Heart and ventilation rate changes during tonic immobility in Ornate Tinamou (*Nothoprocta ornata*) and High Andean chicken (*Gallus gallus*) compared to Chilean Tinamou (*Nothoprocta perdicaria*)

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# Heart and ventilation rate changes during tonic immobility in Ornate Tinamou and High Andean chicken compared to Chilean Tinamou

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**Sammanfattning/Abstract:**

Animals can show different responses to fear for example by playing dead when there is no possibility to escape. This response is called tonic immobility (TI) and is a well-established test of fear to evaluate fearfulness. Long durations of TI are generally considered as high levels of fearfulness. Physiological changes observed during tonic immobility suggest that there are changes in the autonomic nervous system (ANS) strongly involved in this process. The main objective for this study was to analyse duration of tonic immobility and heart and ventilation rate during tonic immobility in three different species; domesticated High Andean chickens (*Gallus gallus*), wild-caught Ornate Tinamous (*Nothoprocta ornata*) and Chilean Tinamous born in captivity (*Nothoprocta perdicaria*). In this study needle electrodes were used to measure heart and ventilation rate. The time following induction of tonic immobility (i.e. after holding the bird on its back for 15 s) was characterized by a large increase in heart and ventilation rate. During tonic immobility a progressive decrease in heart and ventilation rate was observed in all species, significant in all cases except for heart rate between start and end of TI in chickens. The duration of TI was significantly longer in Ornate Tinamou compared to Chilean Tinamou and chickens. The same was observed in latency to first head movement. TI is probably controlled by the autonomic nervous system, but a heart rate variability analysis has to be done in order to determine the different relative contributions of the sympathetic and parasympathetic systems in these species.

**Nyckelord/Keyword:**

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1 Abstract

Animals can show different responses to fear for example by playing dead when there is no possibility to escape. This response is called tonic immobility (TI) and is a well-established test of fear to evaluate fearfulness. Long durations of TI are generally considered as high levels of fearfulness. Physiological changes observed during tonic immobility suggest that there are changes in the autonomic nervous system (ANS) strongly involved in this process. The main objective for this study was to analyse duration of tonic immobility and heart and ventilation rate during tonic immobility in three different species; domesticated High Andean chickens (Gallus gallus), wild-caught Ornate Tinamous (Nothoprocta ornata) and Chilean Tinamous born in captivity (Nothoprocta perdicaria). In this study subcutaneous needle electrodes were used to measure heart and ventilation rate. The time following induction of tonic immobility (i.e. after holding the bird on its back for 15 s) was characterized by a large increase in heart and ventilation rate. During tonic immobility a progressive decrease in heart and ventilation rate was observed in all species, significant in all cases except for heart rate between start and end of TI in chickens. The duration of TI was significantly longer in Ornate Tinamou compared to Chilean Tinamou and chickens. The same was observed in latency to first head movement. TI is probably controlled by the autonomic nervous system, but a heart rate variability analysis has to be done in order to determine the different relative contributions of the sympathetic and parasympathetic systems in these species.

2 Introduction

The most common response for animals to fear or threat is the “fight-or-flight” response which is characterized by an increase in blood pressure, heart rate and dilation of blood vessels in muscles (Alboni et al. 2008). But animals can show different responses to fear and threat for example by playing dead when there is no possibility to escape (Alboni et al. 2008). This response is called tonic immobility (TI) and is characterized by a temporary suppression of the righting reflex (Jones 1986). It may last from seconds to hours and is thought to be a distant-dependent defence mechanism that can reduce the risk of the predator continuing its attack (Thompson et al. 1981, Jones 1986). It has previously been shown by Thompson et al. (1981) that cats choose to attack an active quail more often than an immobile quail.
Tonic immobility is a well-established test of fear and is often used in domesticated animals to evaluate fearfulness (Forkman et al. 2007). A long duration of TI is generally considered as an indication for high levels of fearfulness. It has also been shown that procedures to reduce fear such as handling can reduce TI duration, and vice versa (Gallup et al. 1972, Nash et al. 1976a, Jones et al. 1991). Tonic immobility tests have previously been carried out on domesticated chickens and quail (Valance et al. 2008, Forkman et al. 2007, Gentle et al. 1989). It has also been done in many other different species such as pigs, sheep, rabbits, reptiles and amphibians (Forkman et al. 2007, Nishiumi et al. 2015, Giannico et al. 2014, Santos et al. 2010).

