The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK
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Evaluation of microwave energy as a humane stunning technique based on electroencephalography (EEG) of anaesthetised cattle

J-L Rault*†, PH Hemsworth†, PL Cakebread†, DJ Mellor‡ and CB Johnson‡

† Animal Welfare Science Centre, School of Land and Environment, University of Melbourne, Melbourne, VIC 3010, Australia
‡ Animal Welfare Science and Bioethics Centre, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North, New Zealand
* Contact for correspondence and requests for reprints: raultj@unimelb.edu.au

Abstract

Humane slaughter implies that an animal experiences minimal pain and distress before it is killed. Stunning is commonly used to induce insensibility but can lead to variable results or be considered unsatisfactory by some religious groups. Microwave energy can induce insensibility in rats, and high power equipment has recently been developed for sheep and cattle. We examined the effectiveness of different settings for microwave energy delivery, power and duration, to induce insensibility based on electroencephalography (EEG) of anaesthetised cows, using the minimal anaesthesia model. All applications resulted in the appearance of seizure-like complexes in the EEG, a pattern considered incompatible with awareness. Shorter duration of application resulted in more rapid EEG changes, as quickly as 3 s. Higher power resulted in a longer duration of EEG suppression, at least 37 s and up to 162 s. Microwave energy can induce insensibility in cattle based on seizure-like complexes in the EEG.

Keywords: *animal welfare, electromagnetic, euthanasia, insensibility, pre-slaughter, slaughter*

Introduction

Humane slaughter implies minimal pain and distress on an animal before it is killed. Various factors can influence pain, fear and distress in abattoir settings including: the previous experience and breed of the animal (Grandin 1997); the facilities' design (Grandin 1990); handling techniques (Hemsworth *et al* 2011); and the slaughter method used (Anil 2012). In Western countries, stunning of the animal is a legislative requirement mandated to induce insensibility, defined as the incapability to experience any feeling or sensory experiences, and thus ensure that the animal cannot feel pain. Different stunning techniques can be used depending on factors such as the species and age of the animal, practicality, animal and worker safety and economical considerations (AMI 2010; Anil 2012). Some stunning techniques qualify as reversible, with the animal able to recover from the process (eg head-only electrical stun), whereas others are irreversible if performed correctly (eg captive-bolt stun). However, current stunning techniques can lead to variable results (captive-bolt stunning: Gouveia *et al* [2009] with efficiency of 68.2%; electrical stunning: Zivotofsky & Strous [2012] reporting efficiencies of 60.9 to 90%), hence the search for novel stunning techniques or alternatives.

The current standards around slaughter in Australia (AS 4696: 2007, sub-clause 7.10; Anonymous 2007) and the United States (Humane Methods of Slaughter Act of 1978) for instance require that an animal is rendered unconscious and insensible to pain prior to slaughter and remains so until death. However, Halal and Kosher meat production requires that animals being processed for human consumption are healthy and uninjured at the time of slaughter. Consequently, many of the methods of stunning used in modern commercial abattoirs are not acceptable as the animals are considered injured by the stunning process and the animals cannot recover from stunning (Nakyinsige *et al* 2013). As a result, 'ritual slaughter' without prior stunning is enabled in Australia (AS 4696: 2007; sub-clause 7.12) and the United States (Humane Methods of Slaughter Act of 1978; Section 6).

The pain that an animal experiences at slaughter is central to the legislative requirement to stun in Western countries. Pain is difficult to study because it is an inherently subjective experience. While humans can report pain, only indirect indices of pain are available for use in animals. Furthermore, many of the traditional behavioural and physiological indices that have been used to study pain are also measures of non-painful stressors. For example, measures such as hormone response (eg catecholamines, glucocorticoids) and behaviour are not specific to pain (Mellor *et al* 2000). However, neurophysiological tools are now widely used in humans to assess pain. Studies in humans experiencing pain have demonstrated that in contrast to the more traditional physiological measures, electroencephalographic (EEG) data correlate well with subjective evaluations of pain, indicating the value of quantitative EEG analysis as an

indicator of the degree of pain perceived by humans (Marchand *et al* 2002). Recently, neurophysiological responses of animals, recorded by EEG, have been shown to provide valuable insights into the perception of pain by animals (Murrell & Johnson 2006), and specifically that slaughter of cattle (*Bos taurus*) by ventral-neck incision without prior stunning is painful, based on EEG changes (Gibson *et al* 2009a). Furthermore, this practice is likely to be of more concern in cattle than other species, such as smaller ovine species (sheep [*Ovis aries*], goats [*Capra hircus*]), due to the longer bleeding times required in cattle before loss of consciousness occurs (Newhook & Blackmore 1982; Gregory *et al* 2010). Hence, an important welfare consideration in slaughter is that the animal is insensible at the time of the ventral-neck incision. Alternative stunning techniques that are quick, humane and acceptable by all are needed.

