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Preliminary investigations into the use of microwave energy for reversible stunning of sheep

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Abstract

Stunning prior to slaughter is commonly used to render the animal insensible to pain. However, for certain markets, stunning is disallowed, unless the animal can fully recover if not slaughtered. There are very few available methods of inducing a fully recoverable stun. This preliminary study investigates the potential for microwave energy application to be used to induce a recoverable stun in sheep. Cadaver heads were used to demonstrate that brain temperature could be raised to a point at which insensibility would be expected to occur (44°C). Trials on four anaesthetised sheep confirmed this finding in a live animal model where brain temperatures between 43 and 48°C were achieved with 20 s of microwave energy application. Although the applicator and process variables require some further development, this technology seems eminently suitable for use as an alternative method of inducing a recoverable stun.

Keywords: *animal welfare, electromagnetic, humane slaughter, insensibility stun, sheep, syncope*

Introduction

Pre-slaughter stunning is widely used to render the animal insensible to pain at the time of exsanguination, thereby preventing unnecessary suffering and distress. However, certain markets, particularly some Jewish and Muslim markets that allow pre-slaughter stunning, require that any stun used is fully reversible. This means that commonly used stunning methods, such as mechanical captive-bolt stunning, cannot be used when processing animals for these markets. Head-only electrical stunning is widely used in sheep slaughter for such markets, as the animal will fully recover if not slaughtered (reversible stun). In this method, an electric current is passed through the brain, resulting in insensibility with brain activity similar to that seen in an epileptic fit. However, when applied to cattle, electrical head-only stunning has met with some criticism, particularly in terms of the duration of stun, which may not be long enough to allow death to occur prior to the stun wearing off (Lambooy & Spanjaard 1982; Wotton *et al* 2000). Therefore, there is a need to investigate alternative reversible methods of stunning.

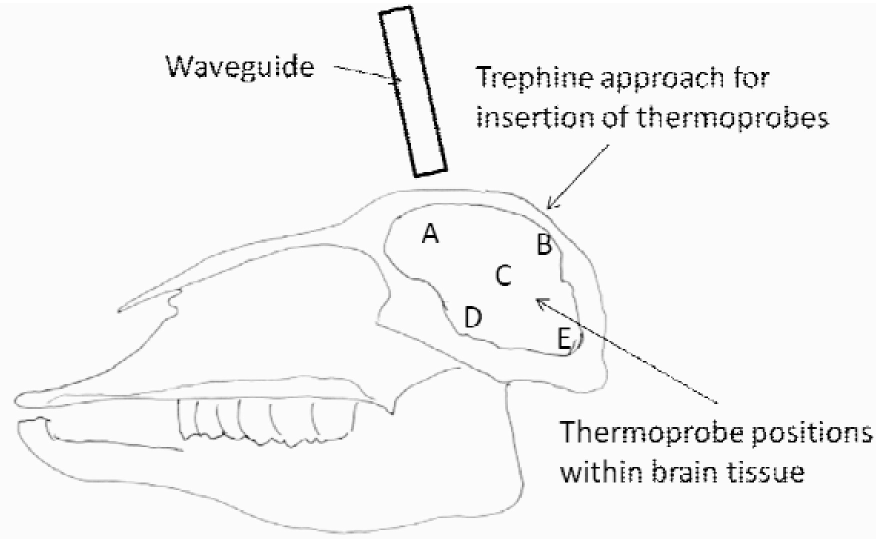
Electromagnetic induction of insensibility has been proposed, using microwave generation of an electromagnetic field within the brain, to raise brain temperature to a level at which consciousness would lapse. Thermal unconsciousness such as that induced by exercise heat stress or fever is reported to occur when core body temperature reaches between 40 and 45°C (Ohshima *et al* 1992), dependant on the species concerned. If the brain temperature could be controlled in such a way that the temperature did not rise beyond the point at which brain damage would occur (likely to be approaching 50°C, a temperature above which cooking of tissues would be expected to begin), then insensibility would be fully recoverable, in a manner not dissimilar to a patient recovering from hyperthermic syncope (heat stroke). This preliminary study investigates the feasibility of utilising microwave energy to raise the brain temperature to a point at which insensibility would be predicted to occur, using sheep as a model.

