Final report

DTS: Diathermic Syncope® controlled trials

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Prepared by: Alison H Small
Commonwealth Scientific and Industrial Research Organisation

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Abstract

DTS: Diathermic Syncope® is a novel dielectric (electromagnetic) stunning system that has the potential to address the requirements of the Halal and Kosher markets, without current disadvantages of existing stunning methods. This project confirms pilot study outcomes in a larger number of cattle and explores the animal handling infrastructure and energy and power input parameters that would be suited to a commercial processing situation. Cattle (234), of a range of age, sex and bodyweight including heavy bulls and Brahman-type animals, were successfully stunned on first application of DTS. Live observations indicated that DTS consistently induces insensibility, with no vocalisation, no evidence of pain or distress, and EEG data confirmed the development of a high-amplitude-low-frequency (HALF) epileptiform state. The duration of insensibility was suited to exsanguination using the Halal cut. There was no requirement to use an immobiliser during application of the exsanguination cuts, and the animal bled rapidly to brain death using the Halal cut alone. It is likely that the animal stunned using DTS can also return to consciousness (based on EEG and returning eye focus), so this method of stunning is likely to be acceptable to the Halal and Kosher markets.

Executive summary

Background

A dielectric (electromagnetic) stunning system has been developed by Wagstaff Food Service Pty Ltd and Advanced Microwave Technologies. This system, trademarked DTS: Diathermic Syncope®, has the potential to address the requirements of the Halal and Kosher markets, without current disadvantages of existing stunning methods (e.g. the potential for cracked skulls associated with percussive stunning, the need to use an immobiliser to improve operator safety in electrical head-only stunning, the need for a second exsanguination cut following the neck cut to remove the risk of the animal regaining consciousness during bleed-out). Pilot trials on live animals have been successful in inducing electroencephalogram traces consistent with unconsciousness, of sufficient duration to allow exsanguination prior to recovery. DTS produced comparable post-slaughter meat quality and physiological responses in treated cattle to those stunned using penetrative captive bolt. The current project comprised trials on live animals in a working controlled environment, validating the previous outcomes in a larger number of animals, demonstrating repeatability. Ultimately the outcomes of this work will be used to gain industry stakeholder agreement on full commercialisation of the technology. The progress of research and development of this technology is evidence of the Australian Industry's commitment to continual improvement in Animal Welfare at processing, which in turn supports the continued social license to operate.

Objectives

The objectives of the project were:

1. Confirmation of previous science works in a commercialised environment.
2. Data to support approval of the technology as a commercial means of inducing insensibility on cattle for the production of meat for human consumption
3. A full report on the development and validation work.
4. A manuscript suited for submission for publication in a peer-reviewed journal.
5. A review of the remaining data required to submit an application to the EU Animal Health and Welfare Panel; for assessment of DTS as an 'alternative stunning intervention', if that were desired.

Objectives 1, 2, 3 and 5 have been met and the details are addressed in this report.

Against Objective 4, the data from the preliminary confirmation study was published in Small et al. (2019), alongside the data from P.PIP.0395. A further technical submission, encompassing the remaining data, is planned.

Methodology

There were three phases to the project – the first was a validation of previous research findings in 20 cattle using the reconfigured applicator system. This was followed by a pre-commercial-scale evaluation in which 181 cattle including a small number of Bos indicus and

heavy bulls were stunned using DTS. Variations in input power and energy parameters and a variety of applicator head designs were tested. Finally, an evaluation of a proposed commercial set-up including a rotary box for handling of the stunned body was carried out, in which 33 cattle were stunned using DTS.

Results/key findings

In the absence of technical problems leading to interrupted delivery of energy, all animals were rendered unconscious using DTS. 234 animals were successfully stunned on first application. At no time was Animal Welfare compromised by technical faults.

Power settings of 20 kW provided a rapid onset of insensibility, while a power setting of 18 kW led to a slower onset of insensibility. Power settings of 25 kW and above led to overheating at the skin surface.

Energy deliveries in the range of 160 to 200 kJ achieved insensibility of sufficient duration to allow exsanguination using a neck cut alone, with operator safety during exsanguination optimised. Energy deliveries of 160 kJ and less resulted in shorter duration of insensibility with a risk of early return to consciousness, while energy deliveries of 220 kJ and above resulted in early onset of intense convulsive activity, reducing operator safety.

Live observations indicated that DTS consistently induces insensibility, with no vocalisation, no evidence of pain or distress, and EEG data confirmed the development of a high-amplitude-low-frequency (HALF) epileptiform state.

There was no requirement to use an immobiliser during application of the exsanguination cuts, and the animal bled rapidly to brain death using the Halal cut alone. The blood flow on exsanguination was strong and rapid.

Visual inspection of the brains of carcases indicated no visible damage when energy deliveries of less than 220 kJ were applied.

Benefits to industry

The project was planned and executed under end-user-centred design principles, with the Halal and Kosher markets in mind. DTS is an effective means of inducing insensibility in cattle, with a duration of insensibility suited to exsanguination using the Halal cut. It does not damage the skull; is effective in heavy animals such as bulls; and there is no need to use an immobiliser or second cut for exsanguination. It is likely that the animal stunned using DTS can also return to consciousness (based on EEG and returning eye focus), so this method of stunning is likely to be acceptable to the Halal and Kosher markets.

Future research and recommendations

A key challenge throughout this project was technical faults in energy delivery, and the stability of the infrastructure. Further work should focus on improving the infrastructure to resolve these issues.

The current project utilised mechanical stunning (penetrative captive bolt) as the back-up stun method. In the event of an interrupted DTS application, a re-stun would be quicker if a second DTS application was immediately applied, as opposed to the need to open the Faraday cage and remove the waveguide to apply the mechanical stun. Validation of this approach is recommended.

To proceed to wider industry adoption, regulatory approval of the technology as a method of pre-slaughter stunning of the animals is required. Regulatory approval will require modifications to the Standards and Guidelines.

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1. Background

Billions of cattle are slaughtered each year around the world to produce meat, a valuable source of protein in our diets. Regardless of culture, it is agreed that slaughter should be carried out in the best possible manner, however, there is disagreement on what constitutes the best manner. Modern slaughter often involves stunning the animal prior to the slaughter cut, rendering the animal unconscious such that it does not feel the pain of the neck cut. However, existing stunning methods are not acceptable to all markets (e.g. Muslim or Jewish), and they are in themselves not perfect. The Jewish and Muslim communities have strict requirements relating to the production of meat, based on the teachings within the Torah and Quran. One of the key issues in current commercial meat production is the requirement of Muslim (Halal) and Jewish (Kosher) laws that animals are healthy, whole and undamaged at the point of slaughter, slaughter being defined as the cutting of the neck such that the animal dies as a consequence of blood loss. A dielectric (electromagnetic) induction of insensibility has the potential to fully comply with these requirements.

At present, in a commercial abattoir setting, cattle are stunned by mechanical or electrical means. Mechanical stunning can be carried out by use of the penetrative captive bolt, or the non-penetrating (mushroom) stun. The penetrative captive bolt produces a hole in the skull (and subsequent brain damage), so is not acceptable according to the Muslim or Jewish laws. Furthermore, although penetrative captive bolt is considered to be the 'gold standard' stunning method, it is not 100% effective. Where the head of the animal is not fixed in position, it can be difficult for the operator to accurately position the 'shot'. Where no head restraint is used, up to 50% of stuns may be misapplied, and not effective (Gouveia et al., 2009). In Europe, where head restraint is commonly used, 7 to 15% of penetrative captive bolt stuns are estimated to be ineffective (Endres, 2005, Marzin et al., 2008, Von Holleben et al., 2010), whereas in the US, failure rates have been reported to be in the order of 0.6 – 1.2% (Grandin, 1994), where effective head restraint is used. The Australian export meat industry has set targets of 96% of animals to be effectively stunned on first shot, which is achievable with the use of appropriate head restraint.

Non-penetrative mechanical stunning has been accepted by the Australian, Malaysian and Indonesian Islamic authorities, and is utilised throughout the Australian meat industry. It is estimated that 80% of cattle slaughtered in Australian Export-registered abattoirs are stunned using non-penetrative mechanical stunning. The challenges with non-penetrative mechanical stunning (also called mushroom stun), are that it can produce large cracks and fractures to the skull, visible when the head is skinned. If these cracks are present, the carcase is deemed unacceptable for Halal meat. Furthermore, percussive stunning can result in extensive, visible brain damage, and thus does not comply with the requirement that the animal be 'undamaged' at the point of slaughter. Potentially, it may be only a matter of time before percussive stunning will be considered unacceptable for the Halal market.

To reduce the number of carcases demonstrating skull fractures, attempts are made to minimise the power (kinetic energy) delivered by the instrument. However, where the power is reduced, the incidence of improper (failed) stuns increases, and animals may need two or more 'shots' to be rendered fully unconscious. The back-up method is often the application of a penetrative captive bolt stun, for welfare reasons, and the carcase is not acceptable as Halal. Reported failure to stun rates with non-penetrative mechanical stunning are high and very variable, particularly where adequate head and neck restraint is not in use. For Example, Lambooij et al. (1981) could only produce immediate unconsciousness in 15 out of 19 veal calves of 200 kilograms live weight (i.e. a 21 % failure rate); Blackmore (1979) found that 80 % of 90 calves between one and 2 weeks of age were effectively stunned as determined by behavioural observations (i.e. a 20 % failure rate); Hoffmann (2003) used either cartridge activated or pneumatic concussion stunning devices and found that 12 % out of 1248 cattle had to be re-stunned (Thesis findings reported in von Holleben et al. (2010); Endres (2005) examined stunning effectiveness in more than 5500 cattle, using two different pneumatic non-penetrative devices. In total only 83.3 % of the cattle were stunned by the first blow (16.7 % failure rate). Highest re-stun rates of 20 % were found in young bulls. Sixty percent of the 548 heads examined showed profound injuries of the frontal bone in the impact area of the bolt including inner and outer bone laminae and partly the dura mater. (Thesis findings reported in von Holleben et al. (2010)); Oliveira et al. (2018) reported a need for back-up stunning in 29 % of cattle stunned using non-penetrative mechanical stunning; and Gibson et al. (2019) reported a failure rate of 18 %, based on EEG data of young bulls, of 550 kg live weight. Based on these findings, the EU has disallowed percussive stunning in animal over 10kg bodyweight (EC 2009). Thus, for welfare reasons, there is an urgent need to provide the Halal market with a more reliable method of inducing unconsciousness prior to slaughter.

Electrical head-only stunning has been accepted by the Australian, Malaysian and Indonesian Islamic authorities. The challenges with electrical head-only stunning applied to cattle are that the electrical current sometimes must be applied for 20 seconds or more in order to achieve the required epileptiform activity in the brain (Devine et al., 1986, Vonmickwitz et al., 1989, Schatzmann and Jaggin-Schmucker, 2000), this can lead to burn marks on the head at the point of application, which are considered unacceptable for Halal meat, and the duration of insensibility can be very short, such that animals may be regaining consciousness during the bleed-out period (EFSA, 2004). Where animals are observed to be regaining consciousness during the bleed-out period, for welfare reasons a penetrative captive bolt stun is applied as a back-up and consequently the carcase is not acceptable as Halal. Furthermore, in order to increase the rate of exsanguination and limit the risk of consciousness returning during bleed-out, the regulator requires a ventral neck incision (thoracic stick procedure) to be carried out after the Halal neck cut. This conflicts with the Halal requirements of some markets.

At present, only in Australia, cattle slaughtered for the Kosher market receive captive bolt stunning immediately after the neck cut, thus reducing the duration of the pain and distress suffered. However, this does not avoid potential pain associated with the initial neck cut. By providing a suitable method of pre-cut induction of insensibility, all animals processed for the Kosher market could be rendered insensible prior to exsanguination.

A dielectric (electromagnetic) stunning system has been developed by Wagstaff Food Service Pty Ltd and Advanced Microwave Technologies (Ralph et al., 2011, Small et al., 2013a, McLean et al., 2017). The mechanism of action is selectively increasing the temperature in the brain to the point that hyperthermic syncope (fainting) occurs. Thermal unconsciousness such as that induced by exercise heat stress or fever is reported to occur when core body temperatures reach between 40 and 45°C (McDaniel et al., 1991, Ohshima et al., 1992, Mohanty et al., 1997, Roccatto et al., 2010, Lerman et al., 2014, Yoshizawa et al., 2016). Trials on live animals have been successful in inducing electroencephalogram (EEG) traces consistent with unconsciousness, of sufficient duration to allow exsanguination prior to recovery, and produces comparable post-slaughter meat quality and physiological responses in treated cattle to those stunned using penetrative captive bolt (Small et al., 2015, Small et al., 2019). The next stage is to carry out trials on live animals in a working controlled environment, validating the previous outcomes in a larger number of animals, demonstrating repeatability; and subsequently to gain industry stakeholder agreement on full commercialisation of the technology. Within this project, we have carried out a controlled pre-commercial-scale series of live animal trials, including a short ‘demonstration’ of recoverability. The outcomes of this project will support use of the technology at the Wagstaff Garfield abattoir through inclusion in the establishment’s Approved Arrangements, and are to be included in a dossier of information to the National Animal Welfare Task Group seeking recognition that “DTS Diathermic Syncope” (the process) constitutes a humane stunning method, i.e. that the animal is unconscious and insensible to pain before being bled out at slaughter.

2. Objectives

The objectives of the project were:

1. Confirmation of previous science works in a commercialised environment.
2. Data to support approval of the technology as a commercial means of inducing insensibility on cattle for the production of meat for human consumption
3. A full report on the development and validation work.
4. A manuscript suited for submission for publication in a peer-reviewed journal.
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3. Methodology and results

3.1. Animal studies

3.1.1. Animal Ethics Committee approval

The animal studies were carried out in three phases.

The first phase aimed to confirm previous laboratory-scale results in a more commercial situation. This phase was approved by the Victorian Department of Economic Development, Jobs, Transport and Resources (DEDJTR) Wildlife and Small Institutions Animal Ethics Committee (WSI AEC), reference 30.16.

A copy of the application and approval are provided in Appendix 1.

The second phase, the pre-commercial scale validation, and third phase (rotary box evaluation) were approved by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Wildlife and Large Animal Animal Ethics Committee (CWLA AEC), reference 2019-17.

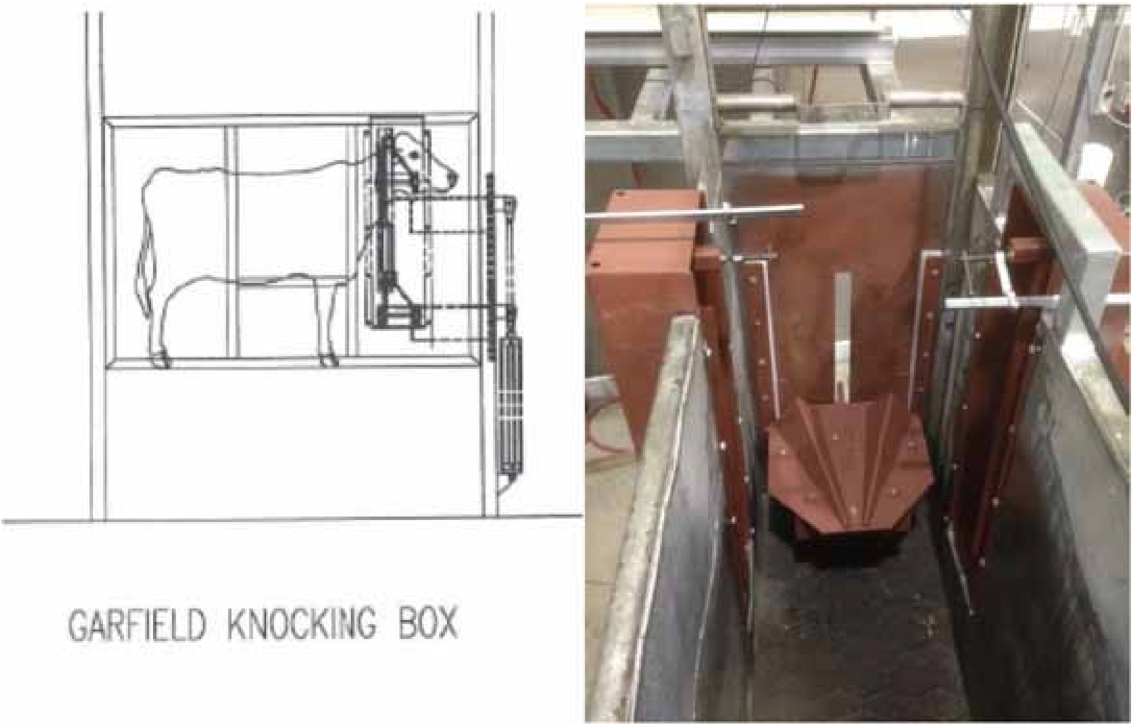
A copy of the application and approvals (the rotary box evaluation was added as a modification to the initial approval) are provided in Appendix 2.

3.1.2. Preliminary confirmation of previous science outcomes

3.1.2.1. Method

A total of 20 cattle (mixed breed, predominantly dairy crosses, some were aged cull cows, and others were poor-quality dairy cross steers. Age and gender of individuals was not recorded) was processed using Prototype 4 of the DTS: Diathermic Syncope system, under Victorian State Government Wildlife Animal Ethics Approval 30.16. Cattle were brought individually to the restraint unit. For application of DTS, the cattle were individually restrained in a stun box with neck yoke, head capture and chin lift (Figure 1), all of which were fully enclosed in a Faraday cage. A thermal image of the forehead was taken and electroencephalogram (EEG) was recorded prior to DTS application.

Figure 1: Engineering concept diagram of restraint unit (left), photograph of chin lift unit, taken during installation, prior to enclosure with Faraday cage shielding (right).



The study was carried out over three days, and on each day, energy application was initiated at 360 kJ, delivered using a power setting of 30 kW, and when each energy setting was confirmed to induce insensibility, the energy setting was sequentially reduced by increments of 25 kJ throughout the 20 animals, with a return to higher energy levels at the beginning of each processing day such that the settings applied were: one animal at 360 kJ, five at 300 kJ, five at 275 kJ, two at 250 kJ, five at 225 kJ, and two at 200 kJ. Towards the end of the study, power settings of 20 kW were attempted, with the total energy delivery set at levels (275 kJ and 200 kJ) that had produced insensibility when delivered using a power setting of 30 kW and finally one animal was processed using an energy delivery setting of 200 kJ and power setting of 15 kW (Table 1).

Post DTS application, corneal reflex was tested, a post-application thermal image taken, and EEG was recorded, with corneal or somatic withdrawal reflex tested at 30-second intervals. When 90 seconds (or 30 seconds of stable, clear signal was observed on the screen) of EEG had been recorded, the leads were removed, and the body released from the restraint unit onto the bleed conveyor, where it was rodded (the oesophagus sealed), and exsanguinated using the ‘thoracic stick method’ which severs the common carotid artery close to the thoracic inlet. If signs of returning consciousness were detected, namely the return of corneal reflex or eye focus and following movements, the animal was stunned using a penetrative captive bolt. Once the body was exsanguinated, it was processed and inspected for human consumption according to the normal practices at this abattoir.

During application of DTS, the animals were monitored using real-time video capture through a security camera system. Observations of animal reaction were recorded from the video footage in real time, then subsequently footage was played back at reduced speed in order to prepare a detailed event log for each individual animal.

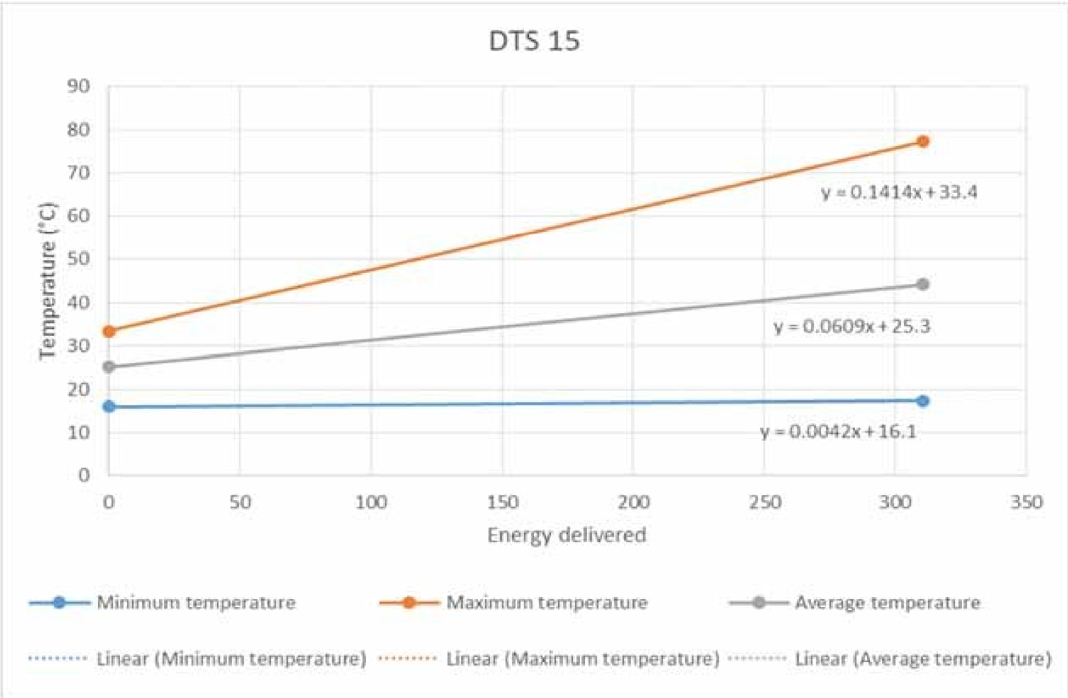
Table 1: Energy parameters delivered during the validation study

ANIMAL	SETTING (KJ, KW)	ENERGY DELIVERED (KJ)
1	360, 30	421
2	300, 30	350
3	300, 30	351
4	300, 30	171
5	300, 30	338
6	275, 30	317
7	250, 30	292
8	250, 30	284
9	225, 30	267
10	225, 30	258
11	225, 30	261
12	225, 30	125
13	225, 20	247
14	300, 30	351
15	275, 30	310
16	275, 20	310
17	275, 20	312
18	275, 20	297
19	200, 20	226
20	200, 15	223

The EEG data were analysed offline using LabChart 8 (ADInstruments, Sydney, Australia). Artefacts were identified and rejected manually, with reference to video footage to identify event-related artefact and edge artefacts. A band-pass filter of 0.1 to 30 Hz was applied to the raw data, and the Spectral Analysis Package within LabChart 8 was used to apply Fast Fourier Transformation, with multiplication using a Hann window in 1-second epochs with a 25% overlap. Total power (Ptot), median frequency (SEF50) and 95% Spectral Edge frequency (SEF95) were extracted. The median value of Ptot in the pre-DTS recording was calculated and this was used as the baseline value. Baseline normalization was then carried out by transforming data for each 1-second epoch into decibel change from baseline according to the formula: dB= 10*log10(value/baseline), to bring all data sets into a comparable format. These data, and data for SEF95 and SEF50 were charted and inspected for EEG suppression and epileptiform activity, and where possible time to resolution of EEG suppression was recorded.

Thermal images were analysed in ResearchIR (FLIR Systems AB, Danderyd, Sweden). On each image, the forehead area was delineated to exclude the ears, eyes and muzzle area. Pre- and post-DTS minimum, maximum and mean surface temperatures were used along with the total energy delivered as recorded by the DTS control software to generate a 'temperature change by energy delivered' chart for each individual (example in Figure 2). Comparing 'total energy delivered by time' from the DTS control software with video annotation of behaviours allowed an estimation of the energy delivered at the point of loss of posture, and the associated surface temperature at that point could then be estimated from the 'temperature change by energy delivered' chart.

Figure 2: Animal 15 estimated temperature change on the forehead by energy delivered.