Microwave energy (also known under the terms 'high energy microwave', or 'focused beam microwave irradiation') has been reported to induce loss of consciousness when applied to conscious rats (*Rattus norvegicus*), causing petit or grand mal seizures for 1 min after exposure and an unconscious state for the following 4 to 5 min with the animal ultimately recovering (Guy & Chou 1982; Lambooy *et al* 1989). However, Lambooy *et al* (1989) deemed this technique unsuitable for pigs (*Sus scrofa*) at that time, partly because of the capacity of the microwave generator being too low to deliver sufficient power. In recent years, microwave technology has developed to the point that high power equipment is available that can focus the energy to produce a rapid rise in temperature in cattle brains (Ralph *et al* 2011). It is expected that raising the brain temperature will stop brain function and result in insensibility, whilst still allowing the animal to regain consciousness after a period of time (for a review, see Small *et al* 2013a). This is supported by preliminary evidence in sheep (Small *et al* 2013b).

The aim of this project was to examine the effectiveness of different settings for microwave energy delivery, power and duration of application, to induce insensibility on anaesthetised cows. Insensibility was assessed by the appearance of seizure-like complexes in the EEG. All animals in this project were kept under minimal anaesthesia for the whole procedure. The minimal anaesthesia model has been previously validated as a suitable model to investigate the effects of noxious stimuli (Murrell & Johnson 2006; Gibson *et al* 2007).

Materials and methods

Animal welfare and ethical considerations

This project was approved by the University of Melbourne Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. All animals used in this project were anaesthetised prior to being subjected to the experimental treatment and kept under anaesthesia for the whole procedure. Hence, it allowed us to study the effects of microwave application without inflicting any pain or distress on the animal. The animals were never given the opportunity to regain consciousness and were ultimately humanely euthanised by lethal barbital overdose. The different settings of microwave delivery, that is, power and duration, were tested based on a stop-go basis to minimise animal use.

Study animals

Ten Hereford × Angus crossbred female cows with a bodyweight estimated around 180 kg were used over a total of six days. The animals were sourced from the daily intake of the abattoir and originally intended to be slaughtered for meat consumption. Food was withdrawn for 24 h and water withdrawn overnight prior to the procedure to avoid regurgitation during induction of anaesthesia. In order to minimise handling stress, no electric prods were utilised when moving the animals.

General handling and anaesthesia procedures

All animals were held in lairage and handled in a similar manner. At the time of treatment, the designated animal was moved from the lairage area through a single chute race and into a restraining box. The head of the animal was restrained and minimal anaesthesia was induced and maintained following a previously validated procedure for cattle, by administrating the anaesthetic agent (a mixture of 3.4 mg kg^{-1} ketamine [Teva Pharmaceuticals Pty Ltd, Macquarie Park, NSW, Australia] and 4.1 mg kg^{-1} propofol [Norbrook Laboratories, UK]) into the jugular vein of the animal (Gibson *et al* 2007). Once the animal was anaesthetised, as indicated by loss of posture, the side of the box was opened and the animal rolled into lateral recumbency. Endotracheal intubation was carried out by a veterinarian by advancing the endotracheal tube to the rima glottidis and confirming correct placement by palpation as the tube advanced into the trachea. Once intubated, the animal was placed in dorsal recumbency onto a specifically designed V-restrainer rolling crate. The palpebral reflex was continuously monitored and additional propofol immediately administered if the animal showed signs of recovery from the anaesthetic. The animal on the rolling crate was finally moved to an adjacent room 15 m away and the endotracheal tube connected to the anaesthetic machine delivering halothane in oxygen via a circle breathing system using standard clinical flow rates and vapouriser settings. The animal was allowed to breathe spontaneously throughout the experiment. End-tidal halothane tension was maintained at 0.9%. Patient stability and depth of anaesthesia was monitored throughout the procedure using an anaesthetic agent monitor (Cardell® Veterinary Monitor Max-12HDim multiparameter monitor, Midmark Animal Health, Versailles, OH, USA), recording the end-tidal carbon dioxide tension, end-tidal halothane tension, respiratory rate and heart rate throughout the anaesthetic procedure. The corneal reflex was also monitored every 5 min, by touching the side of the eye. Anaesthesia under halothane was maintained for at least 30 min to allow for the effects of the anaesthetic induction agents (ketamine and propofol) to wear off and reach a steady state of halothane anaesthesia.

