The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK

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Effect of amount and frequency of head-only stunning currents on the electroencephalogram and somatosensory evoked potentials in broilers

ABM Raj* and M O'Callaghan

Department of Clinical Veterinary Science, University of Bristol, Langford, Bristol BS40 5DU, UK
* Contact for correspondence and requests for reprints: M.Raj@bristol.ac.uk

Abstract

The effectiveness of head-only electrical stunning of broilers, with a root mean square (RMS) current of 100 or 150 mA delivered using either 50, 400 or 1500 Hz sine wave alternating current (AC), was investigated. The changes occurring in the spontaneous electroencephalogram (EEG) were evaluated using Fast Fourier Transformations (FFT) to determine the impact of the amount and frequency of stunning current on total (2–30 Hz) and relative (13–30 Hz) power contents in the EEG. Induction of epileptiform activity and reduction in the EEG power contents to less than 10% of pre-stun levels from the end of epileptiform activity were used as indicators of effective stunning. The duration of unconsciousness and insensibility was determined on the basis of the return of EEG power contents. In addition, the changes occurring in somatosensory evoked potentials (SEPs) were subjectively evaluated to determine the impact of stunning treatments. The results of ANOVA (repeated measures) showed statistically significant effects of interactions between the current frequencies, amount of current and repeated measures on changes in EEG power contents ($P < 0.001$). Stunning broilers with 150 mA delivered using 50 Hz resulted in EEG changes that were indicative of more pronounced neuronal inhibition following epileptiform activity and also lasted longer than was the case when broilers were stunned with 150 mA delivered using 400 Hz. Stunning broilers with 100 mA delivered using 50 Hz resulted in changes very similar to those observed after stunning with 150 mA of 50 Hz, but which lasted for a relatively shorter time. However, these changes were more pronounced and lasted longer than did stunning with 100 mA delivered using 400 Hz. The effects of stunning broilers with 150 mA of 400 Hz were similar to those found after stunning with 100 mA of 50 Hz. By contrast, stunning broilers with 100 mA of 1500 Hz failed to fulfil the criteria set out in this study. Stunning of broilers with 150 mA of 1500 Hz induced epileptiform activity but failed to reduce EEG power contents to less than 10% of pre-stun levels. Therefore, the stunning of broilers with 100 or 150 mA of 1500 Hz may not be adequate to avoid pain and suffering during slaughter. Thus, minimum currents of 100, 150 and 200 mA should be delivered whilst using 50, 400 and 1500 Hz, respectively, to achieve effective electrical stunning in broilers. Severing of the carotid arteries in the neck following head-only electrical stunning, and high frequency (>125 Hz) electrical water bath stunning of broilers should also become a statutory requirement to prevent the return of consciousness during bleeding.

Keywords: *animal welfare, chicken, current, frequency, slaughter, stunning*

Introduction

Electrical stunning is the most commonly used method for rendering animals, including poultry, unconscious prior to slaughter under commercial conditions. This stunning method involves the application of a current of sufficient magnitude across the head to induce grand mal epilepsy, which is a kind of generalised epilepsy accompanied by tonic-clonic seizures, and is always associated with unconsciousness in humans (Hoenderken 1978; Lambooy 1981). Grand mal epilepsy occurs due to hyper-synchronisation of the activity of neurones in the brain, which can be recognised by the predominance of high amplitude (>100 mV), alpha (8–13 Hz) activity in the electroencephalogram (EEG) or electrocorticogram (ECoG). Under the conditions of normal neuronal function, the excitatory amino acid (EAA) neurotransmitters facilitate the excitatory bursts while inhibitory amino acid (IAA) neurotransmitters inhibit such actions. Since the two neurotransmitter systems exist together in the brain, it is believed that they jointly provide a controlled balance of neuronal activity in conscious animals. However, when two fast-acting EAAs, namely glutamate and aspartate, are released excessively into the extra-cellular space, they play important roles in the initiation, spread and maintenance of epileptic activity in the human brain (Meldrum 1994). Gamma-aminobutyric acid (GABA) is the principal IAA neurotransmitter which, when released into the extra-cellular space, inhibits neuronal activity and epilepsy (Meldrum 1984).

The correlation between the neurophysiology and neurochemistry of electrical stunning and slaughter is well established in sheep and calves (Cook *et al* 1992, 1995, 1996). Cook *et al* (1992, 1995) have investigated head-only electrical stunning in sheep, and reported that the development of epileptiform activity following the head-only electrical stunning of sheep is dependent on EAAs, and that the termination of epilepsy and suppression of reflexes is attributed to GABA release. They concluded that EAAs and GABA, synergistically, produce unconsciousness and analgesia required to safeguard animal welfare during the electrical stunning and slaughter process.

By contrast with the red meat species, electrical stunning (head-only or water bath) of chickens seldom produces grand mal epilepsy in the brain; only a small proportion develop epileptiform activity following electrical stunning (Gregory & Wotton 1987, 1990a). About 90% of birds that develop epileptiform activity show low frequency (<3 Hz) polyspike or spike-and-wave activity suggestive of the absence of, or petit mal, seizures, which are not always associated with loss of consciousness in humans (Gregory 1986). It is also true that focal epilepsy may begin with such EEG manifestations before becoming secondarily generalised epilepsy accompanied by loss of consciousness (Dreifuss & Ogunyemi 1992).

We do not yet clearly know the brain mechanism(s) that might be responsible for the inhibition of grand mal epilepsy or for the lack of synchronisation of neuronal activity in electrically stunned poultry. However, it has been proposed that chickens can be assumed to be unconscious provided that the electrical stunning-induced low frequency epileptiform activity leads to a period (at least 30 s) of quiescent or isoelectric EEG (Schutt-Abraham *et al* 1983). In this situation, the duration of unconsciousness induced by stunning could last for about 40 s post-stun (eg 10 s of epileptiform activity plus 30 s of isoelectric EEG). Since the interval between the end of stunning and neck cutting under commercial conditions can be up to 20 s, it can be assumed that exsanguination of birds by cutting all of the major blood vessels in the neck would lead to brain ischemia within the ensuing 20 s, and hence prevent the return of consciousness and sensibility in birds. If the duration of unconsciousness and insensibility is shorter (eg lasts for 30 s), then neck cutting will have to be performed sooner (eg within 10 s post-stun) to avoid recovery.

Further studies aimed at determining the minimum effective stunning current have used the abolition of somatosensory evoked potentials (SEPs) in the brain as an unequivocal and objective indicator of loss of consciousness in chickens (Gregory & Wotton 1989, 1990a, 1991). Despite the differences in the criteria (EEGs or SEPs) used in previous studies, it was concluded that a minimum of 120 mA per chicken in a water bath stunner would be necessary to achieve effective stunning.