Birds that show greater fear responses may have greater physiological responses to stress. Physiological changes observed during tonic immobility suggest that there are changes in the autonomic nervous system (ANS) strongly involved in this process (Alboni et al. 2008). The induction of tonic immobility leads to increased heart rate and blood pressure which suggests an increase in sympathetic activity (Gentle et al. 1989). This is also accompanied by increased lipid breakdown and increased muscle glucose to increase energy availability. These changes allow the animal to mobilize energy resources to prepare for the righting reflex ending TI (Nash et al. 1976b). When induction is over, heart and respiratory rate decrease, which could be the result of either decrease in sympathetic activity or increase in parasympathetic activity (Gentle et al. 1989).

The main objective for this study was to analyse duration of tonic immobility, heart rate and ventilation rate during tonic immobility in three different species; domesticated High Andean chickens (Gallus gallus), wild-caught Ornate Tinamous (Nothoprocta ornata) and Chilean Tinamous born in captivity (Nothoprocta perdicaria). The study was carried out to get more information about the differences in fear and physiological changes of the tonic immobility response. With more information about these differences and changes we can apply the information on animals in the wild to learn more about their behaviours. We can also use the information to give better conditions for animals living in captivity, both wild and domesticated.

The main hypothesis for this study was that heart and ventilation rate would increase due to induction and then decrease during tonic immobility until righting of the bird. Heart and ventilation rates were predicted to be higher in Tinamous than High Andean chickens due to
fearfulness, and the highest in Ornate Tinamous that are wild-caught. The TI duration was hypothesized to be shorter in High Andean chickens than in both Tinamou species.

3 Materials & methods

3.1 Animals
The animals used in this study were nine adult Ornate Tinamous (caught in the wild the year before) and five adult High Andean chickens (4 hens and 1 rooster). The Ornate Tinamous had an average body weight of 460 ± 58 g (mean ± SD) and the chickens and had an average body weight of 1563 ± 619.4 g (mean ± SD). The Ornate Tinamous and chickens were compared with previous collected data from eight adult Chilean Tinamous with an average body weight of 438 ± 42.0 g (mean ± SD).

3.2 Facilities
All animals were facilitated at Universidad Mayor de San Andrés in La Paz, Bolivia. The Ornate Tinamous were held in one cage together (1.9 x 7.8 x 4.8 m). The cage contained nesting material, bushes, stones and grass, and the floor was made of soil. The chickens were separated from the Ornate Tinamous in four cages (2.0 x 4.5 x 1.5 m) separate or in pairs. The cages contained small houses, nesting material, stones and the floor was made of soil. Food and water was provided to all the birds on a daily basis.

3.3 ECG electrode attachment
Before inserting the needle electrodes the skin over the Pectoralis muscles was cleaned with Iodine alcohol (Alcohol Yodado 1 %, Industrias Torrico Antelo, Cochabamba, Bolivia). The two needle electrodes were then inserted subcutaneous into each of the Pectoralis muscles. The electrodes were secured with surgical tape as required. The cords connected to the electrodes were then put in a way to minimize disturbance for the bird and secured with surgical tape.
3.4 Tonic immobility test

Before each tonic immobility test the electrodes were attached, the bird was put in a covered box and the measurements started. The bird was kept in the box for about two hours to obtain baseline heart and ventilation rate. After two hours the bird was taken out of the box and put on a table were the tonic immobility was induced. The tonic immobility was induced by turning the bird onto its back and holding one hand over the chest with light pressure and one hand over the head for 15 seconds. This has been found to be the optimal time for induction (Gallup et al. 1971). The bird was then slowly released and the experimenter backed away. If the bird righted within five seconds the induction was said not to be successful and the induction was retried for a maximum of total three times. During the test the experimenter stood or sat as still and silent as possible until the bird righted itself. Heart and ventilation rates were measured during the whole test. The number of inductions, time to first head movement and time to righting was noted during the test. The protocol was repeated twice for each bird on different non-consecutive days.

3.5 Data recording and analysis

Heart and ventilation rates were measured using the needle electrodes connected to an impedance meter (model 2991, impedance converter, Morro Bay, California). The impedance meter was connected to a Powerlab unit (ADIInstruments Ltd.) that in hand was connected to a HP Pavilion Ultrabook where the signal was acquired using LabChart Pro 7.3 (ADInstruments, 2011).

