Physiological responses to low atmospheric pressure stunning and the implications for welfare

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ABSTRACT In low atmospheric pressure stunning (LAPS), poultry are rendered unconscious before slaughter by gradually reducing oxygen tension in the atmosphere to achieve a progressive anoxia. The effects of LAPS are not instantaneous, so there are legitimate welfare concerns around the experience of birds before loss of consciousness. Using self-contained telemetry logging units, high-quality continuous electroencephalogram (EEG) and electrocardiogram (EKG) recordings were obtained from 28 broiler chickens during exposure to LAPS in a commercial poultry processing plant. Application of LAPS was associated with changes in the EEG pattern in the form of increases in total power, decreases in mean frequency, and in particular, increases in slow-wave (delta) activity, indicating a gradual loss of consciousness. Increased delta wave activity was seen within 10 s of LAPS onset and consistently thereafter, peaking at 30 s into LAPS at which point the EEG signal shared characteristics with that of birds in a surgical plane of anesthesia. During LAPS, heart rate consistently decreased, with more pronounced bradycardia and arrhythmia observed after 30 s. No heart rate increases were observed in the period when the birds were potentially conscious. After an initial quiescent period, brief body movements (presumed to be ataxia/loss of posture) were seen on average at 39 s into the LAPS process. Later (after 120 s on average), artifacts related to clonic (wing flapping) and tonic (muscle spasms) convulsions were observed in the EKG recordings. Based on EEG analysis and body movement responses, a conservative estimate of time to loss of consciousness is approximately 40 s. The lack of behavioral responses indicating aversion or escape and absence of heart rate elevation in the conscious period strongly suggest that birds do not find LAPS induction distressing. Collectively, the results suggest that LAPS is a humane approach that has the potential to improve the welfare of poultry at slaughter by gradually inducing unconsciousness without distress, eliminating live shackling and ensuring every bird is adequately stunned before exansguination.

Key words: electroencephalogram, low atmospheric pressure, poultry, welfare, stunning

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INTRODUCTION

Slaughter methods are only acceptable when they result in minimal signs of agitation and distress in the period during which some degree of consciousness cannot be excluded. Some methods of stunning of poultry [e.g., controlled atmosphere (gas) stunning, **CAS**] do not cause immediate induction of unconsciousness, and measurement of appropriate physiological responses allows an assessment of the potential welfare insults experienced by the birds during the conscious phase. This approach has been successfully applied in previous work examining the welfare consequences of CAS (e.g., Gerritzen et al., 2004; McKeegan et al., 2007, 2011; Coenen et al., 2009). Controlled atmosphere stunning has the potential to improve welfare of poultry at slaughter by eliminating the stress associated with shackling of conscious birds and has the advantage of ensuring effective stunning of every animal. Although there has been a recent trend for increased uptake of CAS in Europe and the United States, some barriers to its use remain and there is some debate over which gas mixtures for CAS are most humane (Raj, 2006). With the advent of EU legislation Regulation (EC) no. 1099/2009 on the protection of animals at the time of killing (OJ L 303, 18.11.2009, pg. 1; European Commission, 2009), which will render many current electrical stunning systems (in Europe) illegal, there is still a need for alternative humane stunning systems.

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Low atmospheric pressure stunning (**LAPS**) is a novel method in which birds are rendered unconscious by being placed in sealed chamber, in which a vacuum pump is used to gradually reduce oxygen tension in the atmosphere. This achieves a gradual anoxia, so as with gas stunning, the effects of LAPS are not instantaneous. As a result, there are legitimate welfare concerns about the experience of birds before loss of consciousness. Decompression has been criticized in the past mainly because of concerns around rapid decompression, which results in pain and distress due to expanding gases trapped in body cavities (American Veterinary Medical Association, 2007). It has been argued however, that when decompression is done slowly, it can be humane (Vizzier-Thaxton et al., 2010), and reports of loss of consciousness resulting from decompression (due to high altitude) in humans suggest that the experience is painless (Smith, 1965). Some previous studies on LAPS are available, and these have identified process variables for a suitably gradual decompression (Purswell et al., 2007) and examined behavior and corticosterone responses (Vizzier-Thaxton et al., 2010) and meat quality (Battula et al., 2008; Vizzier-Thaxton et al., 2010). Damage to organs during decompression has been a concern, but Vizzier-Thaxton et al. (2010) reported that tissue taken from the pectoralis major, lung, and liver after LAPS stunning had no signs of damage or pathology. Together, these studies suggest that gradual hypoxia is promising as a humane method of stunning for poultry. Given the mode of action of hypoxia, this is in agreement with other work examining hypoxia leading to anoxia achieved with gas environments, which has favorable results for welfare (Woolley and Gentle, 1988; Raj et al., 1991).

Recently, the USDA gave the LAPS system a "no objection" status and a full-scale operational system was installed at a commercial slaughter plant in Arkansas. The system is in routine use, providing an opportunity to assess bird welfare during the LAPS process running at a commercial capacity of 360 birds per minute. As in previous work (e.g., Coenen et al., 2009; McKeegan et al., 2011), we used a sophisticated physiological logging system to simultaneously record high-quality electroencephalogram (**EEG**) and electrocardiogram (**EKG**) signals during the stunning process, via a small logging unit worn by the bird in a Lycra backpack, with no trailing wires (described in Lowe et al., 2007). The EEG monitoring allows a determination of global brain activity, which can be analyzed using various techniques to evaluate loss of consciousness. Measurement of EKG allows an evaluation of the physiological impact of the process and can give an indication of stress or fear. Our overall aim was to provide an objective welfare assessment of commercially relevant LAPS by characterizing the timing of loss of consciousness and determining the welfare impact of events occurring in the conscious phase.

MATERIALS AND METHODS

Subjects and Husbandry

The subjects were 28 broilers reared under standard conditions in a commercial rearing farm. The rearing area was furnished with deep wood shavings, and birds had ad libitum access to standard broiler food and water. The experiments were specifically authorized by the University of Arkansas Institutional Animal Care and Use Committee.