Nowadays, poultry are stunned commercially using 5–120 mA of current delivered with 50–1500 Hz of sine wave alternating current (AC) or with a pulsed direct current (DC). In comparison with 50 Hz, electrical water bath stunning of chickens using high frequencies has been reported to result in fewer carcass and meat quality defects and therefore has commercial benefits (Wilkins *et al* 1998). There is no published neurophysiological evidence to suggest that a single current level, whether it be root mean square (RMS) current of AC or average current of pulsed DC, will be sufficient to protect bird welfare when stunning is performed using a variety of electrical frequencies.

Like electrical stunning, the parameters (waveform and frequency of current) used in electroconvulsive therapy (ECT) for drug-resistant psychiatric human subjects also vary; however, the criterion used in predicting the therapeutic response is the occurrence of highly synchronised electrical activity followed by a quiescent EEG, which is also referred to as cortical spreading depression (Krystal & Weiner 1999). The presence of excessive extra-cellular potassium is associated with the depolarisation of neurones that occurs during spreading depression, which is manifested as a quiescent period in the EEG. If electrical stunning-induced epileptiform activity in poultry is indeed indicative of generalised epilepsy, then it should also be followed by a complete depolarisation of neurones in the brain, which could be recognised from the occurrence of a distinct period of quiescent EEG. Indeed, previous studies have shown that effective electrical stunning produces 30 s of isoelectric EEG (Richards & Sykes 1967; Kuenzel & Walther 1978). However, the power contents in the EEGs or ECoGs of electrically stunned poultry have not been quantified previously. In addition, the effects of amount and frequency of stunning current and the magnitude of neuronal inhibition (depth of electronarcosis) occurring during quiescent EEG in electrically stunned animals, including poultry, are not known.

Ideally, the impact of stunning parameters should be evaluated using quantitative EEG (or ECoG), rather than qualitative (subjective) assessment, in order to provide better descriptions of the magnitude of changes occurring in power content of EEG signals during the epileptiform and quiescent periods. This will facilitate better understanding of results and comparison between studies. It is also thought that the impact should be evaluated using head-only electrical stunning with short stun duration (eg 1 s). This is because head-only electrical stunning involves transcranial application of a current directly across the brain and thus is considered to be better than water bath stunning in which the current is applied through the whole body. In the latter, it will be difficult to separate the impact of current on the brain from its effects on the peripheral nervous system. Short stun duration would reveal whether the chosen electrical stunning parameters induce an immediate loss of consciousness, which is a statutory requirement.

This study was carried out to determine the impact of head-only stunning of broilers with a constant RMS current of either 100 or 150 mA delivered for 1s with a frequency of 50, 400 or 1500 Hz sine wave AC. The stunning current was delivered using a variable voltage / constant current stunner developed by the Silsoe Research Institute (Sparrey *et al* 1993). The advantage of using this type of stunner is that it modulates the output voltage according to the electrical resistance in the pathway, such that a pre-set current is delivered constantly during the stun. The changes in the EEG and in SEPs were analysed to determine the effectiveness and duration of unconsciousness induced with the stun.

In this study, the immediate induction of unconsciousness and insensibility was ascertained on the basis of the occurrence of epileptiform activity, and the depth of unconsciousness (electronarcosis) was ascertained on the basis of the total power contents in the isoelectric (or profoundly suppressed) EEG being less than 10% of the pre-stun EEG power content — as has been used in rats to define the onset of G-force induced isoelectric EEG (Lukatch *et al* 1997). The birds were allowed to recover following stunning and the duration of unconsciousness was determined on the basis of time to the return of total (2–30 Hz) and relative (13–30 Hz) power contents in the EEG. Activity in the 13–30 Hz EEG frequency band is believed to reflect corticocortical and thalamocortical transactions related to flow of information through the thalamus to the cortex and specific information processing, at least in mammals (Hughes & John 1999).

Materials and methods

This study was carried out with the approval of an Ethical Review Process, under a UK Home Office (Scientific Procedures) Licence. In total, 90 six to seven week old commercial (ROSS) broilers intended for human consumption were obtained from a processing plant and used in this study. Of the 90 broilers, 11 died at induction of anaesthesia, two died during surgery, two died during recovery, 14 had broken EEG recordings or somatosensory stimulating electrodes during recovery and five broilers had noisy EEG signals. Broilers with broken electrodes and those with noisy EEG signals (n = 19) were not subjected to stunning treatments, but were instead killed by manual neck dislocation. The remaining 56 broilers were randomly allocated to one of the stunning treatments, but the EEG data corresponding to some of the birds were lost due to technical and recording difficulties. The numbers of birds used to evaluate the changes occurring in EEG (n = 46) and SEPs (n = 53) within each treatment group are presented in Tables 1 and 4 respectively.

The broilers weighed on average 2.1 kg (standard deviation [SD] = 0.45). They were sedated by intra-muscular injection of 100 mg of ketamine hydrochloride (Ketaset, Willows Francis Veterinary, UK), and anaesthetised by intravenous administration of on average 17.5 mg (SD = 3.90) of pentobarbitone sodium (Sagatal, Rhone Merieux, USA). The anaesthetised broilers were implanted with EEG recording and somatosensory stimulating electrodes as described by Gregory and Wotton (1989). After overnight recovery from the surgical procedure, the broilers were stunned (head-only) using a pair of tongs fitted with electrodes made of 1 mm^2 wire mesh (Multishield, RFI Shielding Ltd, Braintree, Essex, UK) encasing fine steel wire. These electrodes had an average impedance of 300 Ohms and, when pressed on the head of a chicken, conformed to the shape of the skull and provided a large electrode contact surface area. The broilers were wrapped with Elastoplasts prior to shackling to minimise the damage to electrodes that might occur due to wing flapping. After shackling, the stunning electrodes were placed firmly behind the eyes on either side of the head of broilers and the birds were stunned (head-only) with a constant current of either 100 or 150 mA delivered with either 50, 400 or 1500 Hz sine wave AC for 1 s.

A variable voltage / constant current stunner manufactured by the Silsoe Research Institute (UK) was used to apply the stun. The peculiarity of this stunner was that the voltage always started at zero at the beginning of the stun and it reached the maximum output voltage (up to 620 V) that was sensed by the electrical circuit in the stunner to be necessary to deliver the pre-set current within the first current cycle. Subsequently, the voltage gradually decreased within the first 200 ms of the stun, due to the breakdown of electrical resistance in the pathway by the high voltage, to a level that was sufficient to deliver the current uniformly throughout the stun duration. The voltage and current profiles of each bird were recorded in this study to verify the effective application of stunning.

