Department of Physics, Chemistry and Biology

Master Thesis

Control and Development of the Autonomic Nervous System in Posthatch Broiler (*Gallus gallus domesticus*) and Red Junglefowl (*Gallus gallus*)

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Sammanfattning/Abstract:

Heart rate is tonically regulated by the two branches of the autonomic nervous system (ANS); parasympathetic activation decreases heart rate while sympathetic activation increases it. Previous studies on the ANS in chickens have focused mainly on embryonic development, but it is largely unknown what happens in the weeks following hatching. The present study focused on the development of the autonomic nervous system in 2 and 5 week old broiler and Red Junglefowl (RJF). Since broilers are less fearful, less stressed and less active than their wild ancestor RJF, a possible domestication effect on the sympathetic nervous system was investigated by evaluating both physiological and behavioural responses during stress. I found that the heart is mainly under control from the sympathetic nervous system in 2 and 5 week old broiler and RJF as propranolol significantly decreased heart rate during baseline and stress conditions while injection of atropine had little or no effect on baseline heart rates. When the adrenergic tone was blocked, heart rate still increased during stress, more so in 5 week old birds than in 2 week old birds. This suggests that some other physiological regulatory mechanism with fast recruitment is involved in the stress response and it matures in the weeks following hatch. No differences in behaviour between broiler and RJF were observed when the sympathetic nervous system was blocked. As both breeds show similar responses, a domestication effect on the ANS from these results cannot be confirmed.

Nyckelord/Keyword:

Adrenegic tone, Autonomic nervous system, Broiler, Cholinergic tone, Heart rate, Red Junglefowl (RJF)

Content

1 Abstract

Heart rate is tonically regulated by the two branches of the autonomic nervous system (ANS); parasympathetic activation decreases heart rate while sympathetic activation increases it. Previous studies on the ANS in chickens have focused mainly on embryonic development, but it is largely unknown what happens in the weeks following hatching. The present study focused on the development of the autonomic nervous system in 2 and 5 week old broiler and Red Junglefowl (RJF). Since broilers are less fearful, less stressed and less active than their wild ancestor RJF, a possible domestication effect on the sympathetic nervous system was investigated by evaluating both physiological and behavioural responses during stress. This study found that the heart is mainly under control from the sympathetic nervous system in 2 and 5 week old broiler and RJF as propranolol significantly decreased heart rate during baseline and stress conditions while injection of atropine had little or no effect on baseline heart rates. When the adrenergic tone was blocked, heart rate still increased during stress, more so in 5 week old birds than in 2 week old birds. This suggests that some other physiological regulatory mechanism with fast recruitment is involved in the stress response and it matures in the weeks following hatch. No differences in behaviour between broiler and RJF were observed when the sympathetic nervous system was blocked. As both breeds show similar responses, a domestication effect on the ANS from these results cannot be confirmed.

2 Introduction

The autonomic nervous system (ANS) controls heart rate by antagonistic activity between the stimulatory sympathetic branch of the ANS and the inhibitory parasympathetic branch. At all times, both ANS nerve branches are firing on the sinoatrial node. The constant firing rate from sympathetic neurons is called the sympathetic tone and the same is true for the parasympathetic tone. Consequently, heart rate is regulated by altering the frequency of action potentials in both ANS branches. Tonic control of heart rate is modulated in this way. Events to which an animal is exposed to trigger different ANS responses; eating stimulates the cholinergic tone of the parasympathetic nervous system (rest-and-digest), while escaping a predator stimulates the adrenergic tone of the sympathetic nervous system (fight-or-flight). In a stressful situation, the stress response increases the adrenergic tone. The physiological responses include increased heart rate and respiration, vasodilation and glucose production in the liver to be able to provide the muscles in the body with oxygen and energy during fight or flight (Siegel 1980).

Previous studies on the ANS in chickens have focused mainly on embryonic development. While some studies showed that there is a presence of an adrenergic tone but no cholinergic tone from day 12 onwards in embryonic development until hatch in White Leghorn (Crossly & Altimiras 2000, Saint-Petery & Van Mierop 1974, Tazawa et al. 1992), other studies showed the presence of a cholinergic tone (Höchel 1998, Chiba 2004). The presence or absence of a cholinergic tone in chick embryos has been controversial and what happens the weeks following hatch is largely unknown. In particular it is unclear if the ANS undergoes functional maturation posthatching as it does in other species (Altimiras 2009).

The Red Junglefowl (RJF) is considered the wild ancestor of today's domestic fowls. Domestication causes morphological and behavioural changes in the animals subjected to the domestication process. Broilers, a domesticated fowl bred for meat production, are less active (Bessei 1992) and less fearful than their wild ancestor RJF. This difference in behaviour suggests an underlying physiological change caused by domestication, possibly a decrease in sympathetic innervation due to its involvement in the stress response.

The aims of this study were (i) to investigate the autonomic control of the heart in broiler and RJF after hatch using pharmacological inhibition of the autonomic nervous system. By use of atropine, the parasympathetic nervous system was supressed by blocking cholinergic post-synaptic receptors (Altimiras et al. 1997). In the same way propranolol, a β – blocker, was used to supress the sympathetic nervous system by acting as an antagonist to the adrenergic tone (Altimiras et al. 1997). Furthermore, this study investigated (ii) if there is a maturation process and/or (iii) a domestication effect on the autonomic control of the heart. The last aim of this study was (iv) to investigate if behavioural changes due to the domestication of broiler chickens are associated with differences in sympathetic activity by analysing differences in activity and vocalization during stress while inhibiting the sympathetic nervous system.

3 Material & methods

3.1 Animals and management

47 broiler chickens and 34 RJF chickens were used in this study. The chickens were kept in the animal facility at Linköping University and had

ad libitum access to food and water. All procedures were carried out under ethical application Dnr.9-13.