At 28 to 30 d of age, the broilers underwent surgery to implant EEG electrodes under general anesthesia. They were moved by car to the surgery site in individual cardboard pet carriers, and were allowed a minimum of 30 min to recover before surgery, remaining in their transport container. After an analgesic premedication (Dexmedetomidine 40 μg/kg, administered intramuscularly, Dexdomitor, Pfizer Animal Health, New York, NY), general anesthesia was induced and maintained with Sevoflurane (Seroflo, Abbott Drug, Abbott Park, IL). At the start of surgery, an analgesic was administered to provide postoperative pain relief (Carprofen 40 mg/kg, administered subcutaneously, Rimadyl, Pfizer Animal Health). The EEG implantation approach has been described previously (e.g., McKeegan et al., 2011). Briefly, the EEG was recorded by two 0.35-mm-diameter Teflon-insulated silver electrodes connected to a socket (DIN, RS Components, Corby, UK), placed on the dura through small holes drilled in the skull, one on each of the dorsal surfaces of the right and left telencephalon at their approximate rostro-caudal and medio-lateral midpoints. An indifferent electrode was placed between the skull and the overlying tissue under the comb. The EEG implant was secured to the skull with dental cement, and the surrounding skin was closed with sutures. After initial recovery from the anesthetic, birds were placed in a small recovery pen (equipped with wood shavings litter, a heat lamp, and food and water) and were closely monitored. After a minimum of 4 h of recovery, the birds were returned to the rearing site (as before in individual pet carriers) and were housed in individual pens with ad libitum access to food and water and visual and auditory contact with their neighbors. The birds were allowed to recover for a minimum of 5 d before undergoing any further experimental procedure.

LAPS Process

Each LAPS unit is cylindrical, which provides sidewall strength, measures 20 to 20.5 ft (6.1 to 6.25 m) in length and 7 ft (2.13 m) in diameter, and is designed to accommodate 2 commercial broiler transport cages of the type typically used in the United States. The atmosphere is manipulated via a variable airflow withdrawal process. The time of pressure reduction and any hold

time are controlled via a computer that is programmed to a precise sequence so that human error and climate cannot cause a change that would be stressful for the birds. In the case of a power failure, the unit has a failsafe mechanism that will immediately open the doors so that the chickens are not held in an atmosphere that would produce discomfort over time. Cages move into and out of the unit automatically via a powered transfer conveyor. Each chamber is equipped with a hydraulic door on each end, which opens for cage transfer, and when closed, seals the chamber. Chambers are installed to become an integral part of the plant conveyor system before the dump station, and 4 chambers were in use at the plant, their timings staggered to ensure a continuous flow of birds to the shackle line. Vacuum pumps with a capability of removing 19.82 m^3/min of atmospheric air each are connected to the chamber via pipes, and vacuum is applied via pneumatically actuated valves. The entire LAPS cycle from doors closed to opening again takes 280 s and consists of a gradual curve of reducing pressure over 75% of the cycle, and then the final pressure is maintained over the remaining 25% (the final pressure is no greater than an 80% reduction in ambient pressure). The LAPS technology is patented under multiple international patents with numerous patents pending. Only one LAPS chamber was used for all trials reported here (due to the proximity of that chamber's in-feed conveyor and out-feed conveyor to the perimeter catwalk structure), and the trials took place over 2 consecutive days.

Trial Procedure

On each day of the trial, birds were transferred individually to cardboard pet carriers and transported to the trial site with a journey time of 30 min. On arrival, the birds remained in their transport boxes in an air conditioned room until subjected to LAPS. Immediately before each LAPS run, one bird was fitted with instrumentation as follows. As in previous work, EKG electrodes were attached to skin overlying the pectoralis muscle (McKeegan et al., 2011). These were commercially available disposable self-adhesive EKG electrodes (Blue Sensor, Ambu Ltd., St. Ives, UK), with press-stud electrical connections, which were adhered to cleaned skin overlying the pectoralis muscle on either side of the sternum. Cyanoacrylate tissue adhesive (Vetbond, 3M, St. Paul, MN) was applied to the EKG electrode pads before placement on the skin to improve bonding. Each bird was fitted with a reusable Lycra harness, which was secured using Velcro fastenings behind the bird's head and incorporated a pocket positioned on the bird's back, which contained a telemetry/logging device capable of logging simultaneous EEG and EKG signals and described elsewhere (Lowe et al., 2007; McKeegan et al., 2011). Briefly, the logging units were battery powered, and each was small enough to be worn by a bird in a Lycra backpack, thus requiring no trailing leads. Two physiological waveform input channels were provided and were used to record EKG and EEG (sampling frequency 1,000 Hz). Logging was triggered and stopped with an external switch, and logged data were recorded onto industry-standard micro-SD memory cards. Four identical loggers were used in rotation.

Once each bird was equipped with sensors and a logger, the logger harness was additionally secured around the birds with elastic bandage (Vetrap, 3M), and a strip of bright pink duck tape was applied to aid visibility during retrieval. Signal logging was started and a 2-min period of baseline recording commenced during which the bird was gently held by an experimenter. After this period, the bird was carried to the loading area of the LAPS system and added to a transport module (Livehaul cage, Modern Live Haul, Beaumont, TX) of commercially reared broilers ready to enter the chamber. Only one instrumented bird was added per LAPS cycle. To aid retrieval (and thus avoid interruption of the ongoing slaughter process), instrumented birds were always added to the top tier of the module at the front (proximate to the LAPS chamber), on the side nearest the access walkway. At the end of each LAPS cycle, the module emerged from the chamber and the instrumented bird was retrieved (the other birds were processed as normal). Logged data were downloaded onto a laptop computer using purpose-designed telemetry software. During each trial, the exact times of the following events were noted using a digital timer: baseline start, baseline end, in module, in transit, in chamber, chamber sealed, LAPS start, LAPS stop, bird retrieved, harness off, and logging stopped.

