it into three parts of 24 cm (Figure 6.). The replications were aimed to assess how long the imprinting effect lasts when they turned two and four weeks old. The experiment overall took 180 seconds and was recorded by GoPro.



Figure 4. Imprinting Preference arena covered with curtains.

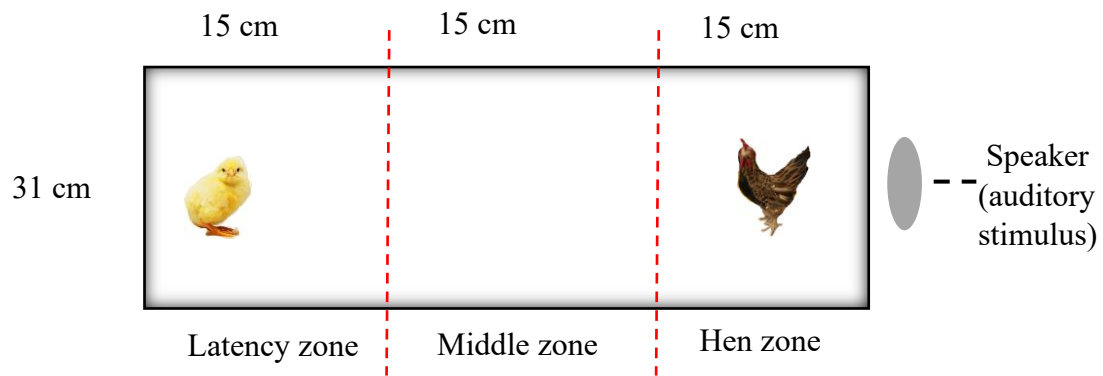


Figure 5. The smaller arena of Imprinting Preference Test

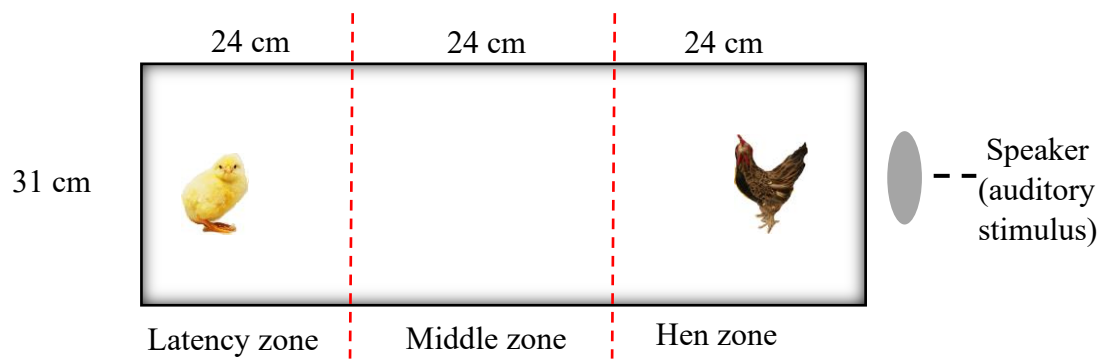


Figure 6. The bigger arena of Imprinting Preference Test.

2.2.3. Experiment 2: Social Preference Test

The test assessed the impact of the presence of a novel stimulus on latency to imprinting stimuli and whether chicks discriminated between imprinting and novel objects. The arena remained the same as in the imprinting preference test (2.2.2) except a novel object was added to the other corner of the arena which was a canned bottle measuring 9.5 cm (Figure 8.). We placed the chicks in the middle of the arena and calculated the time to reach and spent in both imprinting and novel stimuli zones. Chicks were exposed to the social preference test for 360 seconds, for the first time when they were two weeks old, and again when the chicks were eight weeks old. During the second test, we replicated the experiment by using a larger plastic bottle (47 cm in height, Figure 8.) to proportionate the size of the novel object to the size of the chicks.