SEPs were induced by electrical stimulation of the contralateral radial nerve (mean = 2.5 V, SD = 1.03, 2 ms pulses at the rate of 2 stimuli per second). The spontaneous EEG including SEPs were recorded for 2 mins before and for up to 2 mins after stunning on to a magnetic tape (Racal Store 7DS) using a Mingograph EEG 10 recorder (100 mV sensitivity, 0.1 s time constant and 70 Hz upper frequency filter). Owing to overcharging of the amplifier, the EEG signals of some birds were lost for 2–5 s from the end of the stun (Table 1). The occurrence of any post-stun movement artefacts, including convulsions, was recorded on to tapes using the voice channel and was also marked on to the EEG traces for reference. During the recovery period, the presence of consciousness was determined on the basis of spontaneous blinking, constricted pupil and response to comb pinching, or on the ability of the bird to move its head away when its eyeball was touched; however, the exact time to onset of these reflexes was not determined. All of the birds were killed by neck dislocation within 10 mins of recovery from the single stun.

The EEG traces obtained from the Mingograph were subjectively evaluated to determine the changes occurring that were due to stunning and those during recovery. In this regard, the EEG manifestations occurring immediately after stunning and the duration of such manifestations, the duration of occurrence of a profoundly suppressed EEG and the time to onset of EEG activation (regains amplitude) were noted. The analogue EEG signals recorded on magnetic tapes were digitised at a sampling rate of 200 Hz, with AC coupling on, using Vision Data Acquisition System (Nicolet Technology, USA). The digitised data were then transferred to a personal computer using Data Viewer software, in which Fast Fourier Transformations (FFTs) were carried out using Impression software (both supplied by Nicolet Technology, USA). For each bird, six pre-stun and 11 post-stun epochs of 10 s each (up to 120 s from the end of the stun) were separated and used to perform FFT. The FFT was performed on epochs using seamless window, Hanning window and amplitude correction, and energy spectrums (V^2) were derived. During the FFT, six pre-stun epochs were averaged to obtain a single pre-stun energy spectrum for each bird. Thus, one averaged pre-stun and 11 post-stun

Table 1 The effect of electrical stunning current (mA) and frequency (Hz) on the duration (mean ± SD) of epileptiform activity and on the time to activation of EEG.

Treatment	Time to return of EEG (s)	Duration of epileptiform activity (s)	Time to EEG activation (s)
50 Hz, 100 mA (n = 9)	4.3 (0.87)	11.9 (2.85)	42.9 (18.52)
50 Hz, 150 mA (n = 6)	3.3 (0.82)	9.8 (2.14)	35.4 (9.24)
400 Hz, 100 mA (n = 8)	4.2 (1.25)	9.7 (1.60)	36.3 (5.38)
400 Hz, 150 mA (n = 7)	3.7 (0.95)	9.1 (2.12)	36.8 (10.26)
1500 Hz, 100 mA (n = 8)	4.5 (2.07)	12.0 (2.00)	17.6 (4.69)
1500 Hz, 150 mA (n = 8)	3.7 (0.71)	11.9 (1.36)	34.9 (12.97)

epochs of 10 s each were converted into energy spectrums for each bird.

The energy spectrums were 'evaluated' using a built-in option to produce data tables consisting of EEG frequency and corresponding energy (V^2) in two columns. Since the artefacts originating from bird movements (including wing flapping) were found to be of less than 2 Hz, the energy spectrum of EEG frequency bands 13–30 Hz (beta) and 2–30 Hz were extracted. The power contents were then calculated as the area under the curve for each of the EEG frequency bands using the Impression software. A closer evaluation of the power contents revealed that the artefacts originating from background noise or comb pinching affected the power contents in certain EEG frequency bands. Correction for these artefacts resulted in deletion of some data points; however, data were available for a minimum of six birds at any one particular time post-stun in each stunning treatment. The power contents were then exported to a spreadsheet (Microsoft Excel) in which the relative changes (post-stun power × 100 / pre-stun power) occurring during post-stun were calculated for individual birds and then averaged according to treatment.

The evoked potentials were averaged separately using a computer (Acorn RISC PC) program as described previously by Raj *et al* (1998). The averaged SEPs were then plotted and used for subjective determination of the presence or absence of SEPs.

The subjective EEG analysis of data concerning the duration of epileptiform activity, when it occurred and the time to onset of EEG activation were subjected to Kruskal-Wallis and Mann-Whitney *U*-tests, respectively, to determine the effects of the three stunning frequencies and two stunning currents. The total and relative power contents in the EEG of individual birds were subjected to analysis of variance (ANOVA) repeated measures tests to determine the effects of electrical stunning frequency and current. Any missing data were substituted with averages of two adjacent data points. The average power contents of stunning treatments were also subjected to a Friedman's test to determine the significance of differences between the six treatments (three frequencies and two current levels). The impact of stunning treatments on the presence or absence of SEPs were subjected to a Chi-squared (χ^2) test, and the impact of current and frequency on the times to abolition and return of SEPs after stunning was analysed using a two-way ANOVA.

Results

The results of subjective evaluation of EEG traces indicated that all of the broilers stunned with either 50 or 400 Hz sine wave AC, receiving a constant current of either 100 or 150 mA, showed epileptiform activity in their EEG. By contrast, only three of the eight broilers that were stunned with 100 mA of 1500 Hz showed spike-and-wave activity, and the remaining birds showed a mild suppression in the amplitude of EEG signals. However, increasing the stunning current to 150 mA of 1500 Hz resulted in all of the birds showing epileptiform activity in their EEG. The Kruskal-Wallis analysis showed that the stunning frequency had a significant effect on the duration of epileptiform activity ($H = 12.04$; $P < 0.01$; Table 1), and the mean ranks for 50, 400 and 1500 Hz were 24.4, 14.8 and 32.0 respectively. The epileptiform activity lasted on average 1 and 2 s longer after stunning with 1500 Hz than with 50 and 400 Hz respectively. A Mann-Whitney *U*-test revealed that the stunning current had no significant effect on the duration of epileptiform activity. The mean ranks were 24.6 and 21.6 respectively, for 100 and 150 mA ($Z = -0.76$; $P = 0.24$; ns).