3.1.2.2. Results

Energy applications ranged from 200 kJ to 360 kJ. Seventeen animals were assessed as insensible following DTS application. After application, these animals demonstrated behavioural and EEG signs consistent with an electrical stun, loss of posture occurring between 1 and 8 seconds after onset of energy delivery. In 4 animals, which received 300-360 kJ, this insensibility progressed to death. In the remaining 13 animals, absence of corneal reflex persisted for between 100 and 170 seconds, at which point penetrative captive bolt was administered in case of returning consciousness. When these timeframes are compared with those reported for head-only electrical stunning in cattle (application of 4 to >20 seconds, duration 31 to 90 seconds), it is evident that DTS provides a better welfare outcome in terms of duration of insensibility.

For each of the three animals that were not deemed insensible, there had been problems with maintaining contact between the waveguide and the forehead, resulting in leakage of energy into the environment, rather than penetration into the brain. All three appeared sedated, with loss of eye focus and no visual following of movement, and a slow response to a touch on the cornea. All three were stunned using a penetrative captive bolt immediately following assessment of consciousness.

Of the 20 animals processed, 11 EEG recordings were generated: the post-DTS recording was not collected from animal 2 due to accidental shut-down of the laptop running the LabChart software, and in animal 13, corneal reflexes were present and the animal was stunned using the penetrative captive bolt shortly after opening of the Faraday cage, prior to EEG recording. For animals 14 to 20, baseline EEG was not recorded, to reduce the pre-slaughter stress, and post-DTS EEG was recorded on the bleed-conveyor during exsanguination. Thus, baseline EEG for normalisation was not present, and the post-DTS EEG could only be used qualitatively. In the event, once movement artefact was removed, there were very little EEG data available for animals 15-20 prior to the return of the corneal reflex and captive bolt application. All the animals for which usable EEG recordings were collected showed changes from baseline, particularly in terms of Ptot, while for animal 14, for which EEG data was collected post-DTS only and captive bolt was not applied, high amplitude spiking of Ptot was observed. These patterns on EEG are considered to be incompatible with sensibility.

Thermal imaging indicated that the average surface temperature on the forehead at the point of loss of posture would be between 26 and 49 °C, although some animals (animals 6 and 7) that showed a delayed loss of posture also demonstrated hot-spots of up to 109 °C. In context, Australian cattle may experience ambient air temperatures of 45 °C in summer and could experience higher surface temperatures when standing in direct sunlight. Surface temperatures above 50 °C may be uncomfortable to cattle, although no literature on thermal contact sensitivity in cattle could be located to confirm or refute this supposition, while temperatures above 60 °C will result in skin tissue damage, as seen in hot branding which is commonly used for identification of cattle.

During the course of the trial, several modifications to the waveguide positioning apparatus were made, with the aim of improving contact and reducing leakage. Some potential adjustments to the restraint system, to improve animal handling and reduce the risk of leakage (due to the positioning of the animal) were also identified.

The results gathered were confounded by problems with waveguide to forehead contact, resulting in loss of energy to the environment instead of being transmitted into the brain. This variability in energy delivery may account for the variability in latency to loss of consciousness, using loss of posture as the proxy indicator, and problems with waveguide to forehead contact may have contributed to the variability in surface temperatures and the presence of hot spots on the forehead.

3.1.2.3. Key findings

- DTS Prototype 4 successfully rendered cattle insensible based on reflexes, behavioural and EEG data;

- Prolonged duration of insensibility suggests that DTS may be better than the existing commercially available head-only electrical stunning method for cattle;
- Some engineering modifications are required to ensure consistent delivery of energy to the brain and minimise leakage.

Prior to the next stage of development, engineering modifications such that consistent delivery of energy was achieved were carried out.

A full report on this phase is included as Appendix 3.

3.1.3. Pre-commercial-scale validation

For this phase of work, a total of 235 cattle was processed in a series of small batches of between 8 and 50 cattle between October 2017 and October 2019, under the CSIRO Wildlife and Large Animal Animal Ethics Committee (CWLA AEC) approval, reference 2019-17. Between batches, modifications were made to the waveguide apparatus and/or restraint unit in order to optimise stun quality and ease of processing. These modifications led to reductions in the required input power (kW) and elimination of the surface overheating that had been observed during the previous study (section 2.1.2). Data were collected on animal type, energy delivery parameters, presence/absence of indicators of unconsciousness, time of back-up stunning and behavioural observations.

The animals included steers, heifers, cows and bulls, of varying ages from milk teeth only to full mouth cull animals. The animals were predominantly *Bos taurus*, with 5 *Bos indicus* animals, and 13 had horns. Carcase weights ranged from 160 to 413 kg. Power settings for DTS application ranged from 15 to 40 kW, and energy settings from 100 to 371 kJ.

Of 235 animals processed, 6 were assigned to penetrative captive bolt only as a result of their being collapsed in the restraint or overly agitated to securely restrain; and a further 6 were stunned and slaughtered at the end of the final batch using penetrative captive bolt because the current phase of DTS trials was complete and they were 6 remaining animals in the lairage when the plant was being decommissioned for refurbishment in preparation for the rotary box evaluation. These 12 animals were excluded from the evaluation of DTS, leaving 223 animals in the current evaluation.

Of the 223 animals included in the evaluation, a further 16 were excluded from analysis as a result of equipment failure resulting in generator shutdown and the required energy not being applied. These animals were also stunned using a penetrative captive bolt prior to exsanguination.

Of the remaining 207 animals, 201 were rendered fully insensible on first application of DTS (97.1%). This exceeds the expected minimum standard of 96% described in appendix 7 of the National Animal Welfare Standards for Livestock Processing Establishments (AMIC, 2009). The 6 other animals appeared sedated. Analysis of the data indicated that these cases were related to low Power (kW) settings, which were used during the study to determine the lower critical limits of expected operational parameters. In the 201 fully insensible animals, live observations suggested that there was rapid induction of insensibility, which lasted for between 2 and 4 minutes in the majority of cases.

Live observations indicated that low energy levels resulted in shorter duration of insensibility, while energy levels above 220 kJ appeared to result in early onset of intense convulsions. Surface overheating and visible blistering of the skin was evident when power settings were 25 kW and above.

Three entire Angus young bulls, three mature bulls and five Brahman animals (three of which were horned) were included in the data set. Energy applied to bulls ranged from 200 to 250 kJ, and all were rendered insensible using DTS. Forehead skin thickness in the bulls ranged from 5 mm (Brahman) to 24 mm (Angus).

In the absence of technical problems leading to interrupted delivery of energy, all 201 animals were rendered unconscious using DTS. On exsanguination, blood flow was noted to be strong, and the rhythmic pumping of the heart was evident in the first few seconds of blood flow. Both these characteristics are desired by the Halal and Kosher markets.

Visual inspection of a sample of brains, removed from the skull cavity immediately following head inspection, indicated that when energy delivery was 220 kJ or less, there was no visible damage to the brain (Figure 3).

Figure 3: Brains from three cattle processed using DTS. Left 180 kJ; middle 180 kJ; right 320 kJ. Destruction of the brain tissue of the frontal lobe can be seen in the sample on the right, and the brin does not maintain its form when placed on the bench. In the samples at middle and left, the brains maintain their form, and the tissue structure is intact.

The main challenge during this phase was extracting the stunned bodies from the restraint unit, which was a standard abattoir knocking box designed for use with captive bolt. Cattle that are stunned using DTS (or for that matter, electrical stunning) can quickly enter a stiff, tonic ‘rocking-horse’ position, with all four legs rigidly extended. If this happens before the body is rolled out from the box, the body is obstructed by the side door, which cannot open sufficiently to allow ejection from the box. After this ‘rocking horse’ phase, the stunned animal develops a convulsive or kicking phase (similar to that seen in an epileptic episode), followed by a recovery phase. In commercial processing it is important to exsanguinate the animal before the kicking phase begins – both to ensure that the animal does not recover during bleed-out, and because the size of the animal makes handling during that kicking phase very dangerous for the operator. The difficulties in extracting the body from the upright restraint box prevented prompt exsanguination during the tonic phase.

As such, installation of a rotary box was undertaken, so that the stunned animal could be tipped to allow rapid exsanguination.

3.1.4. Rotary box and reconfigured applicator evaluation

A rotary box was installed at the Wagstaff Garfield abattoir, VIC, and fully enclosed in a Faraday cage (Figure 4-6).

Figure 4 (left): Faraday cage enclosing the rotary box. The DTS generator is on the platform seen in the top right-hand corner, and access to the front of the box is provided through a door in the Faraday cage facing the personnel in the image. The stunned animal is released onto the conveyor seen in the bottom left-hand corner of the image.

Figure 5 (right): View of the chin lift apparatus, taken from the access door at the rear of the Faraday cage. The aperture allowing access to the inverted stunned animal can be seen at the bottom left of the image, below the steelwork.

Figure 6: An animal in position, prior to DTS application, with the chin lift applied and the waveguide applicator in position on the forehead. The door allowing access to the inverted stunned animal is now closed.

Over a three-day period from 1st to 3rd December 2020, 35 *Bos taurus* slaughter generation cattle of mixed breeding were processed using DTS: Diathermic Syncope® to induce insensibility, inverted using the rotary box and bled in the inverted position using the Halal cut delivered by a registered Halal slaughterman. The work was approved by the CSIRO Wildlife and Large Animal Animal Ethics Committee (CWLA AEC), reference 2019-17.

The animals were processed to give carcase weights ranging from 242 to 413 kg (10 steers and 25 heifers). Breeds were crosses of Angus, Hereford, and Holstein/Friesian and eight had horns.

Energy applications between 140 and 230 kJ were used in 33 animals, all of which were rendered insensible using DTS. Two animals received energy levels below 100 kJ as a result of equipment failure, these were immediately stunned using a non-penetrating device. Energy levels of 140-160 kJ appeared to result in shorter durations of insensibility, while energy levels above 200 kJ appeared to result in early onset of intense convulsions.

The study reconfirmed consistent induction of insensibility, based on live observations of the animal and assessment of corneal and palpebral reflexes. The insensibility was sustained during bleeding using the Halal neck cut. Electroencephalogram (EEG) data were collected from 19 animals, reconfirming the high-amplitude spiking in Ptot following DTS application (Figure 7). This spiking persists throughout bleeding. In the example shown in Figure 8, sticking was performed at 56 seconds post-DTS application, and spiking in Ptot was still evident some 60 seconds post-sticking. The period between 90- and 110-seconds post-DTS application was characterised by convulsions, so the EEG was obscured by movement artefact.

Use of the rotary box improved handling of the stunned body, and the updated applicator configuration allowed consistent repeatable induction of insensibility, which was sustained during Halal bleeding. When a delay to collect EEG data was not required, sticking was carried out within 30 seconds of DTS application.

After sticking, Ptot rapidly dropped towards baseline, over a period of 20 to 60 seconds.

Figure 7: EEG trace from animal 208 in the current study, showing high amplitude spiking of Ptot post-DTS application.

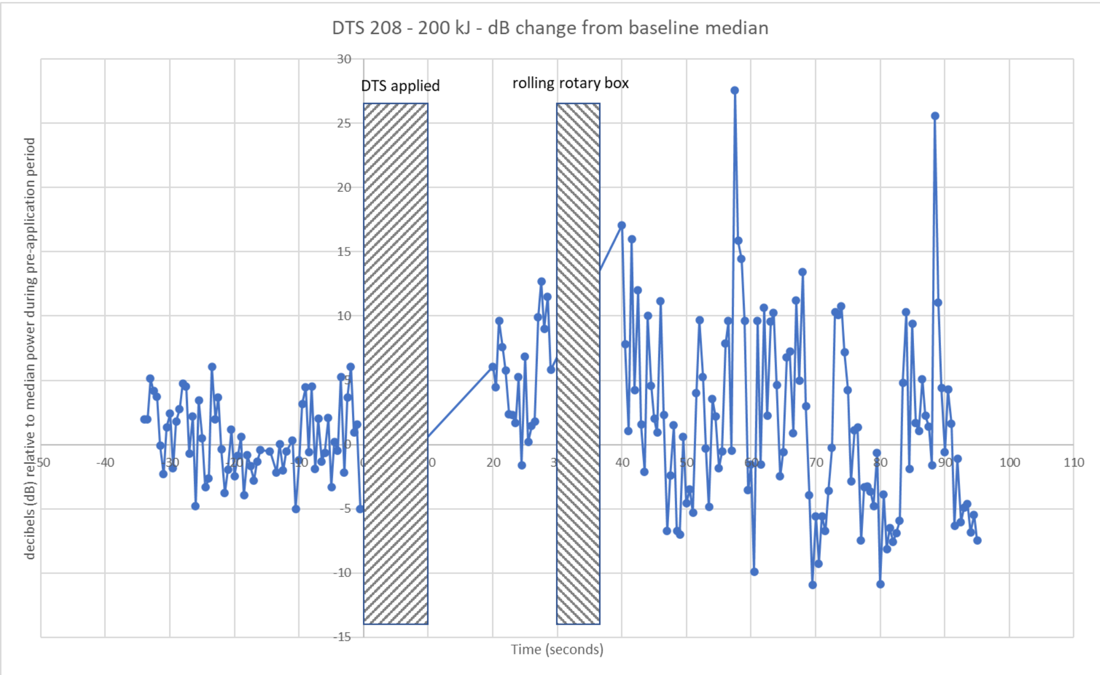
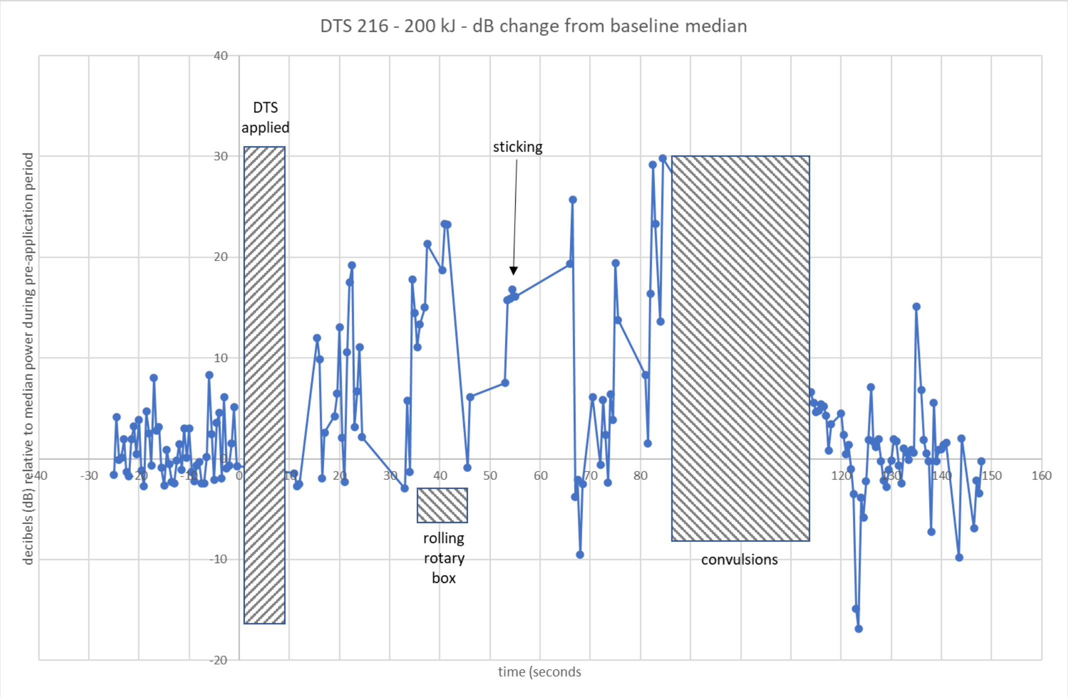


Figure 8: EEG trace from animal 216 in the current study, showing high amplitude spiking of Ptot post-DTS application. This spiking persists throughout bleeding, sticking having been performed at 56 seconds post-DTS application, with spiking still evident some 60 seconds post sticking. The period between 90- and 110-seconds post-DTS application was characterised by convulsions, so the EEG was obscured by movement artefact.



3.2. Assessment of existing data against the EU guidelines for the assessment criteria for studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing

This activity was carried out by Dr Leisha Hewitt (Dr Leisha Hewitt Livestock Welfare) as an independent consultant to the project, in order to mitigate against potential bias. The remit provided to Dr Hewitt was to:

> *Review work to date in the light of the EFSA guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing (EFSA, 2013), and provide recommendations regarding further research activities.*

In this context, 'work to date' meant work done prior to the work described in the body of this current report, i.e. laboratory investigations (McLean et al., 2017, Small et al., 2013a, Small et al., 2013b), anaesthetised animal trials (Rault et al., 2014) and a pilot study on conscious animals (P.PIP.0395 and G.PAW.0009) (Small et al., 2019).

The EFSA guidelines define the assessment process and the criteria that will be applied by the Animal Health and Welfare Panel to studies on known new or modified legal stunning interventions to determine their suitability for further assessment. The criteria that need to be fulfilled are eligibility criteria, reporting quality criteria and methodological quality criteria. The pilot study was conducted in a commercial abattoir, under experimental conditions. The parameters in the study were assessed when applying a stunning intervention based on the application of DTS technology. This technology is not included in Annex I of Council Regulation (EC) No 1099/2009 or the EFSA guidance. The parameters were therefore mapped against the ESFA guidance using previously published scientific findings.

The full report is presented in Appendix 4. The recommendations made under the review were predominantly associated with reporting, particularly with regards to the detailed descriptions of statistical assumptions and parameters. These recommendations were addressed during preparation of the report for the current project.

4. Discussion

During the entirety of the current study, 258 cattle were presented, of which 242 cattle were processed using DTS: Diathermic Syncope® as the stunning method (Table 2). The remaining 16 animals did not receive a DTS application due to technical faults resulting in generator shutdown prior to energy being delivered, and they were stunned using a captive bolt. Of the 242 animals, 8 received low energy deliveries due to technical faults and were deemed inadequately stunned, so were re-stunned using a penetrative captive bolt. Thus 234 of 242 (96.7%) cattle were stunned on first application of DTS, based on loss of posture and loss of corneal and somatic withdrawal reflexes (Von Holleben et al., 2010). Loss of posture, which is considered to be a definitive indicator of unconsciousness (EFSA, 2004, Muir, 2007, Von Holleben et al., 2010, EFSA, 2013), occurred between 1 and 8 seconds after onset of energy delivery.

The latency to onset of physical signs of insensibility was related to power setting. A higher power (kW), providing a more rapid delivery of the energy 'package', led to a shorter time to onset of physical signs of insensibility. The work on anesthetised cattle, carried out by Rault et al. (2014), indicated that shorter durations of applications resulted in more rapid onset of EEG suppression, while greater power settings resulted in a longer duration of insensibility. This is difficult to interpret fully, based on the small number of animals processed in their study and the fact that the animals in their study each received two applications of energy; however there is a relationship between power setting and rate of heating. The units of power (kW) is shorthand for 'kJ per second', so a higher power setting will reduce the time required to reach an energy delivery of 200 kJ than a lower power setting. However, the higher power setting may predispose to focal overheating, as seen in the first phase of the current study.

Based on the outcomes of this project, we recommend that operational guidelines define the desired range of energy application to be between 160 and 200 kJ.

Table 2: Summary of cattle stuns using DTS in all trials to date

Trial	Number presented for DTS	Number DTS delivered	Number stunned on first application	Percentage stunned on first application	Reasons for 'failures'
Rault et al. 2014 *(anaesthetised cattle)*	9	9	9	100	
P.PIP.0395	11	10	10	100	Technical fault 1 animal
P.PIP.0528 *Upright knocking box*	223	207	201	97.1	Technical fault 16 animals; Low energy delivery 6 animals
P.PIP.0528 *Rotary box*	35	35	33	94.3	Technical fault 2 animals - partial energy delivery
Totals	278	261	253	96.9	

The stunned animals demonstrated behavioural signs consistent with an electrical stun – rapid blinking or flicking of the eyelids including the *membrana nictitans* (third eyelid), loss of posture, tonic (stiff) and clonic (convulsive) phases (Table 3), so it is of value to compare the outcome of DTS against those reported for electrical stunning. In cattle, electrical stunning can be applied as head-only, which affects the brain, resulting in an epileptiform seizure, from which the animal can regain consciousness; or as head-to-chest, which incorporates a current passed through the heart, resulting in cardiac arrest, and the death of the animal. As DTS does not directly affect cardiac function, the comparison should be against head-only electrical stunning.

The biggest problem with head-only stunning of cattle is a very short duration of epilepsy, followed by strong convulsions (EFSA, 2004). The electrical current must be applied across the brain for sufficient duration to overcome the inherent impedance (resistance) across the head, and application of electrical stun ranges from 4 to greater than 20 seconds (Devine et al., 1986, Vonmickwitz et al., 1989, Schatzmann and Jaggin-Schmucker, 2000). The duration of unconsciousness in those studies was reported as 31 - 90 seconds. In contrast, DTS induces loss of posture within 8 seconds of onset of energy application, and the duration of unconsciousness recorded during the current study was between 100 and 240 seconds, based on absence of corneal reflex, and duration of EEG changes.

Table 3: Behavioural indicators of unconsciousness following DTS application, as compared with electrical stun.

BEHAVIOURAL SIGN	*DTS*	*EFSA SCIENTIFIC OPINION 2004*	*AMIC ANIMAL WELFARE STANDARD*
IMMEDIATE COLLAPSE	☑*	☑*	☑*
EPILEPTIFORM SEIZURE (DESCRIBED IN DETAIL)	☑†	☑	☑
LACK OF NORMAL RHYTHMIC BREATHING	☑#	☑	☑
NO SPONTANEOUS EYE BLINKING	☑		☑
GASPING (BREATHING IN WITHOUT BREATHING OUT) SOMETIMES OCCURS	☑		☑
UPWARD ROTATION OF EYES		☑	
DILATED PUPILS		☑	
NO RESPONSE TO NOSE PRICK (PAINFUL STIMULUS)	☑	☑	

*: Row 1: With regards to induction of insensibility prior to slaughter, there is much discussion over the 'immediacy' of the lapse into unconsciousness. in electrical stunning, the current must flow for a period of at least 2 (sheep) or 5 (cattle) seconds in order to ensure a sustained loss of consciousness (Warrington, 1974, Von Holleben et al., 2010) .

†: Row 2, Column 1: Due to the fixed head restraint, full body collapse was not evident. However, the haunches initially dropped, followed by a tonic phase, and then convulsive movements or paddling of the limbs.

#: Row 3, Column1: In the current study, a short suspension of respiration was evident in some animals, but not all. However, when respiration was present it was much slower and deeper than in the conscious animal.

Return of reflexes are considered to be the earliest indication that consciousness is returning and was the point at which penetrative captive bolt was applied during the current study for animal welfare reasons. In the current study this occurred between 2 and 4 minutes, 30 seconds from loss of posture, which would allow ample time to exsanguinate the animal prior to recovery. Using the rotary box, when a delay to capture EEG data was not required, sticking was performed within 30 seconds of DTS application.