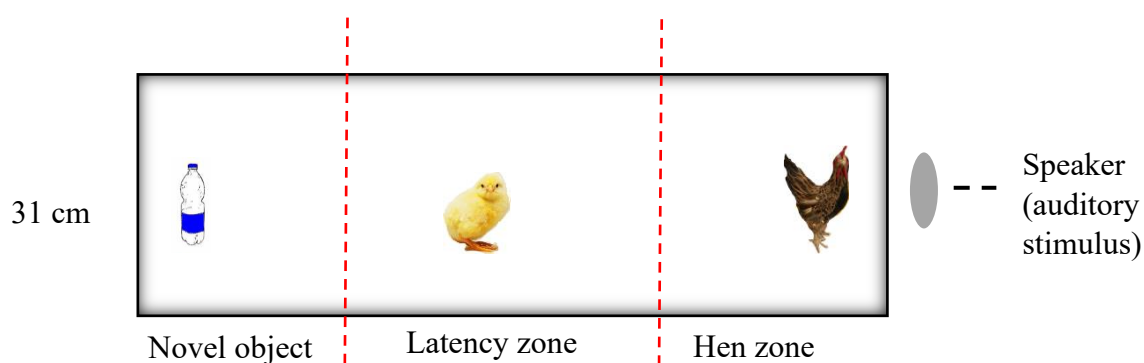


Figure 7. Social Preference Test arena



Figure 8. Novel objects used in Social Preference Test. a) Canned bottle in the first trial, b) Plastic bottle in replication.

2.2.4. Experiment 3: Social Isolation Test

The testing procedure is based on seeing the effect of the imprinting process on distress calls to determine whether socially reared chicks reacted differently when they were separated from the imprinting stimulus and social group, as indicated by changes in their calling frequency (Kaufman, 1961; Hinde, 1961). First, each chick was moved from their home pen and placed into a 21x14x16 cm soundproof box closed by a lid in an empty room. Then, we recorded the chicks' peeps for 300 seconds via a sound recorder. The first experiment occurred when the chicks were three weeks old, and then repeated at eight weeks old. Peeps were analyzed with Python 3.10 (64-bit) and a specific programme called 'WhereMyPeepsAt' which was designed for counting peeps. Each peep was signified with different colours (Figure 9.) The expectation is to see a reduction in distress calling by individuals who are attached to the imprinting stimuli.

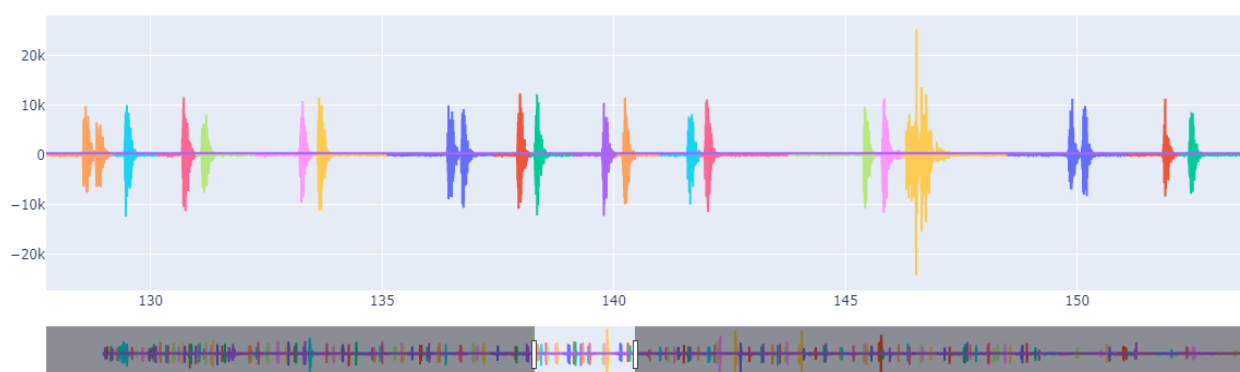


Figure 9. The frequency of peeps. Colours represent different peeps. X-axis shows the trial time in seconds.