Materials and methods

Temperature profiling

Eight sheep heads were sourced from a local abattoir, stored under refrigerated conditions and treated within two days of harvest. A microwave generator (Advanced Microwave Technologies, Wollongong, NSW, Australia) providing power levels of 0–30 kW at 922 MHz; or 0–5 kW at 2.45 GHz was used to heat individual sheep heads. Microwave energy generated was passed through a custom-made waveguide which terminated directly over the frontal

Figure 1



Schematic diagram of cadaver experiment showing position of waveguide and thermoprobes.

lobes of the brain (approximately similar position to the frontal shooting position for captive-bolt stunning). The heads were positioned in a custom-made clamp at the end of this waveguide, emulating the system described by Ralph *et al* (2011). Temperatures were measured using fibreoptic thermoprobes (Neoptix, Canada) from the frontal skin immediately below the waveguide: nose; neck skin behind the jaw; ear (under the skin); eye (subconjunctival); and from five locations within the brain (shown in Figure 1 as positions A, B, C, D and E), immediately prior to and immediately after microwave application. Two heads were treated at 922 MHz, and two at 2.45 GHz, with an exposure time of 20 s in order to identify the optimal frequency for subsequent trials.

In four subsequent trials, the heads were positioned as described above, and the fibreoptic thermoprobes were inserted in five locations: frontal skin surface; immediately below frontal skin; upper levels of frontal lobe of brain (position A); middle of brain mass (position C); base of the brain (position E). Temperatures were measured continuously during microwave energy application. Four heads were treated at power levels of 3–6 kW at 922 MHz, with an exposure time of 15 (± 5) s. The range in exposure time is a result of reliance on manual switching of the microwave transmission in this pilot system, rather than an automated switch.

Animal studies

Animal studies were carried out under the authority of CSIRO Animal Ethics Committee Authority 2-09. Four ewes aged 3–4 years were used for the study. They were maintained at pasture at the CSIRO Cannon Hill facility, Queensland, Australia prior to the trial. The trials were carried out on a single day, with one sheep being anaesthetised, treated and euthanased prior to anaesthesia of a subsequent animal commencing. Briefly, each sheep was anaesthetised using a pre-medication of diazepam (Ilium Diazepam Injection, Troy Laboratories, Glendenning, NSW, Australia) at 0.3 mg kg^{-1} intravenously, followed by thiopentone sodium (Ilium Thiopentone, Troy Laboratories) induction. The sheep was intubated and anaesthesia maintained using isofluorane (Attane Isoflurane Inhalation Anaesthetic, Bomac Animal Health, Hornsby, NSW, Australia) in oxygen, delivered via a circle system with an out of circle vaporiser. Patient stability and depth of anaesthesia was monitored throughout the procedure. Respiratory rate and rhythm were observed and monitored by the anaesthesiologist, while pulse and haemoglobin oxygen saturation were monitored using a pulse oximeter (Nellcor N-595, Covidien, USA).

The anaesthetised sheep was placed into a custom-built cradle, based on a V-restraint design, which supported the body in an upright position. The head was placed into a restraint device which would present the frontal part of the brain to the waveguide. Subdermal needles were inserted at either temple to gather simple EEG traces using a single-channel biopotential unit (Neocardiotrace 4000, Watson Victor Ltd, Liverpool, NSW, Australia). These EEG traces were used merely to give an indication of changes in activity and/or amplitude that may warrant further investigation in subsequent studies. In two sheep, trephine holes were drilled into the brain cavity, and fibreoptic thermoprobes inserted into the brain tissue. The aim was to achieve temperature monitoring at upper (position A; Figure 1), middle (position C; Figure 1) and deep (position E; Figure 1) levels within the brain tissue. Patency of anaesthetic tubing and stability of anaesthesia was confirmed for each animal prior to closure of the surrounding Faraday cage.

Each sheep was exposed to 4 kW of energy at 922 MHz for between 5 and 20 s (Table 1). The two sheep which had thermoprobes inserted into the brain were immediately euthanased. The two sheep that did not have thermoprobes inserted were removed from the cradle, the inhalation anaesthetic agent removed, and the sheep monitored until early indications of recovery from anaesthesia were

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observed (chewing and swallowing reflex; return of corneal reflex), at which point the animals were euthanased. Euthanasia was effected by intravenous administration of an overdose of pentobarbitone sodium (Lethabarb, Virbac, Milperra, NSW, Australia).