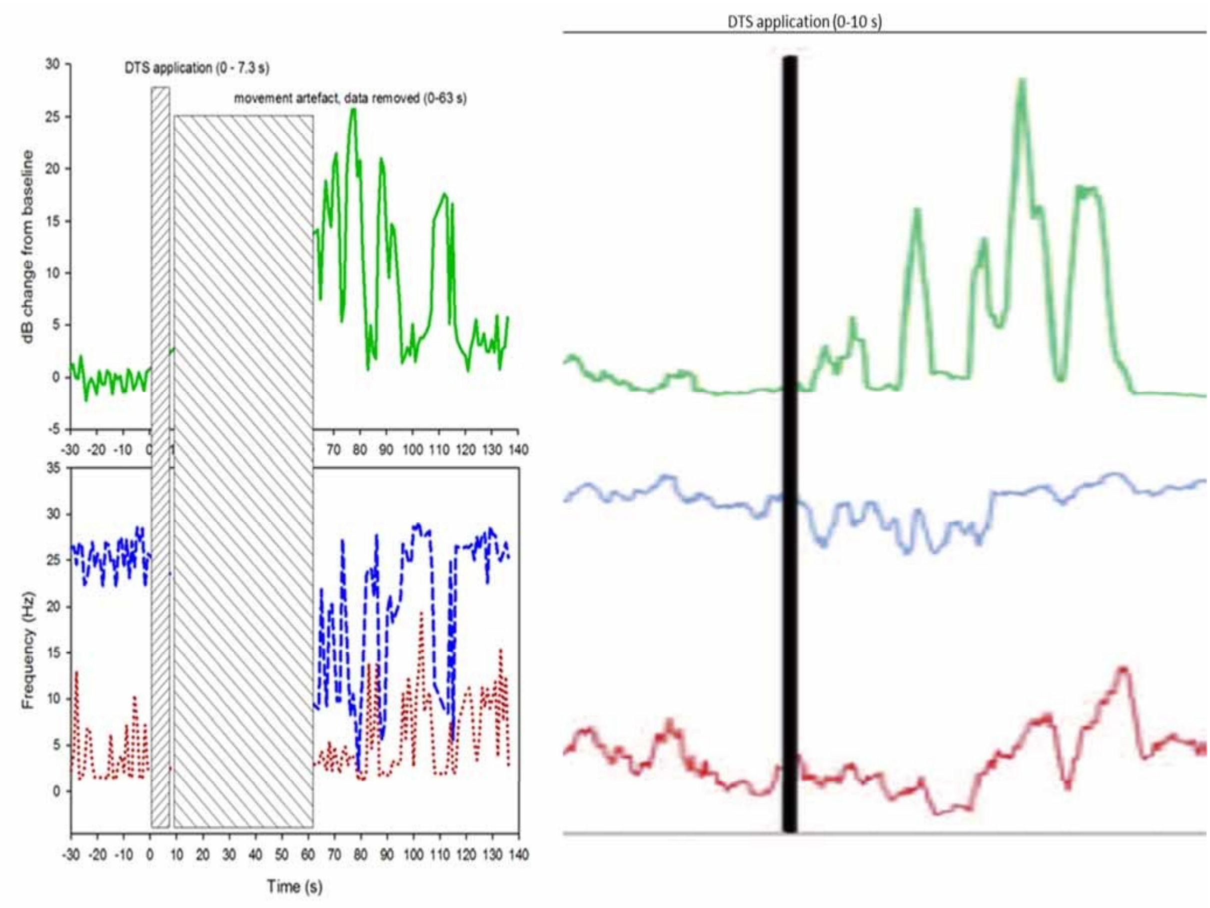
In the early stages of the project, overheating of the skin surface was identified. This was associated with incident power levels of > 25 kW. This was eliminated in the later stages of the project, through optimisation of the auto-tuning device and applicator apparatus such that incident power levels ranged from 18-20 kW. Australian cattle may experience ambient air temperatures of 45 °C in summer and could experience higher surface temperatures when standing in direct sunlight. Surface temperatures above 50 °C may be uncomfortable to cattle, although no literature on thermal contact sensitivity in cattle could be located to confirm or refute this supposition.

Figure 9 shows an example of the EEG data collected during the preliminary confirmation phase, compared against a similar chart reproduced from Rault et al. (2014), both showing a period of 2.5 – 3 minutes post-DTS application, scaled to similar length. Although the software and detail of smoothing methods used to generate these charts differ, clear similarities are evident: total EEG power (Ptot, green line) is dramatically increased and shows high amplitude spiking in the two-minute period post-DTS application; 95% spectral edge frequency (SEF95, blue line) and 50% spectral edge frequency (SEF50, red line) drop following DTS application, and then begin to return towards baseline. These EEG patterns were re-confirmed during the rotary box phase (Figure 6 and Figure 7 in section 3.1.4).

In the current study, movement artefact posed a significant challenge to EEG recording, as the animals began to twitch and convulse, similar to the movements seen during the phases of an electric stun. This movement artefact was not present in the Rault et al. study, as the animals were anaesthetised. Furthermore, in the current study, background electrical noise from the abattoir infrastructure tended to interfere with the EEG signal, so interpretation of the data, particularly the spectral edge frequencies, was challenging.

All the animals for which usable EEG recordings were collected showed changes from baseline, particularly in terms of Ptot, showing high amplitude spiking. These patterns on EEG are considered to be incompatible with sensibility (EFSA, 2004) and during this period the animal cannot feel pain.

Figure 9: Examples of EEG data. Left: Animal 10 in the current study, Right: Animal 6 from Rault et al. 2014 (reproduction of an approximate 3-minute block). Green indicates total EEG power (Ptot) as change from baseline, blue indicates 95% spectral edge frequency (SEF95) and red indicates 50% spectral edge frequency (SEF50).



5. Conclusions

100 % insensibility was achieved when the desired energy package was delivered. Existing stunning techniques in Australia, where conditions are carefully controlled, are expected to deliver a 95-98 % first-stun efficacy (AMIC, 2009) but, particularly where adequate neck and head restraint is not used, can have up to 29 % first stun failure rates (Blackmore, 1979, Lambooy et al., 1981, Hoffmann, 2003, Endres, 2005, Oliveira et al., 2018, Gibson et al., 2019). It is likely that the animal stunned using DTS can also return to consciousness (based on EEG and returning eye focus), so this method of stunning is likely to be acceptable to the Halal and Kosher markets. Furthermore, blood flow was observed to be strong, and the rhythmic pumping of the heart was evident in the first few seconds of blood flow. Both these characteristics are desired by the Halal and Kosher markets.

Use of the rotary box optimised handling of the stunned body, allowing a short stun-to-stick interval without the use of an immobiliser. The updated applicator configuration allowed consistent repeatable induction of insensibility, which was sustained during Halal bleeding, without the need to apply an additional ventral cut.

5.1 Key findings

- In the absence of technical problems leading to interrupted delivery of energy, all animals were rendered unconscious using DTS.
- Power settings of 18 kW led to a slower onset of insensibility, while power settings of 20 kW provided a rapid onset of insensibility. Power settings of 25 kW and above led to overheating at the skin surface.
- Energy deliveries of 160 kJ and less resulted in shorter duration of insensibility with a risk of early return to consciousness, while energy deliveries of 220 kJ and above resulted in early onset of intense convulsive activity.
- Live observations indicated that DTS consistently induces insensibility, with no vocalisation, no evidence of pain or distress, and EEG data confirmed the development of a high-amplitude-low-frequency (HALF) epileptiform state.
- There was no requirement to use an immobiliser during application of the exsanguination cuts, and the animal bled rapidly to brain death using the Halal cut alone. The blood flow on exsanguination was strong and rapid.
- Visual inspection of the brains of carcases indicated no visible damage when energy deliveries of less than 220 kJ were applied.

5.2 Benefits to industry

DTS is an effective means of inducing insensibility in cattle, with a duration of insensibility suited to exsanguination using the Halal cut. The project was planned and executed under end-user-centred design principles, with the Halal and Kosher markets in mind. Key parameters that are likely to be viewed favourably when compared against the current stunning method are:

- There was no requirement to perform a back-up ventral cut (thoracic stick), as the blood flow was strong and exsanguination was rapid;
- Blood flow was visibly strong, and the pulsations associated with heart function were visible in the early stages of bleed-out;
- Exsanguination could be performed safely, without the need for an immobiliser;
- Visual inspection of the brains of carcases indicated no visible damage when energy deliveries of less than 220 kJ were applied, and the stun did not cause cracks in the skull, so eliminates a source of rejection against Halal requirements, and eliminates a potential source of breaches noted on audit when the auditors' interpretation of 'cracked' differs from the regular inspector;
- The stun is effective in heavy animals and bulls, which currently pose challenges to processors using percussive stunning;
- To date, no evidence of blood splash or ecchymosis has been encountered in any of the carcases processed.

It is likely that the animal stunned using DTS can also return to consciousness (base on EEG and retuning eye focus), so this method of stunning is likely to be acceptable to the Halal and Kosher markets.

If deemed acceptable by the Halal and Kosher markets this technology could provide a solution to the current debate in Europe around banning the use of non-stunned (ritual) slaughter.

The progress of research and development of this technology is evidence of the Australian Industry's commitment to continual improvement in Animal Welfare at processing, which in turn supports the continued social license to operate.

6. Future research and recommendations

A key challenge throughout this project was technical faults in energy delivery, and the stability of the infrastructure. For example, the co-axial cable used to transfer energy from the generator to the applicator did not withstand the higher power settings used early in the project. This project identified that incident power levels of 25 kW and above are not required, so that will reduce the pressure on the cable. Further work should focus on improving the infrastructure to resolve these issues, for example, develop a system that does not require the use of co-axial cables.

It should be noted that at no time was Animal Welfare compromised by these technical faults.

The current project utilised mechanical stunning (penetrative captive bolt) as the back-up stun method. In the event of an interrupted DTS application, a re-stun would be quicker if a second DTS application was immediately applied, as opposed to the need to open the Faraday cage and remove the waveguide to apply the mechanical stun. Validation of this approach is recommended, including defining parameters such that the re-stun can maintain compliance with Halal requirements, and confirmation that there would be no detrimental effects on meat quality. The software controlling the system could be configured to automatically select the appropriate energy parameters for the second stun to again reduce the time interval between first and second application.

Notwithstanding the technical challenges encountered during this project, we have achieved consistent induction of insensibility when the desired energy package was delivered to the animal. To proceed to wider industry adoption, regulatory approval of the technology as a method of pre-slaughter stunning of the animals is required. Regulatory approval will require related modifications to the Standards and Guidelines.

Although preliminary observations indicate that it is likely that the animal stunned using DTS can also return to consciousness (based on EEG and returning eye focus), a controlled evaluation of returning consciousness beyond the return of eye reflexes in a restrained animal is warranted.

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8. Appendices

8.1 Appendix 1 Animal Ethics application and approval reference WSIAEC 30.16

Wildlife and Small Institutions Animal Ethics Committee

Dr Alison Small,
Principal Investigator,
Wagstaff Food Service Pty Ltd,
1500 Thompson Rd,
Cranbourne,
Victoria 3977

Ref: Application number 30.16 **28-03-2017**

Dear Dr Small ,

At its meeting of 22-03-2017, The Wildlife and Small Institutions Animal Ethics Committee considered your application 30.16, "DTS: Diathermic Syncope –commercial validation trials". The application was given approval from 22-03-2017 until 21-03-2020 under the following strict conditions:

1/The project is to be conducted in three parts.

Part 1

- Conduct of an initial trial involving no more than 20 animals (*Bos taurus*) to validate the efficacy of the new DTS equipment, prototype 4.

Part 2

- Upon a satisfactory conclusion to Part 1, Stage 6 of the project may be undertaken to optimise the power/time parameters to allow the stun to be reversible. The protocol for stage 6 must be approved by the Committee prior to commencement.

Part 3

- Upon a satisfactory conclusion to Part 2, Stage 5 of the project, Conduct of "Commercial level validation" may be undertaken .

2/ The Executive Officer of the WSIAC must be advised at least two weeks before commencing each part of the project so that that members and representatives of the WSIAEC, including veterinarians can attend.

3/ Submission of a report at the conclusion of each part for review and approval by the WSIAEC before commencing the next part.

General conditions of approval:

- All unexpected adverse events including unexpected deaths, unexpected impacts on animal wellbeing, greater levels than anticipated pain and or distress and unanticipated external events including power failure and extreme weather events that have had an unexpected impact on animal wellbeing must be reported to the WSIAEC as soon as possible. There is an adverse event reporting form that must be used for this purpose to

Privacy Statement *Any personal information about you or a third party in your correspondence will be protected under the provisions of the Information Privacy Act 2000. It will only be used or disclosed to appropriate Ministerial, Statutory Authority, or departmental staff in regard to the purpose for which it was provided, unless required or authorised by law. Enquiries about access to information about you held by the Department should be directed to the Manager Privacy, Department of Economic Development, Jobs, Transport and Resources, GPO Box 4440, Melbourne, 3001.*

allow the WSIAEC to consider the event, review of such events will contribute to the improvement of procedures and animal welfare.

- All work must be conducted in accordance with the approved application including any cited standard operating procedures, the Australian code for the care and use of animals for scientific purposes 8th edition 2013, and all other relevant legislation. Non-compliance with approval is a serious matter, it is not expected that non-compliance will occur, however the WSIAEC must be informed of any breaches of approval immediately.

- Any changes to the approved project or cited standard operating procedures, including the addition of new investigators must be approved by the WSIAEC prior to implementation.

- Continuing approval is subject to annual review of the project by the WSIAEC. In addition to any other reporting requirements, you must submit to the WSIAEC an annual report, due by the first WSIAEC meeting after 22nd March for each year of the project. A final report must be submitted at the conclusion of the project. These reports must be submitted using the appropriate form which is available from the Executive Officer. Please note; Scientific Licence reporting is a separate requirement to reporting to the WSIAEC.

- A final report must be submitted at the conclusion of the project. This report must be submitted using the appropriate form which is available from the Executive Officer. Please note; Scientific Licence reporting is a separate requirement to reporting to the WSIAEC.

- The maximum period of animal ethics approval is three years, after which time a new application must be submitted if the work is to continue. Animal use for scientific purposes without appropriate AEC approval is an offence and must not occur.

If you have any queries regarding this communication please contact me on 0409 143 538 or via email.

All correspondence must include the approval number 30.16 .

Yours sincerely

Carole Webb

Carole Webb
Chair, Wildlife and Small Institutions AEC,
Licensing and Audit, Biosecurity,
Department of Economic Development, Jobs, Transport and Resources,
475 Mickleham Road, Attwood, VIC 3049
DX 211278
Phone: 03 9217 4429
Fax: 03 9217 4416

Email: Max.campbell@ecodev.vic.gov.au

Department of
Economic Development, Jobs, Transport & Resources
WILDLIFE AND SMALL INSTITUTIONS ANIMAL ETHICS COMMITTEE

APPLICATION FOR APPROVAL TO CONDUCT RESEARCH AND TEACHING USING LIVE ANIMALS (PLEASE CHECK WITH THE EXECUTIVE OFFICER THAT THIS IS THE CURRENT VERSION OF THIS FORM.)

Scientific Procedures Licence Number
To be renewed

ECA 202

COMMITTEE USE ONLY

Date Received		**Application Number**	**30.16**
Approval	Yes / No	**Approval Date**	
Chairpersons Signature			

Project Title	**DTS: Diathermic Syncope – commercial validation trials**		
Organisation /Applicant	Wagstaff Food Service Pty Ltd with CSIRO (Commonwealth Scientific and Industrial Research Organisation)		
Proposed Start Date	**1st April 2017**	Proposed Finish Date	**30th December 2018**

The AEC may approve projects for up to three years. Please ensure that adequate time is allocated for the work to be undertaken. You must not commence work without WSIAEC and other approvals in place.

This work may require a Scientific Procedures Licence -You must contact Licence and Audit Sp.Licensing@depi.vic.gov.au or 03 9217 4425 to clarify your licensing requirements– if this project is approved by WSIAEC you must not undertake any work involving animals without an appropriate licence(s) in place if applicable.

You must ensure all other relevant permits and approvals are in place prior to starting work.

Does this work involve the oversight of another AEC? No

AEC	Name of Institution	Contact details

UNLESS OTHERWISE INDICATED ALL SECTIONS OF THIS DOCUMENT ARE TO BE COMPLETED. IF A SECTION IS DEEMED TO BE "NOT APPLICABLE" A BRIEF EXPLANATION MUST BE PROVIDED. PLEASE REMEMBER THIS FORM IS USED FOR MANY DIFFERENT PROJECT TYPES, ADAPT TO YOUR NEEDS TO ENSURE THE AEC RECEIVES ALL THE INFORMATION IT REQUIRES TO MAKE DECISIONS IN COMPLIANCE WITH THE CODE.

Important notice: A service fee applies for consideration of this application.

1. Investigators, including other participants, volunteers and trainees. (Please *add rows for additional investigators.)*

	Name	Position Title and Qualifications
Chief Investigator (Person with Ultimate Responsibility) and Name of Organisation	Alison Small, CSIRO	Senior Research Scientist; BVM&S, CVPH(MH), DVPH(MH), DipECVPH, PhD, GCPS(FSRA), MRCVS
Associated Investigators	1. James Ralph Wagstaff	Study Director
	2. David McLean Advanced Microwave Technologies	Technologist
	3. Dominic Niemeyer CSIRO	Project Officer BTC
	4. James Lea CSIRO	Project Officer BA, BTC
	5. Troy Kalinowski CSIRO	Project Officer Cert III in Laboratory Skills, Cert II in Rural Skills, Cert II in Security Operations
	6. Leisha Hewitt Dr L Hewitt – Livestock Welfare	Animal welfare consultant MSc, PhD
Other Staff/volunteers	1.Tim Barwick Wagstaff	Stockman Farmer Lairage foreman
	2.Mal Webster Wagstaff	Slaughterman Engineer
	3. Andrew Phillips Wagstaff	Works manager
	4. Jim Nolan Wagstaff	Assistant works manager
	5. Cathy Tringham Wagstaff	Group Quality Assurance Manager

2. AIMS, BENEFITS AND JUSTIFICATION OF THE PROJECT

(a) Lay Summary-provide a concise overview of the project, explaining the scientific or educational significance in relation to the project design. Use terms that may be understood by members of the general public. (Plain English)

Use of scientific language should be avoided but if there are scientific terms that must be used provide a glossary in this section or provide explanation within the text throughout.

Billions of cattle are slaughtered each year around the world to produce meat, a valuable source of protein in our diets. Regardless of culture, we all agree that slaughter should be carried out in the best possible manner, however, we disagree on what constitutes the best manner. Modern slaughter often involves stunning of the animal prior to the slaughter cut, rendering the animal unconscious such that it does not feel the pain of the neck cut. However, existing stunning methods are not acceptable to all markets (e.g. Muslim or Jewish), and they are not perfect. The Muslim (Halal) and Jewish (Kosher) laws require that animals are healthy, whole and undamaged at the point of slaughter, slaughter being defined as the cutting of the neck such that the animal dies as a consequence of blood loss.

Mechanical stunning can be carried out by use of the penetrative captive bolt, or the non-penetrating (mushroom) stun. The penetrative captive bolt produces a hole in the skull, so is not acceptable according to the Muslim or Jewish laws. Furthermore, although penetrative captive bolt is considered to be the 'gold standard' stunning method, it is not 100% effective. Where the head of the animal is not fixed in position, it can be difficult for the operator to accurately position the 'shot'. Where no head restraint is used, up to 50% of stuns may be misapplied, and not effective (Gouveia et al., 2009). In Europe, where head restraint is commonly used, 7 to 15% of penetrative captive bolt stuns are estimated to be ineffective (Von Holleben et al., 2010, Marzin et al., 2008, Endres, 2005), whereas in the US, failure rates have been reported to be in the order of 0.6 – 1.2% (Grandin, 1994), where effective head restraint is used. The Australian Export meat industry has set targets of 95% of animals to be effectively stunned on first shot.

Non-penetrative mechanical stunning has been accepted by the Australian, Malaysian and Indonesian Islamic authorities. The challenges with non-penetrative mechanical stunning (also called mushroom stun), are that it can produce large cracks and fractures on the skull, visible when the head is skinned. If these cracks are present, the carcase is unacceptable for Halal. To reduce the number of carcases demonstrating skull fractures, attempts are made to minimise the power (kinetic energy) delivered by the instrument. However, where the power is reduced, the incidence of improper (failed) stuns increases, and animals may need two or more 'shots' to be rendered fully unconscious. The back-up method is application of a penetrative captive bolt stun, for welfare reasons, and the carcase is not acceptable as Halal.

Electrical head-only stunning has been accepted by the Australian, Malaysian and Indonesian Islamic authorities. The challenges with electrical head-only stunning applied to cattle are that the electrical current sometimes must be applied for 3 minutes or more in order to achieve the required epileptiform activity in the brain, this can lead to burn marks on the head at the point of application, which are considered unacceptable for Halal, and the duration of insensibility can be very short, such that animals may be regaining consciousness during the bleed-out period. Where animals are observed to be regaining consciousness during the bleed-out period, a penetrative captive bolt stun is applied as a back-up, for welfare reasons, and the carcase is not acceptable as Halal.

Wagstaff Food Services Pty Ltd and Advanced Microwave technologies have designed a system for rendering an animal unconscious prior to slaughter, using radiofrequency electromagnetic energy (PCT/AU2011/000527). The mechanism of action is essentially selectively increasing the temperature slightly in the brain, by only 7° C, to the point that hyperthermic syncope (fainting) occurs, but below the point at which irreversible brain damage and death occurs. Thermal unconsciousness such as that induced by exercise heat stress or fever is reported to occur when core body temperatures reach between 40 and 45°C (Ohshima et al., 1992).

Following extensive development work, and trials on anaesthetised animals (McLean et al., 2017, Small et al., 2013, Rault et al., 2014), a pilot trial was carried out under AEC 29.13. In that study, ten non-anaesthetized cattle received DTS treatments (high energy <290 kJ, n=3; low energy <200 kJ, n=4; and intermediate, n=3), and seven received captive bolt stunning prior to exsanguination. Based on live observations at the time of stun application, the research team was satisfied that DTS induced a state of insensibility, of a sufficient duration to allow humane slaughter through exsanguination.

Live observations, and detailed assessment of video footage indicated that the process was painless, and evidence of distress was not observed, based on indicators such as grimace, ear postures, tail flicking and struggling. The animals remained unresponsive to stimuli and showed evidence of EEG suppression for 3-4 minutes post DTS. EEG changes for DTS and captive bolt animals did not differ. The clearest indicators of effective induction of insensibility were: Loss of corneal responses; Loss of withdrawal response (pinprick); Eye staring, not following movement. Two animals showed indications of returning reflex responses after 180 seconds post treatment.

There were no significant differences between DTS and captive bolt animals in terms of stress hormone responses (cortisol, ACTH, β-endorphin, adrenaline and noradrenaline), and there were no significant differences between DTS and captive bolt carcases in meat quality attributes (pHu (24 h post slaughter); pH, Warner Bratzler Shear Force and Drip loss at 1or 10 weeks post slaughter). DTS carcases were slightly yellower at quartering (MINOLTA b* 2.71 ± 0.59 DTS; 1.06 ± 0.44 control); DTS loins were slightly redder (MINOLTA a* 23.22 ± 0.92 DTS; 20.89 ± 0.69 control) and slightly yellower (MINOLTA b* 2.79 ± 0.93 DTS; 0.77 ± 0.70 control); and DTS rounds were slightly lighter (MINOLTA L* 43.32 ± 1.05 DTS; 40.94 ± 0.78 control) at week 1, than control samples (P<0.05). There were no differences between DTS and captive bolt meat colour measurements at 10 weeks post slaughter.

The pilot trial concluded that DTS provides a humane method of inducing insensibility prior to exsanguination, of sufficient duration to allow exsanguination prior to recovery, and produces comparable post slaughter meat quality and physiological responses in treated cattle to those stunned using penetrative captive bolt.

In order to progress the technology to a commercially viable proposition, and achieve acceptance of the technology by the regulatory authority, the following four objectives have been identified:

- To re-engineer the restraint, head capture and waveguide set-up to ensure low stress for the animal, efficient processing, and limit leakage and automatic cut-out of the generator;
- To validate the outcomes of the pilot study (AEC 29.13) in terms of induction of insensibility, in a larger sample size;

- To confirm time to unconsciousness from onset of DTS application, using the proxy 'fixed, staring eye' measured on video footage;
- To gather data comparing DTS against non-penetrative mechanical stunning (the Halal-accepted stun method used on Australian animals);
- To understand required stun-stick intervals to ensure that animals are allowed to bleed out fully prior to recovery of sensibility;
- To demonstrate that the technology may be operated at a rate of processing that would be commercially viable (1-2 minutes per animal).

This application addresses the studies proposed to address these objectives.

All references moved to Appendix 5

(b) Describe the aims of the project.