3. Results

3.1. Imprinting Preference Test

Our results showed that chicks that had gone through the imprinting procedure significantly presented a shorter latency value to the imprinting stimuli than the control group (Figure 10. t

(42)= -1.839; $p=0.0.37$). After replications of the test, latency was consistent among imprinted chicks in terms of approaching in a shorter time to the imprinting stimuli than the control chicks (Figure 10. $t(75)=-2.129$; $p=0.018$). The more we replicated the test, after two times replications; the latency got shorter of the imprinted chicks was than in the first experiment (Figure 10. $t(75)=-2.507$; $p=0.007$). The results showed that there were no preferences between groups when it comes to time spent in the middle zone, ($t(42)= 0,361$; $p=0.36$), as well as no changes during replications, 1st replication of $t(75)=0,579$; $p=0.282$); second replication of $t(75)=-1,053$; $p=0.148$). Nevertheless, the preference for staying in the hen zone was significant between treatments (Figure 11. $t(42)=1.689$; $p=0.050$). We did not specify any sex preferences for approaching imprinting stimuli.

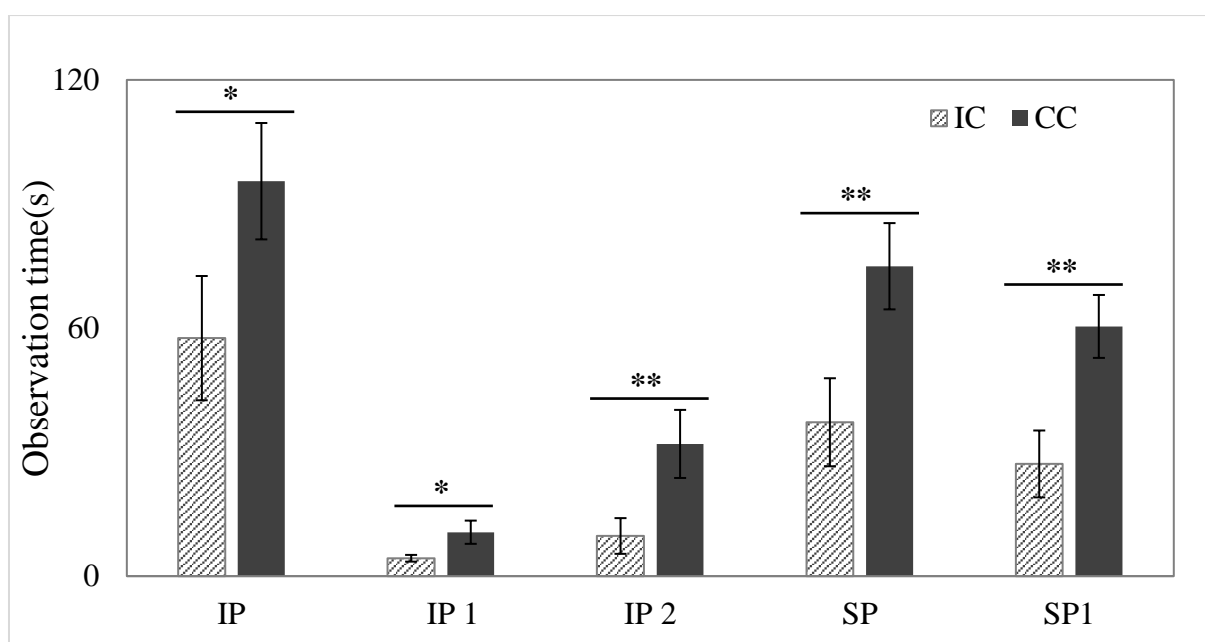


Figure 10. Mean latency for control chicks (CC) and imprinted chicks(IC) to pass the latency zone within the given arena. Comparison of mean latency (s) between Imprinting Preference and replication (IP, 48-72h post-hatch; IP1, 2 weeks old; IP2, 4 weeks old) and Social Preference Test(SP, 10 days old; SP1, 4 weeks old). Asterisk (*) indicates the statistical significance of $p<0.05$ and asterisks (**) indicates the statistical significance of $p<0.01$.