The broiler chicks were sexed at hatch using the feather-sexing method and only female chicks were kept for the study, while male chicks were euthanized. RJF chicks at 2 weeks of age were sexed through dissection after the data collection was completed and 5 week old chicks were sexed by appearance. In total, there were 14 female and 20 male RJF.

The animals were divided into two age groups; 2 and 5 weeks of age, and within each age group there were two treatment groups; control and drug treatment (propranolol). The number of animals varied between groups, see Table 1.

Table 1: Number of individuals in each group.

	2 wks	**5 wks**
Broiler		
Control	12	13
Propranolol	11	11
RJF		
Control	8	8
Propranolol	9	9

3.2 Electrocardiographic monitoring and activity

When placing the electrodes used for ECG-measurements, the birds were placed on their backs and the electrodes were positioned subcutaneously at each side of the carina and secured with surgical tape. The wires were also secured with surgical tape on the birds' back in order to maintain a strong signal throughout the measurement.

LabChart 7 (ADInstruments) was the programme used to record data on heart rate and activity. The electrodes used to measure heart rate were connected to an impedance converter (UFI model 2991), which translates the signal to PowerLab (model 26T LTS) connected to LabChart 7.

The restraint bag, which was used as a stressor during the heart rate measurements, was suspended on a force transducer. The force transducer was also connected to PowerLab, via a Bridge AMP (FE221), and registered the activity of the birds while in the bag.

3.3 Administration of drugs

In this study, two drugs were used; the β-adrenoreceptor propranolol and the muscarinic blocker atropine. Propranolol blocks the sympathetic tone that controls heart rate and atropine blocks the parasympathetic tone. Together they completely block the autonomic nervous systems control on heart rate.

The birds receiving drugs were administered 3 mg kg^{-1} propranolol and 1.5 mg kg^{-1} atropine intraperitoneally. RJF control birds were injected with 0.9 % saline solution to make sure that the effect seen on heart rate was from the drug and not the injection. However, broiler control birds did not get a saline injection due to over-sight.

3.4 Experimental protocol

3.4.1 Short-term ECG measurements

All birds were weighed before the measurement started. Birds in drug treatment groups were infused with propranolol, RJF control birds were infused with saline and broiler control birds were not infused. The electrodes were placed and secured with surgical tape when a strong signal was seen. The birds were then placed in a dark box for 20-30 minutes to obtain a baseline measurement of heart rate. After the baseline measurement, the birds were subjected to a stressor; i.e. restraining in a stress bag for 5 minutes. Vocalizations by the birds while in the restraint bag were also recorded using a dictaphone (Olympus, VN-2100PC) hung in close proximity to the bird.

3.4.2 Long-term ECG measurements

All procedures were as described above but the birds were kept in a dark box for 2 hours instead of 20-30 minutes. After 2 hours the bird was infused with the muscarinic blocker atropine and put back into the dark box for 1 hour for subsequent heart rate measurement. After the atropine measurement, the bird was infused with propranolol, thereby blocking the autonomic nervous systems control on heart rate completely, and once again put back into the dark box 20-30 minutes. 2 week old birds were accompanied by another bird during the entire procedure.

3.5 Data analysis

Baseline heart rate was taken as the average value over 30 seconds towards the end of the heart rate trace when the heart rate was stable. Stress heart rate was taken from a 5 second average directly after the bird was placed in the bag and hung on the force transducer to acquire the initial rise in heart rate due to stress (Figure 1).

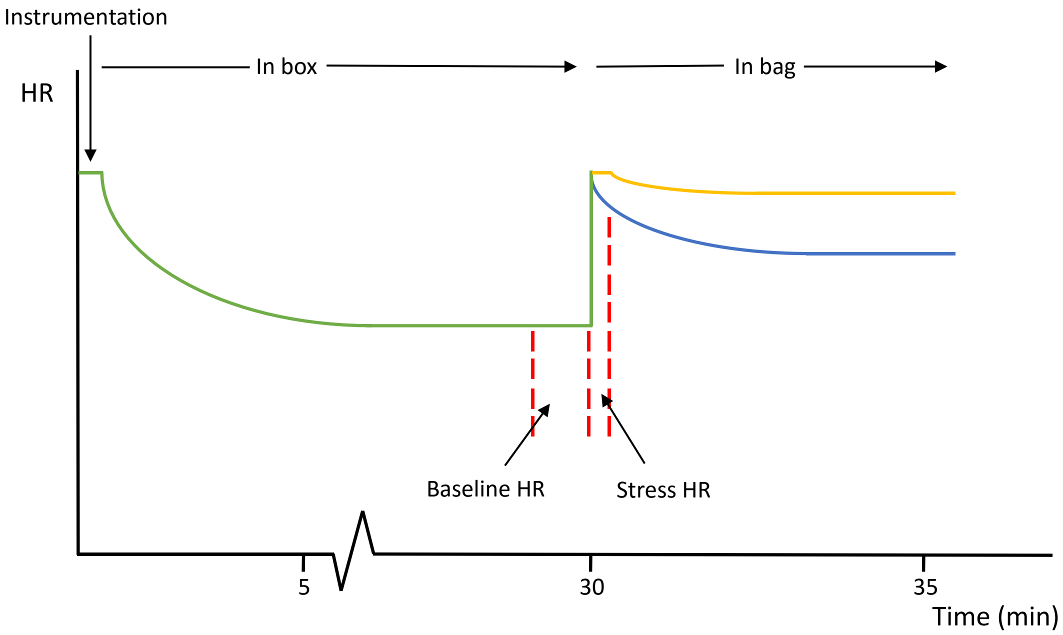

Figure 1: Expected heart rate-trace during short-term ECG measurements; green line represents baseline, yellow line represents RJF and blue line represents broiler. Broken red lines indicate where baseline and stress heart rate data was collected from the trace.

Permutation tests were performed with Statboss Permutation test v1.0 between all group treatments and ages to determine differences. Permutations test is a test used for analysing differences between group means and has been argued to be superior to *F* and *t*-tests in biomedical research (Ludbrook & Dudley 1998, Lew 2008).