Immediately after euthanasia, the heads were removed, and skinned to assess physical appearance of skin and bone at the site of energy application. The heads were stored at 2°C for three days, after which the brains were removed for histological examination, to investigate the potential for physical damage to brain tissue as a result of excessive heating. Histology was carried out by the University of Queensland Veterinary Pathology Laboratory, Australia.

Results

Temperature profiling

The comparison of two frequencies of energy application is shown in Table 2. Energy at 922 MHz gave much greater penetration into the brain tissue than did energy at 2.45 GHz.

Subsequent trials at 922 MHz, with continuous temperature monitoring, showed that heating rates in each area of tissue were linear (Figure 2). The skin experienced greater heating than the brain, and heating rate decreased with depth into the brain. The average curves show that upper brain temperature change was 40% of skin temperature change.

Animal studies

The temperature profiles achieved for sheep 1 and 2 are shown in Figures 3 and 4. In sheep 1, the energy application of 7.2 s, resulted in a temperature rise of 4.4°C at the top of the brain (position A), and 2.15°C at the bottom of the brain (position E). In sheep 2, the energy application of approximately 20 s, resulted in a rise of 9.65°C (maximum 48.5°C) at the top and middle (positions A and C) of the brain and 2.5 to 40.95°C at the bottom of the brain (position E). During and after this period, breathing remained rhythmic, the pulse regular, and haemoglobin oxygen saturation level at around 98%.

On the electroencephalogram (EEG) traces taken from sheep 1, 2 and 3, it could be seen that brain activity changed from low amplitude before application of energy, to high amplitude after application of energy (sheep 2 given as an example in Figures 5 and 6).

The progress of recovery from deep to light anaesthesia in sheep 3 and 4 was uneventful; return of jaw tone and chewing movements were observed in both animals prior to euthanasia.

On dissection, there was no visible effect on the skin or skull where the energy had been applied, apart from one sheep (sheep 4) where excess heating of the skin had been observed, as a result of the prolonged application of energy (20 s compared with 10 s in sheep 3). In this sheep, the surface of the skull showed a light, tan-coloured scorch mark, and the overlying skin was noticeably detached and crisp. The brains were also grossly normal, apart from sheep 4, which showed an area of hyperaemia on the surface of the brain at the point of energy application. Histologically, tissues from sheep 1, 2 and 3 were generally normal, although signs of autolysis were evident. The exception was sheep 4, which had received overheating; two sections of brain were normal, but one section (taken from the hyperaemic area) showed malacia with loss of nuclear detail and fragmentation of the neuropil. The histopathologist concluded that "the malacia is similar to what would be expected with complete and sudden ischaemia and possibly caused by damage to and within associated blood vessels, as well as thermal effects on the parenchymal cells".

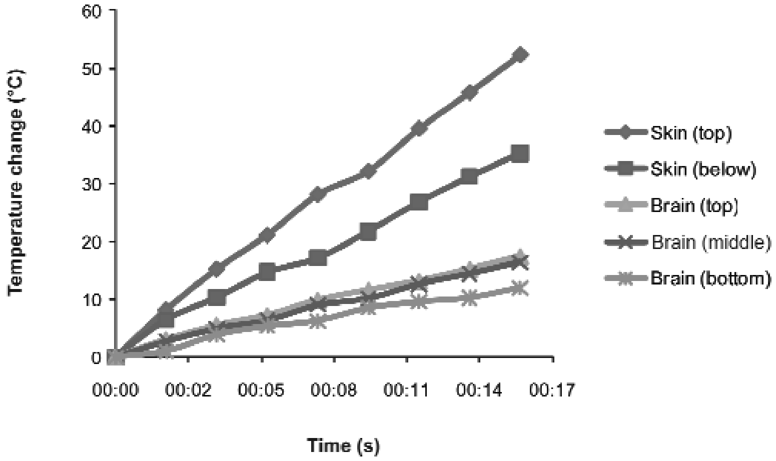
Table 1 Animal trial treatment summary.

Sheep	Microwave parameters	Exposure time	Measurements taken
1	4 kW 922 Hz	7.2 s	Temperature profiles
			EEG
			Histology
2	4 kW 922 Hz	20 s	Temperature profiles
			EEG
			Histology
3	4 kW 922 Hz	10 s	EEG
			Histology
			Observation of onset of recovery
4	4 kW 922 Hz	20 s	Histology
			Observation of onset of recovery

Table 2 Temperature change (°C) in tissues after 20 s microwave energy application to cadaver heads.