1. To re-engineer the restraint, head capture and waveguide set-up to ensure low stress for the animal, efficient processing, and limit leakage and automatic cut-out of the generator;
2. To validate the outcomes of the pilot study (AEC 29.13) in terms of induction of insensibility, in a larger sample size,
    a. To confirm time to unconsciousness from onset of DTS application, using the proxy 'fixed, staring eye' measured on video footage;
    b. and to gather data comparing DTS against non-penetrative mechanical stunning;
3. To demonstrate that the technology may be operated at a commercial rate of processing (1-2 minutes per animal);
4. To understand required stun-stick intervals to ensure that animals are allowed to bleed out fully prior to recovery of sensibility.

(c) Benefits of the project. Is the project expected to achieve any of the following? Please tick as appropriate and provide detail in 2 (d)

☐ Increase our understanding of humans or animals
☐ Produce animals for scientific use (applicable to applications for breeding colonies)
☐ Maintain or improve human or animal health or welfare
☒ Improve animal management or production
☐ Obtain significant information relevant to the understanding, maintenance or improvement of the natural environment
☐ Achieve educational objectives – provide detail of how the attainment of educational outcomes will be assessed in 2(d) see Australian code 2.7.4

(d) Provide detail of the benefits of the outcomes of the project, and how these benefits will be measured? The WSIAEC will consider the ethical acceptability of the proposed use of animals, balancing the potential effects on the wellbeing of animals with the potential benefits of the project.

Billions of cattle are slaughtered every day, around the world, for the production of meat for human consumption. Although many countries use pre-slaughter stunning, some communities do not accept pre-slaughter stunning (Jewish and Muslim). This is because their religious laws require that animals processed for human consumption are healthy, uninjured and normal at the moment of carrying out the slaughter cut. As a result, many methods of stunning used in modern commercial slaughter are not acceptable, because the animals could be considered injured, or because a proportion of animals would not recover from the stun. In electrical stunning, bruising, or blood splashing can occur in the muscles, rendering it poor quality, and unacceptable to Jewish and Muslim communities, because it is not perceived as 'normal'.

Furthermore, there are known welfare problems with existing methods of stunning (Von Holleben et al., 2010).

Head-only electrical stunning is used in sheep, but is not suitable for use in cattle because of concerns over the duration of insensibility being insufficient to allow death through total blood loss to occur prior to recovery from the stun. The duration of insensibility is reported to be between 10 and 90 seconds – but when unstunned cattle are slaughtered by the neck cut prescribed under Muslim or Jewish law, some animals can take 2-6 minutes to lose consciousness (Gregory et al., 2010, Cranley, 2011).

For mechanical stun, failure rates of up to 30% have been reported – i.e. up to 20% of animals require a second shot, or show signs of recovery during exsanguination (Von Holleben et al., 2010). This is particularly noticeable when the mechanical stun used is the non-penetrating type, the type used in Australian Halal slaughter, and again, durations of unconsciousness reported range between 20 and 90 seconds: shorter than the duration of exsanguination required to ensure that no animal returns to consciousness during exsanguination.

Gas stunning (CO_2/N_2 mixtures) is used for pigs and poultry, and has recently been researched in sheep and calves. It is not acceptable to Muslim and Jewish communities, because it is often a kill method, i.e. non-recoverable. Furthermore, there are major concerns over welfare of the animals – pigs have been shown to take on average 38 seconds to lose consciousness, during which time they are seen to hyperventilate, vocalise and attempt to escape from the highly aversive CO_2 experience (Von Holleben et al., 2010).

All existing stun methods have the potential to cause a certain amount of pain, particularly if mis-applied, resulting in an animal that is not rendered insensible. It is widely accepted that the objective of 'humane killing' of any species is the minimisation of 'avoidable pain and suffering' (EC 93/119/EC) (Adams and Sheridan, 2008). A challenge exists in evaluating the experience of an animal undergoing euthanasia: the gold standard method of assessing sensibility versus insensibility is the Electroencephalogram (EEG). However, interpretation of the EEG patterns is difficult. There are patterns known to be incompatible with consciousness, e.g. epileptiform activity or flat-line suppression; and there are patterns known to be indicative of consciousness. However, there is also a transitional state, during which period, the subjective experience of the animal is unknown (Meyer, 2015). This transitional state lasts for 8 seconds or more in mechanically stunned animals (Gibson et al., 2009b), while in un-stunned animals, pain-related patterns can be detected in the EEG trace for up to 30 seconds post neck cut (Gibson et al., 2009a).

There is a need for a new method of rendering animals insensible prior to slaughter, one that is reliably effective, acceptable to the Jewish, and growing Muslim population, and provides a duration of insensibility sufficient to allow exsanguination prior to recovery. The development work carried out to date suggest that DTS: Diathermic Syncope can achieve these criteria – the animals were rendered insensible; there were no detrimental effects on the meat produced; and those that received an energy dose of between 100 and 200 kJ remained unconscious for at least 4 minutes.

All references moved to Appendix 5

(e) *Are there any features of this proposal which raise special ethical considerations?*

None

(f) *Are there any actual or potential interests (including relationship, financial or affiliation) which may affect judgements and decisions regarding the wellbeing of animals involved in this project?*

None

(g) *Has this or similar work been undertaken by other investigators? How is this work different and what will it contribute to existing knowledge? How have the lessons learned from previous, similar work been incorporated into this application?*

This project continues work that has been carried out over the past 30 years (see timeline below).

Activity	**Status**
Cadaver experiments on sheep brains in order to estimate the likely power requirements that would raise the temperature by 8-10°C	**Completed 1986** Conceptually feasible, but generators of predicted output not available Rankin et al 1986
Literature review on potential for use of Microwave Technology for stunning beef cattle	**Completed 2005** Conceptually feasible, and generators of predicted output are now available Owen and Shaw 2005
Microwave stunning technology	**Completed 2009**

development stage 1	Generators of suitable output are now utilised in industrial applications. Work was carried out on cadaver heads to demonstrate that the required rise in temperature in the brain could be achieved. McLean 2009
Microwave induced insensibility for animals stage 2	**Completed 2010** Anaesthetised sheep were used to demonstrate that the expected temperature rise as predicted in stage 1 would occur when there was an active cerebral circulation. Small 2010
Stage 3: Non-recovery anaesthetised animal trials	**Completed 2013** Anaesthetised cattle were used to demonstrate induction of insensibility as measured by EEG Rault et al 2013
Stage 4: Proof of concept demonstration that insensibility can be induced	**Completed 2015** Ten non-anaesthetized cattle received DTS treatments and were rendered insensible. No detrimental effects on meat quality or endocrine responses were demonstrated AEC 29.13
Stage 5: Commercial level validation	The subject of the current application The aim will be to confirm the outcomes of stage 4 in a larger field trial
Stage 6: Optimisation of the power/time parameters to allow the stun to be reversible	To be planned The concept of reversibility is vital to the Kosher and Halal markets This is likely to involve another phase of anaesthetised animal trials Preliminary data from the proposed current study will inform this phase

The recent pilot study (AEC 29.13) identified some technological, engineering and research aspects that required addressing in the next stage of development:

Modifications to the technology

If recoverability is desired, and the animal to return to herd life, as opposed to being immediately slaughtered, the desiccation and skin changes at the application point should be minimised.

Engineering aspects:

Development to improve:

- *Ease of capturing and positioning head;*
- *Maintenance of contact with waveguide;*

- *Consideration of potential for contouring or flexibility within the waveguide tip;*

- *Ease of extraction of body from box;*
    - *Consideration of side clamp and tipping technologies.*

Note: designs must ensure compliance with OH&S regulatory standards and guidelines.

Research aspects

Subsequent to development of Prototype 4, addressing particularly the surface heating aspect, commercial pilot trials are required to understand and define the critical limits of energy application that allow induction of insensibility, but minimise brain tissue damage, such that the animal may recover and function in a normal manner.
Key aspects to consider include:

- *Parameters for recovery;*
    - *Factors that may affect critical limits (e.g. gender, maturity, liveweight);*

In the proposed study, these aspects are being addressed:

Skin changes at the application point

During the pilot study, one adverse finding was blistering and scorching of the skin at the point of application of energy, particularly in those receiving higher energy applications (greater than 200 kJ). Those receiving lower energy applications showed much less tissue damage, the lesion being more akin to those seen when hot branding animals. It is fair to assume that if the animal remained conscious, it would experience the initial phasic (immediate, or 'fast') pain associated with heat stimulus, and this would be subsequently followed by the tonic ('slow' or more slowly developing) pain of inflammation. As the animals are rendered unconscious, and then slaughtered, we can exclude tonic ('slow') pain as a concern because it develops over a period of time following the insult, and focus on the phasic ('fast') pain. We have re-engineered the waveguide and applicator to minimise surface blistering. Our investigation into the issues indicated that the issue was multi-factorial. The interface between air, hair and skin was concentrating energy at the application point, leading to plasma formation (Plasma is a state of matter that is often thought of as a subset of gases, but the two states behave very differently. Like gases, plasmas have no fixed shape or volume, and are less dense than solids or liquids. But unlike ordinary gases, plasmas are made up of atoms in which some or all of the electrons have been stripped away and positively charged nuclei, called ions, roam freely.) At the same time, the vacuum applied to remove dust and dander (loose skin particles) from the waveguide led to dehydration of the hair surface. The combination of charged plasma and a highly dehydrated environment caused the hairs to ignite and burn, and this contributed to excess heating of the skin surface. These issues have been addressed by the insertion of a Teflon window at the interface end of the waveguide (Advanced Microwave Technologies, 2016). This removes the need to apply a vacuum, as dust cannot travel up the waveguide, prevents the concentration of energy at the interface, preventing the plasma formation, and thereby preventing the runaway heating noted during the pilot study. Images of heads treated with Prototype 2 (without the

Teflon window), and prototype 4 (with the Teflon window) are shown below.

After 1 application. Prototype 2

After 3 applications. Prototype 4

We are confident that with the addition of the Teflon window, the time to generation of a painful stimulus will be much greater than that seen with Prototypes 2 (anaesthetised animal trials) and 3 (pilot study). Furthermore, the addition of the Teflon window has resulted in reduced energy 'wastage', and therefore increased efficiency, meaning that the time taken to increase brain temperature by the target 8 °C using Prototype 4 will be reduced relative to Prototype 3. Using the mean heating rates generated during cadaver testing, we can predict that the time to insensibility (defined as reaching a brain temperature of greater than 43 °C) is reduced by nearly a full second (table below, data generated from Advanced Microwave Technologies, 2016a). This means that the duration of application can be reduced, with in turn reduces the total energy supplied by the system. For example, a 10 sec application of 30 kW delivers 300 kJ, and a 10 sec application of 20 kW delivers 200 kJ. During the pilot study subjects receiving greater than 200 kJ demonstrated severe scorching and blistering. Using Prototype 4, we can reduce the overall duration of application to 5 sec, such that a 30 kW application delivers a total of 150 kJ, and a 20 kW application delivers 100 kJ. During the pilot study subjects receiving between 100 and 150 kJ demonstrated lesions similar to those produced by hot branding, and use of the Teflon window in Prototype 4 will further limit this surface heating.

Prototype	Average heating rate	Time taken to raise brain temperature by 8 °C, from 38 °C to 46°C		
		At 20 kW	At 25 kW	At 30 kW
3	0.124 °C /kW /sec	3.23 sec	2.58 sec	2.15 sec
4	0.21 °C /kW /sec	1.90 sec	1.52 sec	1.27 sec

During the pilot study (Prototype 3), we estimated time to insensibility based on the indicator 'fixed staring eye', and a convulsion similar to that demonstrated by animals undergoing electrical stunning. These events occurred between 2 and 3 seconds from the start of DTS application. In some video recordings associated with high energy applications, evidence of blistering was observed from 4-5 seconds from the start of DTS application, and it appears that during the pilot study, it is likely that the animals fell unconscious immediately before, or almost concurrently with the onset of blistering. Using prototype 4 in the upcoming study, we are confident that we have eliminated the risk of blistering occurring until well after the onset of insensibility. However, as a result of the increase in efficiency of energy delivery, if we do continue to utilise the parameters used for the anaesthetised animal trials (i.e. application durations of 10 sec), we are likely to induce overheating, and blistering may be visible on the surface of the head after DTS application. Therefore, in the upcoming study, using Prototype 4, we propose to limit application durations to 5 sec.

To summarise: The cadaver work during development of prototype 4 assessed relative heating rates of the superficial skin under the waveguide against heating rates of brain tissue. In prototype 3, we used a vacuum system to remove steam from the application point. This may have contributed to excessive desiccation of the skin, and production of the skin damage.

During development of prototype 4, the technologists considered and tested a variety of options – e.g. vacuum/ no vacuum; fan/ no fan; use of a microwave-lucent window, use of a high-power coaxial system. A copy of the reports are supplied (Final Commissioning Report – Applicator 08-09-2016.pdf, and High Power Coaxial Report 10-11-16.pdf). The key outcomes of the commissioning trials are:

1. Surface heating, skin damage and depilation have been eliminated by addition of a Teflon window at the contact point
2. Reduction in input energy required to achieve target temperature rise within the brain.
    a. This increase in efficiency is due to the re-shaped footprint and the Teflon widow.
    b. A further increase in efficiency is generated through use of the high powered coaxial system
    c. The temperature at which unconsciousness is achieved is reached faster than with the previous prototype.

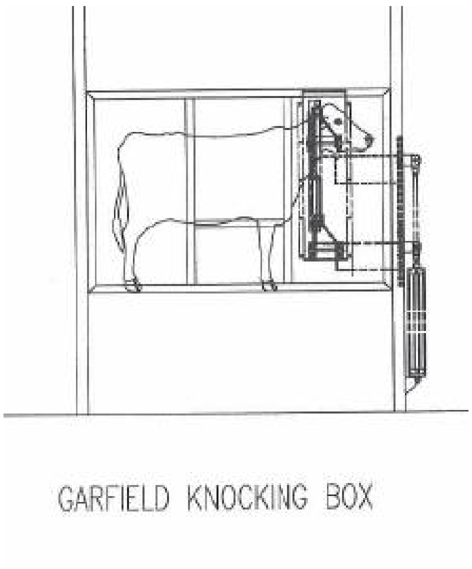
Maintenance of contact with the waveguide

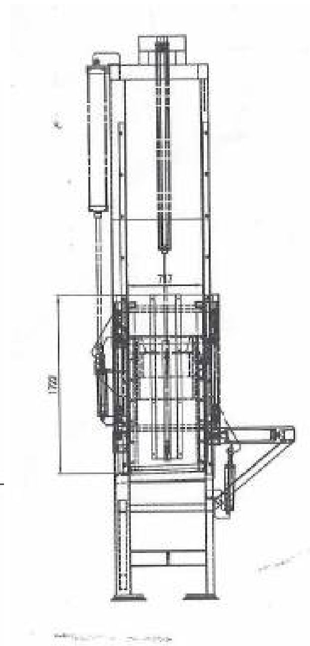
During the pilot study, the automatic cut-out was triggered due to loss of contact between the animal's head and the tip of the waveguide, at the point where the animal convulsed. This will be addressed in two ways:

1. The waveguide will be re-engineered to be more flexible, and the tip shaped so that is fits more snugly to the head. The applicator commissioning report (Final Commissioning Report – Applicator 08-09-2016.pdf) describes the reduced footprint of the applicator contact point. Furthermore, the addition of the Teflon window reduces the risk of arcing, which will reduce the risk of triggering the automatic cut-out. To increase the flexibility of the waveguide, sections of the waveguide will be replaced by high power coaxial cables, so that the waveguide can (i) be more easily positioned on the animal's head, and (ii) move with the animal's head within the head restraint. When the coaxial system was developed and tested using cadaver heads, the efficiency of energy delivery was again improved (High Power Coaxial Report 10-11-16.pdf).

2. The head capture unit will be supported by hydraulic rams, which are more rigid than

the pneumatic rams used during the pilot study, so that the convulsion cannot compress them. The head capture unit has been completely re-designed (report 160606 Head Restraint Installation.pdf supplied). Engineering diagrams and a photograph of the installed head capture unit are shown below.





Ease of capturing and positioning the animal's head

The flexible waveguide arrangement being developed as mentioned above (use of high power coaxial cables) will allow the waveguide to be applied at an angle away from vertical, such that the waveguide can be brought to the animal's head, rather than bringing the animal's head to the waveguide, as was done in the pilot studies. Furthermore, this flexibility allows the waveguide to move with the head within the head restraint, reducing the risk of loss of contact and energy leakage.

Ease of extraction of body from box

The knocking box has been re-designed, with a neck yoke that lifts away, and a slight side-step arrangement. The side-step is commonly used in commercial knocking boxes to help roll the body out, by tipping it off-balance. The box used in the pilot trials did not have the side-step, so the body was balanced, and difficult to tip out.

Commercial-scale validation

The animal phase of this application aims to achieve five key objectives:

1. To validate the outcomes of the pilot study (AEC 29.13) in terms of induction of insensibility, in a larger sample size;

2. To confirm time to unconsciousness from onset of DTS application, using the proxy ‘fixed, staring eye’ measured on video footage;
3. To gather data comparing DTS against non-penetrative mechanical stunning (the Halal-accepted stun method used on Australian animals);
4. To understand required stun-stick intervals to ensure that animals are allowed to bleed out fully prior to recovery of sensibility;
5. To demonstrate that the technology may be operated at a commercial rate of processing (1-2 minutes per animal).

The indicators of insensibility identified during the pilot trials will be utilised to assess insensibility at commercial speeds. The animal is deemed unconscious when the following reflexes do not occur:

- Corneal reflex (eye retracts when touched);
- Palpebral reflex (eyelids blink closed when eye is almost touched);
- Somatic pain reflex (the head flinches when a needle is inserted into the sensitive skin of the nose);
- Focussing and following (the eye follows the movement when a hand is passed across the field of vision).

If any of these reflexes are present the animal will be deemed to be returning to consciousness, and it will be immediately stunned using a penetrative captive-bolt, and exsanguinated (if the exsanguination has not already begun).

3. ANIMAL USE

(a) Indicate the planned numbers of animals to be used (laboratory and field trials) or provide an estimate of the numbers and the species likely to be trapped based on experience, available information and previous trapping success rates (field work). By-catch animals must be included in this estimate and clearly indicated. Please group animal numbers by method of capture. Where privately owned animals are used applicants must comply with 2.4.18 of the Australian code.

Species *Include both scientific and common name*	Source *Include location details*	Number of animals to be used over the duration of the approved project (max 3 years).
Bovine (*Bos Taurus*): cattle	Commercial intake at the Wagstaff Abattoir	90 for pilot study validation + 270 for commercial scale evaluation **Total: 360**

Are any of the animals proposed to be used: Note there are additional legislative or compliance requirements applicable in some cases.

☐ Non-human primates?

☐ Privately owned?

☐ From Municipal Pounds?

☐ Livestock on commercial farms?

☐ Genetically modified – if so provide OGTR certification number and status. Provide details of whether this animal line has been characterised.

☐ Endangered or protected species?

(b) Provide detail of where the animals will be housed and where procedures will be undertaken. Provide evidence that animal use location complies with relevant licences.
Are any of the facilities where animals will be used or housed under the oversight of another AEC?

Animals will be housed in the normal commercial lairage of the Wagstaff Abattoir.
They will be provided water and hay ad-libitum.
Animals will be handled using low-stress handling techniques, by experienced stockpersons.
The animals will be restrained in a modified commercial knocking box with head-capture for application of the DTS treatment.

(c) Provide evidence as to why the use of animals is necessary in this project?
Why this species/strain/sex and age? Please justify.

Live animals are required to validate the outcomes of the pilot study.
24 replicates per group are the minimum required to provide statistically sound data for the validation phase – we have requested 30 in each group (total 90), to allow a comfortable margin, and allow for any exclusions to the study (e.g. if any animals become unwell, or if there are any technological failures resulting in the data from a particular animal being unacceptable for the validation)300 animals are the minimum required to predict with confidence a 1% failure rate during the commercial level evaluation.
The target species for the commercial application of this technology is cattle, at the liveweights normally processed under commercial conditions.
Within the 300 animals utilised for the commercial level evaluation, we expect a range of liveweights between 200 and 600 kg; and a range of sex classes (entire male, steer, heifer and cow). This will assist us in validating our choice of desired energy application.
Cadaver work during the prototype development suggests that there is little difference in energy requirement between bovines from the different sex classes and liveweights routinely processed at the Wagstaff abattoir..

(d) Provide detail of why the use of animals is essential to achieve all the stated aims of this project. What are the potential alternatives to replace animals in all or part of this project? Where these alternatives are not used provide justification as to why they are not suitable. Please indicate information sources on alternatives that have been accessed (eg. web databases and information, literature etc.). Provide details of how this application complies with the principle of replacement as defined in the Code.

Stage 1, development and testing of prototype 4, will use cadaver heads supplied by Wagstaff Abattoir, covering a range of animal liveweights.

Stage 2, validation of pilot study outcomes will require live animals. No suitable alternatives are available, as inert models and cadavers do not have a functioning circulation and physiology.

(e) Will any of the animals be subject to recurrent experimental use (or repeated capture)? If so, please justify the reuse of the animal/s and include details of the previous activity they were involved in (or provide details of strategies to avoid recapture or reduce its impact).

No – the animals will be slaughtered

(f) Will there be an opportunity for the sharing of tissues or animals that have been humanely killed? If so, please indicate how this opportunity will be conveyed and to whom.

We have agreed with the University of New England Meat Science Group, to provide specimens from the *longissimus lumborum* muscle (the sirloin), so that further meat quality analyses can be performed. However, we will be offering tissue specimens to interested researchers from the Jewish and Muslim communities, through our professional networks.

(h) Summary of animal use. Tick the boxes that describe the nature of the proposed work.
(NB ensure compliance with License conditions).

☐ Observation involving minor interference (eg. repeated spot-lighting or intrusive observation, some non-invasive behavioural studies).

☐ Simple trap and release projects. (No biometrical analysis).

☐ Mass capture techniques (e.g. cannon netting) where there this an anticipated, acceptable mortality rate.

☐ The use of Fyke Nets, Gill Nets, Mist Nets, Dip nets, or Opera House Traps.

☐ Use of baited camera traps.

☐ Use of non- baited camera traps.

☐ Use of call play back.

☐ Multiple use /re-use of animals/recapture.

☐ Biometrical analysis – measuring, weighing and prolonged handling.

☐ PIT tagging and/or attachment of monitoring devices.

☐ Intervention under anaesthesia or tranquilizer. (Attachment of devices, removal of devices, tranquilizer dart capture, relocation, biometrical study etc.).

☒ Humane killing for dissection, samples or voucher specimens.

☐ Prolonged restraint or confinement.

☐ Minor changes to husbandry (such as diet interventions and feed trials).

☐ Minor surgery with recovery (biopsies, cannulations or minor procedures under anaesthesia or sedation).

☐ Major surgery under anaesthesia with recovery (eg. orthopaedic, abdominal, thoracic, transplant surgery, mulesing or castration without anaesthesia).

☐ Surgical intervention without anaesthesia – e.g. Mulesing or castration.

☐ Xenotransplantation.

☐ Studies involving repeated irritation such as sensitivity studies or skin testing. A person must not carry out a scientific procedure involving the eye of any animal to determine irritancy of a chemical or biological agent unless the procedure is carried out under terminal anaesthesia.

☐ Stomach tubing or gavage.

☐ Induction of disease (e.g. induction of diabetes).

☐ Induction of tumours.

☐ Implantation of cells, tissues or fluids derived from other animals.

☐ Creating or developing new genetic animal lines.

☐ Minor physiological challenge (e.g. minor -infection, inflammation, phenotypic modification, antiserum production).

☐ Moderate to major physiological challenge (eg. major- infection, inflammation without pain relief, phenotypic modification, prolonged isolation, weight loss, deprivation of food or water, arthritis induction).

☒ Animal unconscious without recovery (eg. non-recovery procedures, anaesthesia).

☒ Minor conscious intervention (eg. tail tipping, injection, blood sampling, minor nutrition studies, shearing, attachment of devices).