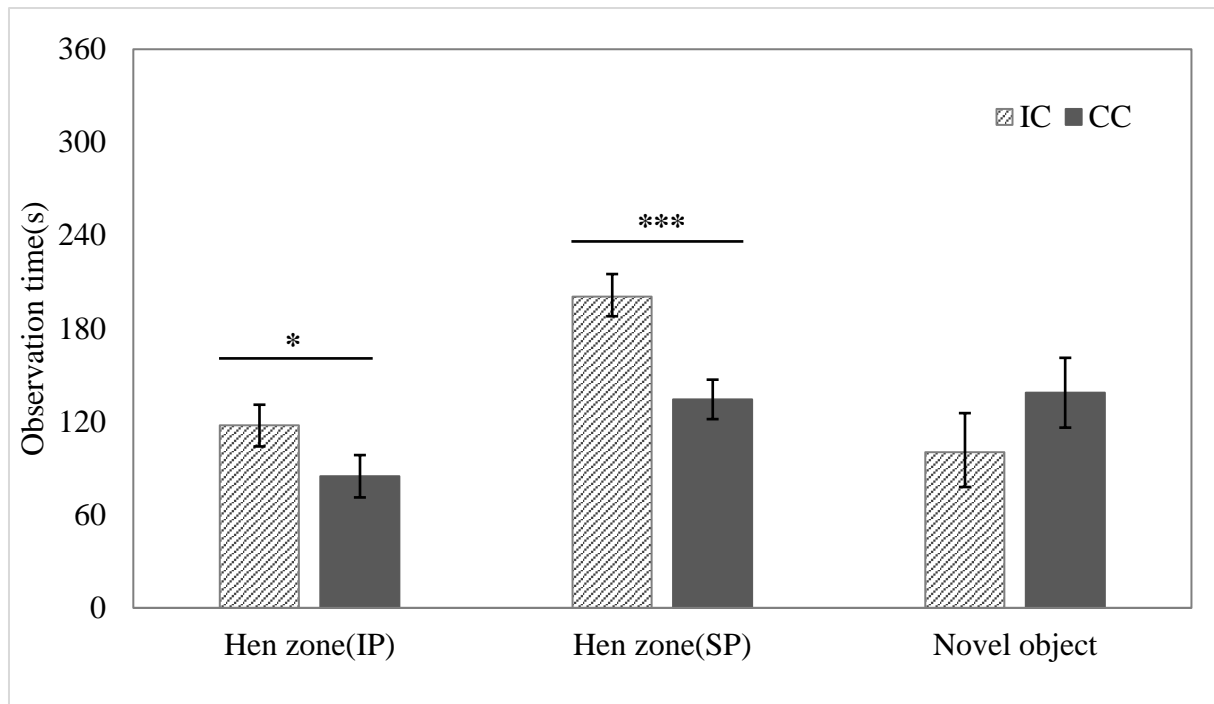


Figure 11. The mean value of time spent in the hen zone and the novel object zone. Imprinting Preference Test (IP), Social Preference Test(SP) and the novel object placed in the Social Preference Test. Asterisk (*) indicates statistical significance of $p < 0.05$; asterisks (**) indicate statistical significance of $p < 0.01$ and asterisks (***) indicate statistical significance of $p < 0.001$.

3.2.Social Preference Test

The latency was highly significant between treatments (Figure 4. $t(75)=2.532$; $p=0.007$) and got shorter after replication(SP1) (Figure 10. $t(75)=2.995$; $p=0.002$). It showed that imprinted chicks had a shorter latency to approach each novel object or imprinting stimuli than the control ones. Both results of time spent in the middle zone and the novel object were not significant regardless of treatment and sex. However, there was a significant difference in time spent in the hen zone (Figure 11. $t(75)=-3.444$; $p < 0.001$). Imprinted chicks tended to stay near imprinting stimuli. After replication, the preference towards the hen zone remained mostly the same despite a slight decrease ($t(75)=-2.161$; $p=0.017$).

Even though sex was not a meaningful measurement for previous assessments (the Imprinting Preference Test), there was a time when we saw sex preferences for females staying in the hen zone (Figure 12. $t(75)=-3.004$; $p=0.002$). Yet, this result is not enough to claim that there is a strong sex preference since the lack of data in the replication as well.