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Electroencephalography (EEG) placement

The EEG electrodes were placed on the animal's head. Subdermal 27-G stainless-steel needle electrodes (Medelec, Radiometer, Auckland, New Zealand) were placed in a four-electrode montage to record two channels of EEG. For each channel, the common non-inverting electrode was placed mid-line between the medial canthi of the eyes, the inverting electrodes placed bilaterally over the mastoid processes, and the ground electrode placed caudal to the poll.

The EEG was amplified using isolated differential signal amplifiers (Iso-Dam isolated physiological signal amplifiers; World Precision Instruments, Sarasota FL, USA), with a gain of 1,000 and pass-band of 0.1 to 500 Hz and digitised at a rate of 1 kHz (Powerlab/4sp, ADInstruments Ltd, Sydney, NSW, Australia). Data were analysed off-line after completion of the experiment.

Experimental treatment

The microwave device has been developed by Advanced Microwave Technologies (Wollongong, NSW, Australia), to deliver power levels of 0–30 kW at 922 MHz specifically designed for the head of livestock using high energy focal microwave energy (Ralph *et al* 2011). It has been successfully used on sheep (Small *et al* 2013b), and since then improved to optimise penetration and focus the energy into the brain. The wand of the device was applied in contact with the top of the front head, on a mid-line half-way between the medial canthi of the eyes and the poll. The exact location varied slightly depending on the head shape.

The microwave application was imposed at different powers and for different durations. The microwave settings used in the first two animals (20 kW for 15 s) replicated those used in a preliminary experiment (Rault *et al*, unpublished results) to confirm that these microwave settings could alter the EEG signal. We then applied 20 kW for 10 s on two animals, 30 kW for 10 s on three animals, 30 kW for 5 s on one animal, and 12 kW for 25 s on one animal, in an order determined by the stop-go basis sequence to minimise animal use.

The microwave application was repeated 35 min later at an identical dose to give a total of two applications per animal, apart from the last two animals. This second application was used in an attempt to deliver multiple applications to a single animal maintained under anaesthesia, hence allowing the collection of additional data while reducing the number of animals needed for the experiment. However, only the first application is discussed because the results of the second application were clearly different to the first application based on a much greater penetration of the microwaves as a result of the first application. Furthermore, one animal did not respond to the anaesthetic agents and had to be euthanised before any treatment could be applied. Hence, we obtained data from nine animals. From the time the microwaves were applied, the animal was never given the opportunity to regain consciousness before death ensued. The animal was euthanised 10 min after the last microwave application by administrating a lethal dose of barbital (Lethabarb, Virbac Australia Pty, Milperra, NSW, Australia) into the jugular vein.

EEG analysis

EEG epochs contaminated by artefacts, overscale or underscale were manually rejected from analysis of raw EEG data, using Chart 4.2.3 (ADInstruments, Castle Hill, NSW, Australia). Refined analyses were then performed by calculating the F50, F95 and Ptot for consecutive non-overlapping 1-s epochs, using purpose-written software (Spectral Analyser, CB Johnson, Massey University, Palmerston North, New Zealand 2002). Data were multiplied using a Welch window. Fast Fourier Transformation was applied to each epoch, generating sequential power spectra with 1-Hz frequency bins and median frequency, 95% spectral edge frequency and total EEG power were derived from the power spectra. Subsequent analysis was performed using Microsoft Excel®.

Four variables were derived from combinations of the raw data and/or the variables derived from the frequency spectra: time to onset of EEG suppression (raw data), time to nadir of EEG suppression (95% spectral edge), duration of EEG suppression (combination of raw data, 95% spectral edge and total EEG power), and maximum effect (95% spectral edge) (Figure 1). Time to onset of EEG suppression was measured from the start of the microwave application until the first appearance of seizure-like complexes in the EEG, hence including the duration of microwave application. Time to nadir of EEG suppression was measured from the start of the microwave application until the maximum depression of 95% spectral edge. Duration of EEG suppression was measured from the time of onset until the re-emergence of a normal EEG pattern similar to that seen prior to the application of the microwaves. The maximum effect was determined by the maximum reduction of the 95% spectral edge frequency.