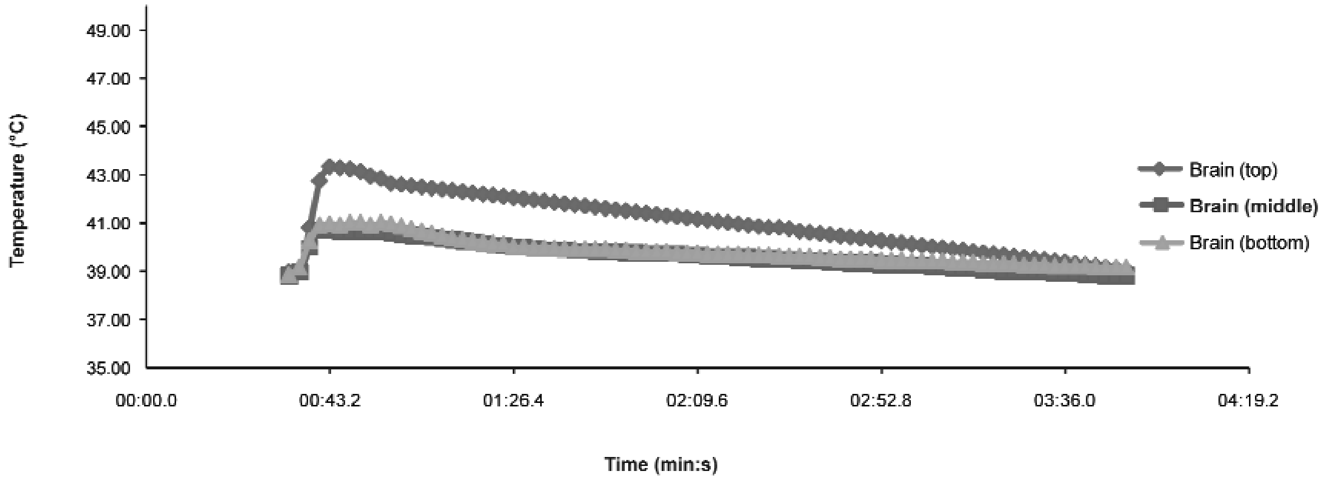
	2.45 GHz		922 MHz	
Tissue	Head 1	Head 2	Head 1	Head 2
Skin surface	21	17.5	17	23
Subdermal	7.5	5	Not recorded	Not recorded
Ear	2	3	Not recorded	Not recorded
Neck	2.5	0	0.5	0
Eye	2.5	1	2	2
Nose	4	2	3	0
Brain, upper rostral (position A, Fig 1)	6	2.5	15	24
Brain, upper caudal (position B, Fig 1)	2	3.5	14	11
Brain, middle (position C, Fig 1)	3	1	9	14
Brain, lower rostral (position D, Fig 1)	0	0.5	12.5	10.5
Brain, lower caudal (position E, Fig 1)	0	0	8	15

Figure 2



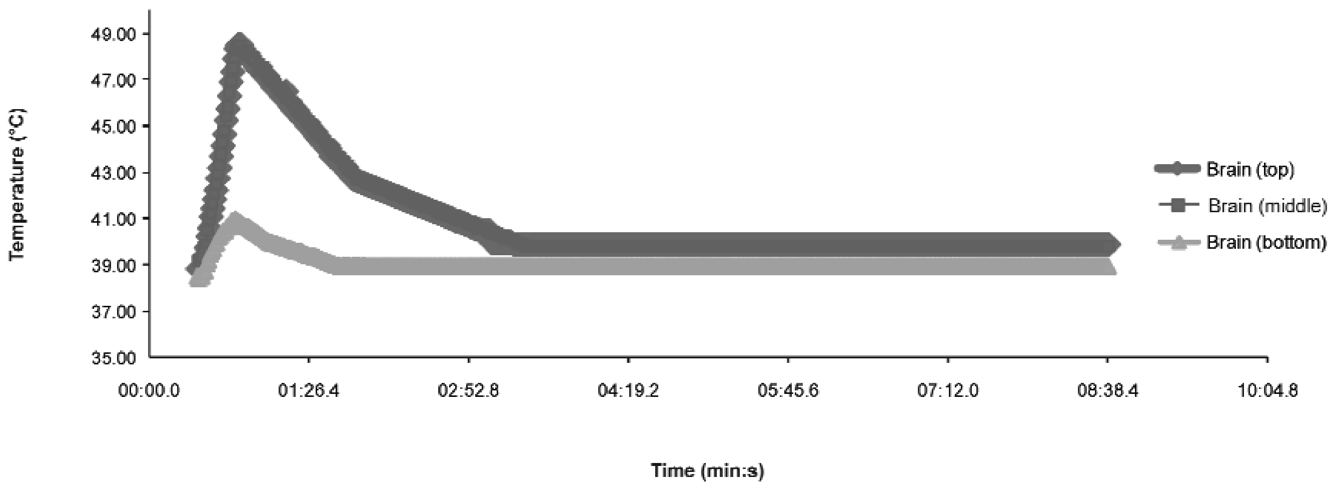
Temperature change in sheep tissues during microwave energy application (average from four cadaver heads treated at 922 MHz).