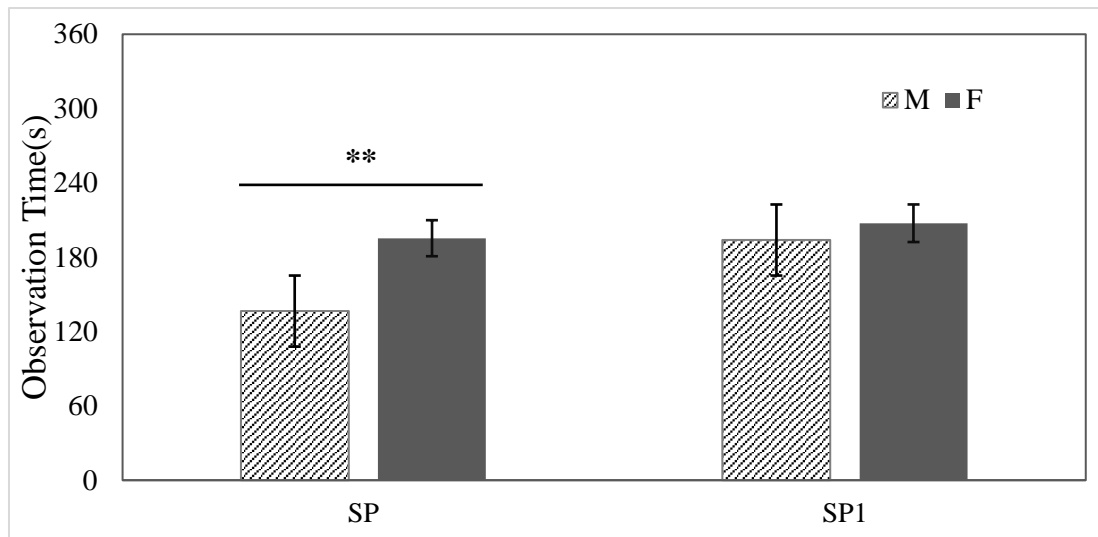


Figure 12. Mean value(s) of time spent in the hen zone according to sex. Social Preference Test (SP) and replication (SP1), the bar graph shows sex preferences for spending time (s) in the hen zone. Asterisks (**) indicate a statistical significance of $p < 0.01$.

3.3.Social Isolation Test

In Social Isolation Test, the results showed that there were not any significant results among treatments as well as after replication of the experiment (Figure 13. $t(75) = -1.466$; $p = 0.073$). We observed a minor, yet significant result between sexes, only during the initial trial of the Social Isolation test (Figure 14. $t(75) = 2.131$; $p = 0.018$) and it came up with males having more peeps after isolation.