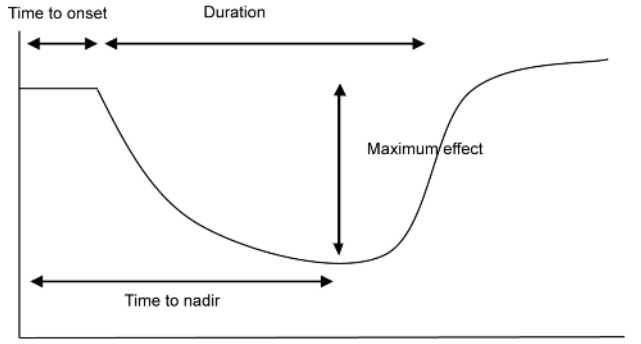
Electrocardiography (ECG) analysis

Electrocardiogram (ECG) data were recorded by placing three ECG electrodes on the animal's body, for the last four animals and only for the first microwave application. The electrodes were placed in a three-electrode montage. Adhesive electrode pads were adhered to the skin; the positive electrode was placed on the chest wall 5 cm behind the left point of the olecranon; the negative electrode was situated 10 cm out from mid-line of the thoracic back on the right-hand side; the ground electrode was placed in the same position as the negative electrode but on the left-hand side. ECG recordings were acquired using Powerlab 4/25T (ADInstruments, Castle Hill, NSW, Australia). The ECG tracings were analysed using Chart 5 software (version 5.5.5, ADInstruments, Castle Hill, NSW, Australia) to produce continuous heart-rate recordings.

Post mortem head histopathological autopsies

Most animals were given two microwave applications apart from the last two animals which were only given one microwave application in order to reliably observe the effects of a single microwave application as its intended use in the field. Post mortem autopsies were performed by a veterinary pathologist on the heads of four animals to determine histological changes in the skin and brain tissues.

Figure 1



Visual representation of the four variables derived from the EEG data: time to onset of EEG suppression (raw data), time to nadir of EEG suppression (95% spectral edge), duration of EEG suppression (combination of raw data, 95% spectral edge and total EEG power), and maximum effect (95% spectral edge).

The entire brain as well as the skin overlying the area of application were removed and fixed in 10% buffered formalin. Sections for histological assessment were obtained from the skin and the brain. For the skin, these regions corresponded to the following areas: central (obtained from margin of full-thickness loss of skin); rostral; caudal; and both lateral margins of the treatment site (as determined by gross examination of the deep surface of the skin). For the brain, these regions corresponded to the following areas: central (frontal lobe obtained from adjacent to medial longitudinal cerebral fissure at the centre of the treatment site); lateral marginal tissue (parietal lobe as determined by gross inspection of the brain); and deep brain structures (one section each of basal nuclei, thalamus, hypothalamus and caudal colliculus). Skin and brain sections were stained with haemotoxylin and eosin. The parts of the brains that were examined consisted of the frontal and parietal lobes (meninges, cortex, white matter), the basal nuclei, the thalamus and hypothalamus, and the caudal colliculus. These tissues were assessed visually by the expert pathologist for different parameters: tissue necrosis; vascular necrosis; cavitation or rarefaction; vascular haemorrhage, vascular congestion or oedema; and thrombosis using a grading scale from none to mild (scattered or rare changes), moderate (widespread changes) or severe (widespread and pronounced changes).

Statistical analysis

The results from animal nine have been discarded from the main EEG results because the application was preceded by two aborted applications due to technical issues with the microwave apparatus, hence the result obtained after the third application was likely to be atypical of the response after a single application. Similarly, the EEG from animal seven was heavily contaminated by ECG artefacts and therefore discarded because it was considered to be of insufficient quality to reach sound conclusions. Thus, seven animals' EEG with four different microwave treatments were analysed.

Due to the potential ethical implications of this novel technique, only ten cows were used, hence only allowing for descriptive analyses. Descriptive analyses are commonly used in the scientific literature to report EEG results. Results are reported as mean (± SD) unless otherwise noted.

Results

EEG results

The microwave application caused artefacts that rendered the EEG recording useless during and until about 3 s after the end of the microwave application. Therefore, the duration is based on the time to visible EEG changes, and consequently some animals could have shown EEG changes before the end of the microwave application.

All of the applications resulted in changes in the EEG pattern indicative of seizure-like activity, as seen in animal six (Figures 2 and 3). The derived variables' time to onset of EEG suppression, time to nadir of EEG suppression, duration of EEG suppression, and maximum effect for each individual animal are presented in Tables 1 and 2.

The duration of the microwave application appeared to influence the time to onset of EEG suppression, as well as time to nadir of EEG suppression, with shorter duration causing more rapidly developing effects (Table 2: 10 s < 15 s < 25 s). On the other hand, the power of the microwave application appeared to influence the duration of EEG suppression, with higher power resulting in longer duration (Table 2: 30 kW > 20 kW > 12 kW). The factors underlying the maximum effect could not be explained from the current data.

Because time of onset included the duration of application of the microwave, this means that the EEG started changing as soon as 3 s after the end of the microwave application, and possibly before since the artefact caused by the microwave rendered the EEG unreadable until that time. However, the low power treatment of 12 kW required almost 2 min (113 s) to take effect. The interval between the time when effects started appearing ('Time to onset') and the maximum effects were seen ('Time to nadir') was within 4 to 22 s depending on the treatments. All treatments could suppress the EEG for at least 37 s and up to more than 2 min (162 s) ('Duration'). The maximum effect remained variable even between two animals administered the same treatment.