The adrenergic and cholinergic tones were calculated using the GII formula as in Altimiras and colleagues (1997).

Activity was analysed in LabChart 7 by summing up peaks from each oscillation made by the birds' movement in the stress bag during 5 minutes. Head movements were excluded, i.e. only peaks made from oscillations made by the entire body were registered (Figure 2).

Vocalizations were analysed in Adobe Audition CC 2014. Previous studies by Collias & Joos (1953), Kruijt (1964), Andrew (1964), Collias (1987) and Marx and colleagues (2001) have shown that chickens have a large vocal repertoire with several different calls occurring dependent on

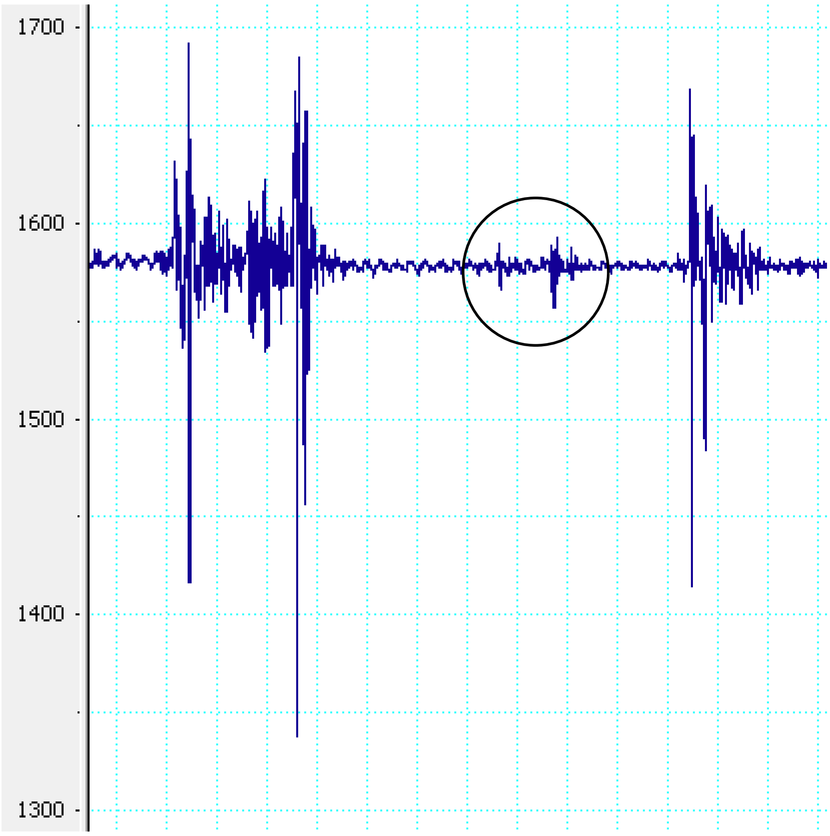

Figure 2: Figure illustrating body and head movement during bag restraint. Head movement (encircled) was excluded.

what situation the chicken is in. In the present study, the chickens' vocalisations were recorded while the chicken was expected to experience stress. Therefore, the calls relevant to the study are Distress calls (DC) and Fear trills (FT) (Figure 3a, 2b). Calls occurring that were not DC or FT were noted as Other calls (OC). Each call made by the birds during the 5 minute period in the stress bag were sorted into one of the three categories DC, FT or OC and summed.

Statistical analysis of activity and vocalization were made with permutation tests, Statboss Permutation test v1.0.

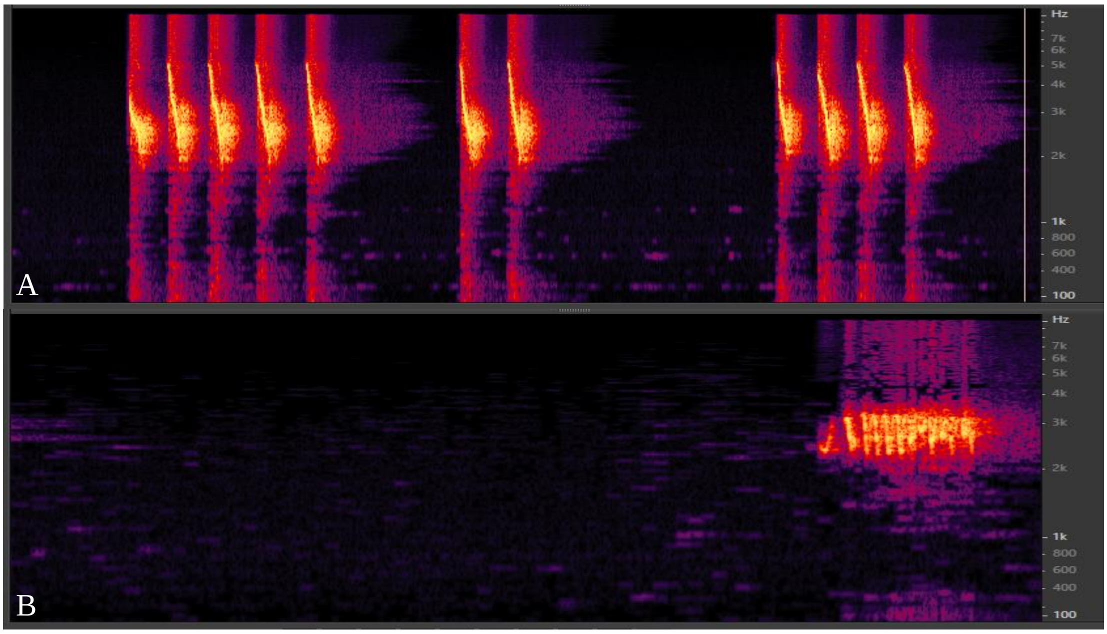

Figure 3: Sonogram of distress call (A) and fear trill (B).