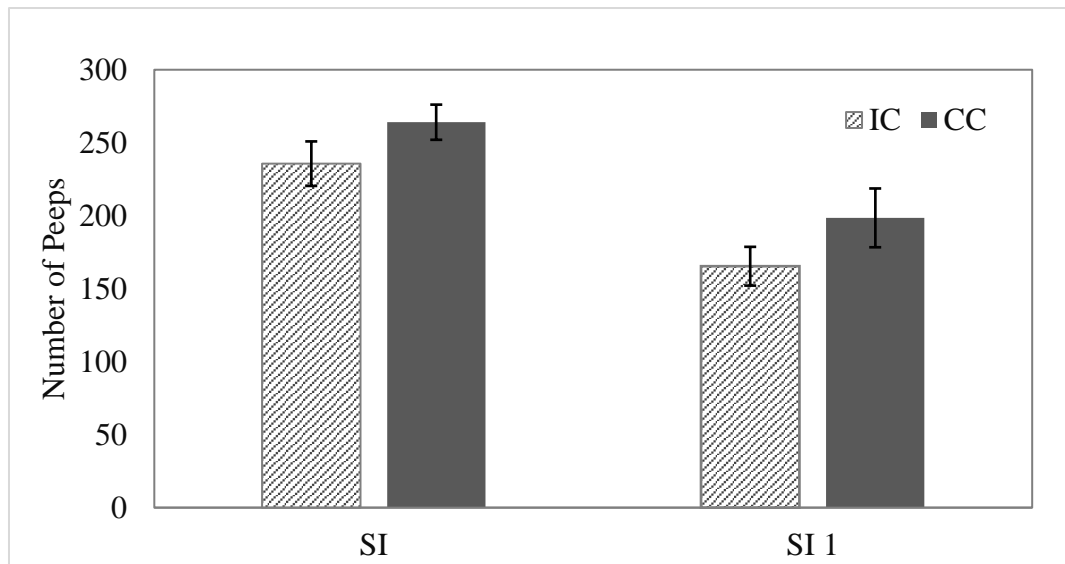


Figure 13. The number of peeps. Social Isolation test (SI) and replication (SI 1, 8 weeks old). The changes in the number of peeps after isolation.

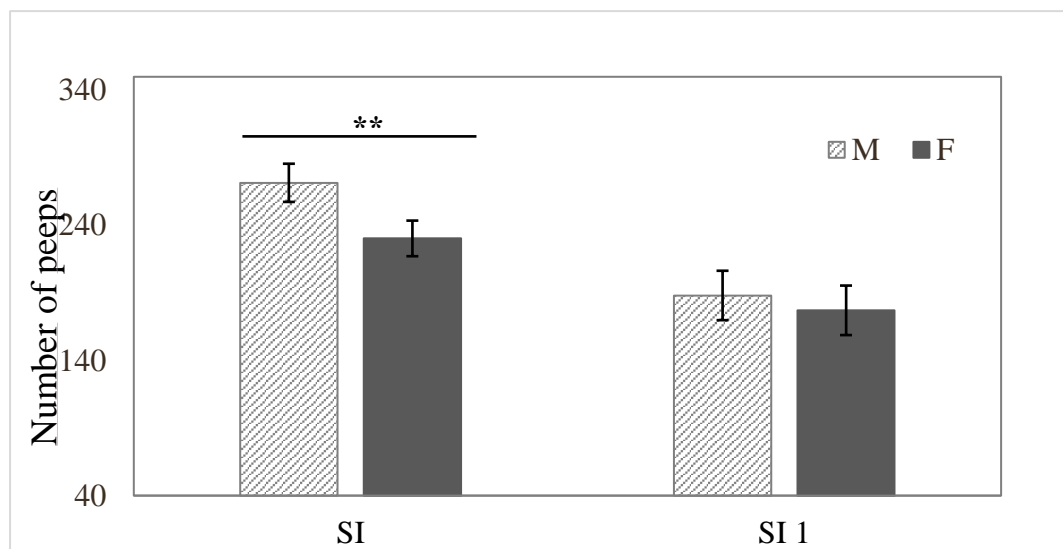


Figure 14. The number of peeps. Social Isolation test (SI) and replication (SI 1, 8 weeks old). Sex differences in the number of peeps after isolation. Asterisks (**) indicate a statistical significance of $p < 0.01$.

4. Discussion

Due to the uncertainties involved in estimating the duration of filial imprinting and its possible long-term effects on chicks' social behaviour, we carried out the current study to illuminate this problem. To investigate this, we ran over different experiments which are all connected to the imprinting procedure.