Analysis

The logged data files were uploaded into a data acquisition and analysis program (Spike 2 Version 4.2, Cambridge Electronic Design, Cambridge, UK). Analysis consisted of examining artifact-free excerpts from the EEG and EKG signals at fixed times throughout the LAPS process. Analysis focused on the first 60 of LAPS because, based on previous work (Vizzier-Thaxton et al., 2010), that is the period when the birds are losing consciousness and is when any welfare issues would be apparent. We also examined EEG and EKG at the end of the LAPS cycle to check the status of birds as they exit the system for further processing. The time points analyzed for both EEG and EKG in each bird were as follows: baseline (final 2 s of 2-min baseline), in transport module (bird placed in the module), in transit (module started moving into the chamber), in chamber (module stopped moving inside the chamber), and then 0 (LAPS chamber doors sealed), 10, 20, 30, 40, 50, 60 s into LAPS, and LAPS end (280 s), see Table 1. In the case of any noise/artifact contamination of the trace at these time points, the nearest clean 2-s epoch of EEG/EKG signal was used. The EEG was

analyzed by producing power spectra of artifact-free 2-s epochs corresponding to each of the time points outlined above, using a fast Fourier transform algorithm (1024, Hanning window, resolution 0.976 Hz bins). Using the power spectrum, the median frequency (**F50**) and total power (**PTOT**) were manually calculated at each time point. Median frequency is the frequency below which 50% of the total power of the EEG is located. Total power is defined as the total area under the power spectrum curve (Murrell and Johnson, 2006). These values were calculated because it is well recognized that decreases in EEG F50 and increases in PTOT are correlated to clinical signs of loss of consciousness and anesthesia (Schwilden, 1989; Martín-Cancho et al., 2006). Additionally, the relative power (voltage) contribution of different frequency ranges (delta <4 Hz, theta 4 to 7 Hz, α 8 to 12 Hz, β 13 to 30 Hz) at each time point was determined. Delta waves were specifically examined because these are associated with sleep, anesthesia, and loss of consciousness. Time series analysis in the form of one-way ANOVA with time interval as a factor was performed for heart rate, PTOT, F50, and mean contribution of delta frequency waves, followed by post-hoc paired *t*-tests to highlight differences between each time point and the baseline. Because multiple post-hoc tests were carried out, a Bonferroni correction was applied (the corrected significance level applied was $P = 0.001$).

Clean EKG signal was used to determine heart rate (based on the number of QRS complexes in 4-s epochs) at each time point. In this trial it was not possible to observe instrumented birds directly to record their behavior, but artifacts on the EKG trace (relating to movement and electromyogram activity) have been used previously to identify wing flapping behavior and substantial body movements (McKeegan et al., 2011) and were also noted here. Specifically, we examined the EKG trace and for each bird noted time to first movement artifact, time to first artifact with duration >5 s, number of artifact bouts, and total artifact duration. Finally, we cross referenced EEG and movement artifact data on an individual-bird basis and analyzed 2-s portions of EEG (as above) for each bird immediately preceding first movement (ataxia), mid flap (between flapping bouts), and after wing flapping had ceased.

RESULTS

EEG Responses

High-quality EEG signals were collected for all 28 birds for the duration of LAPS. During LAPS a series of consistent changes in the visual appearance of the EEG were apparent. Figure 1 shows a representative series of 2-s EEG excerpts from the baseline and at 10-s intervals during the first 60 s, and up to 200 s into the LAPS process (data from bird 12). Baseline EEG waveforms consisted of low-amplitude, high-frequency activity, as we would expect given the birds' alert state at this time. As LAPS progressed, there were clear changes in the appearance of the EEG from 10 s onward, with increased slow wave activity (reflecting synchronization of neuronal firing) becoming apparent and more pronounced with time. Slow wave activity is associated with sleep, anesthesia, and unconsciousness, and the appearance of this pattern indicates that LAPS induces unconsciousness. By 60 s into LAPS, the appearance of the EEG indicated deep unconsciousness, as evidenced by a suppressed EEG signal (transient periods of electrically suppressed brain activity). At the end of the LAPS cycle, the EEG was always isoelectric (residual low-level noise reflecting a lack of EEG activity, Figure 1).

To examine changes in the EEG in more detail, power spectrum analyses were carried out to quantify the spectral characteristics of the signal for each bird at each time point. Figure 2 shows the mean PTOT of the EEG signal at each time point, including baseline, in module, in transit, and in the chamber before LAPS began, the first 60 s of LAPS at 10-s intervals and LAPS end (n = 28). Before LAPS during handling, and transport into position, the total power did not change (~300 μV^2), as the birds were conscious during this time, but during LAPS total power of the signal progressively increased ($P < 0.001$, one-way ANOVA, n = 28). The PTOT was higher than baseline at 10, 20, 30, and 40 into LAPS; all $P < 0.001$ paired *t*-test, n = 28). The PTOT values increased as birds shifted from conscious (low-amplitude, high-frequency EEG wave activity) to unconscious states where EEG wave

Table 1. Sequence of events for electroencephalogram (EEG) data collection including baseline recording and low atmospheric pressure stunning (LAPS)-related procedures from bird loading to the end of the LAPS process

Event	Code	Description
Baseline	B	Bird fully awake/conscious, gently held by a researcher sitting calmly for 2 min
Module	M	Bird placed into top compartment[1] of a commercial transport module with other birds at standard stocking density
Transit	T	Transportation via conveyer to the LAPS chamber
Chamber	C	Transportation module in LAPS chamber (stopped moving)
LAPS 0	0	LAPS chamber doors sealed
LAPS 10, 20, 30, 40, 50, 60		10-s time intervals during LAPS procedure
LAPS end	END	End of LAPS procedure (280 s)

[1]Compartment location: uppermost level at the end proximal to the LAPS chamber on the side nearest the access walkway.