4 Results

4.1 No sex differences between male and female RJF

As RJF of both sexes were used in this study, differences between the sexes were investigated. No significant differences were found between males and females in the treatments or in mass as expected since they were not sexually mature (appendix, Table 2).

4.2 Blockade of the sympathetic nervous system does not affect the activity and vocalization of chickens during stress

There was no significant difference in activity during bag restraint between the breeds (Figure 4). There is large inter-individual variation within the groups, while some animals did not struggle at all during the 5 minute bag restraint, other moved much more.

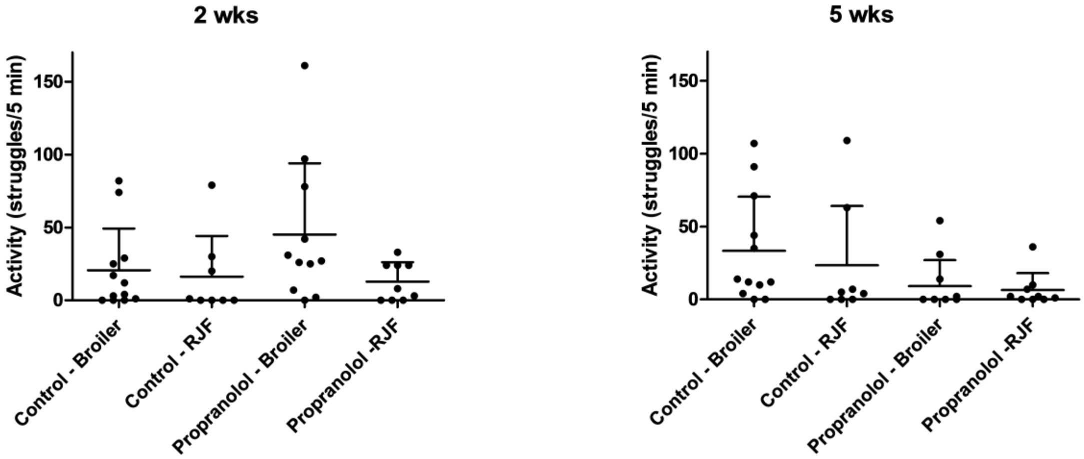

Figure 4: Activity, number of struggles registered in 5 minutes during stress in the restraint bag. Data shown as mean (standard deviation).

In general, distress calls were the most common and 36% of all birds produced them, while only 8% of the birds made fear trills. An important note is that not all birds vocalized while in the restraint bag; 49 % of all broilers and 26 % of all RJF chickens vocalized. Put together 38 % of all chickens vocalized and out of these birds, 93 % made distress calls and 20 % made fear trills. Broilers treated with propranolol vocalized more FT than RJF at 2 week of age (Permutation test, p=0.02, Figure 5C). The difference might appear to be outlier driven, however, when removing the outlier the difference was still significant. No other significant differences were found in DC, FT or OC between the two breeds at different ages. The vocalization data has, similarly to the activity data, large individual variation within the groups.