☐ Death as an end point (see POCTA Act for definition, e.g. toxicity testing, fatal disease progression).

☐ Single housing of a social species.

☐ Use of neuromuscular blocking agents

☐ Unsure/Other (specify)...............................

4. Statistical Justification and Project Design (Principle of Reduction)

Provide an overview of how the project is designed in relation to its aims. Use flow charts or timelines to assist the AEC as applicable.

If multiple experimental cohorts are used provide a justification for the inclusion of each group and the experimental intervention (for example a drug trial with two doses of drug – why two doses and why was each dose chosen).Include control groups as applicable.

Provide justification for the numbers (including repetitions), based on experimental design and statistical considerations. Provide a table if this will assist the AEC to understand the request. Wherever possible, projects must be designed to minimise animal usage and must ensure adequate numbers of animals are allocated to each group or cohort to reach statistical significance, failure to do so will result in animal wastage and is not consistent with the principle of reduction. NB Reducing the number of animals used must not result in greater harm, including pain and distress, to the animals used.
If the potential impact to animal wellbeing cannot be predicted on the basis of available evidence a pilot study must be incorporated into the experimental design.
For teaching projects include the ratio of students to animals, the number of times each animal will be used in each class and or handled per day or week.

Stage 1: Development of prototype 4

Cadaver heads from a range of liveweights and cattle classes will be utilised to generate thermal curves for superficial skin tissue at the point of application, and the brain tissue. Liveweights in the range 200-600 kg will be selected from the Wagstaff abattoir commercial process for these cadaver studies. Steers/heifers, cows and bulls will be selected as available. A minimum of three heads from each sex class, and liveweights 200-400 and 400-600 will be assessed. This will give an initial statistical representation of the thermal curves associated with these parameters. From this data, the required energy delivery for each sex class and liveweight grouping will be predicted. Cadaver work during the prototype development suggests that there is little difference in energy requirement between bovines from the different sex classes and liveweights routinely processed at the Wagstaff abattoir.

Stage 2: Animal trials

Part 1 – confirmation of pilot trial results and comparison against non-penetrative mechanical stun

The aim of this part is to collect detailed behavioural and electro-encephalographic (EEG) data pertaining to DTS as compared with penetrative captive bolt and non-penetrative mechanical stun. This data will be utilised to address the study objectives:

1. To validate the outcomes of the pilot study (AEC 29.13) in terms of induction of insensibility, in a larger sample size;
2. To confirm time to unconsciousness from onset of DTS application, using the proxy ‘fixed, staring eye’ measured on video footage;
3. To gather data comparing DTS against non-penetrative mechanical stunning (the Halal-accepted stun method used on Australian animals);
4. To understand required stun-stick intervals to ensure that animals are allowed to bleed out fully prior to recovery of sensibility.

The pilot study (AEC 29.13) utilised a small number of replicates, and the data were not statistically representative. We propose to:

- Generate statistically valid data by increasing the number of replicates to 30 in each group;
- Incorporate an evaluation of DTS against non-penetrative mechanical stun (mushroom-stun), which is the Halal-accepted method of stunning of Australian cattle, both within Australia and overseas (Indonesia and Malaysia). By

incorporating this treatment group in the current study, we reduce the number of experimental animals utilised overall, as this question will be raised by Halal authorities, and the comparison will be required at some point in the future if not performed now.

Planned treatment groups:

DTS – 30

Penetrative captive bolt – 30

Non-penetrative stun - 30

Number of animals requested for Part 1: **90**

Sample size calculations were carried out using data from the pilot study (AEC 29.13), and a summary output is shown below:

To have a 95% power in the analysis, targeting a P-value of 0.05 – i.e. having a 95% chance of being 95% confident of finding a difference or not, we need 24 individuals in each treatment group. This level of power and P is the normal accepted statistical power in biological studies. We have planned 30 in each group which gives us 95% power and P of 0.02 – i.e. having a 95% chance of 98% confidence in detecting a difference between treatment groups.

I - Sample Size Calculations for Means						
	Anticipated Values					
	Mean	**Stan. Dev**				
Group 1	-22.7	12.14		Difference in m	-61.1	%
Group 2	-8.83	14.64				
The cells in the table below show the estimated number of subjects needed in each						
group in order to demonstrate a statistically significant difference at "p" values						
ranging from 0.10 - 0.01and at varying levels of "power".						
	Power is the probability of finding a statistically significant difference at					
a given "P" value with the specified number of subjects in each group.						

	Sample Size Needed in Each Group			
alpha level		**Power**		
("p" value)	**95%**	**90%**	**80%**	**50%**
0.10	20	16	12	5
0.05	24	20	15	7
0.02	30	24	19	10
0.01	33	28	22	12

Table for (Z1-alpha/2+Z1-beta)squarec				
		beta		
alpha	0.05	0.1	0.2	0.5
0.1	10.8	8.6	6.2	2.7
0.05	13	10.5	7.8	3.8
0.02	15.8	13	10	5.4
0.01	17.8	14.9	11.7	6.6

Part 2 – pre-commercial level validation

This part aims to satisfy the requirements of the Chief Veterinary Officer for approval of the technology as a recognised method of rendering animals insensible prior to slaughter.

The data gathered are binary (effective or ineffective) data on the success rate of DTS in inducing insensibility in a commercial situation. Data gathered are based on proxy indicators of insensibility (no corneal reflex, no somatic withdrawal reflex – pin-prick or pinch), and use of back-up captive bolt stun (indicator of ineffective induction of insensibility, or onset of recovery). This data will be utilised to address the study objectives:

1. To validate the outcomes of the pilot study (AEC 29.13) in terms of induction of insensibility, in a larger sample size;
4. To understand required stun-stick intervals to ensure that animals are allowed to bleed out fully prior to recovery of sensibility;
5. To demonstrate that the technology may be operated at a commercial rate of

processing (1-2 minutes per animal).

Live cattle will be used to validate, under commercial-type conditions (aiming to process one animal every 2-3 minutes) the repeatability of induction of insensibility demonstrated during the pilot study (AEC 29.13). A number of 300 replicates is required to detect a failure rate of 1%. The current gold standard for stunning prior to slaughter is the penetrative captive bolt. The lowest reported failure rate for penetrative captive bolt stun is 2%. The lowest reported failure rate for non-penetrative mechanical stun is 15%, however, in Australia, failure rates are considered to be less than 10%.
We propose to reduce the number of animals required in this part by utilising the data from 30 DTS processed cattle in Part 1 of the current study to contribute to the 300 replicates.
Number of animals requested for Part 2: **270**

Sample size calculations were carried out using published data on failure rates in captive bolt and non-penetrative mechanical stun, and a summary output is shown below:

Binary data is data where the measured response is a simple yes-no outcome. This type of data would be used in the commercial validation or 'ground-truthing' phase, e.g. using presence or absence of a reflex as the yes/no criterion. The problem with binary data is that many more individuals are required to test for a difference between treatments, particularly if the treatment groups are very similar.
For example:
If we assume that captive bolt has a 98% success rate (two in every 100 animals require re-stun), and mushroom-stun has a 90% success rate (10 in every 100 animals require re-stun), the sample sizes required to detect a difference between these treatment groups are summarised as:

To have a 95% chance of being 95% confident of finding a difference or not, we need 250 individuals in each treatment group.
To have a 90% chance of being 95% confident of finding a difference or not, we need 207 individuals in each treatment group.
To have a 95% chance of being 90% confident of finding a difference or not, we need 212 individuals in each treatment group.
To have a 90% chance of being 90% confident of finding a difference or not, we need 174 individuals in each treatment group.

II - Sample Size Calculations for a Difference in Proportions (frequency)

	Anticipated Values	
	Proportion with	**(without)**
Group 1	0.98	0.02
Group 2	0.9	0.1

The cells in the table below show the estimated number of subjects needed in each group in order to demonstrate a statistically significant difference at "p" values ranging from 0.10 - 0.01and at varying levels of "power".
Power is the probability of finding a statistically significant difference at a given "P" value with the specified number of subjects in each group.

	Sample Size Needed in Each Group			
alpha level	**Power**			
("p" value)	**95%**	**90%**	**80%**	**50%**
0.10	212	174	133	73
0.05	250	207	161	92
0.02	298	250	198	119
0.01	332	282	227	140

Table for (Z1-alpha/2+Z1-beta)squared

	beta			
alpha	0.05	0.1	0.2	0.5
0.1	10.8	8.6	6.2	2.7
0.05	13	10.5	7.8	3.8
0.02	15.8	13	10	5.4
0.01	17.8	14.9	11.7	6.6

We have requested 300 animals, in the event that the failure rate of non-penetrative stun

under trial conditions is less than 10%.

Endorsement of Statistician/ Biometrician*

**** Wherever possible, applications must be endorsed by a Statistician / Biometrician or Reference to an appropriate statistical text provided. Please explain if endorsement is not provided***

- I am aware of and support the proposed research outlined in this application.

Statistician /Biometrician	Sonja Dominik	Date	

5. DESCRIPTION OF THE DESIGN AND PROCEDURES

(a) Provide sequential details of what happens to the animal/s from the time that they are captured/received until the conclusion of the project, detailing the sequence and timing of events (including any repeat procedures or studies). Flow charts, clearly set out tables and time lines will assist in making this information clear. Consideration must be given to the Principle of refinement as defined in the Code in all stages of project design- methods that alleviate or minimise potential pain and distress, and enhance animal wellbeing. Please include the following (if applicable):

Note: The application must be a "stand alone" document and where reference is made to Standard Operating Procedures (SOPs), brief overviews of methods must be provided on the application form with precise cross-reference to detailed documentation. Where reference is made to SOPs they must be provided with the application for approval by the AEC; citation and use of SOPs must comply with the code 2.2.33.

Stage 1: Development of prototype 4

This stage does not involve live animals, so the detail is not presented here. Two reports on the development to date (Final Commissioning Report – Applicator 08-09-2016.pdf and High Power Coaxial Report 10-11-16.pdf) is supplied.

The data from the cadaver evaluations suggest that energy levels as low as 40 kJ (8 kW for 5 sec) can provide the desired temperature increase to achieve insensibility. However, we propose to deliver energy in the range of 50-150 kJ during this phase of the study, by utilising settings such as 10 kW for 5 sec (50 kJ); 20 kW for 5 sec (100 kJ), or 30 kW for 5 sec (150 kJ). As a result of the increase in efficiency between Prototypes 3 and 4, if we do continue to utilise the parameters used for the anaesthetised animal trials (i.e. application durations of 10 sec), we are likely to induce overheating, and blistering may occur.

Stage 2: Animal trials

Part 1 – confirmation of pilot trial results and comparison against non-penetrative mechanical stun

The animals will be delivered to the abattoir and cared for in the lairage facility under normal commercial conditions. Animals will be individually identified by their NLIS ear tag.

90 cattle will be used for this part of the study. The treatment groups are:

DTS – 30
Penetrative captive bolt – 30
Non-penetrative stun - 30

Baseline Electroencephalogram, heart rate and respiratory rate data will be collected from each animal on the day prior to slaughter.

<u>On the trial day.</u>

For each animal:

1. Restraint in standard crush facility, modified to allow accurate DTS application, at the abattoir
2. Application of the assigned treatment (Captive bolt, non-penetrative mechanical stun, or DTS, the dose given according to the predictions calculated under stage 1)
3. Behavioural monitoring of 'animal during application' of the stunning method by real-time video
4. Reflexes (corneal reflex and somatic withdrawal reflex) checked to confirm unconsciousness
5. Roll-out of unconscious animal
    a. Thermal imaging of the head
    b. Post treatment EEG recording – continuous until exsanguination
6. Recheck reflexes
7. Heart rate and respiratory rate measurement
8. Exsanguination using neck stick (halal procedure)
9. Reflexes re-assessed during exsanguination
10. Carcase processed according to commercial practice

Steps 1 to 9 will be completed for each animal, and the animal confirmed dead, prior to beginning work on the subsequent animal.

Note: in the event that any animal subject to DTS demonstrates behaviours indicative of severe pain or severe adverse welfare, it will be immediately euthanased, with due consideration of operator safety, using penetrative captive bolt followed by exsanguination by thoracic sticking method.

Detail:
Animals will be sourced from the commercial intake at Wagstaff Abattoir, and held in the lairage prior to the study. They will be cared for by experienced lairage personnel, handled using low-stress handling techniques, and provided water and hay ad libitum. Animals are individually identified by their NLIS ear tag.

Preparation of animals

Animals will be treated individually. Baseline Electroencephalogram data will be collected from each animal on the day prior to slaughter. For this activity, the animal will be brought individually to a crush within the lairage, using low-stress handling techniques. Once the animal is standing quietly in the crush, heart rate will be measured using a heart rate monitor or stethoscope, with due regard to operator safety. Respiratory rate will be evaluated visually. EEG pads will be glued onto the head – one either side of the head just below the horns, one on the flat part between the nostrils and eyes, and one on the neck, slightly to one side, behind the neck bails.

> The EEG pads provide contact points to the skin overlying the skull, allowing us to measure electrical activity within the brain. Medical researchers would criticise this approach, and advise that for ideal EEG recording, we should use needle electrodes inserted under the skin. However, this is a painful procedure, and to minimise stress to the animals, we have been using glue-on pads for our humane slaughter research. These give less detail in the EEG than needle electrodes would, but we are assessing the EEG merely for evidence of epileptiform activity or suppression; and not for nuances of sleep or cognition, the glue-on pads provide the data we require. We consider this to be a 'Refinement' step in terms of the 3 R's of Animal Ethics.

The baseline EEG will be recorded for approximately 1 minute: Once we have 20 seconds of clear EEG readings, the recording can cease. The animal will return to its home pen. The EEG pads will remain on the animal's head – we have utilised this methodology before, with no adverse reactions.

Trial day

Each animal will be restrained in a standard slaughter restraint crush that has been modified to incorporate a head restraint unit that allows accurate application of DTS. This head restraint lifts the forehead to allow accurate positioning of the waveguide applicator. The animal will be allowed to settle in the restraint before application of the treatment (Captive bolt, non-penetrative mechanical stun or DTS).

Treatment application

Animals will be monitored throughout the procedures by researchers experienced in humane slaughter assessment and in animal behaviour assessment.

Captive bolt or non-penetrative mechanical stun application

After the head is captured, the assigned treatment will be applied, by a trained slaughter person, according to industry guidelines.

DTS Application

After the head is captured, the faraday cage (essential operator safety screen) will be closed, and DTS immediately applied. The total dose delivered will be set based on cadaver thermal curves generated during Prototype 4 development*, and is expected to be in the range of 50-200 kJ. The control system of the microwave generator measures input power and automatically adjusts the electromagnet to give the level called for. Minor voltage supply variations do not affect it.

**During cadaver head trials the weight of the whole cattle animal corresponding to each test head will noted. The relationship between animal weight and temperature change gradient* *can be established and used to plot power prediction curves taking into account cow weight (McLean et al, 2009). This increases the accuracy of power level prediction and allows for estimation based on cattle weight.*

The data from the cadaver evaluations suggest that energy levels as low as 40 kJ (8 kW for 5 sec) can provide the desired temperature increase to achieve insensibility (High Power Coaxial Report 10-11-16.pdf). However, we propose to deliver energy in the range of 50-150 kJ during this phase of the study, by utilising settings such as 10 kW for 5 sec (50 kJ); 20 kW for 5 sec (100 kJ), or 30 kW for 5 sec (150 kJ). As a result of the increase in efficiency between Prototypes 3 and 4, if we do continue to utilise the parameters used for the anaesthetised animal trials (i.e. application durations of 10 sec), we are likely to induce overheating, and blistering may occur.

A comparison of the heating efficiency of Prototype 4 (to be used in the proposed study) and Prototype 3 (used in the pilot study) is shown below.

Prototype	Average heating rate	Time taken to raise brain temperature by 8 °C, from 38 °C to 46°C		
		At 20 kW	At 25 kW	At 30 kW
3	0.124 °C /kW /sec	3.23 sec	2.58 sec	2.15 sec
4 without coaxial system	0.21 °C /kW /sec	1.90 sec	1.52 sec	1.27 sec
4 with coaxial system	0.29 °C /kW /sec	1.38 sec	1.10 sec	0.92 sec

Post treatment application

Following treatment application, the animal will be assessed for lack of response to the following reflexes:

- Corneal reflex (eye retracts when touched);
- Palpebral reflex (eyelids blink closed when eye is almost touched);
- Somatic pain reflex (the head flinches when a needle is inserted into the sensitive skin of the nose, or a pair of pliers used to pinch the soft tissue between the toes);
- Focussing and following (the eye follows the movement when a hand is passed across the field of vision).

Note: If any of these reflexes are present the animal will be deemed to be returning to consciousness, and it will be immediately euthanased using a penetrative captive-bolt, and exsanguinated (if the exsanguination has not already begun).

The animal will then be rolled out of the box, and post-treatment EEG collected. EEG recording will continue until at least one minute of good quality trace is obtained, before exsanguination takes place. Reflexes will be reassessed at the beginning of EEG recording, and every 30 seconds. If any reflexes return, captive bolt will be applied. During EEG measurement, heart rate (stethoscope) and respiratory rate will be measured, and thermal images will be taken of the head.

Exsanguination will be performed using the halal neck cut. If any reflexes have returned, captive bolt will be applied prior to exsanguination, and exsanguination will be by the thoracic method.

Reflexes will be rechecked at 30 second intervals throughout exsanguination, to assess the possibility of return to consciousness during exsanguination. If any reflexes have returned, captive bolt will be applied immediately.

Normal carcase dressing will proceed after 2 minutes of exsanguination, 2 minutes being the recommended delay under Halal processing conditions.

Part 2 – pre-commercial level validation

Part 2 will not proceed if the principal investigator has any concerns that DTS application is not consistently resulting in induction of insensibility, based on the progress of Part 1 above.

This part aims to satisfy the requirements of the Chief Veterinary Officer for approval of the technology as a recognised method of rendering animals insensible prior to slaughter.

The animals will be delivered to the abattoir and cared for in the lairage facility under normal commercial conditions. Animals will be individually identified by their NLIS ear tag.

270 cattle will be used for part 2

Animals will be treated individually. Each animal will be restrained in the standard slaughter restraint crush that has been modified to incorporate a head restraint unit that allows accurate application of DTS. The animal will be allowed to settle in the restraint before application of DTS.

After the head is captured, the faraday cage (essential operator safety screen) will be closed, and DTS immediately applied. The total dose delivered will be set based on cadaver thermal curves generated during Prototype 4 development*, and is expected to be in the range of 50-200 kJ. The control system of the microwave generator measures input power and automatically adjusts the electromagnet to give the level called for. Minor voltage supply variations do not affect it.

**During cadaver head trials the weight of the whole cattle animal corresponding to each test head will noted. The relationship between animal weight and temperature change gradient can be established and used to plot power prediction curves taking into account cow weight (McLean et al, 2009). This increases the accuracy of power level prediction and allows for estimation based on cattle weight.*

Animals will be monitored throughout the procedures by abattoir QA staff trained and experienced in humane slaughter assessment and in assessment of reflexes used as proxy indicators of insensibility.

Following treatment application, the animal will be assessed for lack of response to the following reflexes:

- Corneal reflex (eye retracts when touched);
- Palpebral reflex (eyelids blink closed when eye is almost touched);
- Somatic pain reflex (the head flinches when a needle is inserted into the sensitive skin

of the nose, or a pair of pliers used to pinch the soft tissue between the toes);

- Focussing and following (the eye follows the movement when a hand is passed across the field of vision).

Note: If any of these reflexes are present the animal will be deemed to be returning to consciousness, and it will be immediately euthanased using a penetrative captive-bolt, and exsanguinated (if the exsanguination has not already begun). If an animal is deemed to be conscious after DTS application, it will be stunned using a penetrative captive-bolt and exsanguinated. Consideration of animal parameters (e.g. gender and liveweight) and technical parameters (e.g. energy delivered) will be undertaken, to assess possible reasons for failure to induce insensibility, before any further animal is processed using DTS. Energy parameters will be adjusted to account for differing animal parameters when this is deemed to be the root cause of failure to induce insensibility.

The animal will then be rolled out of the box, and exsanguination will be performed immediately using the halal neck cut. The body will be hoisted for bleeding, and the next animal will then be moved into the restraint box, and the next application sequence begun. Normal carcase dressing will proceed after 2 minutes of exsanguination, 2 minutes being the recommended delay under Halal processing conditions.

Reflexes will be rechecked, by a separate individual to the slaughter person, at 30 second intervals during bleed-out to assess the possibility of return to consciousness during exsanguination. If any reflexes have returned, captive bolt will be applied immediately.

(b) Complete the following table if any substances are to be administered (e.g. anaesthetic, analgesic, tranquillising agents, treatments).The total volume to be injected must be compatible with the size of the animal and the route of injection (see NHMRC Guidelines to promote the wellbeing of animals used for scientific purposes: The assessment and alleviation of pain and distress in research animals (2008) for guidance. It is a requirement of the AEC that where substances are given by injection a new sterile needle and syringe (as applicable) is used for each injection. NB section (g) requires information regarding agents used for humane killing. Do not list these agents here.

Name of agent	Route of administration	Dose	Volume to be injected.	Needle gauge	Approximate duration of experimental procedure Indicate if repeat administrations, etc
NONE					

(c) Complete the following table if any devices are to be attached (e.g. tracking devices, tags).The size (mass and volume) of the device must be compatible with the size of the animal and its behaviour and ecological requirements.

Name of device	Place of attachment	Method of attachment	Size (Mass and Volume)	Needle gauge if applicable	Approximate duration of experimental procedure. Indicate if repeated use, etc
EEG Electrodes	EEG: common non-inverting	glue	Each electrode	Not applicable	Pads applied once, for baseline EEG

(Red Dot®, 3M)	electrode placed midline 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.		is 1.6g mass, and 3 cm in diameter		measurement, on the day prior to slaughter. The process of application lasts a maximum of 2 minutes. The pads are not removed from the animal – previous trials have demonstrated that the animals tolerate the pads well

(d) Housing and care:
Where care and management does not accord with current best practice – provide detail of why current best practice cannot be used in this project and justification as to why this is necessary.

Short term housing/containment e.g. wildlife post trapping held for short periods prior to release where justified, provide details of how long (anticipated and maximum) the animals will be held, the type of containment and provision of suitable food, water and environmental conditions as applicable. Provide details of how it will be determined that the animal may be safely released including species specific strategies that take into account ecology and predation factors. Consider referring to the NHMRC Guide to the care and use of Australian native mammals in research and teaching for species specific recommendations as applicable.

Long including housing/containment , Provide details of housing type and husbandry including provision of food, water and enrichment, breeding and care of juvenile animals (if applicable). If special diets are to be used provide detail of the diet.

Ensure compliance with relevant Codes of Practice, including the Victorian Code of Practice for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits and farm animal codes of practice.

Animals will be cared for under normal commercial lairage practices, by trained lairage personnel. Animals will be restrained in a modified crush for application of the stunning treatment, by personnel trained and experienced in the operation of cattle crushes and slaughter boxes.

(e) What is the fate of the animals upon completion of the study? Detail and provide justification for the experimental endpoint and the maximum length of time animals will be held in this project.

Indicate if 'death as an end point' (as defined in The Code and POCTA Act) is intended and if so provide the means to prevent or minimise harm, including pain and distress, and how this will be considered, implemented and reviewed at all stages of the project.

The animals will be slaughtered and processed according to the Australian Standard for the

Hygienic Production of Meat and Meat Products for Human Consumption (AS4696/2007)

(i) Humane Killing

If animals are to be humanely killed as part of the project (experimental endpoint) or euthanased as a consequence of expected (for example impact of the experimental model) and unexpected circumstances (for example injuries and disease) (humane endpoint) please complete the following table. Where animals are bred as part of this project provide details of the fate of animals surplus to requirements. Humane killing must be in accordance with species specific requirements and current best practice. If more than one method is required include details of all methods.