ECG results

The microwave application had similar effects on the heart rate of the four animals for which data were collected (Figure 4 and Table 3). The heart-rate baseline recorded over the 5 min prior to the microwave application was 92.2 (± 8.4) bpm. After the start of the microwave application, the heart rate dropped within 5.0 (± 2.4) s to 65.8 (± 24.0) bpm, and then rebounded after 23.75 (± 1.9) s from the start to 82.3 (± 10.3) bpm. It stabilised within 160 (± 37.4) s to 73.2 (± 12.1) bpm, a lower level than the initial baseline obtained prior to the microwave application.

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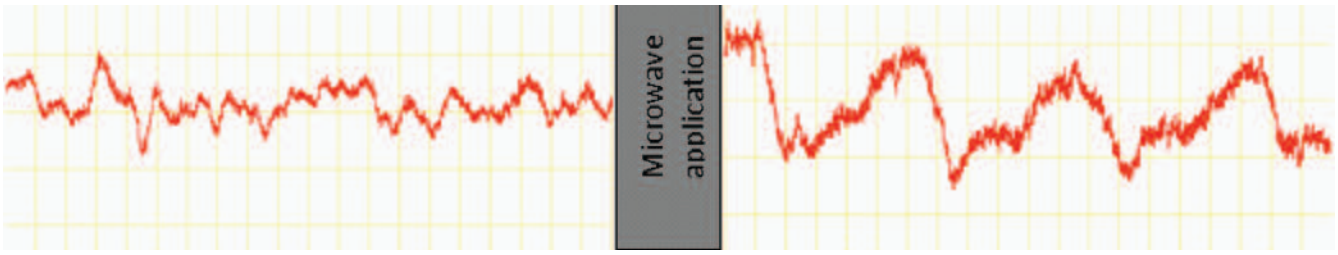
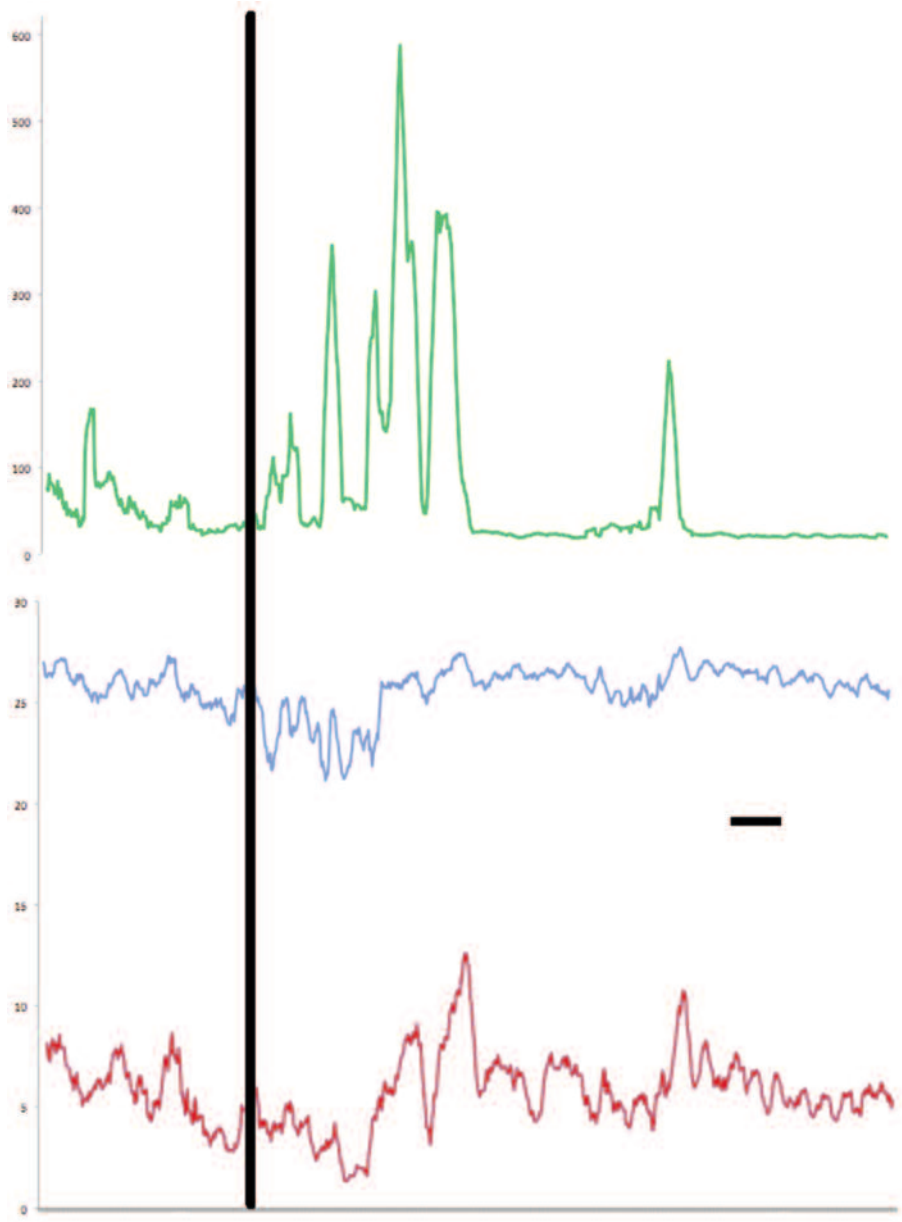
Figure 2