4.1. Imprinting Preference Test

Results showed that chicks imprinted on stimuli that we presented, and the duration of imprinting remained constant. The imprinting procedure had a greater impact on both Imprinting Preference and Social Preference Tests in terms of approaching imprinting stimuli. As expected, latency was highly consistent between individuals, as well as after replicating each test, imprinted chicks took less time to move towards imprinted stimuli than the control chicks. This is also in line with previous studies where they stated that chicks make an attachment to visually moving objects and show predisposed to reach moving objects after being exposed to stimuli for more than 30 minutes (Bateson, 1966; Martinho, 2016; Kacelnik, 2016). Thus, this behaviour of imprinted chicks would have an impact on the permanency of imprinting. By this I mean, after the imprinting procedure, we placed the imprinted chicks into pens where we also put stuffed hens. Therefore, one possibility is that chicks continued to imprint visually. Another reason for the reduction in latency could be the presence of two different stimuli in the imprinting procedure (e.g. tidbitting sound and visual stuffed hen). Previous studies claimed that exposure to auditory stimuli with visual stimuli during the imprinting procedure had significantly influenced chicks' preferences toward visual stimuli. Therefore, during the imprinting procedure, the combination of several stimuli has a stronger effect on learning than being presented alone. (Van Kampen, 1991). Pitz & Ross (1961) addressed the impact of being exposed to several stimuli. The study focused on visual cues on imprinting behaviour by associating moving objects with loud sounds. They trained three-day-old chicks under three conditions. One of the experimental group were exposed to the loud clapper in the presence of a moving object whereas the other experimental group were unable to see the moving object where the sound occurred. Their findings showed that chicks could see the object when the bang was sounded and displayed the highest following behaviour towards moving objects. Therefore, we can understand the importance of multiple stimuli that contribute to the early learning process in the context of imprinting behaviour. Regards to incubation in the darkness could also affect the asymmetry since the last stage of

incubation is where the lateralization occurs by exposure to the light. So light experience is important for the development of chicks' visual discrimination. In our experiments even though there is a possible impact of the absence of light during incubation, it would not affect our results since the light conditions were the same for either control and the imprinted chicks.

4.2. Social Preference Test

In the Social Preference test, we ran the test in the arena where the novel object involved imprinting stimuli. According to the findings, the imprinted chicks displayed shorter latency in the replication compared to those in Experiment 1. Moreover, the latency to approach decreased for the control chicks. The reason would be the reaction of fear or coming across an undefined object for the first time (Bateson, 1976). Therefore, it is reasonable to suggest that the avoidance behaviour displayed by control chicks toward novel objects could be a result of endogenous processes such as inheritability of filial imprinting or characterization of predisposition by neural mechanism. A previous study which focused on newly hatched chicks had not been exposed to any visual stimuli before testing in darkness, shows a correlation with our findings. In that situation, it showed that chicks had spontaneous preferences for face-like configurations compared to artificial objects or distorted versions of the same visual stimulus (Di Giorgio, 2017).

In the hen zone, imprinted chicks were more tend to stay here in both the first trial of the experiment and its replication, compared to the time chicks spent in Experiment 1. It shows that chicks could clearly discriminate imprinting stimuli as soon as they come across novel stimuli. This could mean that approaching and spending more time in the hen zone would be the reflection of the aversive behaviour towards novel objects with in contrast to the previous study explained that prolonged exposure to novel stimuli can alter chicks' preference towards novel objects (Salzen, 1968; Meyer, 1968). Unlike the Imprinting Preference Test, we observed minor sex preferences, with females spending more time in the hen zone which has been previously underlined that females prefer familiar stimuli more than males (Lemaire, 2021). Recent studies claimed that sex differences can be observed in the social behaviour of chicks when they are placed in an environment designed to mimic their natural surroundings. Workman & Andrew (1989) reported that females are more likely to stay within the sight of a hen compared to male chicks of the same age, which indicates females have a stronger social

attachment to parents than males. Therefore, we can interpret that females have a stronger tendency to build social contact with imprinted stimuli and it indicates that there might be sex differences in the presence of a novel object which is correlated with our findings. However, since we did not have the same result in the replication, it is not enough to report sex differences in reacting to undefined objects.

4.3.Social Isolation Test

In the Social Isolation test, we tested the number of chicks peeping when socially isolating them. We hypothesized that imprinted chicks and being exposed to imprinting stimuli during the study would make less distress calling. Our results showed that there is no strong correlation between treatments; we only saw a slight difference in peeping frequency, with control chicks, having more distress calling. Even though we calculated peeping frequency to observe the imprinting effects in the absence of companion or imprinting stimuli, we observed that distress call was not the only reaction when they are in a fear-related situation in this test. Besides peeping, flying over to the box or freezing is also an important response to be considered, which is categorized as a depressive-like syndrome (Katz, 1981). Also, a pharmacological study done by E. Lehr, showed that the application of antidepressant compounds on socially isolated chicks induced an approximately 50% decrease in distress calls (Lehr, 1989). Therefore, another fragment in our results that the imprinting procedure had no substantial effect on distress calls or indicating stress itself could be the changes in main factors during the entire study(e.g. temperature, changes in age, lack of companion). Kaufman (1961) found in his study that distress calls in naive chicks decreased with age at 60 °F which is related to our findings even though, the current study does not bear on the effect of temperature changes. Thus, considering previous research, claiming that social bond is still formed at 72-96 hours after hatching, it is likely to be the period when we separated each chick for applying other experiments in the current study.