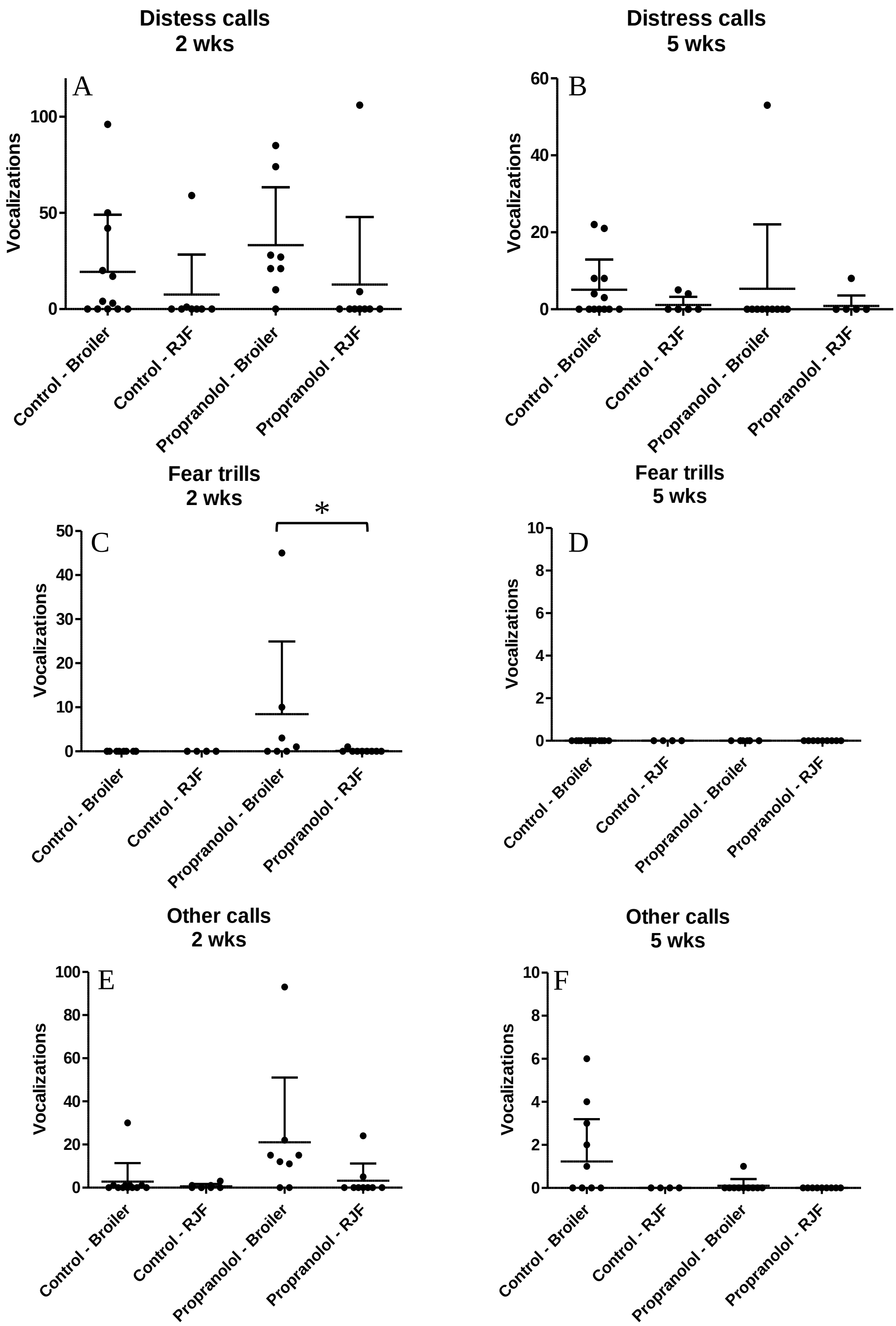

Figure 5: Number of vocalizations while in the restraint bag for 5 minutes. Data shown as mean (standard deviation).

4.3 Heart rate is largely controlled via stimulation of the adrenergic tone

Short-term measurements were done in order to investigate the stress response after blocking the adrenergic tone with propranolol. Heart rate decreased in all groups during both baseline and stress conditions after injection of propranolol. In most groups, heart rate increases significantly during stress when comparing to the baseline heart rate at 20-30 minutes and the heart rate obtained during the initial stress in the restraint bag. As expected, stress heart rate increased from the baseline heart rate in control conditions in all cases except for RJF 2 weeks (Figure 6A and B). After injection of propranolol the expectation was that the difference between baseline and stress heart rate would decrease or even potentially disappear. Indeed, the difference between heart rates did decrease significantly in broiler chicks (Figure 6C and D). In RJF, a clear numerical decrease was observed between baseline and stress heart rate, but not a significant one (Figure 6C and D). To clarify the effect propranolol has on heart rate during stress, how much of the heart rate increase propranolol blocked in percent was calculated. In broiler propranolol blocks 70% of the heart rate increase at 2 weeks of age and 46% at 5 weeks. In RJF propranolol blocks 88% of the heart rate increase in 2 week old birds and 33 % in 5 week old birds.

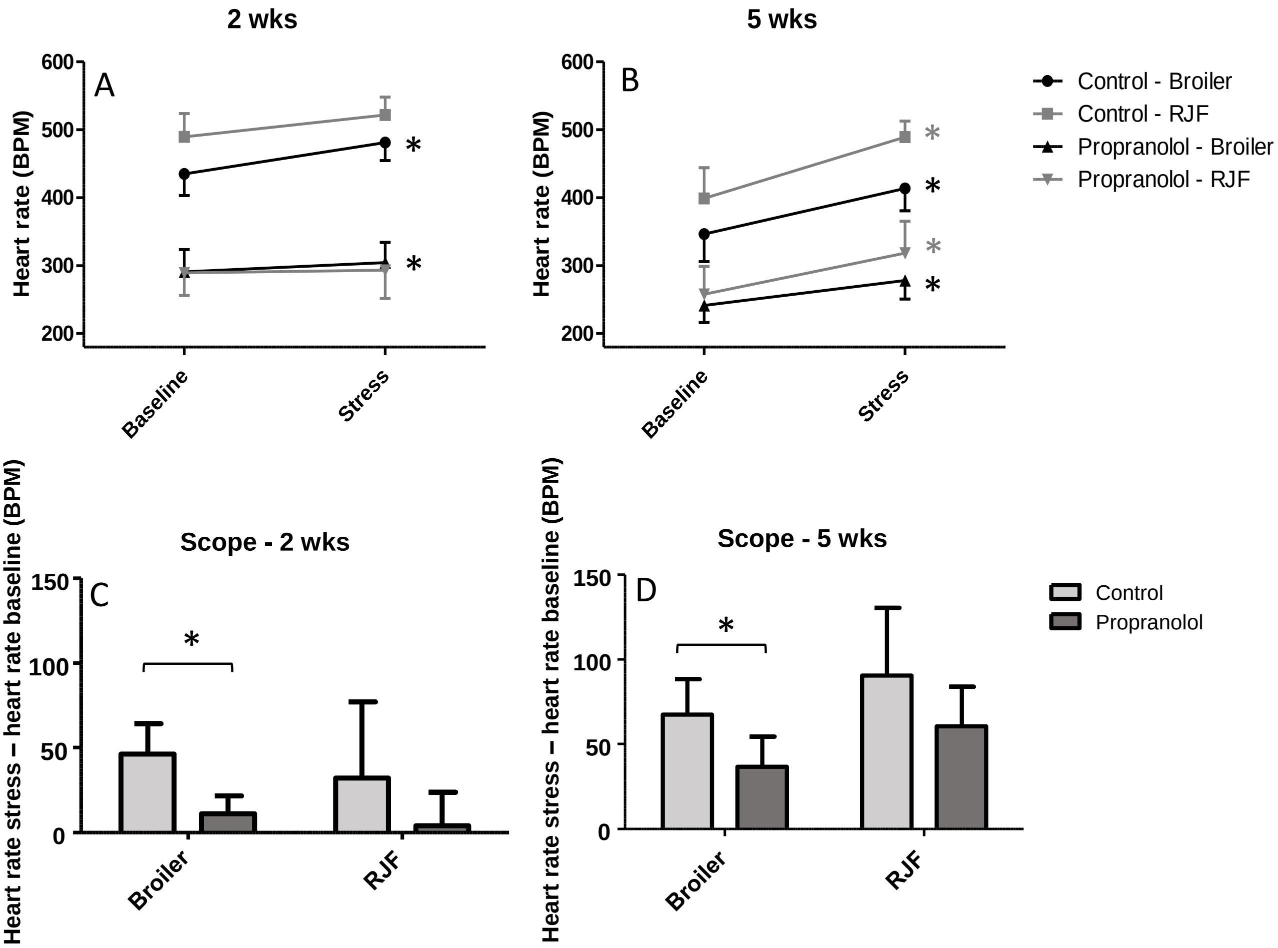

Figure 6: Heart rate at baseline and stress in 2 week old (A) and 5 week old (B) broiler and RJF chickens. Black stars represent significant differences in broiler and grey stars represent significant differences in RJF. Heart rate scope; difference between heart rates during baseline and stress in 2 week old birds (C) and 5 week old birds (D). Data shown as mean (standard deviation).