A 4-s sample of EEG data prior to and after microwave application, recorded from animal six receiving 20 kW for 10 s. Horizontal divisions represent 200 ms, vertical divisions represent 100 mV.

Figure 3



Total EEG power (top; arbitrary units), 95% spectral edge (middle, Hz units) and median frequencies (bottom; Hz units) derived from the EEG frequency spectra of animal six, receiving 20 kW for 10 s. The time of microwave application is indicated by the black vertical line, the horizontal bar represents a 1-min duration. A low pass filter has been applied to the traces (ten-point moving average) to make them easier to interpret visually.

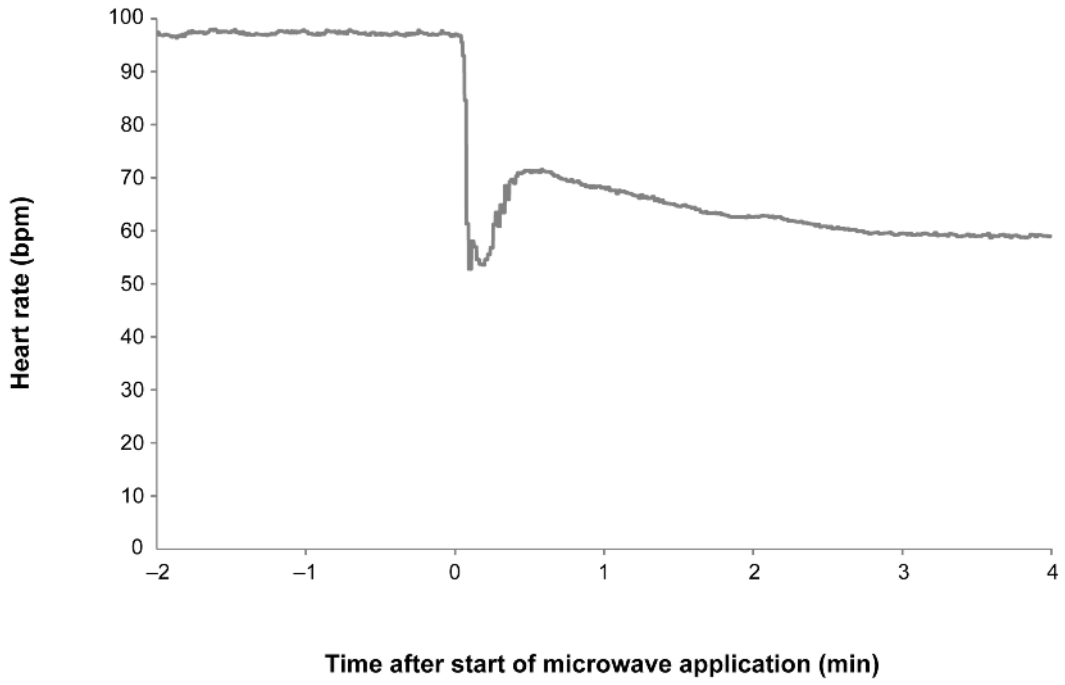
Table 1 EEG results for each individual animal. Time was measured from the start of the microwave application.

Factor	Animal ID								
	1	2	3	6	4	7	8	5	9
Power (kW)	20	20	20	20	30	30	30	12	30
Duration (s)	15	15	10	10	10	10	10	25	5
Time to onset (s)	24	50	14	12	16	27	14	138	12
Time to nadir effect (s)	65	52	22	28	24	34	37	142	20
Duration (s)	129	81	140	78	109	80	215	37	50
Maximum effect (% reduction in 95% spectral edge frequency)	25	18	29	21	31	11	59	6	8

Table 2 EEG results pooled by treatment. Results are presented as average and (value1, value2) corresponding to each animal if the treatment was applied to two animals (n = 2). Time is counted from the start of the microwave application.