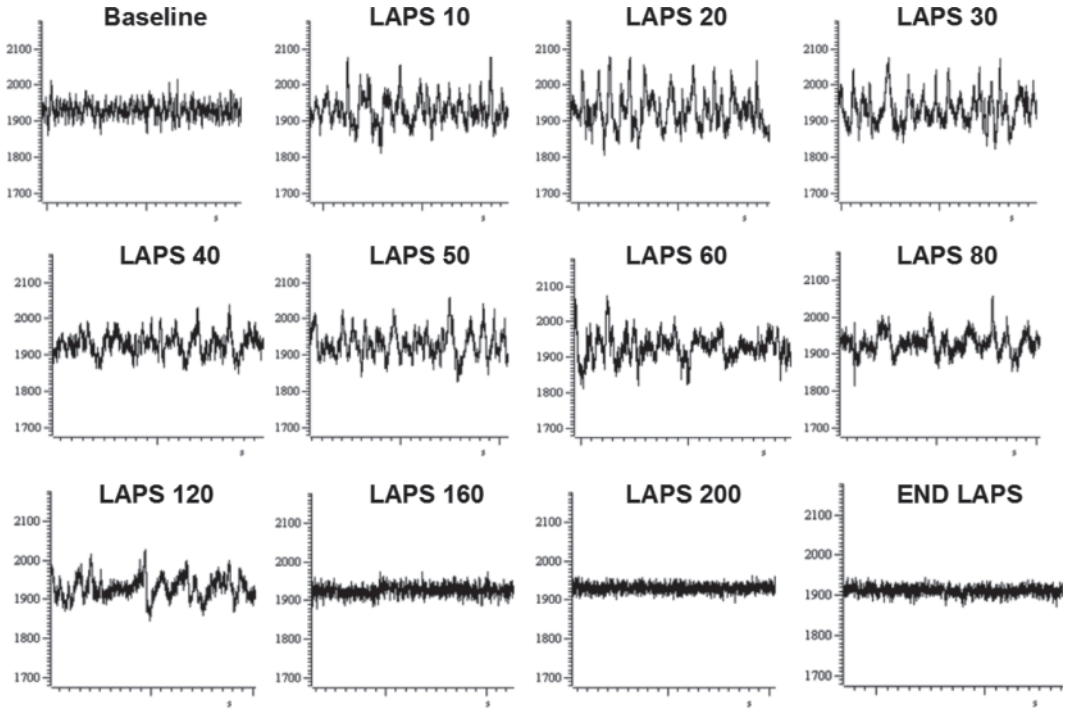

Figure 1. A representative series of electroencephalogram (EEG) trace excerpts (each 2-s duration, data from bird 12) illustrating the typical appearance of the EEG at 12 time points [baseline, low atmospheric pressure stunning (LAPS) 10 to 60, 80, 120, 160, 200, and end LAPS]. The y-axis units are microvolts, and the x-axis units (large tick marks) are seconds.

activity was synchronized and the cumulative power was at its highest (characterized by high-amplitude, low-frequency wave forms). Power rapidly increased in the early part of LAPS and peaked at 30 s into LAPS (2.4-fold increase in power relative to baseline), indicating that the birds were losing consciousness during this time. After 30 s the power decreased as the EEG gradually became suppressed as the birds began to die due to the effects of hypoxia. Total power did not increase after 60 s of LAPS, and at the end of LAPS there was very little power in the signal (<15% of baseline, $P < 0.001$, paired t-test, n = 28); this is because the EEG was isoelectric and the signal represents low level noise that is nonbiological in origin.

Plotting F50 against time (Figure 3), higher median frequency in the alert part of the cycle (before LAPS during handling) was apparent (approximately 26 Hz), followed by decreased F50 during LAPS (especially after 10 s), exhibiting a maximum reduction in median frequency of approximately 75% compared with base-

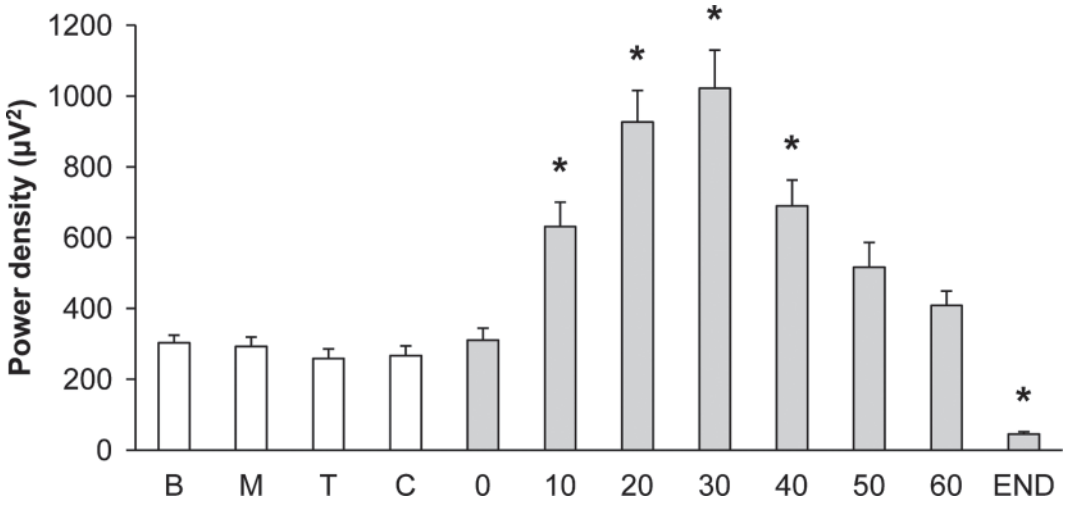

Figure 2. Histogram showing the mean (±SE) total power of the electroencephalogram (EEG) constructed using 2-s epochs of EEG activity from 12 time points [B, baseline; M, in module; T, in transit; C, in chamber; 0 to 60, 0 to 60 s after low atmospheric pressure stunning (LAPS) start; END, LAPS end]. n = 28. *Asterisks indicate time intervals that are different from baseline ($P < 0.001$).