To understand the mechanisms regulating heart rate, long-term ECG measurements were performed on RJF. In the long-term experiments the administration of cholinergic and adrenergic antagonists allowed the calculation of cholinergic and adrenergic tones. The cholinergic tone in 2 week old birds is 3 ± 6 % and -10 ± 6 % in 5 week old birds (Figure 7). The adrenergic tone on the other hand is 33 ± 8 % and 22 ± 8 % respectively in 2 and 5 week old birds (Figure 7).

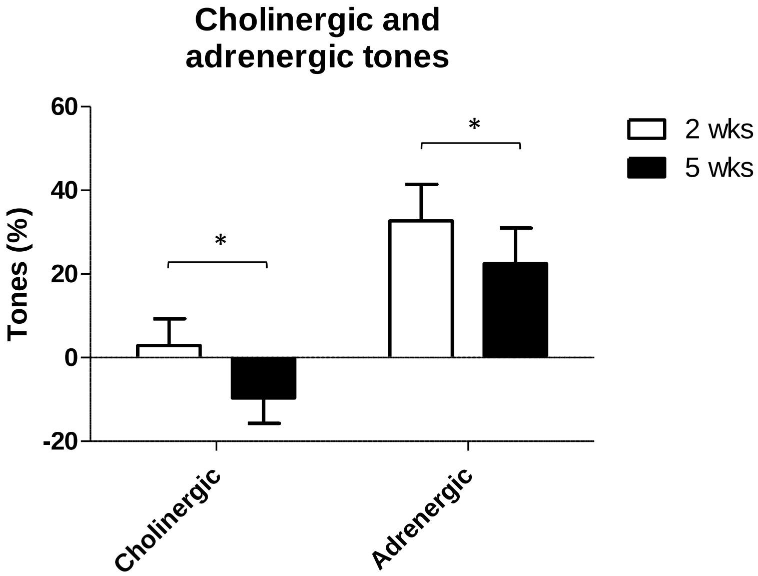

Figure 7: Cholinergic and adrenergic tones in percent in RJF. White bars represent 2 week old birds and black bars 5 week old birds. Data shown as mean (standard deviation).

In the long-term measurement baseline heart rate was taken after 20 minutes and 2 hours. When comparing the heart rate at these two times, it is significantly lower after 2 hours than 20 minutes in both 2 and 5 week old birds (2w: $p=0.03$; 5w $p=0.02$, Figure 8).

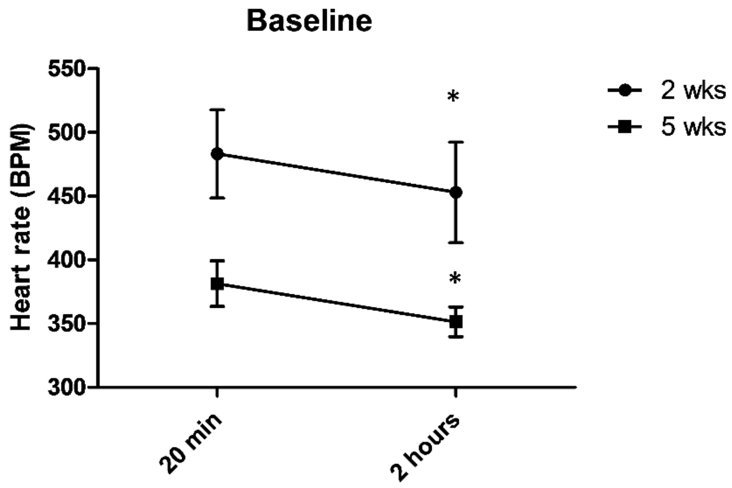

Figure 8: Baseline heart rate after 20 minutes and 2 hours. Circles represent 2 week old birds and squares 5 week old birds. Data shown as mean (standard deviation).

The second part of the protocol for the long-term ECG-measurement was injection of atropine. The expectation was that this would increase heart rate due to blockade of muscarinic receptors, however, 1 hour after injection of atropine heart rate was not significantly different from the 2 hour baseline in 2 week old birds (p=0.2, Figure 9A). Furthermore, in 5 week old birds, heart rate had decreased significantly 1 hour after the atropine injection in comparison to the 2 hour baseline (p=0.02, Figure 9B).

Lastly, propranolol was injected and the decrease in heart rate was significant for both ages as expected (2w: p=0.008; 5w: p=0.016, Figure 9A and B).

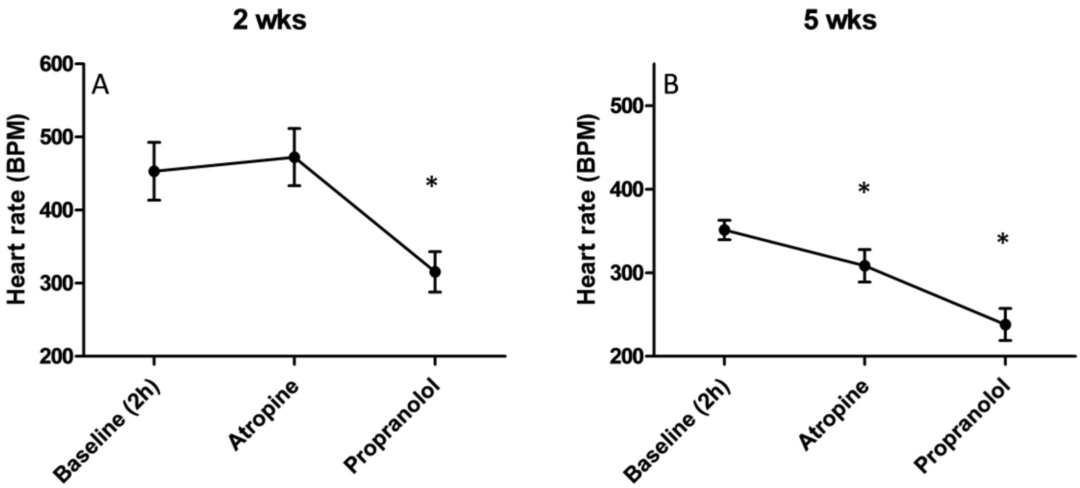

Figure 9: Baseline heart rate after 2 hours, atropine heart rate after 1 hour and propranolol heart rate after 20-30 minutes in 2 and 5 week old RJF birds (A and B resp.). Data shown as mean (standard deviation).