A profoundly suppressed EEG ensued epileptiform activity in all broilers that were stunned with 100 or 150 mA of 50 and 400 Hz, and in those stunned with 150 mA of 1500 Hz. Three of the eight broilers stunned with 100 mA of 1500 Hz, which showed epileptiform activity following stunning, had profoundly suppressed EEGs and the others showed only a mild EEG suppression. The recovery of neuronal activity (EEG activation) was clearly recognisable from the intermittent appearance of the 'small spikes on a dome' pattern in the profoundly suppressed EEG or the intermittent burst of high frequency activity in the mildly suppressed EEG. Typical examples of EEG activation are presented in Figure 1 using EEG traces of broilers showing epileptiform activity or mild suppression after stunning with 100 mA of 1500 Hz. This EEG pattern started to appear more frequently before the EEG looked similar to that of conscious broilers. The times to onset of the first appearance of EEG activation were significantly affected by the stunning frequency, in the sense that it was shorter in broilers stunned with 1500 Hz than in those stunned with 50 or 400 Hz ($H = 8.34$; $P < 0.05$; Table 1). The mean ranks were 27.0, 27.3 and 15.8 for 50, 400 and 1500 Hz respectively. Although the average time to activation of EEG in broilers that were stunned with

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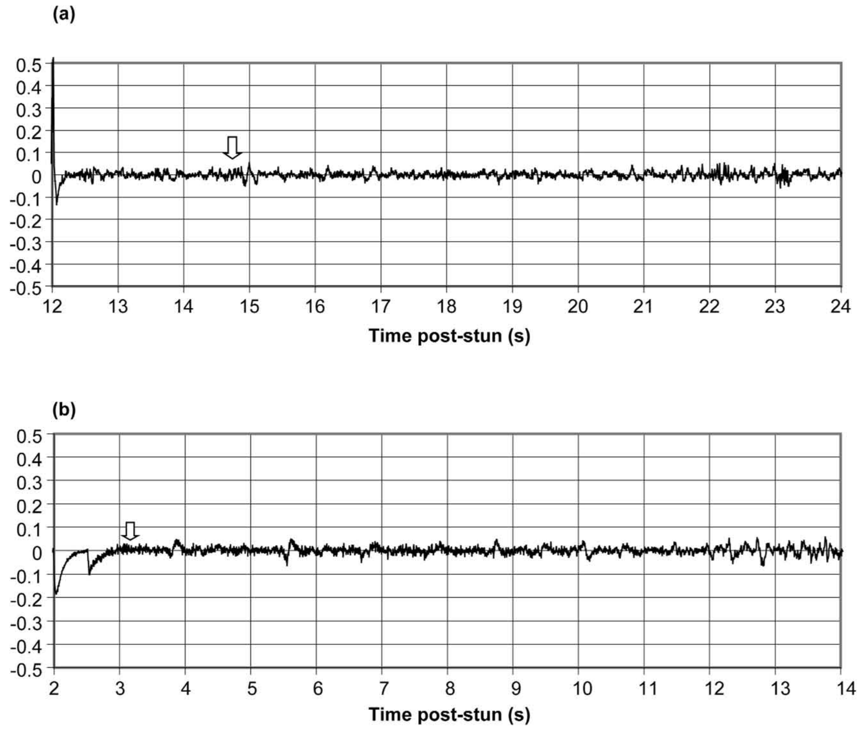
Figure 1



Time to onset of EEG activation in broilers stunned with 100 mA of 1500 Hz (arrow indicates onset of EEG activation). (a) Broiler with epileptiform activity in the EEG. (b) Broiler with suppressed EEG.

Table 2 Statistical significance (*P*-values) of the effects of frequency, current, temporal changes in the EEG power contents (repeated measures) and the interactions between variables.

Variable	Total power 2–30 Hz	Relative power 13–30 Hz
A. Frequency	0.77	0.99
B. Current	0.31	0.31
C. Repeated measures	0.001	0.001
AB interaction	0.02	0.14
AC interaction	1	1
BC interaction	0.43	0.41
ABC interaction	0.001	0.002

100 mA of 1500 Hz was found to be half of that recorded after stunning with 150 mA of 1500 Hz, statistical evaluation showed that stunning current did not affect the time to EEG activation, and the mean ranks were 19.8 and 25.4 respectively for 100 and 150 mA ($Z = -1.43$; $P = 0.15$; ns).

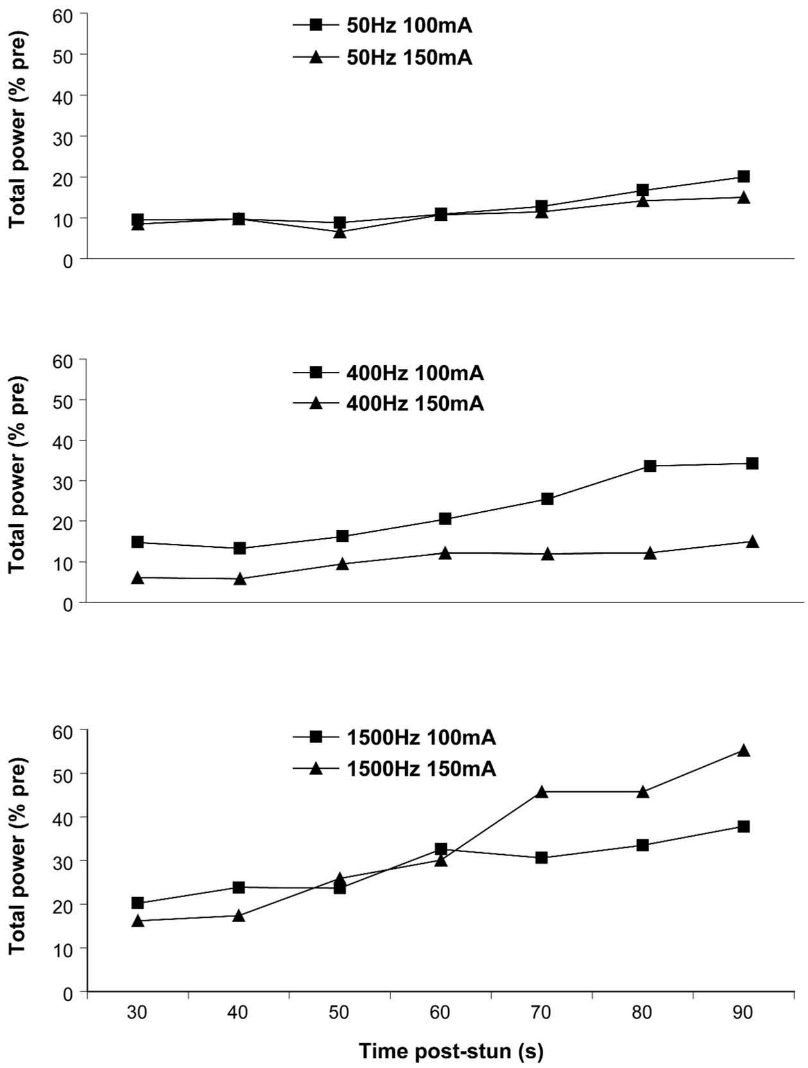
On average (n = 46), the relative power contents in various EEG frequency bands of conscious broilers hung on shackles (pre-stun) were 13.17% (SD = 5.70), 35.56% (SD = 7.10), 20.54% (SD = 3.38) and 30.73% (SD = 9.94) respectively, in 2–4, 4–8, 8–13 and 13–30 Hz.

The results of ANOVA tests (Table 2) showed that neither stunning frequency nor current had a statistically significant effect on the total and relative power contents. As would be expected, the repeated measures on the total and relative power contents in the EEG frequency were significant ($P < 0.001$).