What is the experimental endpoint? This must be as early as possible to enable collection of required data but minimise the impact to animal wellbeing. If animals are humanely killed at the experimental endpoint complete the table below.

Humane slaughter for human consumption

What are the criteria that will be used to define the humane endpoint? Complete the table below.

This is commercial slaughter, animals are healthy and normal up to and including the point of slaughter

NB Humane killing by C02 for laboratory rodents: current accepted practice is the slow fill method of a flow rate of 20% CO2 of chamber volume per minute. Provide details of how the flow rate will be measured in this circumstance, the AEC does not consider use of CO2 as an appropriate method of humane killing in the field.

Method of euthanasia *include agent/dose/route/volume*	Criteria for euthanasia	Are the personnel responsible experienced in this technique?	Will animals be anaesthetised when euthanased?
Slaughter using DTS – electromagnetic stun with exsanguination	All animals assigned to DTS treatment	Yes	No
Slaughter – captive bolt stun with exsanguination	All animals assigned to captive bolt treatment, and Any 'emergency' euthanasia: selected if any animals shows behavioural indicators of distress e.g. bellowing vocalisations, struggling in restraint, alarm ear postures; return of reflexes	Yes	No
Slaughter – non-penetrative mechanical stun with exsanguination	All animals assigned to non-penetrative mechanical stun treatment	Yes	No

(g) If animals are to be transported, please complete the following table. For genetically modified animals this must be compliant with OGTR requirements.

Method of transport	Container/cage type and description.	What are the risks to the animals?	How are risks managed

6. Animal welfare

This section is intended to identify any adverse effects (expected or unexpected) on the animals' well being and how these impacts will be minimised and the welfare of the animal monitored and managed. ***For wildlife studies, this section must include the welfare of by-catch and non-target species affected by the activities associated with the project.***

(a) What, if any, adverse effects may there be on the animals as a result of the procedures to be carried out or drugs administered? Provide a list or table if this will assist the AEC to determine the impact of each procedure (see (c) for measures to reduce impact and (i) for overall impact of the project).

1. Handling and Restraint is stressful.
2. Application of the electromagnetic field may cause localised skin heating at the point of application. However, this is not considered likely utilising the re-designed waveguide.

(b) What, if any, adverse effects may there be on the animals as a result of the phenotype or age of the animal? (See (c) for measures to reduce impact).

NONE

(c) What measures are in place to reduce the impact of any adverse effects on the animals?

1. Personnel are trained in low-stress handling of livestock, and the restraint facility is a modified cattle crush, designed for religious slaughter of the species and size of animal. It includes belly support and head restraint.
2. The application system for prototype 4 is designed to minimise surface heating.
3. The animals will be monitored at all times during the procedure by experienced livestock welfare researchers and veterinarians.

(d) Detail the methods (including parameters monitored) and frequency of monitoring of animals during all aspects of the study including capture, implementation of procedures, holding, release and tracking as applicable.
Where capture devices are used (such as nets and traps) you must provide detail of frequency of trap monitoring and how access to traps, by animals, will be prevented during times when monitoring is not undertaken or environmental conditions are unfavourable.
Trap and net monitoring must be consistent with the survival requirements of both by-catch and target animals.

Laboratory animals and field trial stock must be adequately monitored throughout the project and after all treatments and procedures.
The strategy for monitoring animals is to be provided for all work. Remember you are responsible for the animal throughout the life of the project. Ensure monitoring specified covers the entire project.
Examples of monitoring score and associated actions applicable to laboratory rodents appendix (4) and wildlife appendix (5) are provided. If applicable these may be modified to suit or an alternate score and action system used.
Include the monitoring sheet(s) you will use for the project in this section include parameters you will monitor and what action will be taken in response to identification of abnormalities.
Records of monitoring must be maintained and available for audit or inspection.

Animals will be inspected and fed daily during the lairage period prior to the study.

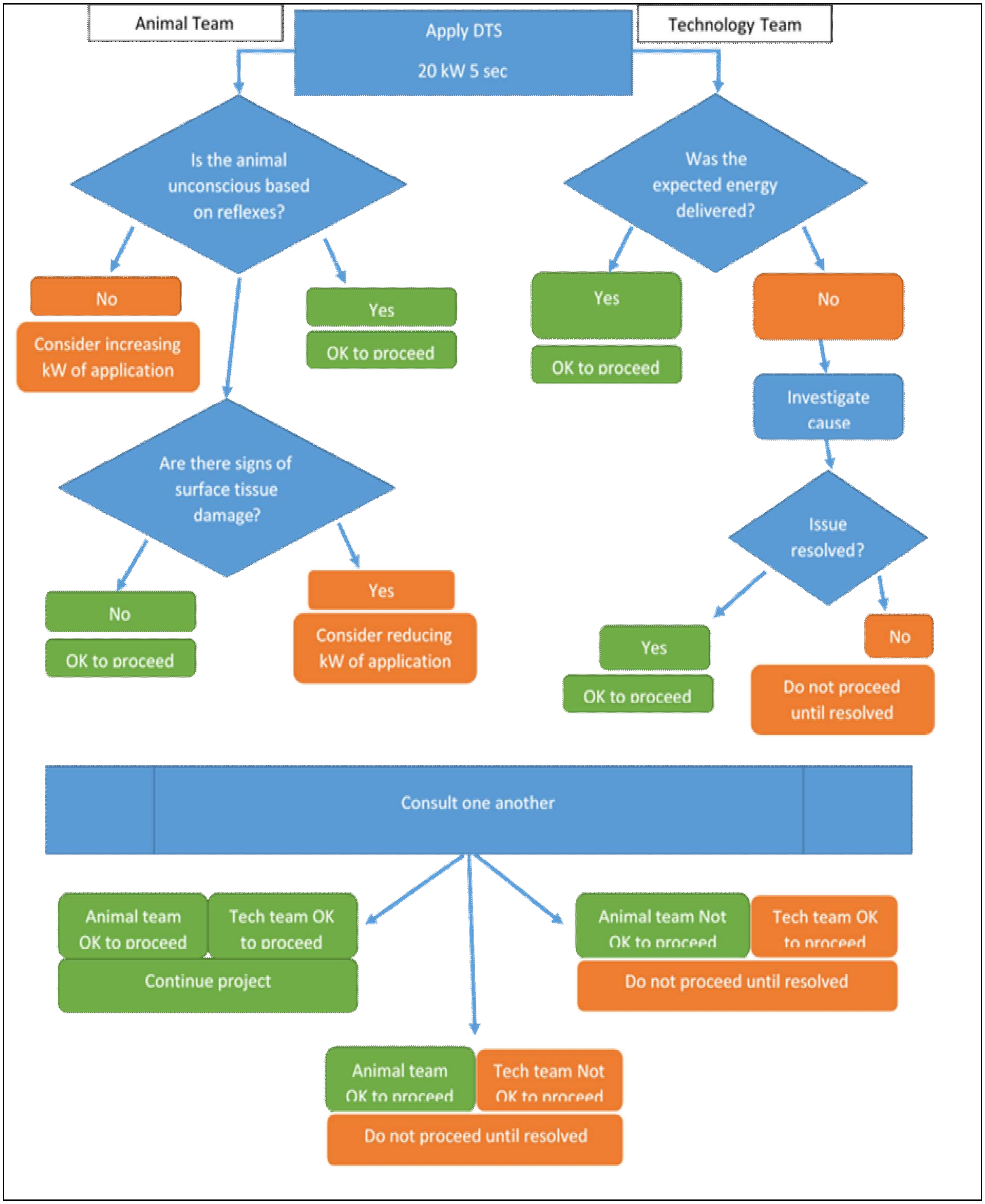
Animals will be restrained for a maximum of 15 minutes for both baseline EEG measurement, and again for the application of DTS on the following day. They will be monitored at all times during the procedure by experienced livestock welfare researchers and veterinarians.

The research is contracted through Meat and Livestock Australia (MLA) and overseen by the Program Manager, Dr Ian Jenson. It is also known to the regulator (overseen by Dr Mark Schipp of the Department of Agriculture [DA], and Dr Charles Milne (VIC)), and is the subject of an application for the method to be approved as a humane method of slaughter. The entire data set and final report will be made available to MLA, the DA, and to the AEC if requested.

We welcome independent monitoring of our work, although we do recommend that the monitor has some prior experience of meat processing and humane slaughter, as the everyday environment alone can be rather confronting to the uninitiated, without the additional factor of developmental research going on.

(e) *Detail what will be done if unforeseen complications or other problems are identified including criteria for intervention, treatment, or removal of animals from the project. It must include the signs and symptoms that will initiate intervention and the nature of the action to be carried out. Monitoring record sheets are to be maintained. In the event of animal welfare impacts that were not anticipated in this application an adverse event report must be submitted.*

In the event that any animal demonstrates behaviours indicative of severe pain or severe adverse welfare, or reflexes associated with returning consciousness are observed, it will be immediately euthanased using captive bolt followed by thoracic stick exsanguination.
On the assumption that such severe adverse welfare events are not observed (other than returning reflexes, they were not observed during the pilot study), a decision tree has been developed to clarify expectations with regard to study progress:



(f) Provide names and contact details of authorised persons responsible for day-to-day monitoring (if applicable) and for dealing with emergencies. (Emergency contacts must be consulted and agree to be listed prior to submission of an application).

Name	Contact phone	Role	Please indicate if day-to-day and/or out of hours responsibility.
Alison Small	0437 349 087	Principal investigator, veterinarian	On trial day
James Ralph	0419 038 904	Study Director	Day-to-Day responsibility, and

Tim Barwick		Stockman Foreman	out of hours

(f) Does the project present any health risks to other animals (non-target species)? If so, what precautions will be employed to minimise this risk?

NONE

(j) Impact to animal wellbeing
Discuss anticipated disease states, morbidity, stress levels and the extent to which the animals may experience pain and or distress as a result of this project.

This is rather difficult to interpret in the case of a slaughter project – we have assessed the changes relative to standard commercial slaughter processes, in which the animal is restrained for a short period while the stun is applied, rather than related to the situation on the farm/feedlot. The maximum period of restraint will be 30 minutes (two occasions of 15 minutes). The predicted interval from start of DTS application to unconsciousness is less than 4 seconds. The maximum period from DTS application to exsanguination or euthanasia will be a maximum of 4 minutes (to allow time for EEG collection).

The AEC is required to assess the overall impact to animal wellbeing of the proposed activity against the justification for the activity. Taking into account all aspects of the proposal ***complete the following table*** *by placing a cross in the relevant section of each row. Ensure consideration is given to phenotype, model/interventions and age of the animals used. This table is based on the Five Domains Model (Mellor et al) see appendix 3. The following table does not need to be completed for Wildlife Studies in the Field.*

Criteria	Normal/ No change	Slight change		Moderate change		Severe change	
		Short term	Long term	Short term	Long term	Short term	Long term
Access to correct food for the species	X						
Access to water or appropriate form of hydration for species	X						
Ability to rest comfortably	X						
Ability to maintain normal body temperature		X					
Ability to move normally	X						
Lack of impairment to function related to wounds or trauma	X						
Lack of impairment to function related to disease	X						
Lack of impairment to function related to pain	X						
Lack of impairment to function related to reduction of circulating blood volume	X						
Ability to express normal social behaviours	X						
*Ability to care for dependent offspring	X						
Ability to freely manipulate it's environment	X						
Lack of impairment to function related to physical restraint	X						
Lack of impairment to function related to changes to nervous system				X			
Lack of impairment to function related to restriction of respiration	X						

**only applicable for animals with dependant offspring at time of procedure(s) or capture.*

7. HUMAN WELFARE

(a) Does the project present any health risks to humans? If so, please provide details and describe what precautions will be employed to minimise this risk? Zoonosis and physical injuries from bites and scratches must be identified. Have all participating personnel been fully advised of the hazards to health and provided with appropriate equipment, training, vaccinations and monitoring?

Working with cattle involves a level of inherent risk, personnel may be kicked, or have limbs crushed against the facility. Personnel have all been trained in safe handling of large animals; they will not enter the pen with the animals; the facility has been designed so that there is no need for personnel to enter the pen with the animals.

Q-fever is a potential zoonotic risk. All personnel have been vaccinated against Q-fever; or have been tested and proven immune.

The risk of exposure to electromagnetic energy is minimised through use of a faraday cage around the head of the animal, and personnel retire around a screening wall (as occurs when x-ray imaging is carried out e.g. in a dental surgery). RF energy meters are used when commissioning the equipment to ensure leakage is minimised. We envisage that a commercial system will be fully enclosed in a faraday cage, and the process automated, including a back-up captive bolt attachment on the waveguide. However, while working on development activities, we require rapid access to the animal, such that we can assess the outcomes, so the current compromise is as described.

8. BIOSECURITY

(a) Discuss the potential risks of the transfer of infectious disease between animals involved in the project and detail the means by which containment is assured. Provide details of current best practice procedures, disinfection agents and control strategies.

Normal abattoir and lairage hygiene procedures are in use.

(b) Provide details of procedures and materials used to control the risk of spreading invasive or infectious organisms during fieldwork.

Not Applicable

9. DECLARATION – *Must be completed by all persons involved with the use of animals within this application. If additional investigators are required to join the project after approval Appendix 2 must be completed and approved by the AEC prior to this person commencing work on the project.*

Individual declaration sheets appended after page 25

- I hereby declare that I have the appropriate qualifications and experience to perform the procedures described in this project or to ensure that they are done correctly. Or have arranged for appropriate training and will be assessed as competent prior to undertaking procedures unsupervised.
- I have read and understand my obligations under the 2013 Australian Code for the Care and Use of Animals for Scientific Purposes. *(The Code)* the Prevention of Cruelty to Animals Act (POCTA) 1986 and regulations2008 and other relevant legislation.
- I accept responsibility for the ethical conduct of the project proposed above, according to the principles contained in that document.
- I acknowledge that the care and maintenance of animals for scientific or educational purposes is the ultimate responsibility of the chief investigator but this work may be delegated to suitably trained and experienced staff listed in this document.
- I have read this application and fully understand the nature, limitations and extent of the proposed work described within.
- I will keep such records as required under the Australian Code and other relevant legislation.
- I will report to the AEC as required by the Australian Code and any conditions set by the AEC.
- I will continuously review this project applying the principles of replacement, reduction and refinement and inform the AEC and request a modification to this project as appropriate.
- Adequate resources are available to undertake this project.

Signature of Chief Investigator (Person with ultimate responsibility)	Alison Small, CSIRO	Date	
Signature of Associate Investigator	1. James Ralph Wagstaff	Date	
Signature of Associate Investigator	2. David McLean Advanced Microwave Technologies	Date	
Signature of Associate Investigator	3. Dominic Niemeyer CSIRO	Date	
Signature of Associate Investigator	4. James Lea CSIRO	Date	
Signature of Associate Investigator	5. Troy Kalinowski CSIRO	Date	
Signature of Associate Investigator	6. Leisha Hewitt Dr L Hewitt Livestock Welfare	Date	
Signature of Associate Investigator	1.Tim Barwick Wagstaff	Date	
Signature of Associate Investigator	2.Mal Webster Wagstaff	Date	
Signature of Associate Investigator	3.Andrew Phillips Wagstaff	Date	
Signature of Associate Investigator	4. Jim Nolan Wagstaff	Date	
Signature of Associate Investigator	5. Cathy Tringham Wagstaff	Date	

(Add additional rows as required)

Endorsement of Organisation

- I am aware of and support the proposed activities involving live animals outlined in this application and I fully understand and accept my institution's responsibilities under the Code and other relevant legislation.
- Adequate resources are available to implement the proposed work in accordance with the agreed protocols and methods and to bring it to completion.

Organisation CEO or President		Date	

References:

The 2013 Australian Code for the Care and Use of Animals for Scientific Purposes.
The Victorian Prevention of Cruelty to Animals Act 1986 and Regulations 2008.

– COPY AND PASTE, ONE FOR EACH INVESTIGATOR. NB PERSONS PROVIDING TRAINING USING ANIMALS WITHIN THIS APPLICATION MUST BE LISTED AS INVESTIGATORS AND COMPLETE THE DECLARATION.

NAME__Alison Small______________________________

QUALIFICATIONS______BVM&S, CVPH(MH), DVPH(MH), DipECVPH, PhD, GCPS(FSRA), MRCVS

EMPLOYEE ☒ **VOLUNTEER** ☐

CURRENT AEC APPROVED PROJECTS IN WHICH YOU ARE INVOLVED (specify if any are approved by another AEC) __

Welfare assessments of analgesic options in female lambs for surgical mulesing and its alternatives – Field studies, young lambs ARA 16/18 CSIRO Armidale Animal Ethics Committee

Welfare assessments of analgesic options in female lambs for surgical mulesing and its alternatives – Field studies, weaner lambs 16/06 CSIRO Armidale Animal Ethics Committee

Case study to examine impact of equine dysmaturity at birth on musculoskeletal and cortical development - 2016 ARA 16/02 CSIRO Armidale AEC

Lambing Rounds to supply welfare group - 2016 ARA 16/07 CSIRO Armidale AEC

Halal stunning of buffalo – validation study ARA 2016-21 CSIRO Wildlife and Large Animal AEC

PLEASE PROVIDE DETAILS OF YOUR EXPERIENCE OF THE PROCEDURES TO BE CARRIED OUT IN THIS PROJECT. (DETAILS OF TRAINING, FORMAL OR OTHERWISE AND ITS CURRENCY)

Technique/procedure Nominated – list each procedure separately	Species Nominated	No. of times you have performed this procedure with this species.			Trainers Name Records of training must be maintained (Specify if experience <20 or not competent)
		<20	20>100	>100	
Cattle handling and restraint	cattle			X	
EEG recording,	cattle			X	
Humane slaughter (captive bolt and exsanguination)	cattle			X	
Welfare assessment at slaughter	cattle			X	
Behaviour assessment	cattle			X	

Alison Small graduated as a veterinarian from the Royal Dick School of Veterinary Studies, Edinburgh, Scotland, in 1993 and spent 12 years working in mixed, mainly livestock practice. During this time she worked as an On-Plant Vet in a number of abattoirs, under contract to the UK Meat Hygiene Service, and holds the UK Animal Welfare Officer certificate. She gained the Royal College of Veterinary Surgeons Diploma in Veterinary Public Health (Meat Hygiene) in 2003, and the Diploma of the European College of Veterinary Public Health in 2005.

In May 2007, she completed her PhD with the University of Bristol, UK, in microbiology, titled “The spread and control of foodborne pathogens in the lairage, with particular reference to cattle”. During her time at the University of Bristol, she was involved in teaching a variety of subjects in the veterinary undergraduate, “meat inspectors”, “environmental health officer”, “official veterinarian” and “taught masters in meat science” courses, including meat hygiene, animal welfare and pathology.

Since January 2006, she has been working for CSIRO, first at Food Science Australia, QLD as a Research Scientist and Meat Industry Adviser, where she completed the University of Maryland Graduate Certificate in Food Safety Risk Analysis. CSIRO projects include review of microbial interventions for meat production, storage life of vacuum packaged beef, and sources of contamination of carcasses. During this time, she led the Australian research team in a CSIRO-UPM collaborative project on the impact of Halal slaughter and stunning on welfare and meat quality.

In July 2010, she transferred to CSIRO Livestock Industries in Armidale, NSW, and is currently working on animal welfare research areas including pain mitigation for livestock; cognitive bias and animal welfare at slaughter. She maintains strong links with the Australian meat industry and with the Halal industry through consultancy activities and regular conference participation, both as a speaker and as a delegate.

– COPY AND PASTE, ONE FOR EACH INVESTIGATOR. NB PERSONS PROVIDING TRAINING USING ANIMALS WITHIN THIS APPLICATION MUST BE LISTED AS INVESTIGATORS AND COMPLETE THE DECLARATION.

NAME____James Ralph____________________________

QUALIFICATIONS______Company Director, Abattoir Operator, Design and Implementing Standards for Meat Processing, Member of Meat Standards Committee, Member of AMIC Animal Welfare Committee

EMPLOYEE ☒ **VOLUNTEER** ☐

CURRENT AEC APPROVED PROJECTS IN WHICH YOU ARE INVOLVED (specify if any are approved by another AEC) ____

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Technique/procedure Nominated – list each procedure separately	**Species Nominated**	**No. of times you have performed this procedure with this species.**			**Trainers Name Records of training must be maintained (Specify if experience <20 or not competent)**
		<20	**20>100**	**>100**	
Abattoir and meat processing plant manager	**Beef**			**X**	40 years industry experience

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NAME___David McLean________________________________

QUALIFICATIONS______Bachelor Engineering (Elec)______________________________________

EMPLOYEE ☒ **VOLUNTEER** ☐

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Technique/procedure Nominated – list each procedure separately	Species Nominated	No. of times you have performed this procedure with this species.			Trainers Name Records of training must be maintained (Specify if experience <20 or not competent)
		<20	20>100	>100	
Laboratory research and development – cattle cadaver heads	**Cattle**		**X**		Carried out all development work throughout this project
Application of DTS to cattle	**Cattle**		**X**		Operated DTS equipment of anaesthetised animal trials (Rault et al 2014); and for pilot trials under AEC 29.13

– COPY AND PASTE, ONE FOR EACH INVESTIGATOR. NB PERSONS PROVIDING TRAINING USING ANIMALS WITHIN THIS APPLICATION MUST BE LISTED AS INVESTIGATORS AND COMPLETE THE DECLARATION.

NAME_____ Dominic Niemeyer_______________________________

QUALIFICATIONS______Associate Diploma in Biological Techniques____________

EMPLOYEE ☒ **VOLUNTEER** ☐

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Lambing Rounds to supply welfare group - 2016 ARA 16/07 CSIRO Armidale AEC

Halal stunning of buffalo – validation study ARA 2016-21 CSIRO Wildlife and Large Animal AEC___

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Technique/procedure Nominated – list each procedure separately	**Species Nominated**	**No. of times you have performed this procedure with this species.**			**Trainers Name Records of training must be maintained (Specify if experience <20 or not competent)**
		<20	**20>100**	**>100**	
Cattle handling and restraint	**Cattle**			**X**	
EEG recording,	**Cattle**		**X**		
Humane slaughter (captive bolt and exsanguination)	**Cattle**			**X**	
Welfare assessment at slaughter	**Cattle**			**X**	
Behaviour assessment	**Cattle**			**X**	

Dominic Niemeyer is a senior research technician at CSIRO, with nearly 20 years experience in livestock handling and research. His current work includes research in meat quality, land transport, lamb survival, temperament testing in sheep and cattle, live export and stocking density in sheep and cattle, sheep and cattle behaviour and cognitive bias as well as sheep and cattle responses to painful husbandry behaviours.
Dom also runs a family farm, producing quality Angus beef and sheep.

– COPY AND PASTE, ONE FOR EACH INVESTIGATOR. NB PERSONS PROVIDING TRAINING USING ANIMALS WITHIN THIS APPLICATION MUST BE LISTED AS INVESTIGATORS AND COMPLETE THE DECLARATION.

NAME James Lea

QUALIFICATIONS BTC, BA

EMPLOYEE ☒ **VOLUNTEER** ☐

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		<20	20>100	>100	
Cattle handling and restraint	**Cattle**			**X**	
EEG recording,	**Cattle**		**X**		
Humane slaughter (captive bolt and exsanguination)	**Cattle**		**X**		
Welfare assessment at slaughter	**Cattle**		**X**		
Behaviour assessment	**Cattle**			**X**	

James Lea graduated for the Biological Technicians certificate in 1986 and has worked in small and large ruminant research since then, In this time he has worked at CSIRO in Armidale and Perth as well as the University of New England. He is currently studying for a Masters of rural science at the University of New England.

His work at Perth and UNE involved research into internal parasites, wool quality, immunology, methanogen and methane emission reduction in cattle and feed choice in sheep.