From the acquired data different time segments were extracted from different time periods, see Figure 1 for example traces. The lowest 1 minute mean for heart and ventilation rate was extracted from the end of the 2 hours of resting to obtain a baseline value. 10 second means were extracted from the first and last minute of the tonic immobility period to obtain mean heart and ventilation rates from the start and end of tonic immobility.

3.6 Statistical analysis

The heart and ventilation rate was measured twice in all individuals. A mean value of the first and second run was calculated since no difference
was found between the two runs with the exception of the end TI heart rate of the chickens.

The mean values were then compared with one-way analysis of variance (ANOVA) and when significant a Tukey’s post-hoc test. To compare the number of inductions a non-parametric Kruskal-Wallis H test was used. Heart ventilation rate at different times were compared within species’ with paired t-tests. The variables TI duration and latency to first head movement were ln-transformed to fulfill the assumption of normal distribution. All statistical analyses were performed with SPSS v.22. Graphs were created with Minitab 17 or Excel 2013. A significance level of p<0.05 was used.

![Graph](image)

**Figure 1.** Example of measured heart (HR) and ventilation rate (VR) traces from a Tinamou in mean beats per minute (BPM). A) Traces for HR and VR during resting to obtain baseline values. B) Traces for HR and VR during tonic immobility.
4 Results

4.1 Heart and ventilation rate

Heart rate was not different between species at baseline ($F_{(2,21)}=3.124$, $p=0.067$), start of TI ($F_{(2,21)}=0.254$, $p=0.779$) or end of TI ($F_{(2,21)}=1.015$, $p=0.381$) (Table 1).

Baseline ventilation rates on the other hand was significantly different between species ($F_{(2,21)}=19.021$, $p<0.001$) (Table 1). A Tukey post-hoc test revealed that chickens had a significantly lower ventilation rate than both Ornate Tinamou and Chilean Tinamou ($p<0.001$). But there was no difference between Ornate Tinamou and Chilean Tinamou ($p=0.915$).

Ventilation rate at start of TI was also significantly different between species ($F_{(2,21)}=9.365$, $p=0.001$) (Table 1). A Tukey post-hoc test revealed that Chilean Tinamou had a significantly higher ventilation rate than Ornate Tinamou ($p=0.045$) and chickens ($p=0.01$). There was no difference between Ornate Tinamou and chicken ($p=0.117$).

Further, the ventilation rate at end of TI was also significantly different between species ($F_{(2,21)}=20.124$, $p<0.001$) (Table 1). A Tukey post-hoc test revealed that chickens had a significantly lower ventilation rate than both Ornate Tinamou ($p=0.011$) and Chilean Tinamou ($p<0.001$). Also, the Chilean Tinamou had a significantly lower ventilation rate than Ornate Tinamou ($p=0.005$).

Comparing different time periods the Ornate Tinamou showed a significant difference between heart rate at baseline, start of TI and end of TI (for mean values see Table 1, Figure 2). Induction of TI induced a significant increase in heart rate ($t_{(8)}=-12.318$, $p<0.001$). After induction the heart rate decreased significantly ($t_{(8)}=-3.377$, $p=0.010$) but still remained over baseline levels.

Ventilation rate in the Ornate Tinamou also showed a significant differences between baseline, start of TI and end of TI (for mean values see Table 1, Figure 2). Induction of TI induced a significant increase in ventilation rate ($t_{(8)}=-6.959$, $p<0.001$). During TI the ventilation rate then significantly decreased until righting ($t_{(8)}=4.177$, $p=0.003$) but remained over baseline levels.
Table 1. Heart and ventilation rate in beats per minute (BPM) during rest, start of TI and end of TI observed in Ornate Tinamou (N. ornata), chicken (G. gallus) and Chilean Tinamou (N. perdicaria). All values shown as means with standard deviation (SD). P values obtained from one-way ANOVAs comparing all species. Different letters indicate a significant difference (p<0.05) obtained from Tukey post-hoc tests.