Factor	Treatment			
	1	2	3	4
N	2	2	2	1
Power (kW)	20	20	30	12
Duration (s)	15	10	10	25
Time to onset (s)	37 (24, 50)	13 (12, 14)	15 (14, 16)	138
Time to nadir effect (s)	59 (52, 65)	25 (22, 28)	31 (24, 37)	142
Duration (s)	105 (81, 129)	109 (78, 140)	162 (109, 215)	37
Maximum effect (% reduction in 95% spectral edge frequency)	22 (18, 25)	25 (21, 29)	45 (31, 59)	6

Figure 4



Typical ECG pattern after microwave application, recorded from animal six receiving 20 kW for 10 s. Time is counted from the start of the microwave application.

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Table 3 ECG results for each individual animal. Time is counted from the start of the microwave application.

Factor	Animal ID				Mean (± SD)
	6	7	8	9*	
Power (kW)	20	30	30	30	
Duration (s)	10	10	10	5	
Baseline heart rate (HR) pre-application (bpm)	97.1 (± 0.6)	88.0 (± 0.2)	82.6 (± 0.2)	101 (± 0.2)	92.2 (± 8.4)
Minimum HR (bpm) and (s) reached	52.8 (3)	83.6 (6)	38.5 (3)	88.2 (8)	65.8 (5)
Rise post-application (bpm) and (s) reached	71.3 (21)	93.1 (24)	75.9 (25)	88.7 (25)	82.3 (23.8)
Baseline HR post-application (bpm) and (s) reached	59.1 (210)	81.4 (150)	67.2 (160)	85.0 (120)	73.2 (160)

* The ECG for animal 9 was collected for the third application, after two failed attempts.

Post mortem autopsies results

The first two animals (animals six and seven) submitted for post mortem analyses were given two microwave applications. The subsequent two animals (animals eight and nine) were only given one microwave application in order to reliably observe the effects of a single microwave application as its intended use in the field. However, it should be noted that animal nine received two failed attempts before the third successful application, hence perhaps this is not representative of the results of a single application.

Skin results

All animals displayed similar skin lesions. Centrally, over the forehead, there was an area of complete skin loss, surrounded by a larger region displaying grey-tan discolouration of the subcutaneous tissue. The skin within this area displayed full-thickness, coagulative necrosis extending down to the skull. Beyond this area, there was rapid progressive decrease in the severity of necrosis, with normal unaffected skin present within 0.5 cm of the margin of the placement of the wand of the device.

Brain results — two consecutive microwave applications

Animals six and seven, submitted to two microwave applications, showed severe lesions at various levels (meninges, cortex and white matter) in the frontal and parietal lobes, with severe tissue and vascular necrosis, cavitation, haemorrhage and vascular congestion. However, only minor if any changes were apparent in the basal nuclei, thalamus, hypothalamus, and the caudal collicus. The lesions in this more basal region consisted mainly of haemorrhage and vascular congestion.

Brain results — single microwave application

Animal eight submitted once to 30 kW for 10 s had medium to a few severe lesions in the frontal and parietal lobes. Animal nine submitted to 30 kW for 5 s had relatively minor to no lesions in the same regions. For both animals, the basal nuclei, thalamus, hypothalamus, and the caudal collicus were relatively unaltered except for mild to moderate vascular congestion.

Discussion

The microwave application induced EEG changes in all nine animals studied. Microwave application induced a pattern of seizure-like activity in the EEG, a pattern that is not considered to be compatible with continued awareness (Devine *et al* 1986; Coenen 1998; Velarde *et al* 2002). Hence, this pattern of seizure-like complexes in the EEG is interpreted as evidence of insensibility in this study. The anaesthetic agent used (halothane) would have prevented the motor activity associated with these seizures, but motor effects such as limb rigidity and possibly tonic or clonic movements may be seen in unanaesthetised animals, similar to what has been reported with rats (Guy & Chou 1982: irradiating heads and shoulders; Lambooy *et al* 1989: irradiating heads only). The EEG changes observed in the present study following microwave application occurred rapidly, as soon as 3 s after the end of the microwave application. As expected, the microwave application caused artefacts that rendered the EEG unreadable until about 3 s after the end of treatment. Therefore, the duration was based on the time to certain EEG changes, and consequently, some animals could have shown EEG changes before the end of the microwave application.