There was also a significant interaction between the effects of stunning frequency and current on the total power content ($P < 0.05$). Increasing the stunning current from 100 to 150 mA, while using 50 or 400 Hz, resulted in a decrease in overall total power content in the EEGs (227 versus 139% and 158 versus 130% respectively). This was because, in comparison with broilers that were stunned with 100 mA of 50 or 400 Hz, broilers stunned with 150 mA of these two current frequencies had prolonged periods of profoundly suppressed EEG. By contrast, the overall total power content increased when stunning current was increased while using 1500 Hz (44 versus 202%). This was because, in comparison with broilers that were stunned with 150 mA of 1500 Hz, fewer broilers (3 out of 8) that were stunned with 100 mA of this current frequency showed epileptiform activity. The duration of epileptiform activity was also longer with 150 mA than with 100 mA of 1500 Hz.

There were also significant interactions between the effects of electrical stunning frequency, current and repeated measures for the total power content ($P < 0.001$). When compared with the pre-stun levels, the total power content increased as a result of the epileptiform activity. The increase in total power content during the period of epileptiform EEG (0–10 s post-stun epoch) in broilers that were stunned with 100 mA, reduced significantly as the stunning frequency was increased (2463, 1690 and 136% respectively,

Figure 2



The effects of the amount and frequency of stunning current on the total power content (2–30 Hz) in the EEG of broilers.

in 50, 400 and 1500 Hz). By contrast, when the stunning was performed with 150 mA, the increases in total power contents during the period of epileptiform activity were significantly less in broilers that were stunned with 50 and 400 Hz than in those stunned with 1500 Hz (1501, 1369 and 3292% respectively).

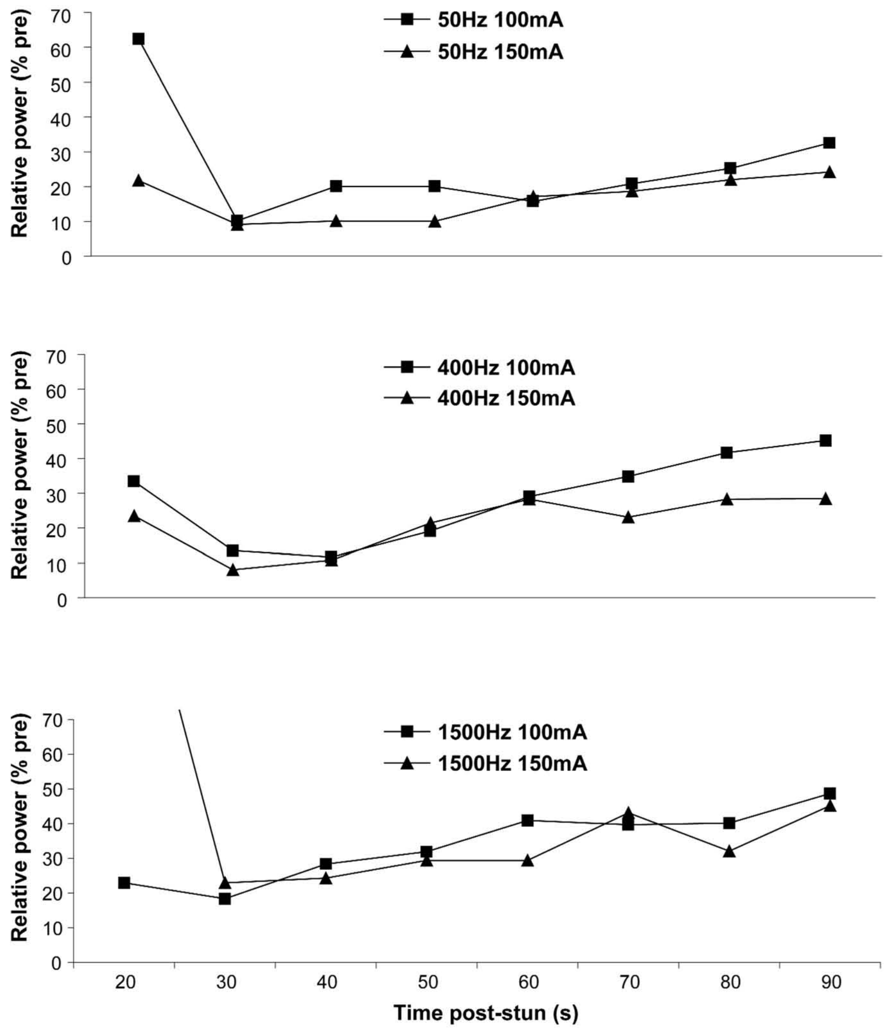
The magnitude and duration of EEG suppression also varied between the stunning treatments. For the sake of clarity, changes occurring in the total power content during recovery after the epileptiform activity, between 30 and 90 s, are presented in Figure 2. At 100 mA, the total power content in the EEG of broilers that were stunned with 50 Hz decreased profoundly, reaching the ultimate low levels of 10% or less of the pre-stun level, and remained at that level for up to 60 s post-stun. In broilers that were stunned with 100 mA of 400 Hz, the total power content decreased to an ultimate low level of 13% and remained at that level for up to 40 s post-stun. In broilers that were stunned with 100 mA of 1500 Hz, the total power content decreased to an ultimate low level of only 17% in one epoch at 11–20 s, and then increased to 20% or more of the pre-stun level.

When stunning was performed with 150 mA of 50 or 400 Hz, the total power content decreased profoundly, reaching the ultimate low levels of 10% or less of the pre-stun level, and remained at that level for up to 60 and 40 s post-stun respectively, in 50 and 400 Hz (Figure 2). Whereas, in broilers that were stunned with 150 mA of 1500 Hz, the total power content decreased to an ultimate low level of 17% and remained close to that level for up to 40 s post-stun. Owing to this, the increase in total power content in the EEG during the recovery period varied according to the amount and frequency of stunning current. At both of the current levels, the increase in total power content during the recovery period was faster in birds that were stunned with 1500 Hz than in those stunned with 50 or 400 Hz, and it was also relatively faster with 400 than with 50 Hz.

The results of ANOVAs (Table 2) showed that the relative power content in 13–30 Hz was also affected significantly by the interactions between the effects of stunning frequency, current and repeated measures ($P = 0.002$; Figure 3). Since stunning of broilers with 100 mA of 50 or 400 Hz resulted in epileptiform activity in the EEG of all of the birds, significant increases in the relative power contents

Figure 3

The effects of the amount and frequency of stunning current on the relative power in 13–30 Hz EEG signals in broilers.



occurred during the epileptiform activity. In comparison with the pre-stun levels, the relative power contents in the epileptiform EEGs (0–10 s post-stun epochs) were 1801 and 1618% respectively, in 50 and 400 Hz. In broilers that were stunned with 100 mA of 1500 Hz, the relative power content during the period of epileptiform EEG increased to 715% of the pre-stun level. This was due to the fact that only three of the eight birds showed epileptiform activity and the others showed mild EEG suppression after stunning with 100 mA of 1500 Hz. The relative power content in this EEG frequency band fell to ultimate low levels of below 10% of pre-stun levels only in broilers that were stunned with 150 mA of 50 and 400 Hz; however, they remained at those low levels for longer in broilers that were stunned with 50 than with 400 Hz. In broilers that were stunned with 100 mA of 1500 Hz, the relative power content in 13–30 Hz fell momentarily between 20–30 s post-stun to 18% of the pre-stun levels, which is significantly higher than for those recorded after stunning with 100 mA of 50 and 400 Hz, and it then increased to 20% and more of the pre-stun level.