<table>
<thead>
<tr>
<th></th>
<th>N. ornata (n=9)</th>
<th>G. gallus (n=5)</th>
<th>N. perdicaria (n=8)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HR (BPM)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>241.9 (28.8)</td>
<td>208.7 (14.8)</td>
<td>226.3 (22.2)</td>
<td>ns (0.067)</td>
</tr>
<tr>
<td>Start of TI</td>
<td>370.6 (34.3)</td>
<td>363.2 (23.6)</td>
<td>375.4 (28.1)</td>
<td>ns (0.779)</td>
</tr>
<tr>
<td>End of TI</td>
<td>333.2 (34.6)</td>
<td>321.9 (40.1)</td>
<td>351.0 (38.8)</td>
<td>ns (0.381)</td>
</tr>
<tr>
<td><strong>VR (BPM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>26.4 (1.0)</td>
<td>a 12.5 (1.0)</td>
<td>b 25.6 (6.3)</td>
<td>a * (0.000)</td>
</tr>
<tr>
<td>Start of TI</td>
<td>61.3 (15.1)</td>
<td>b 38.3 (9.6)</td>
<td>b 86.2 (27.3)</td>
<td>a * (0.001)</td>
</tr>
<tr>
<td>End of TI</td>
<td>45.0 (12.5)</td>
<td>b 26.0 (4.6)</td>
<td>c 63.4 (10.2)</td>
<td>a * (0.000)</td>
</tr>
</tbody>
</table>

* P<0.05, ns non-significant

The chickens did not show significant differences in heart rate between any time period (for mean values see Table 1, Figure 2). Induction of TI induced a significant increase in heart rate ($t(4)=-9.260$, p=0.001) compared to baseline (rest). Although, during TI the progressive decrease in heart rate was visible but not significant ($t(4)=1.970$, p=0.120).

The ventilation rate of the chickens was significantly different comparing ventilation rate between baseline, start of TI and end of TI (for mean values see Table 1, Figure 2). The ventilation rate increased significantly due to induction ($t(4)=-6.403$, p=0.003) and then significantly decreased during TI ($t(4)=4.162$, p=0.014) remaining over baseline levels.

Chilean Tinamous showed similar statistical results as Ornate Tinamous. Heart rate between baseline, start of TI and end of TI showed a significant difference (for mean values see Table 1, Figure 2). The heart rate increased significantly from baseline to start of TI ($t(7)=-12.332$, p<0.001). During TI the heart rate then decreased significantly ($t(7)=2.702$, p=0.031) but remained over baseline levels.
The ventilation rate for the Chilean Tinamou it was also significantly different between baseline, start of TI and end of TI (for mean values see Table 1, Figure 1). Following induction the ventilation rate had increased significantly from baseline ($t(7)=-5.618$, $p=0.001$) and then decreased significantly during TI ($t(7)=2.712$, $p=0.030$) but remained over baseline levels.

![Graph showing heart and ventilation rate in beats per minute (BPM) for Ornate Tinamou (N. ornata), chicken (G.gallus) and Chilean Tinamou (N. perdicaria) at baseline, start of TI and end of TI. Bars show means and 95% confidence intervals. * P<0.05, ns (non-significant).]
4.2 Induction, TI duration and latency to first head movement

The number of inductions was not different between groups as shown by a Kruskal-Wallis H test ($x^2_{(2)}=1.883$, $p=0.390$). The mean number of inductions for Ornate Tinamou was $1.2 \pm 0.3$ (mean ± SD) inductions, for chickens $1.7 \pm 0.8$ (mean ± SD) inductions and for Chilean Tinamou $1.4 \pm 0.7$ (mean ± SD) inductions.

The duration of TI was significantly different comparing all species ($F_{(2,21)}=15.7$, $p<0.001$). The TI duration of Ornate Tinamou ($2632.4 \pm 1921.7$ s) was significantly longer than both chicken ($p<0.001$) and Chilean Tinamou ($p=0.006$). Moreover, there was no difference in TI duration between chickens ($244.0 \pm 217.7$ s) and Chilean Tinamou ($619.4 \pm 445.2$ s) ($p=0.088$).

The results from latency to first head movement were consistent with the results from TI duration. There was a significant difference between all species ($F_{(2,21)}=22.751$, $p<0.001$). A Tukey post-hoc test revealed that the latency to first head movement in Ornate Tinamou ($1166.1 \pm 578.9$ s) was significantly longer than both chickens ($p<0.001$) and Chilean Tinamou ($p<0.001$). There was no difference between chickens ($74.4 \pm 57.9$ s) and Chilean Tinamou ($207.8 \pm 145.1$ s) ($p=0.102$).