Figure 3



Temperature profile for anaesthetised sheep 1 (exposed to 4 kW at 922 MHz for 7.2 s). Microwave energy was applied between 00:36.0 and 00.43.2 s on the time trace.

Figure 4



Temperature profile for anaesthetised sheep 2 (exposed to 4 kW at 922 MHz for 19.8 s). Microwave energy was applied between 00:27.9 and 00.47.7 s on the time trace.

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Figure 5

Anaesthetised sheep 2 EEG prior to energy application.

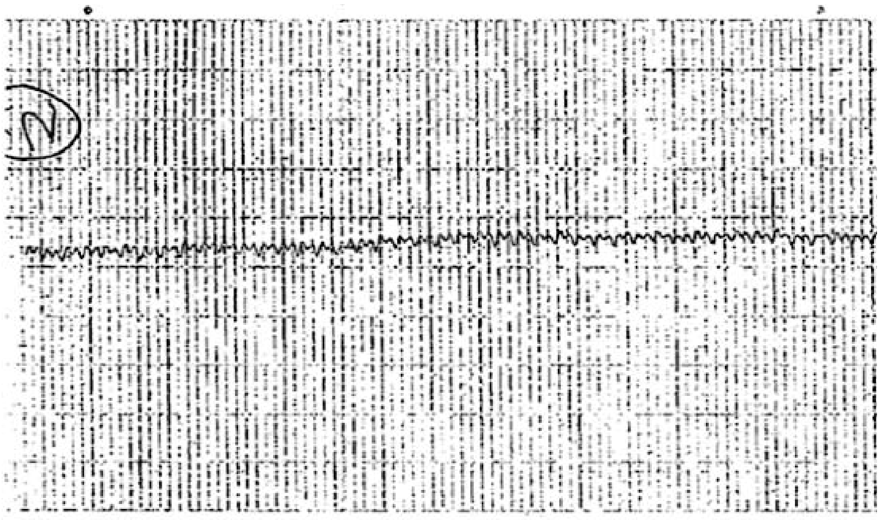
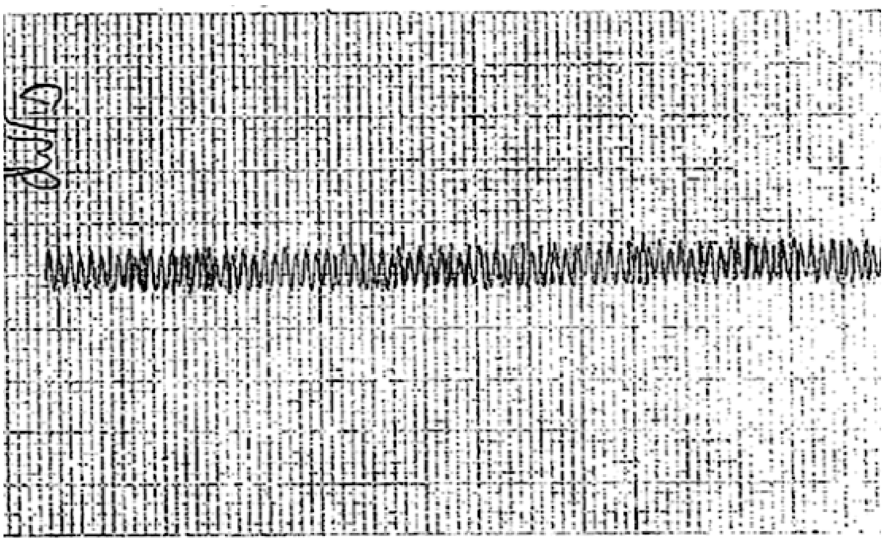


Figure 6

Anaesthetised sheep 2 EEG after energy application.