Increasing the amount of current to 150 mA in all of the three electrical stunning frequencies, significantly altered the magnitude and time course of the changes occurring in the relative power contents. When compared with 100 mA, the relative power contents in the 13–30 Hz EEG frequency band remained at the lowest levels for longer periods (up to 50 s post-stun) after stunning with 150 mA of 50 Hz. Stunning of broilers with 150 mA, in comparison with 100 mA, of 400 Hz resulted in a slight reduction (about 4%) in the relative power content of 13–30 Hz. However, the recovery of relative power content in this EEG frequency band for up to 60 s post-stun was very similar in these two groups. In contrast with 100 mA, stunning broilers with 150 mA of 1500 Hz resulted in a significant increase in the relative power content during the epileptiform activity, and the magnitude of this increase was similar to that which occurred with 150 mA of 50 or 400 Hz. Although epileptiform activity lasted longer in birds stunned with 150 mA of 1500 Hz, the magnitude of reduction and recovery of relative power contents were very similar in broilers stunned with 100 or 150 mA of 1500 Hz. As can be seen in Figure 3, the relative power content was considerably higher and the loss of power also lasted for a significantly shorter time after stunning with 150 mA of 1500 Hz than with 150 mA of 50 or 400 Hz. A closer examination of the relative power contents in the EEGs of broilers that showed epileptiform

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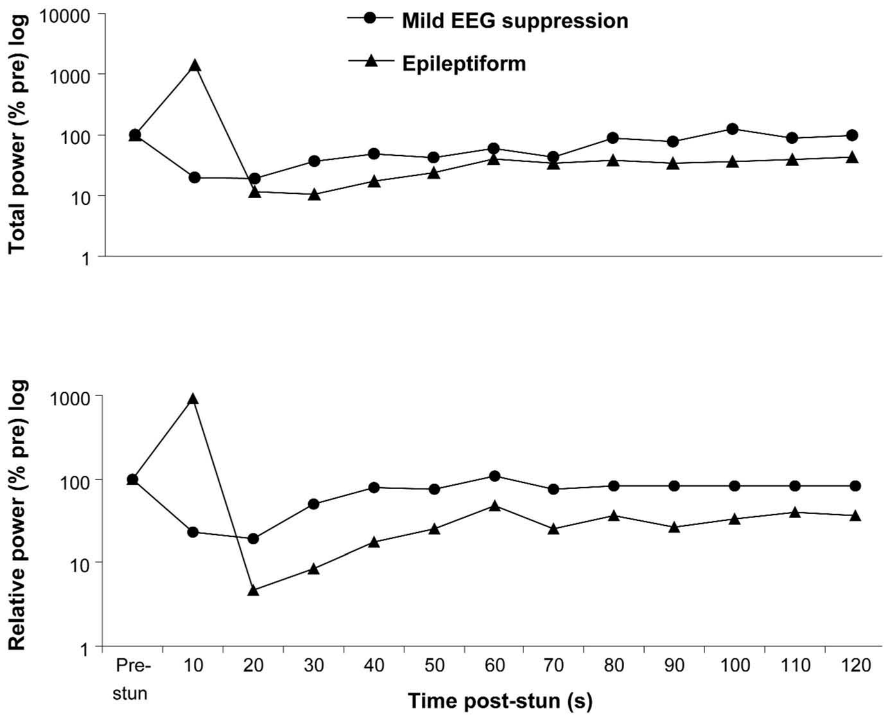
Figure 4



Changes in the total (2–30 Hz) and relative (13–30 Hz) power contents due to manifestation of polyspike or suppression in the EEGs of broilers stunned with 100 mA of 1500 Hz.

Table 3 The effect of the amount and frequency of stunning current on the total power and relative power contents of EEG signals presented as mean ranks (Friedman's test).

Treatment	Total power (2–30 Hz)	Relative power (13–30 Hz)
50 Hz, 100 mA	3.4	3.4
50 Hz, 150 mA	2.0	1.6
400 Hz, 100 mA	3.8	3.5
400 Hz, 150 mA	2.2	3.2
1500 Hz, 100 mA	5.0	5.0
1500 Hz, 150 mA	4.6	4.2
χ^2	25.05	21.13
Significance	$P < 0.001$	$P < 0.001$

activity (n = 3) and those that showed a mild suppression in the EEG (n = 5) after stunning with 100 mA of 1500 Hz, indicated that the latter group of birds contributed to this effect. The total and relative power contents of EEG frequency bands of broilers that showed mild suppression in the EEG were in total contrast with the power contents of broilers that showed epileptiform activity after stunning with 100 mA of 1500 Hz (Figure 4).

Owing to these differences in trends, the statistical evaluation of the treatment averages of total power contents by a Friedman's test also showed significant differences between the stunning treatments (Table 3). Broilers stunned with 150 mA of 50 or 400 Hz had very similar and the lowest ranks and, when compared with these two groups, broilers stunned with 100 mA of 50 and 400 Hz had slightly higher ranks. When compared with these four treatments, stunning with either 100 or 150 mA of 1500 Hz resulted in significantly higher ranks.

The results of subjective evaluation of SEPs were grouped into three categories: (1) SEPs were present throughout the 2 min post-stun period; (2) SEPs were present initially but lost for a period of time and then returned; (3) SEPs were abolished immediately after stunning and returned later. The number of birds in each of these categories and the time to the return of SEPs in different stunning treatments are presented in Table 4. Statistical analysis of the distribution of birds in these categories revealed no significant difference between the stunning treatments ($\chi^2 = 14.67$; $P = 0.14$; ns). ANOVA tests showed that neither the amount nor the frequency of stunning current had a significant effect on the duration for which the SEPs were present following stunning or on the time to the return of SEPs during recovery. Analysis of the EEG data indicated that the presence or abolition of SEPs immediately after stunning was not related to the epileptiform activity; SEPs were present after stunning until the onset of a profound EEG suppression and were

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Table 4 The effect of the amount and frequency of stunning current on the somatosensory evoked potentials in broilers.