5 Discussion

This study shows that the heart is largely controlled by the sympathetic branch of the autonomic nervous system in the weeks following hatching, without an important contribution of the parasympathetic nervous system and this is the case for broiler and RJF.

5.1 Posthatch heart rate is mostly driven by an adrenergic tone on the heart

As shown in Figure 6A and B, when the adrenergic tone is blocked with propranolol there is a significant decrease in heart rate both during baseline and stress conditions in 2 and 5 week old broiler and RJF. Calculated tones on RJF also show an evident adrenergic tone in both 2 and 5 week old birds (Figure 7).

Previous studies on the embryonic development of the autonomic nervous system in chickens show that there is a presence of an adrenergic tone from day 12 until hatch in White Leghorn (Crossly & Altimiras 2000, Saint-Petery & Van Mierop 1974, Tazawa et al. 1992). These studies also showed that there is no presence of a cholinergic tone from day 12 of development (Crossly & Altimiras 2000, Saint-Petery & Van Mierop 1974, Tazawa et al. 1992). In contradiction, Höchel and colleagues (1998) and Chiba and colleagues (2004) show the presence of a cholinergic tone in chick embryos. Whether or not there is a cholinergic tone acting on heart rate in chick embryos is controversial, but all seem to agree that there is an adrenergic tone early on in development. The results from this study shows that after hatch, heart rate is mostly driven by the adrenergic tone.

5.2 Posthatch chickens lack cholinergic tone

After blockage of the cholinergic tone with atropine, the expectation would be for heart rate to increase (Pappano & Löffelholz 1947, Altimiras et al. 2009), but this does not occur in this study. In some groups a decrease in heart rate (Figure 9B) was found. One could argue that the reason heart rate does not increase after injection of atropine is because there is no time for returning to a true baseline but this does not seem to be the case. Control baseline values obtained in the present study are 435 ± 32 beats/min for 2 week old broiler chicks and 346 ± 40 beats/min for 5 week old broiler chicks. In a previous study, Olkowski and collegues (1997) measured baseline heart rate in male broiler with needle electrodes. The heart rate baseline acquired in their study was 385 and 364 beats/min for 2 and 5 week old broilers respectively. The value for 2 week old broilers is slightly higher in the current study however, the opposite is true for 5 week old broiler chicks. In another study on 5 week old female broilers the baseline value obtained via surgical implant was 337 beats/min (Wideman 1999). The conclusion is therefore that the baseline heart rates in the current study can be considered true baseline values as they are similar to heart rate values in previous studies. No previous studies on baseline heart rate in RJF were found. Since the baseline of the two strains was obtained in the same manner, the assumption is that the acquired values for RJF also can be considered as baseline heart rate. It is important to note that the lack of a cholinergic tone cannot be attributed to an insufficient dose of atropine. As shown in a previous study by Crossley & Altimiras (2000), 1 mg kg^{-1} is enough to block the parasympathetic nervous system. In the present study, a dose of 1.5 mg kg^{-1} was administered. Tests were also made on adult RJF showing that atropine blocked the effects of a subsequent acetylcholine bolus (unpublished).

Calculated cholinergic tones show that there is virtually no or a negative tone (Figure 6) in 2 and 5 week old RJF. The lack of a cholinergic tone is puzzling but there are two potential explanations that can account for its' absence. (i) Atropine blocks the cholinergic tone which prevents the decrease of heart rate due to parasympathetic stimuli (Altimiras 2009, Chiba et al. 2004, Crossley and Altimiras 2000, Höchel et al. 1997). Blocking this pathway without observing an increase in heart rate could indicate that the heart is not under control of the parasympathetic nervous system. However, it is unlikely that there would be no parasympathetic control of the heart. (ii) Blocking the cholinergic pathway inhibits acetylcholine (ACh), which is involved in nitric oxide-mediated vasodilation (Strauss & Persson, 2000). The inhibition of ACh may impede vasoconstriction, which leads to increased blood pressure. The baroreflex detects the increase in blood pressure and responds by decreasing heart rate in order to decrease blood pressure. However, the baroreflex is a fast acting reflex and its' effect diminishes with time (Altimiras & Crossley 2000, Elfwing et al. 2011, Strauss & Persson 2000). Therefore, its' effect on heart rate should be gone after one hour, yet the results of this study shows that there is no increase in heart rate after injection with atropine. However, as no measurements of blood pressure were made, it is unclear if the baroreflex was involved in the regulation of heart rate during the experiments.

The most likely scenario is that the heart, in fact, is under control of the parasympathetic nervous system, but the system is not being used for tonic control. Parasympathetic stimuli can, however, be recruited if needed i.e. the system is under phasic control, which is why atropine had little or no effect. Previous studies on chick embryos have shown that although the nervous system is fully developed (Pappano & Löffelholz 1974), some studies found no presence of a cholinergic tone in embryonic White Leghorn chicks (Crossly & Altimiras 2000, Saint-Petery & Van Mierop 1974, Tazawa et al. 1992). The experiments performed in the current study may simply not have been under conditions that would elicit the recruitment of a cholinergic tone in the birds.