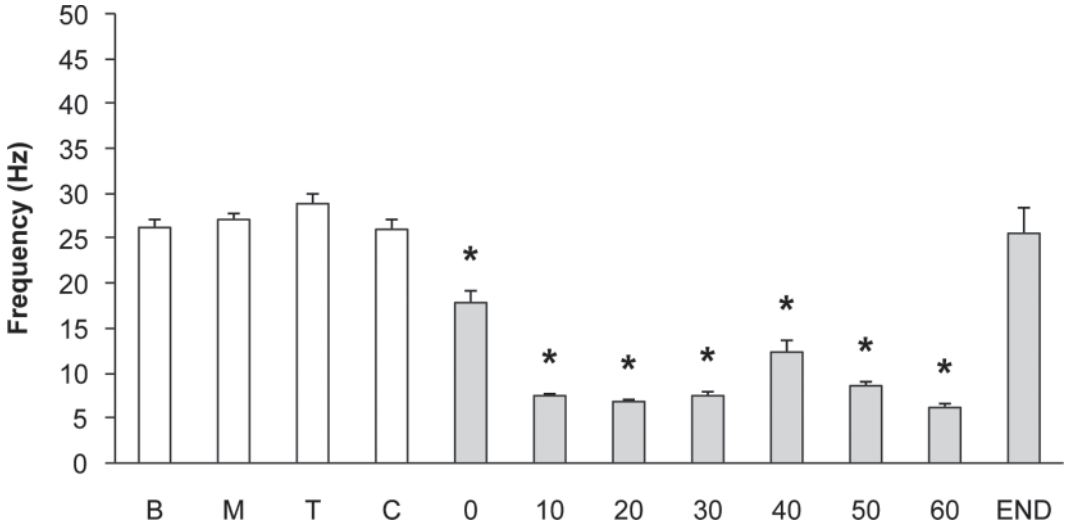

Figure 3. Histogram showing the mean (±SE) median frequency of the electroencephalogram (EEG) constructed using 2-s epochs of EEG activity from 12 time points [B, baseline; M, in module; T, in transit; C, in chamber; 0 to 60, 0 to 60 s after low atmospheric pressure stunning (LAPS) start; END, LAPS end]. n = 28. *Asterisks indicate time intervals that are different from baseline ($P < 0.001$).

line (approximately 7 Hz at 10 s into LAPS). Effects of time interval on F50 were seen ($P < 0.001$, one-way ANOVA, n = 28), and decreases compared with baseline were seen at 0, 10, 20, 30, 40, 50, and 60 s (all $P < 0.001$, paired *t*-test, n = 28). This confirms the dominance of slow-wave, high-amplitude EEG activity, which has a lower mean median frequency than EEG waveform activity associated with consciousness. The transient increase in median frequency at 40 s into LAPS is consistent with global suppression of EEG activity as the birds become deeply unconscious (i.e., intermittent cessation of brain electrical activity), leading to the incorporation of more high-frequency (isoelectric) waveforms in the EEG power spectrogram. Similarly, the increase in F50 at LAPS end is attributable to the presence of high-frequency background noise present in the signal at the end of the LAPS process; this is corroborated by PTOT data revealing the presence of a low power signal consisting of high-frequency, low-amplitude background noise at the end of LAPS.

We also examined the contribution of brain delta waves (0.5 to 4 Hz) to the EEG signal before, during, and after LAPS. The mean relative contribution of delta EEG frequencies at each time point is shown in Figure 4. Delta wave contribution was low (5 to 8% of total) before LAPS during handling because the birds were conscious at these times. The onset of LAPS was associated with a progressively increasing delta contribution up to 30 s (maximum) of LAPS (58% of total), in a pattern closely matching that seen with total power (Figure 2). The delta contribution to the signal changed with time ($P < 0.001$, one-way ANOVA, n = 28) and was higher than the baseline at 10, 20 30, 40, 50, and 60 s (all $P < 0.001$, paired *t*-test, n = 28). After 30 s, the delta contribution decreased as the EEG became suppressed (a state also incompatible with consciousness) but remained elevated compared with baseline.

Heart Rate Responses

Clear EKG waveforms were obtained from all birds during baseline, but in one bird (bird 5), the EKG trace was of poor quality after transfer to the module, so no data were available for that individual during the LAPS process. Figure 5 shows mean heart rate before, during, and after LAPS, with an additional time point before LAPS included to indicate heart rate immediately after handling for instrumentation (H) and heart rate effects of time interval on heart rate were apparent ($P < 0.001$, one-way ANOVA, n = 27). Figure 5 shows that the birds had relatively high heart rates following handling for instrumentation. Between handling for instrumentation and baseline, there was a decrease in mean heart rate (433 to 401 beats per minute, **bpm**), as birds recovered from the stress of handling. Heart rate increased ($P < 0.001$, paired *t*-test, n = 27) when birds were placed in the module (436 bpm) and during transit, which was likely to be associated with stress of being carried and the novelty of the module environment. Nonsignificant decreases in mean heart rate during transit and in the chamber before LAPS started (421 and 412 bpm, respectively) also probably reflect recovery from handling. Sealing of the chamber to start the LAPS process (Figure 5, LAPS 0) did not increase heart rate, but during LAPS there was a steady decrease in heart rate (lower than baseline at 20, 30, 40, 50, and 60 s into LAPS, $P < 0.001$, paired *t*-test, n = 27). This trend for decreasing heart rate was apparent throughout the first 60 s of LAPS (399 to 254 bpm); thus, the most obvious response to LAPS was bradycardia, often associated with arrhythmia. There was no evidence of heart rate increase in the early part of the process when birds are still potentially conscious. At the end of the LAPS process, mean heart rate was low (148 bpm) at which time the traces indicated the