His current work at CSIRO in Armidale (2003 –present) includes research in meat quality, land transport, lamb survival, temperament testing in sheep and cattle, live export and stocking density in sheep and cattle, sheep and cattle behaviour and cognitive bias as well as sheep and cattle responses to painful husbandry behaviours

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NAME____Troy Kalinowski_______________________________

QUALIFICATIONS______Cert III in Laboratory Skills, Cert II in Rural Skills, Cert II in Security Operations. Other plant operation certificates and licenses held

EMPLOYEE ☒ **VOLUNTEER** ☐

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		<20	20>100	>100	
Cattle handling and restraint	**Cattle**			X	
Humane slaughter (captive bolt and exsanguination)	**Cattle**			X	
Welfare assessment at slaughter	**Cattle**		X		
Behaviour assessment	**Cattle**		X		

Troy Kalinowski is a senior research technician, with 15 years' experience in livestock handling, care and research. He is the animal house manager at CSIRO FD McMaster Laboratory, Chiswick, and provides routine slaughter services to research projects as required.

– COPY AND PASTE, ONE FOR EACH INVESTIGATOR. NB PERSONS PROVIDING TRAINING USING ANIMALS WITHIN THIS APPLICATION MUST BE LISTED AS INVESTIGATORS AND COMPLETE THE DECLARATION.

NAME____Leisha Hewitt______________________________

QUALIFICATIONS______BSc (Hons), MSc, PhD_________

EMPLOYEE ☒ **VOLUNTEER** ☐

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Technique/procedure Nominated – list each procedure separately	Species Nominated	No. of times you have performed this procedure with this species.			Trainers Name Records of training must be maintained (Specify if experience <20 or not competent)
		<20	20>100	>100	
Welfare assessment at slaughter	**Cattle**			**X**	
Behaviour assessment	**Cattle**			**X**	

Leisha Hewitt is a consultant on animal welfare practice, industry standards and quality assurance frameworks, with a particular emphasis on animal welfare at slaughter. She has held research and lecturer positions at Murdoch University (WA) and Bristol University Vet School, where she managed the Veterinary Continuing Education Unit and contributed to the Animal Welfare Training program. As part of the Animal Welfare Training Group at Bristol she was responsible for the implementation of training programs and consultancy activities, particularly in the area of livestock production, transportation, stunning and slaughter.

Leisha holds a PhD in Clinical Veterinary Science, an MSc Meat Science, BSc Agriculture and Environmental Science (Honours in Animal Science), Cert IV in Training and Assessment, Post Graduate Certificate of Education, and Diploma in Quality Management. Leisha is a qualified Lead Auditor (ISO9000 Series Auditor/Lead Auditor) and Animal Welfare Assessor with experience in the development, implementation and assessment of animal welfare standards. Previous to her work at Bristol, she worked for a UKAS accredited inspection body (ISO 17020 - responsible for third party animal welfare inspections of farms, abattoirs, hatcheries and packing stations) where she was responsible for the recruitment and management of technically competent inspection staff, supplier auditing and authorisation of all reports and corrective action. She is also a Qualified Maritime Internal Auditor and Instructor.

In the past, Leisha has been called upon in a technical expert capacity by OIE, DAFF, LiveCorp, MLA, RSPCA and EFSA panel. She continues to provide technical advice to a UK retailer and independent inspection body. This involves approving and managing corrective action plans, updating animal welfare standards and providing guidance to auditors.

– COPY AND PASTE, ONE FOR EACH INVESTIGATOR. NB PERSONS PROVIDING TRAINING USING ANIMALS WITHIN THIS APPLICATION MUST BE LISTED AS INVESTIGATORS AND COMPLETE THE DECLARATION.

NAME___Tim Barwick____

QUALIFICATIONS______Livestock Foreman, Farmer, Slaughter floor techniques________

EMPLOYEE ☒ **VOLUNTEER** ☐

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		<20	20>100	>100	
Livestock handling and restraint	**Cattle**			**X**	40 years' experience
Humane slaughter and exsanguination	**Cattle**			**X**	40 years' experience
Assessment of reflexes used as proxy indicators of insensibility	**Cattle**			**X**	40 years' experience

– COPY AND PASTE, ONE FOR EACH INVESTIGATOR. NB PERSONS PROVIDING TRAINING USING ANIMALS WITHIN THIS APPLICATION MUST BE LISTED AS INVESTIGATORS AND COMPLETE THE DECLARATION.

NAME____Mal Webster__________________________

QUALIFICATIONS Slaughterman, Abattoir works engineer

EMPLOYEE ☐ **VOLUNTEER** ☐

CURRENT AEC APPROVED PROJECTS IN WHICH YOU ARE INVOLVED (specify if any are approved by another AEC) ____

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		<20	20>100	>100	
Livestock handling and restraint	**Cattle**			**X**	35 years' experience
Humane slaughter and exsanguination	**Cattle**			**X**	35 years' experience
Assessment of reflexes used as proxy indicators of insensibility	**Cattle**			**X**	35 years' experience

– COPY AND PASTE, ONE FOR EACH INVESTIGATOR. NB PERSONS PROVIDING TRAINING USING ANIMALS WITHIN THIS APPLICATION MUST BE LISTED AS INVESTIGATORS AND COMPLETE THE DECLARATION.

NAME____Andrew Phillips__________________________

QUALIFICATIONS Abattoir Works Manager, Slaughterman,__________

EMPLOYEE ☐ **VOLUNTEER** ☐

CURRENT AEC APPROVED PROJECTS IN WHICH YOU ARE INVOLVED (specify if any are approved by another AEC) ____

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Technique/procedure Nominated – list each procedure separately	Species Nominated	No. of times you have performed this procedure with this species.			Trainers Name Records of training must be maintained (Specify if experience <20 or not competent)
		<20	20>100	>100	
Livestock handling and restraint	**Cattle**			**X**	30 years' experience
Humane slaughter and exsanguination	**Cattle**			**X**	30 years' experience
Abattoir operations	**Cattle**			**X**	30 years' experience
Assessment of reflexes used as proxy indicators of insensibility	**Cattle**			**X**	30 years' experience

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NAME____Jim Nolan___________________________

QUALIFICATIONS **Abattoir Assistant Works Manager, Slaughterman,**

EMPLOYEE ☐ **VOLUNTEER** ☐

CURRENT AEC APPROVED PROJECTS IN WHICH YOU ARE INVOLVED (specify if any are approved by another AEC) ____

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		<20	20>100	>100	
Livestock handling and restraint	**Cattle**			**X**	30 years' experience
Humane slaughter and exsanguination	**Cattle**			**X**	30 years' experience
Abattoir operations	**Cattle**			**X**	30 years' experience
Assessment of reflexes used as proxy indicators of insensibility	**Cattle**			**X**	30 years' experience

– COPY AND PASTE, ONE FOR EACH INVESTIGATOR. NB PERSONS PROVIDING TRAINING USING ANIMALS WITHIN THIS APPLICATION MUST BE LISTED AS INVESTIGATORS AND COMPLETE THE DECLARATION.

NAME____Cathy Tringham_________________________

QUALIFICATIONS Quality Assurance Manager, Animal Welfare Officer______

EMPLOYEE ☐ **VOLUNTEER** ☐

CURRENT AEC APPROVED PROJECTS IN WHICH YOU ARE INVOLVED (specify if any are approved by another AEC) ____

____

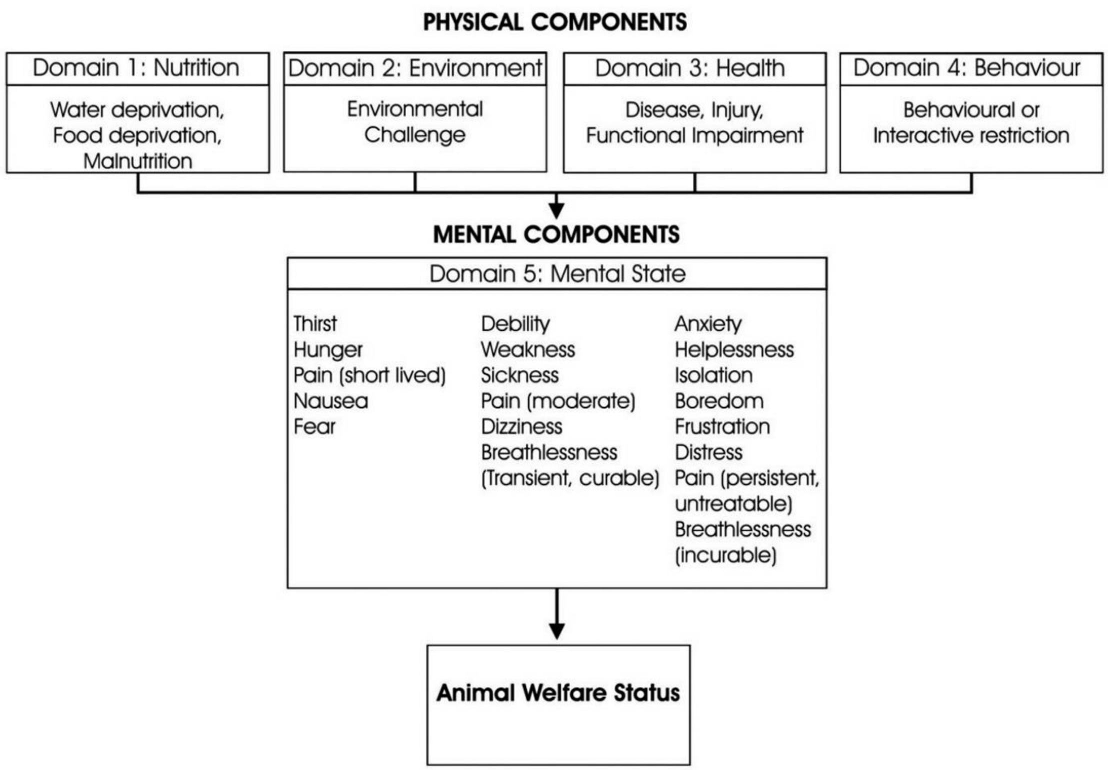
PLEASE PROVIDE DETAILS OF YOUR EXPERIENCE OF THE PROCEDURES TO BE CARRIED OUT IN THIS PROJECT. (DETAILS OF TRAINING, FORMAL OR OTHERWISE AND ITS CURRENCY)

Technique/procedure Nominated – list each procedure separately	Species Nominated	No. of times you have performed this procedure with this species.			Trainers Name Records of training must be maintained (Specify if experience <20 or not competent)
		<20	20>100	>100	
Livestock handling and restraint	**Cattle**			**X**	25 years' experience
Humane slaughter and exsanguination	**Cattle**			**X**	25 years' experience
Abattoir operations	**Cattle**			**X**	25 years' experience
Assessment of reflexes used as proxy indicators of insensibility	**Cattle**			**X**	25 years' experience

Appendix 2

5 Domains Model- David Mellor

Further information may be found at
http://www.altex.ch/resources/445449_Mellor121.pdf



Appendix 3 – Example Laboratory Rodent score and associated action.

(Applicable to experimental models where animals are expected to be normal).

Clinical sign	Score 0 (normal)	Score 1 (minor change from normal)	Score 2 (moderate change from normal)	Score 3 (severe change from normal)
Activity (Act)	normal	isolated, abnormal posture	Huddled/ active only when provoked OR overactive	Inactive when provoked Or moribund OR fitting
Alertness (Alt)	normal	dull or depressed	little response to handling	unconscious
Body condition score (BCS)	normal	Thin; BCS <3	loss of body fat, BCS 2	loss of muscle mass BCS <2
Breathing (B)	normal	rapid, shallow	rapid, abdominal breathing	laboured, irregular, skin blue
Coat/skin (C)	normal	coat rough, minor hair thinning with otherwise normal skin	Unkempt, wounds, hair thinning with skin changes ,minor superficial wound	Deep or large bleeding or infected wounds, or severe self mutilation
Hydration (H)	normal	skin less elastic	skin tenting	skin tenting & eyes sunken
Movement/(M) gait	normal	slight incoordination OR abnormal gait	uncoordinated OR walking on tiptoe OR reluctance to move	staggering OR limb dragging OR paralysis
Body weight (BW)	normal weight compared with known growth curve or cage mates	Weight loss 5-10% compared with highest recorded weight or known growth curve or cage mates	Weight loss 10-15% compared with highest recorded weight or known growth curve or cage mates	Weight loss >15% compared with highest recorded weight or known growth curve or cage mates

Score	Action
Single score 3	Unless immediate supportive care or treatment can be provided that is likely to result in significant improvement the animal must be euthanased immediately. If supportive care or treatment is instigated the animal is to be checked after 30 mins and if significant improvement is not seen the animal is to be euthanased. If showing improvement monitoring is as per score 1 or 2 below
Single score 2	Monitor and score the animal three times daily with at least 3 hours between monitoring time points. An animal that is showing signs of improvement may be left overnight. An animal that is failing to improve at end of day is to be euthanased. Provide supportive care such as mash, jelly, provision of warmth. The exception to the requirement for twice daily monitoring is animals with single score 2 skin/coat changes where once daily monitoring is sufficient.
Single score 1	Monitor and score the animal twice daily with at least 5 hours between monitoring time points. Provide supportive care such as mash, jelly, provision of warmth.
Cumulative score >9	The animal must be euthanased immediately
Cumulative score 3-9	Monitor and score the animal three times daily with at least 3 hours between monitoring time points. An animal that is showing signs of improvement may be left overnight. An animal that is failing to improve at end of day is to be euthanased. Provide supportive care such as mash, jelly, provision of warmth.
Cumulative score 1-3	Monitor and score the animal twice daily with at least 5 hours between monitoring time points. Provide supportive care such as mash, jelly, provision of warmth.

Appendix 4 – Example Wildlife Field Study score and associated action.

Clinical sign	Score 0 (normal)	Score 1 (minor change from normal)	Score 2 (moderate change from normal)	Score 3 (severe change from normal)
Activity (Act)	normal	isolated, abnormal posture, possibly vocalising	Huddled/ active only when provoked OR overactive, strongly vocalising	Inactive when provoked Or moribund OR fitting
Alertness (Alt)	normal	dull or depressed	little response to handling	unconscious
Body condition (BC)	normal	Thin	loss of body fat	Emaciated*
Breathing (B)	normal	rapid, shallow	rapid, abdominal breathing	laboured, irregular, skin blue
Coat/skin (C)	normal	coat rough, minor hair /feather thinning with otherwise normal skin	Unkempt, wounds, hair/feather thinning with skin changes ,minor superficial wound, wet (non-aquatic)	Deep or large bleeding or infected wounds, or severe self mutilation
Hydration (H)	normal	skin less elastic	skin tenting	skin tenting & eyes sunken
Body openings (B)	normal	Slight swelling or redness	Swelling or redness but no impact to normal function	Bleeding from any orifice including- mouth, ears, anus, cloaca.
Movement/(M) gait	normal	slight incoordination OR abnormal gait	Uncoordinated/lameness	staggering OR limb dragging OR paralysis
Skeleton/bones/wings (exoskeleton if applicable) (S)	normal	Healed fractures or deformities slight impact to normal function.	Healed fractures or deformities slight impact to normal function.	Fractures or deformities where normal function is not possible.

Score	Action
Single score 3	Unless immediate supportive care or treatment can be provided that is likely to result in significant improvement, the animal must be euthanased immediately. If supportive care or treatment is instigated the animal is to be checked after 30 mins, or an appropriate time taking into account species specific needs and if significant improvement is not seen the animal is to be euthanased. If showing improvement continue monitoring until the animal and time is suitable for release. Animals suspected of carrying disease must be appropriately stored after euthanasia and a veterinary pathologist opinion sought. *Emaciated – animals that are emaciated due to current environmental conditions may be released if there is reasonable expectation of survival after release.
Single score 2	If there is a reasonable expectation the animal will survive after release it may be immediately released. If supportive care such as food, water or warmth will increase improve the wellbeing of the animal, provide this and then release. If the animal is not expected to survive after release it must be euthanased.
Single score 1	The animal may be released.
Cumulative score >9	If there is a reasonable expectation that the clinical sore reflects the impact of trapping and confinement and the animal will survive on release the animal may be released. If supportive care that will improve the animals wellbeing on release can be provided this must be provided before release. Otherwise the animal must be euthanased immediately. It is unacceptable to release an animal that is unconscious, fitting, has obvious recent broken bones or large deep wounds or significant bleeding from any orifice – all such animals must be immediately euthanased.
Cumulative score 3-9	If there is a reasonable expectation the animal will survive after release it may be immediately released. If supportive care such as food, water, warmth or expert veterinary care will increase or improve the wellbeing of the animal, provide it and then release if and when appropriate. If the animal is not expected to survive on release it must be euthanased by a trained operator.
Cumulative score 1-3	The animal may be released

Appendix 5 - References

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8.2 Appendix 2 Animal Ethics application and approval reference CWLA 2019-17

CSIRO Wildlife and Large Animal AEC

Authority for the use of Animals in Research

WHERE POSSIBLE, THIS AUTHORITY TO BE DISPLAYED IN THE PROXIMITY OF ANIMALS BEING USED IN RESEARCH

The CSIRO Wildlife and Large Animal, Animal Ethics Committee has authorised the following people to use animals in the research project described.

Principal Investigator (PI):	Dr Alison Small
Associate Investigator(s):	Dominic Niemeyer, James Lea, Stephen Humphries, Troy Kalinowski, Sue Belson, Leisha Hewitt, James Ralph, David McLean, Tim Barwick, Mal Webster, Andrew Phillips, Jim Nolan, Cathy Tringham

It is the responsibility of any investigator from an institution other than CSIRO, to notify their AEC in writing of their involvement in this project – Code 2.4.9 and 2.6.8

AEC Application Number:	2019-17
Project Title:	Factors affecting duration of insensibility in cattle stunned using DTS: Diathermic Syncope
Location of Research Site:	Wagstaff Garfield Abattoir, Lovers lane, Garfield VIC 3814
Date of Authorisation:	11 April 2019, updated 12 Dec 2019 – additional numbers of animals approved, updated 28 April 2020 – extension of approval period.
Number and species of animal:	250 cattle
Publications:	You are encouraged to become aware of the ARRIVE Guidelines. These guidelines are intended to improve the reporting of research using animals by maximising the information published and minimising unnecessary studies. Your adherence to these guidelines is expected when publishing your study findings.
Annual Review Due:	1 February 2020
Authorisation Valid Until:	30 Dec 2020, final report due 1 Feb 2021
Condition	Please include a photo or video of the rotary knocking box with the final report

CSIRO Wildlife and Large Animal AEC

Approval is strictly limited to the research proposal as submitted in your application. Any variations or modifications must be considered and approved by the CSIRO Wildlife and Large Animal, Animal Ethics Committee before they are implemented. If the proposed changes are deemed significant, you will need to submit a new application.

Research projects of duration greater than one year will be reviewed annually. This authorisation will be cancelled by the AEC if the requirements of the Australian code for the care and use of animals for scientific purposes, NHMRC, 8th edition 2013 or the conditions of this Authority, are not observed.

CSIRO Wildlife and Large Animal AEC

Authority for the use of Animals in Research

WHERE POSSIBLE, THIS AUTHORITY TO BE DISPLAYED IN THE PROXIMITY OF ANIMALS BEING USED IN RESEARCH

The CSIRO Wildlife and Large Animal, Animal Ethics Committee has authorised the following people to use animals in the research project described.

Principal Investigator (PI):	Dr Alison Small
Associate Investigator(s):	Dominic Niemeyer, James Lea, Stephen Humphries, Troy Kalinowski, Sue Belson, Leisha Hewitt, James Ralph, David McLean, Tim Barwick, Mal Webster, Andrew Phillips, Jim Nolan, Cathy Tringham

It is the responsibility of any investigator from an institution other than CSIRO, to notify their AEC in writing of their involvement in this project – Code 2.4.9 and 2.6.8

AEC Application Number:	2019-17
Project Title:	Factors affecting duration of insensibility in cattle stunned using DTS: Diathermic Syncope
Location of Research Site:	Wagstaff Garfield Abattoir, Lovers lane, Garfield VIC 3814
Date of Authorisation:	11 April 2019
Number and species of animal:	200 cattle
Publications:	You are encouraged to become aware of the ARRIVE Guidelines. These guidelines are intended to improve the reporting of research using animals by maximising the information published and minimising unnecessary studies. Your adherence to these guidelines is expected when publishing your study findings.
Annual Review Due:	1 February 2020
Authorisation Valid Until:	30 May 2020, final report due 1 August 2020

CSIRO Wildlife and Large Animal AEC

Approval is strictly limited to the research proposal as submitted in your application. Any variations or modifications must be considered and approved by the CSIRO Wildlife and Large Animal, Animal Ethics Committee before they are implemented. If the proposed changes are deemed significant, you will need to submit a new application.

Research projects of duration greater than one year will be reviewed annually. This authorisation will be cancelled by the AEC if the requirements of the Australian code for the care and use of animals for scientific purposes, NHMRC, 8th edition 2013 or the conditions of this Authority, are not observed.

CSIRO Wildlife and Large Animal

Animal Ethics Committee

Application for the use of laboratory or captive animals in research

AEC approved 11/4/2019
Amend 1 approved 19 June 2019
Amend 2 approved 12.12.2019 (additional numbers)
Amend 3 approved 28.4.2020 – extension of approval period

V10 – Jan 2019

Commercial in confidence

You must be aware of the responsibilities of investigators section (section 2.4) in the Australian Code for the Care and use of Animals for Scientific Purposes (2013) **https://www.nhmrc.gov.au/guidelines/publications/ea28**

The application must provide clear evidence of the steps taken to comply with the general principles for the care and use of animals for scientific purposes by addressing the issues of:

- the replacement of animals with other methods;
- the reduction in the number of animals used; and
- the refinement of techniques to reduce the impact on animals.

Subject to the approval of this protocol you will be expected to:

a) ensure the welfare of your experimental animals is respected at all times

b) ensure the staff engaged in this project are appropriately trained and skilled in the procedures they are to perform

c) ensure proper records are maintained to provide project progress reports and completion or extension reports to the AEC

d) ensure any unexpected adverse events to experimental animals are thoroughly investigated and reported to the AEC

e) complete annual reports on progress of proposals exceeding 12 month's duration, and a report at the completion of the proposal

f) **ensure all project records are retained for a minimum of 10 years as required by the National Archives Act of Australia. The legal destruction of records is controlled by CSIRO Records Services.**

AEC number:

2	0	1	9	-	1	7	

Use plain English throughout. Where the use of a scientific term is unavoidable, it must be explained by a suitable lay description. A glossary of terms may be included with the application.

A. Principal Investigator/Responsible Scientist:

Name: Alison Small
Contact details:
phone (work): 02 6776 1435
phone (after hours): 0437 349 087
e-mail: Alison.small@csiro.au

B. Application title: (Use a unique title that reflects the specific objective of this proposed research)

Factors affecting duration of insensibility in cattle stunned using DTS: Diathermic Syncope

C. O2D number

OD-200361

D. Australian State(s)/Territory(ies)/Overseas location of project

Victoria

E. Project duration

Commencement date:	**Day and Month:** 15 April	**Year:** 2019
Completion date:	**Day and Month:** 30 December	**Year:** 2020

F. Provide a summary of the project <u>in plain English</u> (no more than 200 words). Include the reasons why this research needs to be conducted, the context of the research, the expected results and what you plan to do.

A new technology for stunning cattle prior to slaughter has been developed (DTS:Diathermic Syncope®), and preliminary studies have indicated that the animals may recover from the stun. This means that the technique may be suitable for the Halal market. However, to be sure that animal welfare can be maintained we need to be sure that the unconsciousness will last for at least as long as the time required for bleed-out to ensure death due to loss of blood pressure using a Halal cut procedure. This time period has been previously determined to be 2 minutes from the Halal cut.

In this project we will use stopwatches to time the duration between induction of unconsciousness (collapse) and the first signs of recovering consciousness (return of the blink reflex) in 200 cattle of varying size and class, recording a range of animal parameters (body weight, gender, breed type), and technology parameters (energy and power settings). This will allow us to run a multivariate regression analysis to assess which (if any) of the factors strongly affect duration of insensibility; and will also allow us to estimate the mean and 95% confidence interval around duration of insensibility, in order to assess the potential suitability of the technique for the Halal market.