5.3 Non-Adrenergic Non-Cholinergic regulation of heart rate

In 2 week old chickens propranolol has a major blocking effect on the stress response and it accounts for 70% of the increase of heart rate in broiler and 88% in RJF (Figure 6C and D). In 5 week old birds the effect of propranolol is only 46% and 30% in broiler and RJF respectively (Figure 6C and D). This suggests that some other component with fast recruitment is also involved in the stress response. As this other component has a larger effect in 5 week old birds, it is likely that it was not yet fully mature in 2 week old birds.

Cholinergic and adrenergic tones act together on the heart to regulate heart rate by increasing or decreasing tones. Therefore, a withdrawal of cholinergic tone would elicit an increase in heart rate which could be a possible explanation for the increase in heart rate seen when the adrenergic tone is blocked. However, as shown in this study, RJF lack a cholinergic tone in the experimental conditions. Thus, cholinergic withdrawal cannot account for the increase in heart rate upon stress after propranolol blockade. Other mechanisms that regulate heart rate include catecholamines from the adrenal medulla, whose effect is blocked by propranolol, and non-adrenergic non-cholinergic control mechanisms such as systems regulating blood pressure (Altimiras et al. 2009).

Part of the stress response is an increase in blood pressure (Siegel 1980) and although I did not measure it in the chickens, it is quite likely that bag restraint caused a pressure increase as other stressful procedures do. The renin-angiotensin-aldosterone system is a hormonal system which includes angiotensin II (ANG II) which regulates blood pressure through direct and indirect pathways. Among other things, ANG II stimulates an increase in heart rate and cardiac output to increase blood pressure. In adult chickens, blood pressure falls (hypotension) after injection of propranolol (Butler 1967, Nishimura 1981). The blood pressure increasing effects of ANG II might be the cause of the increase in heart rate seen in the propranolol treated birds during stress. The larger increase in heart rate in 5 week old birds than in 2 week old birds suggests that there is a maturation process of the renin-angiotensin-aldosterone system in chickens. Further investigation into the effects blocking the adrenergic and cholinergic tone has on blood pressure and the potential involvement and maturation of the renin-angiotensin-aldosterone system is needed.

5.4 Activity and vocalization

This study investigated whether or not the inhibition of the sympathetic nervous system would affect the activity of broiler and RJF during a stressful situation i.e. in the restraint bag. The results show no influence of the sympathetic nervous system on the activity of the birds as there were no differences between untreated birds and birds with their adrenergic tone inhibited. Thus, this study cannot confirm that the physiological stress response is involved in the control of the behavioural responses to stress since the experiment used showed no effect.

A previous study by Marx and colleagues (2001) showed that the number of distress calls increased during stepwise isolation of chicks. Chickens are social animals and being alone can be considered stressful for them. Thus, we know that chicks produce distress calls when stressed. But we do not know the underlying mechanism(s) that causes a bird to vocalize. As chicks produce distress calls more often in a stressful situation it is possible that the stress response is involved. When exposed to a stressor (the restraint bag) in this study, 36 % of all birds vocalized and 93 % of these birds made distress calls. Birds with their adrenergic tone inhibited did not vocalize less than untreated birds. Interestingly, when looking at breed differences, broilers vocalized more than RJF. 49 % of all broilers vocalized, while only 26 % of all RJF vocalized. This is most likely due to a domestication effect.

5.5 Conclusions

During early life in broiler and RJF chickens, the heart rate is largely controlled by the sympathetic nervous system as seen by the evident effect blocking the adrenergic tone has on heart rate. However, when the sympathetic nervous system is inhibited by propranolol there is still an increase in heart rate during stress. A suggested mechanism causing the increase in heart rate is the renin-angiotensin-aldosterone system, although further investigation into this matter is needed. The heart rate response to stress is larger in 5 week old birds than in 2 week old birds, suggesting a maturation process.

The current study also found that the birds in this study had no cholinergic tone on heart rate because inhibitors of parasympathetic tone had no effect on heart rate. It is unlikely that there would be no parasympathetic control of the heart and the most likely explanation is that the heart is not under tonic control from the parasympathetic nervous system, but phasic control.

The activity and vocalization responses to stress are most likely controlled by some other mechanism(s) than the autonomic nervous system as no differences were found in birds with their adrenergic tone inhibited.

5.6 Societal & ethical considerations

Broiler is the chicken breed bread for meat production. They have a short lifespan and are slaughtered when they are around 8 weeks old. There are a number of well know welfare issues concerning broilers due to the selective breeding for rapid growth and high energy conversion.

As with all production animals it is important to be able to provide good welfare for broiler chickens. One aspect of animal welfare is to make sure that the animal is not stressed. To be able to asses this, it is important to understand the basic underlying physiology of the animal and its' responses. The present study can contribute with some understanding of how the ANS works and responds in a stressful situation.

Furthermore, this research can be used in connection to determining which would be a suitable temperature to keep these animals in. A broiler with low heart rate scope is also expected to have a low metabolic scope. To provide the animals with the appropriate ambient temperature in order to minimise the amount of metabolic energy allocated to thermoregulation may not only improve animal welfare but may also provide a financial advantage for the poultry industry. Housing the animals at its’ thermo neutral zone would render the feed from which the energy is used for thermoregulation superfluous and hence that feed is not necessary to provide, which results in a reduction in feed costs without compromising the growth-rate of the broilers.

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8 Appendix

Table 2: No significant differences found in RJF between sexes in any groups.

Groups - RJF	P-value
Baseline	
Control 2 wks	0,6429
Control 5 wks	0,4857
Propranolol 2 wks	0,6429
Propranolol 5 wks	0,4127
Stress	
Control 2 wks	0,2143
Control 5 wks	0,1714
Propranolol 2 wks	0,9127
Propranolol 5 wks	0,8016
Mass	
Control 2 wks	0,1786
Control 5 wks	0,1143
Propranolol 2 wks	0,0952
Propranolol 5 wks	0,119