![Figure 3](image-url) 

**Figure 3.** TI duration and latency to first head movement for Ornate Tinamou (N. ornata), chicken (G. gallus) and Chilean Tinamou (N. perdicaria). Bars represent means, 95% confidence intervals and dots represent individual values. Different letters indicate a significant difference ($p<0.05$).
5 Discussion

The purpose of this study was to give a picture of the physiological changes in Tinamous and High Andean chickens during tonic immobility. The time following induction was characterized by a large increase in heart and ventilation rate in all species (Table 1, Figure 2). During tonic immobility a progressive decrease in heart and ventilation rate was also observed in all species, significant in all cases except for heart rate between start and end of TI in chickens (Table 1, Figure 2). The duration of TI was significantly longer in Ornate Tinamou compared to Chilean Tinamou and chickens (Figure 3). The same was observed in latency to first head movement (Figure 3).

Heart rate represents the net interactions between vagal (which reduces heart rate) and sympathetic (which increases heart rate) regulation, referred to as sympathovagal balance. At rest, both the sympathetic and parasympathetic systems are tonically active regulating cardiac activity with a dominance of vagal regulation (von Borell et al. 2007). Stress responses, like TI and the “fight-or-flight” response, are associated with increased heart and ventilation rates (Alboni et al. 2008). An increase in heart rate is mainly caused by increased sympathetic activity, but it may also result from a decrease in parasympathetic activity, or changes in both systems. In a previous study comparing quail selected for long (LTI) and short TI duration (STI) they found that the intrinsic heart rate (during total ANS blockade) did not differ between lines, but the vagal activity was higher in STI quail and sympathetic activity was higher in LTI quail (Gaudiniere et al. 2005). In another study they found similar results at rest, in STI quail the parasympathetic activity was dominant and in LTI quail the sympathetic and parasympathetic activities were in balance (Valance et al. 2007). Moreover, Valance, et al. (2008) found that induction of TI lead to an increased sympathetic activity relative to parasympathetic. Also, more fearful individuals or species’ could, as hypothesized, be expected to have higher heart rate during stressful situations associated with increased sympathetic activity, but it was not found in this study (Table 1). There were no differences in heart rate at any times between species (Table 1). The relative sympathetic and parasympathetic activity could be different between species, but we cannot know this just by analyzing heart and ventilation rate. The reason that the heart rate was not different at start of TI (i.e. after induction) could been due to the chickens being more struggling. During induction the chickens were by far the most struggling, the Ornate Tinamous were much easier to induce in TI. By being more struggling the heart rate
might have increased in the chickens and erased any potential differences between species. This has also been discussed previously by Valance et al. (2008) where the LTI quail had higher parasympathetic activity than STI quail and one explanation was the lack of repetition of induction in LTI quail.

On the other hand, the baseline ventilation rate was significantly lower in chickens compared to both Ornate Tinamou and Chilean Tinamou (Table 1). This is in line with results from a previous study on handling stress in Great tits where they found that shy individuals showed higher ventilation rates than bold individuals (Carere et al. 2004). Ventilation rate is not studied as much as heart rate in birds. Ventilation rate could respond like heart rate to stressful events because both are, at least in part, controlled by the autonomic nervous system (Carere et al. 2004). Moreover, during start of TI the ventilation rate was instead significantly higher in Chilean Tinamou (86.2 ± 27.3) than both Ornate Tinamou and chicken (Table 1). These results are not consistent considering fearfulness with the results observed in TI duration (Figure 3) or the results and suggestions by Carere et al. (2004). At the end of TI all species were significantly different in ventilation rate. Chilean Tinamou with the highest (63.4 ± 10.2), Ornate Tinamou in the middle (45.0 ± 12.5) and chickens with the lowest (26.0 ± 4.6). This was not expected either. The High Andean chicken could have been expected to have higher ventilation rate due to the hypoxic conditions in La Paz, Bolivia (>2400 m.a.s.l) (Ivy et al. 2014).