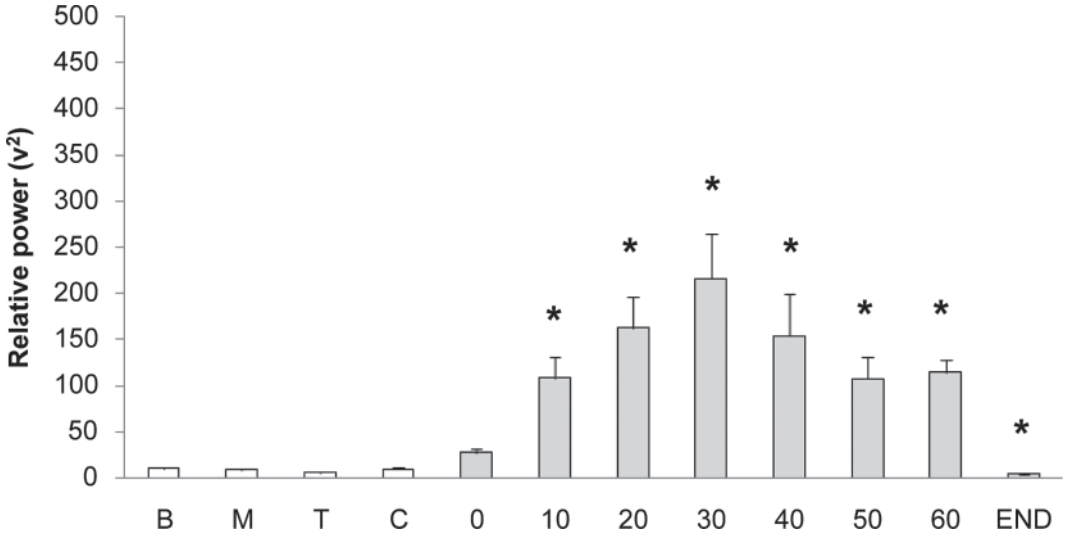

Figure 4. Histogram showing the mean (±SE) power contribution of the delta (<4 Hz) frequency range constructed using 2-s epochs of electroencephalogram (EEG) activity from 12 time points [B, baseline; M, in module; T, in transit; C, in chamber; 0 to 60, 0 to 60 s after low atmospheric pressure stunning (LAPS) start; END, LAPS end]. n = 28. The y-axis units are arbitrary. *Asterisks indicate time intervals that are different from baseline ($P < 0.001$).

onset of heart failure, visible as strong arrhythmia, very low and fluctuating amplitudes and fibrillation. Throughout recording, EKG waveforms were sometimes obscured due to electromyogram activity arising from the pectoral muscles or movement artifacts. Heart rate could not be measured at these times, but these disturbances were used to determine patterns of body movement (see below).

Bird Movement

Table 2 summarizes the timings of movement artifacts exhibited by the birds during LAPS. After LAPS started, all of the birds exhibited a quiescent period before the first onset of movement (first artifact seen 20 to 69 s into LAPS, mean 39 s). These early movements were brief and are likely to be related to loss of

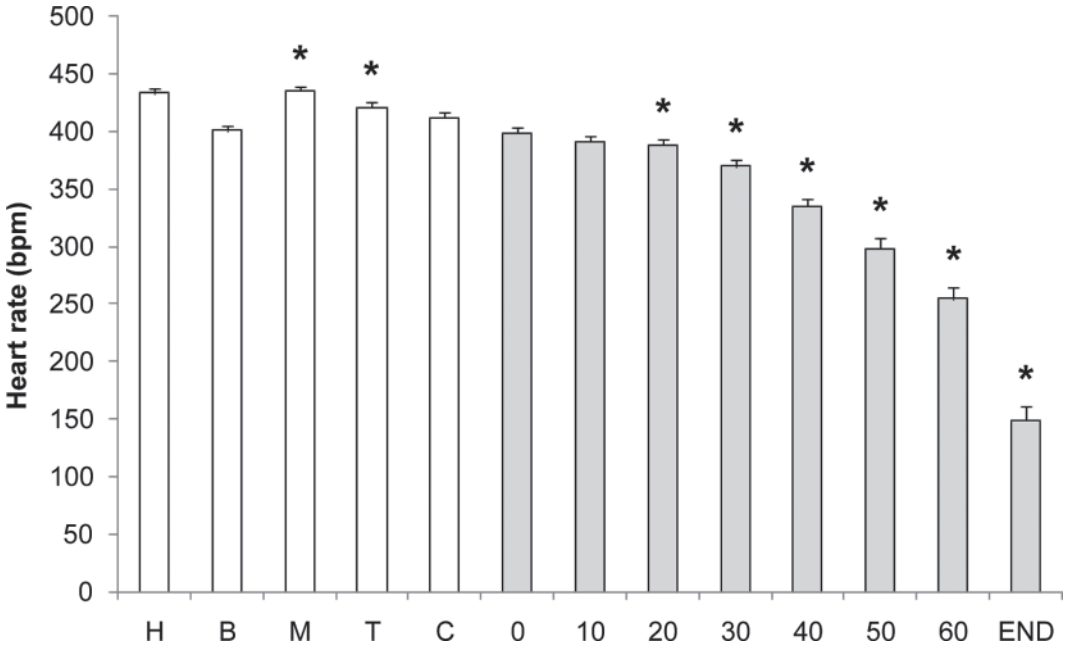

Figure 5. Histogram showing the mean (±SE) heart rate (bpm) during 4-s epochs at 12 time points (H, immediately posthandling for instrumentation; B, baseline; M, in module; T, in transit; C, in chamber; 0 to 60, 0 to 60 s after LAPS start; END, LAPS end). n = 27. *Asterisks indicate time intervals that are different from baseline ($P < 0.001$).

Table 2. Mean (±SE) and range of timings of movement artifacts on the electrocardiogram trace (n = 28)[1]

Item	First artifact (s)	Artifact bout onset >5 s (s)	Total artifact bouts	Total artifact duration (s)
Mean ± SE	39 ± 2	128 ± 3	9 ± 0.5	38 ± 1
Range	20 to 69	96 to 159	4 to 16	27 to 51

[1]First artifact, onset of sustained artifact (>5 s, thought to indicate wing flapping), total artifact bouts, and total artifact duration relative to start of low atmospheric pressure stunning.

balance and posture and the bird's attempt to regain these. Longer artifact bouts lasting more than 5 s [assumed based on previous work (McKeegan et al., 2011) to be related to clonic convulsions/wing flapping] were seen between 96 and 159 s (mean 128 s) into the LAPS process. The mean number of movement bouts was 9 (range 4 to 16 s). Total duration of movement ranged from 27 to 51 s (mean 38 s).