Treatment	Number of birds in which the SEPs were…			
	Present throughout (A)	Present initially and then lost (B)	Abolished and then returned (C)	Time to return (s)* (D)
50 Hz, 100 mA (n = 10)	2	5	3	49.8 (14.49)
50 Hz, 150 mA (n = 7)	2	5	0	47.0 (10.34)
400 Hz, 100 mA (n = 7)	3	3	1	45.6 (6.75)
400 Hz, 150 mA (n = 10)	1	9	0	44.8 (11.37)
1500 Hz, 100 mA (n = 10)	2	7	1	36.0 (13.24)
1500 Hz, 150 mA (n = 9)	0	9	0	46.6 (9.04)

* Includes mean (SD) time to return of SEPs in categories B and C.

abolished during the occurrence of a profound EEG suppression. The time to the return of SEPs was significantly correlated with the time to the onset of EEG activation ($r^2 = 0.40$; $P < 0.001$).

Discussion

Although the results of this study show that neither electrical stunning frequency nor the amount of stunning current had a significant effect on the total (2–30 Hz) or relative (13–30 Hz) power contents in the EEG, the significant interactions between the effects of these variables suggest that the induction of epileptiform activity and the magnitude and time course of profound EEG suppression are significantly influenced by the amount and frequency of the stunning current.

Stunning broilers with 100 or 150 mA of 50 Hz induced epileptiform activity in the EEGs of all of the broilers and with corresponding increases in the total power contents after stunning. Following the epileptiform activity, the total power content decreased to ultimate low levels of below 10% of the pre-stun values and remained at those levels for up to 50 s post-stun. These changes are considered to be characteristic of generalised epilepsy and hence they fulfilled the criteria set out in this study. However, since the relative power content in the 13–30 Hz band remained at less than 10% of pre-stun levels for a relatively shorter time after stunning with 100 than with 150 mA (30 versus 50 s post-stun), the stunning of broilers with 150 mA of 50 Hz may be considered to be better than stunning with 100 mA of 50 Hz.

Although stunning broilers with 100 mA of 400 Hz produced epileptiform activity in all of the broilers, it failed to reduce the total and relative power contents in the EEG to below 10% of the pre-stun level. This implies that complete neuronal depolarisation failed to occur after the epileptiform activity. Increasing the stunning current of 400 Hz to 150 mA, reduced the total power content to less than 10% of the pre-stun level for up to 40 s, but the reduction in the relative power content lasted for up to only 30 s post-stun, which is comparable to the effect of 100 mA of 50 Hz. Nevertheless, this stunning treatment also fulfilled the criteria.

Stunning with 100 mA of 1500 Hz failed to induce epileptiform activity in five out of eight broilers, which is one of the criteria used to determine the effectiveness of stunning. This treatment also failed to induce a profound EEG suppression in any of the chickens, as indicated by the levels of total power content in the EEG. This implies that stunning with 100 mA of 1500 Hz failed to induce complete depolarisation of neurones, as would be expected to occur after generalised epilepsy. Owing to this, the possibility of consciousness and sensibility in these birds could not be dismissed. Increasing the amount of current to 150 mA of 1500 Hz induced epileptiform activity in all of the chickens, caused relatively more suppression in the EEG and prolonged the time to onset of EEG activation when compared with 100 mA of this current frequency. Subjective interpretations of the EEGs of broilers suggested that a profoundly suppressed EEG occurred after stunning with 150 mA of 1500 Hz. However, neither the total power nor the relative power content decreased below 10% of the corresponding pre-stun levels to suggest that complete neuronal depolarisation occurred after stunning with 150 mA of 1500 Hz. Clearly, stunning of broilers with 150 mA of 1500 Hz failed to fulfil the criteria and therefore it is arguable whether unequivocal loss of consciousness and sensibility occurred in these birds. Thus, a minimum current of more than 150 mA (eg 200 mA) may be necessary to achieve unequivocal and sustained loss of consciousness after stunning with 1500 Hz.

The results of this study clearly suggest that the EEG changes and hence the depth and duration of unconsciousness occurring after electrical stunning are significantly influenced by the amount and frequency of stunning current. At 100 mA, the magnitude of increase in total power content in the epileptiform EEG declined as the frequency of the stunning current increased. The magnitude of neuronal inhibition, and hence EEG suppression, also declined as the stunning frequency increased. The duration of this inhibition, and hence the occurrence of profoundly suppressed EEG, was also influenced by the stunning current frequency. In comparison with 50 and 400 Hz, the extended duration of epileptiform activity observed in broilers stunned with 150 mA of 1500 Hz did not influence the magnitude and duration of EEG suppression. The time to EEG activation was also shorter in broilers stunned with 1500 Hz than in those stunned with 50 or 400 Hz. Within 1500 Hz, the time to EEG activation was shorter in broilers stunned with 100 mA than with 150 mA. These effects are probably due to variations in the neurotransmitter release responses. There are two pools of synaptic vesicles: a readily releasable pool at the plasma membrane and a reserve pool. Electrical stimulation of the mouse auditory system with 100 or 300 Hz has shown that the synapse can sustain very high frequencies of neurotransmitter release for many seconds without depleting the vesicle pool (Wang & Kaczmarek 1998). In addition, stimulation of presynaptic terminals of mouse auditory brainstem neurones with 100 Hz resulted in the refilling of the synaptic neurotransmitter pool at a slower rate than did stimulation with 300 Hz (Wang & Kaczmarek 1998). Therefore, Wang and Kaczmarek (1998) concluded that high frequency stimulation of presynaptic terminals significantly enhances the rate of neurotransmitter replenishment.

The depth of unconsciousness, as determined from the magnitude of neuronal inhibition induced by electrical stunning current frequency in chickens, on the other hand could be determined by the duration for which the current stays at the maximum within each cycle, otherwise known as period (period = 1000 / frequency). In this regard, electric currents of 50, 400 and 1500 Hz sine wave AC have periods of 20, 2.5 and 0.67 ms respectively. Owing to the longer periods of a 50 Hz current, the effectiveness of this frequency was found to be more pronounced than 400 Hz, and stunning with 1500 Hz resulted in a mild suppression. It is therefore possible to suggest that the impact of a stunning current depends upon the period of current used and that it decreases markedly when the period is below the threshold limit necessary to induce depolarisation of neurones.