The induction of tonic immobility induced a strong increase in both heart and ventilation rate for all species which is consistent with previous studies in chickens (Valance et al. 2008, Gentle et al. 1989, Eddy et al. 1990) (Figure 1). This has also been observed in rabbits (Carli 1974). The increase in heart rate induced by induction of TI was explained by Valance et al. (2008) as due to a shift of the sympathovagal balance towards sympathetic dominance relative to parasympathetic activity. This is a typical response to stress seen in the “fight-or-flight” response to prepare for the increased metabolic needs (Alboni et al. 2008). There has also been studies in mammals showing that the relative parasympathetic activity decreased in response to stress, which is associated with the increase in sympathetic activity (Sgoifo et al. 2006, Visser et al. 2002). The induction of TI has been suggested to cause animals’ strong negative stress that is characterized by the observed increase in heart rate (Gentle et al. 1989, Eddy et al. 1990). This suggestion is in line with the
understanding of TI as being the ultimate response to prey being confronted with a predator (Gentle et al. 1989).

During tonic immobility heart rate decreased progressively in all species but still remained over baseline levels (see example in Figure 1, Figure 2). The decrease was significant in both Tinamou species but not in chickens, although it was a visible decrease of about 40 BPM. This is consistent with previous studies in quail (Valance et al. 2008) and chicken (Nash et al. 1976b, Gentle et al. 1989). The same results has also been seen in rabbits (Hatton et al. 1978). The ventilation rate also decreased progressively and significantly in all species (see example in Figure 1, Figure 2). Valance et al. (2008) explained the progressive decrease in heart rate by a decrease in relative sympathetic dominance which has previously also been suggested by Gentle et al. (1989) and Nash et al. (1976). Valance et al. (2008) found that during TI the parasympathetic activity was the highest in the beginning in LTI quails and that it remained stable until righting. But in STI quail the parasympathetic activity was lower in the beginning and then increased at the end of TI. They reason that since sympathetic dominance reflects the animal’s preparation for a “fight-or-flight” reaction this could reflect behavioral differences between quail. Valance et al. (2008) also suggest that the increase in parasympathetic activity at the end of TI in STI quail could be related specifically to the end of TI, perhaps to activate the baroreflex to facilitate righting. They conclude that the changes involved in preparing the righting reflex of quail is linked to an autonomic process, a reinforcement of the parasympathetic activity. Since the chickens didn’t have a significant decrease the heart rate and time righting might also have something to do with fearfulness. Because they are not as fearful, with a shorter duration of TI, they might not need an as big decrease in heart rate as more fearful species. The changes in relative autonomic activity might occur earlier when the bird is less fearful and prepare the bird for righting. A more detailed study of the changes in autonomic nervous system activity has to been done in order to determine this.

There has also been studies suggesting that only the parasympathetic system is involved in the TI response (Giannico et al. 2014). When tonic immobility is induced you change the body position which changes the blood flow. This change is detected by the baroreceptors. Some of these receptors are located in the carotid sinus, and in birds the aortic arch is also innervated by afferent vagal fibers. These fibers have nerve terminals that transmit wall stretch signaling central arterial blood pressure (Valance et al. 2008). Stimulation of the baroreceptors activate the
baroreflex that regulates heart rate for example. This reflex is controlled by the autonomic nervous system, particularly the parasympathetic branch. Hatton et al. (1978) did an experiment in rabbits where they suppressed the baroreflex via denervation of the carotid sinus. This lead to an increased number of inductions needed to induce TI. As Valance et al. (2008) says the baroreflex is mainly under parasympathetic influence, then Hatton’s results would suggest that the parasympathetic nervous system plays a major role in the induction progress. In this study there was no difference between species in the number of inductions needed to induce TI, although it could have been expected according to previous studies. LTI quails have been shown to be more susceptible (i.e. easier to induce TI in) than STI quail (Valance et al. 2008). In the study by Valance et al. (2008) TI was induced after the first attempt in all long TI quails, but short TI quails required more attempts.

The separate effects of the sympathetic and parasympathetic nervous systems cannot be determined by just analyzing heart rate (von Borrell et al. 2007). An increase in heart rate can, as mentioned, be the result of increased sympathetic activity, decreased parasympathetic activity or both. Heart rate variability allows analysis and determination of the separate and relative effects of the autonomic nervous branches. This would allow to determine if there is a difference between the three species, wild-caught, domesticated and born in captivity. It would also give an interesting insight in what role the autonomic nervous system has in TI and what determines the time of righting in these species. All these species might also be affected by the high altitude of La Paz, Bolivia. The sympathetic activity might be higher in species acclimatized to high altitude. Sympathetic activity remains high after prolonged periods at high altitude (Dhar et al. 2014) but heart rate usually returns to sea level values after acclimatization (Vogel et al. 1967).