EEG in Relation to Bird Movement

Figure 6 shows mean total power (A) and delta wave contribution (B) in the EEG while in the chamber before LAPS, immediately pre-ataxia, during flapping (between bouts), and after flapping based on one data point for each bird for each time period (the mid-flap time interval was the first epoch of clean EEG that could be obtained after flapping started). The data show that the birds exhibit slow-wave activity (and hence greater total power) immediately before ataxia, and that during flapping the EEG is suppressed (as evidenced by lower power and lower delta contribution). Compared with baseline, PTOT was increased pre-ataxia ($P < 0.001$) and was lower than the baseline at the end of wing flapping ($P < 0.001$, paired t-tests, n = 28). Delta wave contribution to the EEG signal was higher than baseline at pre-ataxia and mid flap ($P < 0.001$, paired t-test, n = 28). On both graphs it can also be seen that after flapping the EEG is isoelectric.

DISCUSSION

This study provides the first high-quality physiological data to be recorded from birds undergoing LAPS and was enabled by the use of self-contained, portable mini-loggers worn by each bird. These loggers allow signals to be continuously recorded from the birds without restricting their movement or the need for trailing wires in situations where measurements would otherwise be very difficult or impossible to obtain (Lowe et al., 2007).

The EEG data clearly show that LAPS gradually induces unconsciousness, as demonstrated by changes in total power, median frequency, and delta wave contribution. In fact, changes in EEG pattern were seen within 10 s of LAPS onset (Figures 1, 2, 3, and 4), but it is possible that the very early changes in EEG pattern relate to the fact that inside the chamber it is completely dark and darkness/eye closure can have a rapid effect on the EEG signal (D. E. F. McKeegan, unpublished observations). In any case, all birds showed further changes in EEG frequencies and power spectrum indices associated with reduced vigilance/loss of consciousness by 10 s into LAPS, peaking at 30 s into the stunning procedure. Because LAPS is a gradual process, it is not possible to directly pinpoint an exact time that consciousness is lost—indeed the loss of consciousness is in itself a process—but the dominance of delta (slow) waves at 30 s into LAPS shows that this process was well underway at this time. In previous work on stunning (McKeegan et al., 2007), we have used visual inspection of the EEG trace to determine time to suppressed EEG as a conservative measure of time to loss of consciousness. That method was difficult to apply in the current work because of the very gradual nature

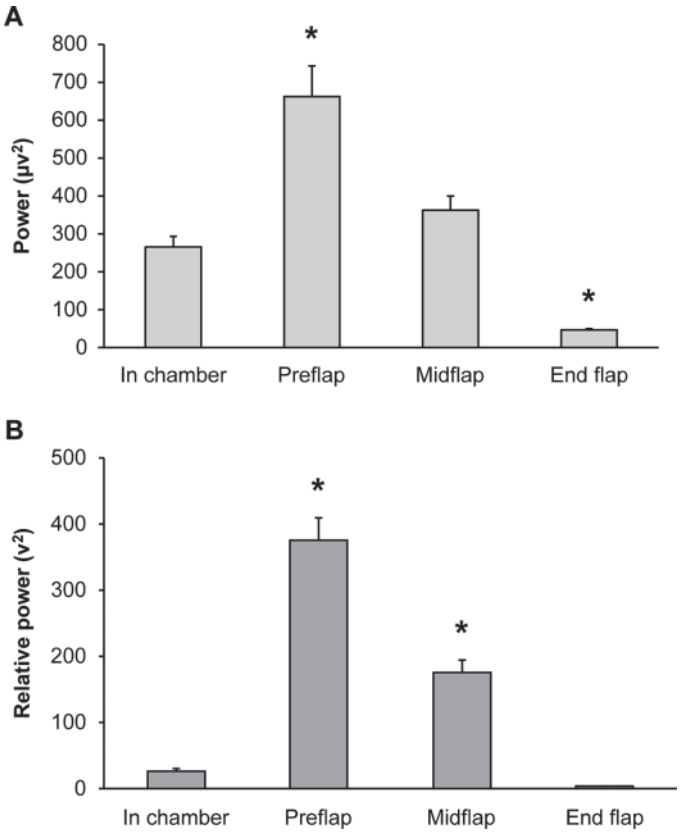

Figure 6. Histograms showing A) the mean (±SE) total power of the electroencephalogram (EEG) and B) the power contribution of the delta (<4 Hz) frequency range constructed using 2-s epochs of EEG activity from 4 time points from each bird (in chamber, preflap, midflap, and end flap). n = 28. *Asterisks indicate time intervals that are different from baseline ($P < 0.001$).

of the changes observed. The well-established approach of spectral analysis using fast Fourier analysis (Cooley and Tukey, 1965; Kaiser, 2005, Johnson et al., 2005a,b; Murrell and Johnson, 2006; Murrell et al., 2007; Benson et al., 2012) was applied, which allowed us to characterize the nature of the frequency changes as LAPS progressed, and examining the delta (slow wave) frequency contribution was particularly useful in the early part of the LAPS process.

Recently, using identical methods to those described here, we measured EEG characteristics in different vigilance states in hens including unconsciousness under anesthetic (surgical plane, maintained with Sevoflurane at 2% 1.25 L/min, D. A. Sandercock and D. E. F. McKeegan, unpublished data). Under general anesthesia, the F50 (approximately 7 Hz) was identical to that observed here at 30 s into LAPS (also approximately 7 Hz), confirming that the EEG pattern observed is consistent with unconsciousness. After 30 s into LAPS, the delta contribution decreased as the brain electrical activity became suppressed, a state also incompatible with consciousness (Hansen and Claasen, 2005) and similar to the EEG pattern observed in a deep hypnotic state induced with anesthesia (D. A. Sandercock and D. E. F. McKeegan, unpublished data). The characteristics of the EEG response were consistent between birds, but some variation was apparent. This is likely to be due to several factors including variation in body size and resistance to anoxia, and these factors are likely to result in variation in responses to a range of stunning methods and slaughter processes. In our previous work, death has been defined by various factors such as presence of isoelectric EEG, respiratory arrest, and heart failure (McKeegan et al., 2007). We did not attempt to determine a time to death in the current study, being content that the EEG was isoelectric for all birds on exit from the LAPS chamber. In addition, by the end of LAPS, all birds had undergone respiratory arrest, preventing any recovery.