For any stunning technique, the search for the minimum duration of application necessary to induce insensibility is crucial. Reducing that interval is likely to improve animal welfare by reducing the time that the animals may experience pain and distress. Our results indicate that an application for 10 s resulted in insensibility within 3 s. In comparison, non-penetrative captive-bolt stunning induced EEG changes in about 8 s in calves in a study which also used the minimal anaesthesia model (Gibson *et al* 2009b). The present results confirm findings on other species (rodents: AVMA 2013; sheep: Small *et al* 2013b) that microwave energy is a relatively quick process in comparison to other reversible stunning procedures, such as cattle electrical stunning, for which applications of up to 15 s can be performed in order to over-ride the spinal reflex arc and reduce kicking (AMI 2010), or carbon dioxide stunning in pigs, with latency of about 25 s to loss of posture (Velarde

et al 2007). Furthermore, our results indicated that a shorter duration of application induced more rapidly developing EEG changes in the range of duration tested (10–25 s), as evidenced by shorter 'time to onset' and 'time to nadir'.

Another consideration for reversible stunning techniques is the duration of insensibility, or the time before the animal regains consciousness. Insensibility should last until death ensues through exsanguination. Following microwave application, EEG suppression lasted for at least 37 s and up to more than 2 min. Our results also indicated that applying higher power extended the duration of insensibility. The search for a long period of insensibility is useful for cattle because consciousness can persist from 1 to 2 min after exsanguination (Newhook & Blackmore 1982; Gregory *et al* 2010).

As expected, the microwave application caused artefacts that rendered the EEG unreadable until after the end of treatment. This is an inherent limitation of using the electroencephalographic technique to assess the microwave technique since both techniques interact with electric activity. This leaves a window of uncertainty regarding the aversiveness of the microwave technique during its application and the experience of the animal during that short period of time. It is also not clear when the skin burns on the surface of the head appeared relative to loss of awareness. The animal's perception of the procedure up to the induction of insensibility (possibly in the order of 10–15 s or less) should be investigated with alternative scientific methods that allow for data collection during microwave application.

The abrupt bradycardia (ie drop in heart rate) observed following microwave application is in agreement with the literature on the heart-rate response to noxious stimuli (Johnson *et al* 2005; Woodbury *et al* 2005; Gibson *et al* 2007). The magnitude of that drop differed between animals, but the heart rate rebounded within 24 s, irrespective of the treatment applied. Interestingly, the heart rate stabilised to a different, lower level, following microwave application. The most plausible explanation is that this may be the result of temporary and longer persisting effects on the brainstem or thalamus (Benarroch 2001).

To prevent pain and distress, microwave energy should cause rapid loss of consciousness. The brain regions underlying consciousness are still debated, but the cerebral cortex and the brainstem, especially the thalamus, are believed to play major roles (Coenen 1998; Shaw 2002; Gaetz 2004; Alkire *et al* 2008). The EEG pattern observed in this study is considered incompatible with continued awareness. Based on the post mortem autopsies, most histological changes appeared in the upper regions of the brain, with the frontal lobe, adjacent to the zone of application of the microwave, being the most affected, closely followed by the parietal lobes which are located on the sides of the animal's brain. However, the regions of highest interest in regards to consciousness, the deeper regions of the brain, namely the basal nuclei, thalamus, hypothalamus, and the caudal collicus, appeared to be relatively unaffected by microwave application, even following two microwave applications. Further research is warranted regarding the dissipation of microwave energy throughout the brain and whether this is a homogeneous process or not. The lesions observed would suggest that animals may be able to regain consciousness following microwave application, although the frontal regions of the brain would unlikely be intact.

Practical implications for the use of microwaves as a stunning technique

Three parameters can be considered essential for the welfare implications of a stunning technique: the time to onset of insensibility ('how quickly the stun acts', the shorter the better), the duration of insensibility (underlying the 'stun-stick interval', the longer the better), and the variability between animals (the lower the better). Among the treatments that have been tested within the present experiment, 20 kW for 10 s yielded the best outcomes: these two animals were judged insensible in less than 14 s from the start of the microwave application and remained insensible for at least 78 s. These outcomes are promising for commercial implementation. A microwave application of less than 10 s may be sufficient, although this requires further rigorous testing.

Furthermore, the identification and validation of method-specific behavioural signs of insensibility following microwave application on conscious animals (eg, absence of rhythmic breathing, behavioural response to a corneal reflex, spontaneous eye blinking) would be valuable for use in both research and industry settings. Further research is also needed using larger sample sizes in order to determine the inter-individual variability in response to microwave application and whether characteristics such as head shape, age or gender influence the efficiency of the microwave technique. Such characteristics can affect stunning efficiency for other widely used techniques such as captive-bolt gun (Gouveia *et al* 2009).

Conclusion

These findings indicate that microwave energy can induce insensibility based on the appearance of seizure-like complexes in the EEG of all nine animals studied. The parameters' power and duration influenced different aspects of the EEG changes. The duration of application influenced the onset of insensibility whereas the power delivered influenced the length of time during which the animal was insensible.

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