Discussion

In order to induce insensibility, the brain tissue must be heated to a temperature at which hyperthermic syncope would be expected to ensue. This is predicted to be around 43°C (Ohshima *et al* 1992), while Guy and Chou (1982) rendered rats insensible after raising brain temperature to above 44°C using a microwave device, and Lambooy *et al* (1989) also stunned rats using microwaves. In this latter study the brain temperature immediately after exposure was recorded to be above 47.3°C. A sheep's core body temperature is around 38°C, so the temperature change desired is in the order of 6°C or more. The cadaver work demonstrated that energy at a frequency of 922 MHz gave better penetration into the brain than 2.45 GHz, and indicated that temperature changes of 8°C or more are indeed achievable.

However, the living brain does have an inherent cooling mechanism (Niemark *et al* 2007) to protect the animal from hyperthermic syncope, and to effect a stun, it is imperative that this cooling mechanism is rapidly overcome by the energy applied. The animal studies were carried out to confirm that this can indeed be the case.

The target outcome was to achieve a temperature of greater than 43°C, but less than 50°C in the upper parts of the brain within a matter of seconds. These temperatures would be expected to induce recoverable insensibility in a conscious sheep. The aim was also that the brainstem — base and back of the brain — temperature would not be raised above 43°C, as this part of the brain is essential for cardiac and respiratory function. In the anaesthetised sheep, the required temperature range was achieved between 10 and 20 s of

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energy application, dependant on which region of the brain was studied. Furthermore, the EEG showed high amplitude activity similar to the epileptiform activity induced by the current practice of electrical stunning, and similar to those demonstrated by Lambooij *et al* (2011) when stunning chickens using transcranial magnetic stimulation. This suggests that the sheep, if they had not been anaesthetised, would have noticeably lapsed into unconsciousness.

Further work would be needed to understand the biological variation between animals, and to set critical limits for energy application in sheep. Also, similar work would need to be carried out on cattle. One of the primary concerns in the first instance is the development of an applicator that does not result in excessive surface heating. The particular microwave set-up used in the work so far only allowed 40–50% of the generated energy to be transferred as net power to the brain, and this may be able to be improved, thus shortening the required duration of energy application. In the cadaver trials it was shown that use of 2.45 GHz resulted in excessive skin heating compared with brain heating; while 922 Hz gave better penetration, and therefore relatively less superficial heating. Further development is required to optimise penetration and 'focus' the energy into the brain rather than onto the skin. It would be expected that skin temperatures of 53°C and above would be uncomfortable to the animal, based on cat studies (Rice & Kenshalo 1962).

Further research

Higher power would be recommended for commercial use to allow the required temperature rise to be effected in less than 1 s. However, the applicator used in the current trial would not be suitable for higher power application, due to excessive surface heating and arcing, ie energy sparking between hairs on the skin surface. For commercial use, an applicator designed to minimise surface heating and arcing will need to be developed.

Further work will be needed to fully understand the underlying neurophysiology and set critical limits for energy application. Furthermore, to allow this technology to be used in a commercial situation, guidelines for assessing efficacy and animal welfare status will need to be developed, as it would appear from the anaesthetised animal trials that rhythmic breathing remains present throughout. The applicator set-up used in this study is a basic prototype and further refinements will be required for the development of a commercial prototype, to increase efficiency of energy transfer and reduce surface heating. There is still a need to demonstrate that application of microwave energy at the power levels calculated does indeed render a conscious animal insensible.

Animal welfare implications

Preliminary trials on sheep suggest that microwave energy application could be a suitable method of inducing a fully reversible stun, which may meet the requirements of those Jewish and Muslim markets that allow pre-slaughter stunning.

Conclusion

Cadaver trials indicated that microwave energy could be used to induce hyperthermic syncope in sheep.

Application of microwave energy caused rapid increases in brain temperature to a point above which insensibility would be expected to occur (43°C), and below that which protein denaturation and damage would be expected to occur (50°C).

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