From the point of view of welfare, what matters is what the birds experience before loss of consciousness. Behavioral responses to LAPS have been reported previously (Vizzier-Thaxton et al., 2010) and consisted of minimal response until the birds became ataxic, lost posture, and began wing flapping. This is similar to observations of anoxia-induced gas stunning where no negative behavioral responses were seen before ataxia (Woolley and Gentle, 1988; McKeegan et al., 2007). The mode of action of the LAPS process is hypoxia leading to anoxia, and the physiological and behavioral responses reported previously (Vizzier-Thaxton et al., 2010) and seen here are consistent with this. In response to LAPS, birds showed a pronounced reduction in heart rate (bradyarrthythmia) and vigorous wing flapping (clonic convulsions) as would be expected with loss of brain function due to anoxic death.

Moderate heart rate increases were observed associated with handling, particularly the extensive handling required to instrument the birds. Following this, heart rate decreased throughout the baseline period, followed by transient increases associated with further handling (either being carried by hand or automatic movement of the transport cage). It should be noted that the instrumented birds that were monitored here were handled by humans much more than birds entering routine LAPS, so our measurements are relative to an elevated baseline. In the time between handling for instrumentation and just before LAPS began, the average heart rate decrease was 20 bpm, so further elevation was possible once LAPS began. In any case, stress associated with handling is a relevant consideration in any slaughter system, as slaughter processes routinely occur following transportation and manual handling, so it is commercially relevant to examine physiological responses relative to an elevated baseline. Handling-related stress is minimized in the routine LAPS system by stunning the birds in their transport containers and smooth automatic transition of the transport cages into the chamber, without jolts or loud noises. Importantly, no heart rate increases were observed during LAPS in the period when consciousness was a possibility (which would be expected if the birds were experiencing fear or distress during induction). During LAPS, heart rate consistently decreased, with more pronounced bradycardia and arrhythmia observed after 30 s. This is an expected and widely reported response to hypoxia (Woolley and Gentle, 1988; McKeegan et al., 2007).

Behavior was not directly observed in this study, but characteristic movement artifacts on the EKG trace provided some information about bird movements. Care is required when interpreting these artifacts, but their use is supported in our previous work in which behavior and movement artifact data have been corroborated (Coenen et al., 2009; McKeegan et al., 2011). As already stated, behavioral responses to anoxia consist of ataxia, loss of posture, and clonic convulsions (wing flapping). Based on preliminary behavioral observations in a single bird chamber, we assumed that the first substantial movement after LAPS onset indicated ataxia/loss of posture, and our mean estimated time to ataxia in the current experiment (39 s) fits almost exactly with directly observed mean time to ataxia in a previous pilot trial in broilers of the same age subjected to the same pressure reduction curve (38 s, D. E. F. McKeegan and M. A. Gerritzen, unpublished observations). However, in that pilot trial, the observed wing flapping duration was only 14 s compared with 38 s here, which suggests that some of the artifact on the EKG trace in the current trial was due to both clonic (wing flapping) and tonic (muscle spasms) convulsive activity. Another possible explanation of the differences is the presence and subsequent movement of other birds in the vicinity of the bird under study in the commercial trial, which was not the case for single birds in the pilot study. In general, compared with previous reports of anoxic gas killing (in nitrogen gas) under laboratory conditions, the onset

of the expected behavior events was more gradual. For example, McKeegan et al. (2007) reported that broilers undergoing anoxia exhibited wing flapping after 40 s compared with an average of 120 s with LAPS. Raj et al. (1991) reported loss of posture at 11 s and onset of convulsions at 22 s in hens exposed to argon containing 2% residual oxygen, and McKeegan et al. (2013) recently reported loss of posture at 9 s and convulsions at 15 s in broilers exposed to anoxic gas filled foam containing less than 1% residual oxygen. Thus, it would appear that LAPS induces hypoxia more slowly than these other anoxia-based stunning approaches, which is not necessarily unacceptable providing the lengthened induction to unconsciousness is not accompanied by distress. The results of this study would suggest that this is not the case for LAPS.

Strong behavioral responses, convulsions, or both could be a cause for welfare concern if this activity happens at a time when the birds might be conscious. To investigate this, we cross referenced the EEG and EKG artifact data and examined portions of EEG immediately preceding ataxia and during and after artifacts relating to convulsions (primarily clonic wing flapping). These results indicated that the EEG is dominated by slow-wave activity during the LAPS process before body movements indicating loss of posture are observed (pre-ataxia), and is suppressed during periods of clonic convulsion and is essentially isoelectric after this period of activity (after cessation of wing flapping). These observations are consistent with EEG and behavioral responses reported in previous studies on anoxic gas killing (McKeegan et al., 2013), and suggest that these responses occur after loss of consciousness.

In conclusion, the findings support the notion that the LAPS method is a humane approach for rendering poultry unconscious and causing minimal stress until death by anoxia. Based on EEG and body movement responses, a conservative estimate of time to loss of consciousness is around 40 s. The EEG data provide strong evidence that loss of consciousness is gradually induced by LAPS and has been achieved before convulsions take place. The lack of even transient heart rate elevations in any of the birds during the conscious period strongly suggests that the birds did not find LAPS induction to an unconscious state distressing. Collectively, the results show that LAPS has the potential to improve the welfare of poultry at slaughter by gradually inducing unconsciousness without distress, thereby eliminating the need for live shackling and ensuring every bird is adequately stunned before exsanguination.

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