In terms of sex differences in peeping frequency, our findings showed higher distress calling in males than females. Regarding the results, we can interpret that female may imprint stronger and this connection let them cope with a stressful situation. However, as we mentioned above, the indication of distress can be interpreted in different ways. Jonas (1977) claimed that females have higher distress calling than males after isolation whereas males are more tend to be in a lying or sitting position. In addition to that, another open-field study done

by Faure (1975) suggested an inverse relationship between fear and distress calling by proposing that fear has an inhibitory effect on birds' distress calling or an action.

4.4. Conclusion

It can be concluded from the present study that the imprinting procedure with 90 minutes of exposure to chicks was successful in terms of the first indication of imprinting stimuli. The prolonged exposure to imprinting stimuli such as placing imprinted chicks inside the pens with visual stuffed hen and replicating the experiment several times influenced chicks' filial imprinting preferences. Our observation showed that imprinting shortened the chicks' approaching time to the stimuli rather than staying in the stimuli zone (hen zone with tidbitting sound). Also, The latency period consistently decreased over time. However, using novel objects had more effect on the time spent imprinting stimuli between treatments. Imprinted chicks were interacting for a longer time with imprinting stimuli instead of exploring the novel object. Similarly, control chicks were also more prone to be in the stimuli zone in the presence of novel objects. Based on the results, the effect of imprinting on peeping frequency could be in line with our hypothesis, associating distress calls as a sign of coping with stressful conditions. On the other hand, the relation between imprinting and peeping frequency can be interpreted in different ways which need to be focused more. Regarding the effect of the imprinting procedure on chick learning, focusing on more artificially stimulated features for further studies, imprinting can be a practical way of improving chicks' welfare.

5. Societal and Ethical Considerations

The use of animals in scientific research creates major societal and ethical concerns. It is crucial to be certain about the welfare of animals is taken into account. As contribute the animal research, we are responsible to link scientific knowledge to the practical movement that turns out to be beneficial for animals and humans. Regarding ethical concerns, in my study, I ran the process by covering the education in Laboratory Animal Science, LAS, correlated with current Swedish animal welfare laws. My priority in this study to treat chickens with respect and be ensure that I am providing the all needs for their welfare.

Even though chickens as research animals could be categorized as convenient to work with because of being resilient animals and easy to handle, there are several aspects that must

consider; avoiding stressful conditions during hatching (temperature, humidity), providing appropriate housing, including the extension of pens when chickens get bigger, the exposure of light and darkness as in nature and preparing a steady feeding schedule. For two months experimental period, it was important for me to take my steps carefully without discomforting or putting the chicks in an unnecessarily stressful situation. Since the study has collaborated with another research where chicks were also used in different tests, avoiding testing on the same day was an integral part of my research plan.

From a societal standpoint, imprinting is a sensitive process after hatching that is affected any possible exposure and might influence chickens' development. Thus, as in my study, researchers are conducted with imprinting procedures that could have manipulative implications on chickens which affects their welfare. By this I mean, for example, handling during the imprinting period would change the dynamics during commercial egg production. Unappropriated handling or environment is likely to result in stress-related behaviour and eventually negatively affect commercial egg production.

On the other hand, well-treatment could increase productivity as well as social cohesion at the group level. Regarding this, it can also shed light on another societal consideration which is related to the science world. Since imprinting behaviour is a process that both humans and animals experience, further studies in different fields can bring alternative techniques for understanding imprinting itself from various perspectives concerning welfare improvement.

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