This interpretation is supported by the results of another study in which the impact of water bath stunning hens with a constant RMS current of 100 mA of AC, delivered using 100, 200, 400, 800 and 1500 Hz, on the total power content was investigated (Raj & O'Callaghan 2004). The results of that study showed that increasing stunning frequency resulted in greater power contents after the epileptiform activity. In another study, the impact of head-only electrical stunning of broilers with three pulse widths (5, 10 or 15 ms) of 50 Hz pulsed DC (with a period of 20 ms) delivering a constant average current of 130 mA, was evaluated (A B M Raj unpublished). The results of that study showed that increasing pulse width (ie increasing the duration of current 'on' time within each cycle) increased the percentage of birds showing epileptiform activity, decreased the total power content to less than 10% of pre-stun levels at the maximum pulse width and also prolonged the time to recovery of total power content in the EEG. The results of *in vitro* studies of the release of radio-labelled (3H) noradrenaline and 5-hydroxy-tryptamine (5-HT) indicated that, at a stimulation frequency of 50 Hz pulsed DC (with a period of 20 ms) and a constant current of 120 mA, increasing pulse widths from 1 to 19 ms produced greater increases in the release of neurotransmitters from chicken brain slices (G Jackson 2002, personal communication).

From a stunning and slaughter point of view, it has been reported that the blood vessels severed at slaughter vary considerably under commercial conditions and that the time to onset of brain death depends upon which blood vessels have been severed (Gregory & Wotton 1986). The situation is likely to be worse if only one or two jugular veins in the neck, as opposed to carotid arteries, are severed at slaughter. At 150 mA current level, the total and relative power contents in the EEG frequency bands remained suppressed for 10 s longer after stunning with 50 (50 s) than 400 Hz (40 s). This implies that the electrical stunning of broilers with 150 mA of 50 Hz is better than with 150 mA of 400 Hz. As explained previously, this difference between 50 and 400 Hz could also be due to the phenomenon of synaptic depression induced with 400 Hz (Wang & Kaczmarek 1998). However, the effectiveness of stunning induced by 100 mA of 50 Hz and 150 mA of 400 Hz may be sufficient to ensure bird welfare, provided that the carotid arteries supplying oxygenated blood to the brain are severed at slaughter.

It is worth mentioning that a considerably higher current (minimum of 240 mA) than those used in the present study was found to be necessary to stun broilers with the conventional head-only electrical stunning tongs fitted with pins, in which the contact area would have been smaller than was the case in the present study (Gregory & Wotton 1990b). The results of the present study also showed that the SEPs were abolished only during the occurrence of a suppressed EEG, which is in agreement with the report of Gregory and Wotton (1990a). Gregory and Wotton (1989) reported a close association between the occurrence of epileptiform activity in the EEG and abolition of SEPs after electrical stunning in chickens. This relationship is probably dependent upon the magnitude of EEG suppression occurring after the epileptiform activity. On the other hand, the results also showed that the magnitude of EEG suppression necessary to result in abolition of SEPs is less than that required to induce a profound EEG suppression indicative of unconsciousness in electrically stunned chickens. This scenario certainly occurred in broilers that were stunned with 100 mA of 1500 Hz in which the SEPs were abolished in all of the broilers irrespective of the magnitude of suppression and of whether or not they showed epileptiform activity after stunning. However, there were considerable differences in the EEG power contents between the birds that showed epileptiform activity and profound EEG suppression and those that showed only a mild suppression in the EEG after stunning. This is probably due to the fact that the neurones in the somatosensory cortex of the chicken brain are predominantly dopaminergic (Durstewitz *et al* 1999), and direct application of dopamine to the somatosensory cortex has been reported to attenuate neuronal excitation induced by somatosensory stimulation in conscious monkeys (Rolls *et al* 1984). Together, these reports imply that low levels of dopamine release occurring locally in the somatosensory cortex as a result of stunning with low currents could abolish SEPS, but are insufficient to induce a widespread inhibition in the brain required to assume loss of consciousness. This is supported by the fact that the

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brain extra-cellular levels of homovanillic acid (HVA), a metabolite of dopamine, and 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of serotonin, increased significantly during stressful handling, and that the stress of compressing a metatarsal bone by metal shackle reduced the amplitude of SEPs in unrestrained chickens (Sparrey & Kettlewell 1994; Gruss & Braun 1997). Therefore, abolition of SEPs alone may not be a reliable indicator of the effective electrical stunning of poultry.

The power contents in the EEG frequency bands remained on average below 50% of the pre-stun levels well beyond the recovery of consciousness in broilers. Thus, consciousness can exist in the presence of this level of neuronal inhibition and a profound neuronal inhibition is required to assume unconsciousness in chickens. Since the time to the return of behavioural indicators of consciousness was not recorded in this study, it is not possible to suggest exact levels of total or relative power contents necessary to assume consciousness. Further studies involving the calculation of power contents in short (eg 1 s) epochs and reliable behavioural indicators of consciousness in poultry, such as resumption of breathing, are needed to establish such relationships. For example, Velarde *et al* (2002) found that the time to the return of rhythmic breathing in electrically stunned sheep coincided with the time to the return of signs of consciousness in their EEG.

However, in spite of the unconvincing neurophysiological changes that occurred after stunning broilers with 100 mA of 1500 Hz, all of the broilers showed clonic seizures (wing flapping) during the application of stunning and the early part of the epileptiform activity in EEGs, which terminated as tonic seizure. Although the birds were restrained with Elastoplasts, the onset and severity of seizures appeared to be similar to those that occurred with the other stunning treatments. Since dopamine acts on D1 receptor sites with proconvulsant effects (Godbout *et al* 1991; Cepeda *et al* 1992), it can be suggested that the threshold current necessary to stimulate this mechanism, and hence to induce seizures, is less than that required to achieve unconsciousness. This also implies that the induction of seizure (wing flapping) alone cannot be used as a criterion for determining the effectiveness of electrical stunning treatments in chickens. More importantly, since a relationship between brain dopaminergic system and motor activity exists in chickens (Gruss & Braun 1997), it may not be wise to use the time to recovery of motor function to determine the duration of unconsciousness induced by electrical stunning in chickens.

It is worth mentioning that the variable voltage / constant current stunner used in this study, by virtue of having the capacity to output high voltage (>620 V peak) and being capable of modulating the voltage according to changes in the electrical resistance in the pathway, delivered the pre-set current from the beginning of the stun (within the first current cycle). The situation is likely to be worse when constant voltage stunners supplied with low voltages (<250 V) are used for stunning. This is due to the fact that low voltages will take longer to break down electrical resistance in the pathway and therefore unconsciousness may not be induced without causing pain and suffering.

Conclusion and animal welfare implications

The effectiveness of electrical stunning in chickens is dependent on the amount (mA) and frequency (Hz) of current. The specific implications of the results of this study for animal welfare are:

1. Minimum currents of 100, 150 and 200 mA should be delivered whilst using 50, 400 and 1500 Hz respectively, to achieve adequate depth and duration of neuronal depolarisation and hence unconsciousness in broilers.

2. Severing both of the carotid arteries in the neck after head-only electrical stunning and high frequency (>125 Hz) electrical water bath stunning of broilers should also become a statutory requirement to prevent the return of consciousness during bleeding.

3. The impact of electrical stunning current and frequency on the depth and duration of unconsciousness in other farm animals needs to be investigated using quantitative EEGs.

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