The duration of TI was significantly longer in Ornate Tinamou than both chicken and Chilean Tinamou (Figure 3) which is in line with what was expected. The TI duration in Ornate Tinamou was almost as long as two hours for some individuals. A previous study comparing wild and domesticated finches showed that the domesticated birds had shorter durations of TI (Suzuki et al. 2013). They suggest that the reduced fearfulness in domesticated finches may be due to selective pressure during domestication. They also suggest that the domesticated finches may have been able to increase the investment of energy in reproduction in exchange for the reduced costs of predation and coping necessary to survive in the wild. These behavioral changes may have been a major
target of domestication effects in this species of finches, and maybe other species. Since the duration of TI was not different between chickens (244.0 ± 217.7 s) and Chilean Tinamous (619.4 ± 445.2 s) it could indicate that some domestication has occurred in the Chilean Tinamou (Figure 3). This is also consistent with the much longer duration of TI in Ornate Tinamou (2632.4 ± 1921.7 s) since all individuals used were caught in the wild. There has also been a recent study in pure and hybrid partridges showing that the pure partridges had longer durations of TI (Campo et al. 2015). This is consistent with the results in this study and further strengthens the hypothesis.

It is difficult to know how to define a long or short duration of TI, but we can conclude from the TI duration results in this study that the chickens and Chilean Tinamou are the least fearful and that the Ornate Tinamou is the most fearful. You could also compare duration with previous studies but many previous studies have a maximum time of TI duration where they ended the TI if the animal had not yet righted. This does not allow to study the full response or the real TI duration and therefore how fearful the individual or species are. Although, Gentle et al. (1989) recorded TI durations in chickens that varied from 2 to 20 minutes. This is similar to the results observed in the chickens in this study were the duration varied from less than 1 minute to about 12 minutes.

The latency to first head movement showed similar results as the TI duration (Figure 3). It was significantly shorter in both chicken and Chilean Tinamou compared to the Ornate Tinamou (Figure 3). This suggests that head movements are also associated to the level of fear in the individual. During tonic immobility the head movements were very different from individual to individual. There were also much more head movements in the Tinamous than the chickens. Some chickens didn’t move their heads at all before righting. Gentle et al. (1989) mentioned the issue of disturbances that affect head movements and duration of TI. Some disturbances are difficult to control, such as loud sounding heavy raining. Something that was also observed in the Ornate Tinamou in almost every individual, especially those with long TI duration, was leg tremors. This has been documented previously in chickens (Gentle et al. 1989, Gallup et al. 1971).
5.1 Conclusion

I conclude that the physiological changes during tonic immobility are characterized by an increase in heart and ventilation rate due to induction and then a progressive decrease until righting. These changes are most likely controlled by the autonomic nervous system. The induction of TI indicates a sympathovagal balance shifted towards sympathetic activity. The following decrease is probably due to different relative contributions of the autonomic nervous branches. The decrease in heart rate in chickens was not significant which might reflect the behavioral differences between domesticated and wild birds. No differences were found between species in heart rate at the different times, although this might have been due to different behaviors during induction. The ventilation at rest is lower in less fearful birds and higher during TI in more fearful birds. As predicted the duration of TI was the longest in Ornate Tinamou. The same was observed in latency to first head movement which seems to also be associated with the levels of fear in an individual. This indicates that the wild-caught Ornate Tinamou is more fearful than both Chilean Tinamou and chickens, with some individuals having a lot longer durations of TI. Latency to first head movement is probably also closely associated to fear. Since the Chilean Tinamou was closer in TI duration to the chickens it might have been a subject of some domestication.

5.2 Social & ethical aspects

The experiment was supposed to give an insight in how fear is expressed in different species using the tonic immobility method. Also to describe the physiological changes during this fear response by studying heart and ventilation rate. The results can be useful for evaluating animal handling in for example farming and production when animals are exposed to stressful situations. It is also valuable for giving a picture of how stressful situations affect wild-caught animals. This can also be used in the future to study stress in animals in the wild.

Caution was taken to cause as little stress as possible for the birds. This is done for example by giving the bird time to relax after being removed from the flock and by practicing the method beforehand to make it as efficient as possible.
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