We will also use the opportunity to gather a bank of photographs, thermal images and video footage that could be used to present the technology to the Halal authorities, and, if safe, measure heart rate post stun (another valuable criterion for the Halal authorities).

To ensure that animal welfare is maintained throughout the study, animals will be handled using low-stress handling procedures by experienced stockpersons; DTS will be applied by experienced operators, and assessment of blink reflex carried out by an experienced slaughterperson, who will stun the animal using a penetrating captive bolt immediately the blink reflex returns.

G. What are your null and alternate hypothesis:

For statistical analysis, the hull hypothesis is that animal parameters (body weight, gender, breed type), and/or technology parameters (energy and power settings) do not influence duration of insensibility as assessed using absence of blink reflex.

The alternate hypothesis is that one or more of animal parameters (body weight, gender, breed type), and/or technology parameters (energy and power settings) do influence duration of insensibility as assessed using absence of blink reflex.

SECTION 1. PARTICULARS OF PERSONNEL INVOLVED IN THE PROJECT AND DECLARATION OF RESPONSIBILITIES

1.1 Name and qualifications of all persons involved in the research.

All personnel involved in the project must be listed (in order of degree of responsibility in this project) and the **AEC must be notified of any personnel changes**. *If the names of Volunteers or staff and there appropriate skills and qualifications are not available at the time of preparation of the application, they must be provided to the AEC BEFORE the person undertakes any activities using animals.*

Inexperienced personnel **must** receive appropriate training before undertaking any activities involving animals.

Has any person named in this application had any animal research authority or animal supplier's licences cancelled? If so, please provide details of the date of cancellation, who cancelled the authority or licences and the reason(s).

No

Name	Alison Small
Contact details (phone & email)	02 6776 1435; Alison.small@csiro.au
Qualifications and CSIRO position	BVM&S, CVPH(MH), DVPH(MH), PhD, GCPS(FSRA) Principal Research Scientist
Role(s) in this project	Principal Investigator
Experience in this role	Over 20 years of clinical veterinary and meat industry experience. Humane slaughter research carried out over the past 10 years.
List techniques to be performed on live animals by this person	Assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex.
For each technique, describe the level of expertise, how recent the experience and the number of years of experience this investigator possesses	Alison was first involved in neurophysiological measurements as a veterinary undergraduate in the early 1990s. Thereafter, while supervising the Bristol University research abattoir as On-Plant Veterinarian, and Animal Welfare Officer between 2001 and 2005, she assisted the humane slaughter research team in EEG collection from livestock prior to slaughter. Working as an on-plant veterinarian between 1995 and 2005, and subsequently during her research role, and providing veterinary support to the CSIRO Chiswick research farm (2005 to date), she has carried out many assessments of insensibility, the most recent occasion of which was October 2018.
If no experience, provide details of who will be providing training, how this will occur and how competence will be ascertained.	Not applicable
Provide the details, including the date, of any animal ethics training you have participated in.	Annual refresher training held at CSIRO, Armidale. Latest occasion was June 2017

Signature/declaration of responsibility	I acknowledge that I am aware of the contents of this application, my role in the project and my obligations under the Australian Code for the Care and Use of Animals for Scientific purposes (8th edition, 2013). I agree to comply with the requirements of the Code and all relevant commonwealth, state and territory legislation and any conditions imposed by the AEC

Name	Dominic Niemeyer
Contact details (phone & email)	02 6776 1355; dom.niemeyer@csiro.au
Qualifications and CSIRO position	Associate Diploma in Biological Techniques Project officer
Role(s) in this project	Senior research technician
Experience in this role	Over 20 years ruminant research experience
List techniques to be performed on live animals by this person	Assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex.
For each technique, describe the level of expertise, how recent the experience and the number of years of experience this investigator possesses	Dom has been assessing insensibility for humane slaughter research for the past 3 years, most recently in December 2018. Dom has 20 years of ruminant research techniques, including 15 years in the animal behaviour and welfare team. He has extensive experience in low-stress livestock handling, sample and data collection.
If no experience, provide details of who will be providing training, how this will occur and how competence will be ascertained.	Not applicable
Provide the details, including the date, of any animal ethics training you have participated in.	CSIRO Animal Ethics Training, Armidale. 20th February 2018

Signature/declaration of responsibility	I acknowledge that I am aware of the contents of this application, my role in the project and my obligations under the Australian Code for the Care and Use of Animals for Scientific purposes (8th edition, 2013). I agree to comply with the requirements of the Code and all relevant commonwealth, state and territory legislation and any conditions imposed by the AEC

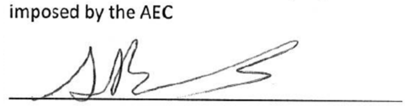
Name	James Lea
Contact details (phone & email)	02 6776 1419; jim.lea@csiro.au
Qualifications and CSIRO position	BTC, BA Project Officer
Role(s) in this project	Senior research technician
Experience in this role	Over 25 years research experience
List techniques to be performed on live animals by this person	Assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex.
For each technique, describe the level of expertise, how recent the experience and the number of years of experience this investigator possesses	Jim has been assessing insensibility for humane slaughter research for the past few years, most recently under AEC 2015-25, AEC2019-05 and VIC DEDJTR WSIAEC 30.16 in October 2018. Jim has over 25 years of ruminant research experience, including 15 years in the animal behaviour and welfare team. He has extensive experience in low-stress livestock handling, sample and data collection.
If no experience, provide details of who will be providing training, how this will occur and how competence will be ascertained.	Not applicable
Provide the details, including the date, of any animal ethics training you have participated in.	CSIRO Animal Ethics Training, Armidale. 20th February 2018

Signature/declaration of responsibility	I acknowledge that I am aware of the contents of this application, my role in the project and my obligations under the Australian Code for the Care and Use of Animals for Scientific purposes (8th edition, 2013). I agree to comply with the requirements of the Code and all relevant commonwealth, state and territory legislation and any conditions imposed by the AEC

Name	Stephen Humphries
Contact details (phone & email)	0418 736 251; Steve.humphries@csiro.au
Qualifications and CSIRO position	Slaughterman-butcher; Meat Inspector
Role(s) in this project	Slaughterman
Experience in this role	Over 40 years as slaughterman-butcher Over 15 years research experience
List techniques to be performed on live animals by this person	Application of stun (non-penetrative or penetrative captive bolt) Assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex. Exsanguination by severance of the common carotid artery (thoracic stick)
For each technique, describe the level of expertise, how recent the experience and the number of years of experience this investigator possesses	Stephen is an experiences slaughterman-butcher, with over 40 years of experience in the meat industry, including acting as the CSIRO research slaughterman for over 15 years. During this time he was involved in a large range of humane slaughter and meat processing technology projects. His most recent experience of slaughtering was in December 2018.
If no experience, provide details of who will be providing training, how this will occur and how competence will be ascertained.	Not applicable
Provide the details, including the date, of any animal ethics training you have participated in.	CSIRO Animal Ethics Training, Armidale. 19h February 2019

Signature/declaration of responsibility	I acknowledge that I am aware of the contents of this application, my role in the project and my obligations under the Australian Code for the Care and Use of Animals for Scientific purposes (8th edition, 2013). I agree to comply with the requirements of the Code and all relevant commonwealth, state and territory legislation and any conditions imposed by the AEC

Name	Troy Kalinowski
Contact details (phone & email)	02 6776 1300; troy.kalinowski@csiro.au
Qualifications and CSIRO position	Cert III in Laboratory Skills, Cert II in Rural Skills Project Officer
Role(s) in this project	Reserve research technician, in the event that James Lea or Dominic Niemeyer are unavailable for the study.
Experience in this role	Over 15 years research experience.
List techniques to be performed on live animals by this person	Assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex.
For each technique, describe the level of expertise, how recent the experience and the number of years of experience this investigator possesses	Troy has recently joined the animal behaviour and welfare team, from another team within CSIRO. He has extensive experience in low-stress livestock handling, sample and data collection, and is rapidly learning new skills and techniques within the humane slaughter research space, having participated in 3 slaughter studies over the past four years (including AEC 2015-25, AEC 2019-05 and VIC DEDJTR WSIAEC 30.16)
If no experience, provide details of who will be providing training, how this will occur and how competence will be ascertained.	Not applicable – Troy has proved adept at the skills listed in previous studies.
Provide the details, including the date, of any animal ethics training you have participated in.	CSIRO Animal Ethics Training, Armidale. 20th February 2018
Signature/declaration of responsibility	I acknowledge that I am aware of the contents of this application, my role in the project and my obligations under the Australian Code for the Care and Use of Animals for Scientific purposes (8th edition, 2013). I agree to comply with the requirements of the Code and all relevant commonwealth, state and territory legislation and any conditions imposed by the AEC

Name	Sue Belson
Contact details (phone & email)	02 6776 1447; sue.belson@csiro.au
Qualifications and CSIRO position	Veterinary Nursing Qualification Project Officer
Role(s) in this project	Reserve research technician, in the event that James Lea or Dominic Niemeyer are unavailable for the study.
Experience in this role	Over 10 years research experience.
List techniques to be performed on live animals by this person	Assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex.
For each technique, describe the level of expertise, how recent the experience and the number of years of experience this investigator possesses	Sue has extensive experience in low-stress livestock handling, sample and data collection, and is rapidly learning new skills and techniques within the humane slaughter research space, having participated in VIC DEDJTR WSIAEC 30.16.
If no experience, provide details of who will be providing training, how this will occur and how competence will be ascertained.	Not applicable – Sue has proved adept at the skills listed in previous studies.
Provide the details, including the date, of any animal ethics training you have participated in.	CSIRO Animal Ethics Training, Armidale. 20th February 2018
Signature/declaration of responsibility	I acknowledge that I am aware of the contents of this application, my role in the project and my obligations under the Australian Code for the Care and Use of Animals for Scientific purposes (8th edition, 2013). I agree to comply with the requirements of the Code and all relevant commonwealth, state and territory legislation and any conditions imposed by the AEC 

Name	Leisha Hewitt
Contact details (phone & email)	0420302131; leisha.hewitt@gmail.com
Qualifications and CSIRO position	BSc (Hons), MSc, PhD Dr Leisha Hewitt Livestock Welfare
Role(s) in this project	Leisha will assist in data collection, and assessment of the technology in terms of industry fit. Leisha has extensive experience in the International standards around Livestock Slaughter.

Experience in this role	Leisha Hewitt is a consultant on animal welfare practice, industry standards and quality assurance frameworks, with a particular emphasis on animal welfare at slaughter. She has held research and lecturer positions at Murdoch University (WA) and Bristol University Vet School, where she managed the Veterinary Continuing Education Unit and contributed to the Animal Welfare Training program. As part of the Animal Welfare Training Group at Bristol she was responsible for the implementation of training programs and consultancy activities, particularly in the area of livestock production, transportation, stunning and slaughter.
List techniques to be performed on live animals by this person	Assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex.
For each technique, describe the level of expertise, how recent the experience and the number of years of experience this investigator possesses	Leisha was first involved in neurophysiological measurements as a PhD student and research technician in 1999, where responsibilities included electrode preparation, assistance with surgery, electrode implantation and analysis of data. Her PhD research project involved an examination of the neurophysiological effects of neck dislocation in turkeys. As an animal welfare researcher and consultant she provides training in the assessment of stunning and slaughter and is consequently experienced in the assessment of behavioural and physiological indicators of effective stunning and slaughter and validation of slaughter techniques.
If no experience, provide details of who will be providing training, how this will occur and how competence will be ascertained.	Not applicable
Provide the details, including the date, of any animal ethics training you have participated in.	Home office licence training (UK) – Technician (1999) Home office licence training (UK) – Principal Investigator (2001) Update and refresher provided by Dr Alison Small during AEC 2017-05
Signature/declaration of responsibility	I acknowledge that I am aware of the contents of this application, my role in the project and my obligations under the Australian Code for the Care and Use of Animals for Scientific purposes (8th edition, 2013). I agree to comply with the requirements of the Code and all relevant commonwealth, state and territory legislation and any conditions imposed by the AEC  ____________________________________

Name	James Ralph
Contact details (phone & email)	0419036904; jhralph@bigpond.net.au

Qualifications and CSIRO position	Wagstaff Company Director, Abattoir Operator, Design and Implementing Standards for Meat Processing, Member of Meat Standards Committee, Member of AMIC Animal Welfare Committee
Role(s) in this project	Livestock procurement, oversight of abattoir personnel and practices
Experience in this role	Over 40 years industry experience
List techniques to be performed on live animals by this person	Mainly supervisory, but may position DTS applicator, and assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex.
For each technique, describe the level of expertise, how recent the experience and the number of years of experience this investigator possesses	Over 40 years industry experience, Oversaw all development of the DTS technology (animal trials under University of Melbourne AEC 1212620-1, VIC DEDJTR WSIAEC 29.13 and WSIAEC 30.16)
If no experience, provide details of who will be providing training, how this will occur and how competence will be ascertained.	Not applicable
Provide the details, including the date, of any animal ethics training you have participated in.	Update and refresher provided by Dr Alison Small
Signature/declaration of responsibility	I acknowledge that I am aware of the contents of this application, my role in the project and my obligations under the Australian Code for the Care and Use of Animals for Scientific purposes (8^{th} edition, 2013). I agree to comply with the requirements of the Code and all relevant commonwealth, state and territory legislation and any conditions imposed by the AEC

Name	David McLean
Contact details (phone & email)	dmclean@uow.edu.au
Qualifications and CSIRO position	Bachelor Engineering (Elec). Advanced Microwave Technologies
Role(s) in this project	Operation of DTS equipment
Experience in this role	David carried out the engineering development of the technology (2006 to date) and has applied DTS to over 50 cattle and 100 cadaver heads during that period

List techniques to be performed on live animals by this person	Operation of DTS equipment
For each technique, describe the level of expertise, how recent the experience and the number of years of experience this investigator possesses	David carried out the engineering development of the technology (2006 to date) and has applied DTS to over 50 cattle and 100 cadaver heads during that period (animal trials under University of Melbourne AEC 1212620-1, VIC DEDJTR WSIAEC 29.13 and WSIAEC 30.16)
If no experience, provide details of who will be providing training, how this will occur and how competence will be ascertained.	Not applicable
Provide the details, including the date, of any animal ethics training you have participated in.	Update and refresher provided by Dr Alison Small
Signature/declaration of responsibility	I acknowledge that I am aware of the contents of this application, my role in the project and my obligations under the Australian Code for the Care and Use of Animals for Scientific purposes (8th edition, 2013). I agree to comply with the requirements of the Code and all relevant commonwealth, state and territory legislation and any conditions imposed by the AEC 

Name	Tim Barwick
Contact details (phone & email)	(03) 5996 0488
Qualifications and CSIRO position	Wagstaff Garfield Livestock Foreman, Farmer, Slaughter person
Role(s) in this project	Livestock care and feeding; moving livestock into restraint, positioning DTS applicator, assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex., application of captive bolt
Experience in this role	Over 40 years' experience
List techniques to be performed on live animals by this person	Livestock care and feeding; moving livestock into restraint, positioning DTS applicator, assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex., application of captive bolt
For each technique, describe the level of expertise, how recent the experience and the number of years of experience this investigator possesses	Over 40 years' experience Carried out these duties throughout the development of the DTS technology (animal trials under University of Melbourne AEC 1212620-1, VIC DEDJTR WSIAEC 29.13 and WSIAEC 30.16)

If no experience, provide details of who will be providing training, how this will occur and how competence will be ascertained.	Not applicable
Provide the details, including the date, of any animal ethics training you have participated in.	Update and refresher provided by Dr Alison Small
Signature/declaration of responsibility	I acknowledge that I am aware of the contents of this application, my role in the project and my obligations under the Australian Code for the Care and Use of Animals for Scientific purposes (8th edition, 2013). I agree to comply with the requirements of the Code and all relevant commonwealth, state and territory legislation and any conditions imposed by the AEC

Name	Mal Webster
Contact details (phone & email)	(03) 5996 0488
Qualifications and CSIRO position	Wagstaff Slaughterman, Abattoir works engineer
Role(s) in this project	Livestock care and feeding; moving livestock into restraint, positioning DTS applicator, assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex., application of captive bolt, exsanguination
Experience in this role	35 years' experience
List techniques to be performed on live animals by this person	Livestock care and feeding; moving livestock into restraint, positioning DTS applicator, assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex., application of captive bolt, exsanguination
For each technique, describe the level of expertise, how recent the experience and the number of years of experience this investigator possesses	35 years' experience Carried out these duties throughout the development of the DTS technology (animal trials under University of Melbourne AEC 1212620-1, VIC DEDJTR WSIAEC 29.13 and WSIAEC 30.16)
If no experience, provide details of who will be providing training, how this will occur and how competence will be ascertained.	Not applicable

Provide the details, including the date, of any animal ethics training you have participated in.	Update and refresher provided by Dr Alison Small
Signature/declaration of responsibility	I acknowledge that I am aware of the contents of this application, my role in the project and my obligations under the Australian Code for the Care and Use of Animals for Scientific purposes (8th edition, 2013). I agree to comply with the requirements of the Code and all relevant commonwealth, state and territory legislation and any conditions imposed by the AEC

Name	Andrew Phillips
Contact details (phone & email)	(03) 5996 0488
Qualifications and CSIRO position	Wagstaff Abattoir Works Manager, Slaughterman
Role(s) in this project	Livestock care and feeding; moving livestock into restraint, positioning DTS applicator, assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex., application of captive bolt, exsanguination
Experience in this role	30 years' experience
List techniques to be performed on live animals by this person	Livestock care and feeding; moving livestock into restraint, positioning DTS applicator, assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex., application of captive bolt, exsanguination
For each technique, describe the level of expertise, how recent the experience and the number of years of experience this investigator possesses	30 years' experience Carried out these duties throughout the development of the DTS technology (animal trials under University of Melbourne AEC 1212620-1, VIC DEDJTR WSIAEC 29.13 and WSIAEC 30.16)
If no experience, provide details of who will be providing training, how this will occur and how competence will be ascertained.	Not applicable
Provide the details, including the date, of any animal ethics training you have participated in.	Update and refresher provided by Dr Alison Small

Signature/declaration of responsibility	I acknowledge that I am aware of the contents of this application, my role in the project and my obligations under the Australian Code for the Care and Use of Animals for Scientific purposes (8th edition, 2013). I agree to comply with the requirements of the Code and all relevant commonwealth, state and territory legislation and any conditions imposed by the AEC

Name	Jim Nolan
Contact details (phone & email)	(03) 5996 0488
Qualifications and CSIRO position	Wagstaff Abattoir Assistant Works Manager, Slaughterman
Role(s) in this project	Livestock care and feeding; moving livestock into restraint, positioning DTS applicator, assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex., application of captive bolt, exsanguination
Experience in this role	30 years' experience
List techniques to be performed on live animals by this person	Livestock care and feeding; moving livestock into restraint, positioning DTS applicator, assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex., application of captive bolt, exsanguination
For each technique, describe the level of expertise, how recent the experience and the number of years of experience this investigator possesses	30 years' experience Carried out these duties throughout the development of the DTS technology (animal trials under VIC DEDJTR WSIAEC 30.16)
If no experience, provide details of who will be providing training, how this will occur and how competence will be ascertained.	Not applicable
Provide the details, including the date, of any animal ethics training you have participated in.	Update and refresher provided by Dr Alison Small

Signature/declaration of responsibility	I acknowledge that I am aware of the contents of this application, my role in the project and my obligations under the Australian Code for the Care and Use of Animals for Scientific purposes (8th edition, 2013). I agree to comply with the requirements of the Code and all relevant commonwealth, state and territory legislation and any conditions imposed by the AEC

Name	Cathy Tringham
Contact details (phone & email)	(03) 5996 0488
Qualifications and CSIRO position	Wagstaff Quality Assurance Manager, Animal Welfare Officer
Role(s) in this project	Oversight of abattoir personnel and practices, assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex., data recording
Experience in this role	25 years' experience
List techniques to be performed on live animals by this person	Oversight of abattoir personnel and practices, assessment of insensibility using animal-based indicators: corneal reflex, palpebral reflex; somatic withdrawal reflex., data recording
For each technique, describe the level of expertise, how recent the experience and the number of years of experience this investigator possesses	25 years' experience Carried out these duties throughout the development of the DTS technology (animal trials under VIC DEDJTR WSIAEC 30.16)
If no experience, provide details of who will be providing training, how this will occur and how competence will be ascertained.	Not applicable
Provide the details, including the date, of any animal ethics training you have participated in.	Update and refresher provided by Dr Alison Small

Signature/declaration of responsibility	I acknowledge that I am aware of the contents of this application, my role in the project and my obligations under the Australian Code for the Care and Use of Animals for Scientific purposes (8th edition, 2013). I agree to comply with the requirements of the Code and all relevant commonwealth, state and territory legislation and any conditions imposed by the AEC

Other staff members from the abattoir and DAWR meat program will be present, but they will be carrying out their normal duties and none of the specific procedures required for this research activity. They will not be involved in any decision-making with regards to the research.

SECTION 2. DECLARATIONS

2.1 Principal Investigator

I believe the project is justified in terms of the potential value of the experiments in obtaining or establishing significant information relevant to the understanding of humans or animals, to the maintenance and improvement of human or animal health and welfare, to the improvement of animal management or production or to the achievement of educational objectives.

I certify that the proposed experimental, teaching or diagnostic use of animals in this project will conform to the governing principles of the Australian Code for the Care and Use of Animals for Scientific Purposes, 8th Edition 2013.

It is acknowledged that the maintenance of experimental animals will ultimately be my responsibility but this work may be delegated to staff suitably trained or experienced in animal care. All staff involved in the experiments are accredited or qualified and have suitable recent experience in the necessary technology and procedures or will receive training and supervision.

Signature: **(print name): …Alison Small Date: …6th March 2019**

2.2 NEXT LEVEL MANAGER to sign. Note: The Principal Investigator cannot sign this section. If the next level manager is a member of the research team, then their manager must sign.

I have read this research application and am satisfied that the use of animals is justified on scientific grounds and that adequate resources are available to undertake the project as described.

If project resources change significantly and there is likely to be a detrimental impact on this project I will advise the AEC immediately.

Signature: .... (print name): CAROLINE LEE Date: 8/3/19

Note: The CSIRO Animal Ethics Committee may apply specific requirements in approving this application. Investigators and teachers must not begin a scientific or teaching activity involving the use of animals before written AEC approval is obtained, and must adhere to all requirements of the AEC and the Code.

SECTION 3. THE PROPOSED RESEARCH PROJECT

Use plain English. Where the use of a scientific term is unavoidable, it must be explained by a suitable lay description. A glossary of terms may be included with the application if necessary.

SCIENTIFIC PURPOSE FOR WHICH THE ANIMALS ARE USED. (Select the major purpose for the activity from the list below. *Refer to Appendix 2 for detailed listing.)*

CATEGORY OF PROCEDURE. (Select a category from the list below. If animals are subjected to different categories of procedure simultaneously, you must record the highest impact category for the combined activity. *Refer to Appendix 3 for detailed listing.*)

Scientific Purpose	Tick	Category of Procedure	Tick
1. The understanding of human or animal biology.		1. Observational studies involving minor interference.	
2. The maintenance and improvement of human or animal health and welfare: activities that aim to produce improvements in the health and welfare of animals, including humans.		2. Animal unconscious without recovery.	√
3. The improvement of animal management or production.	√	3. Minor conscious intervention without anaesthesia.	
4. The achievement of educational objectives.		4. Minor operative procedures with recovery.	
5. Environmental study.		5. Surgery with recovery.	

		6.	Minor physiological challenge.	
		7.	Major physiological challenge.	
		8.	Death as an endpoint.	

The Animal Research Ethics Resources page contains current SOPs, example monitoring sheets, information on necropsy and advice on the 3R's. See:
https://teams.csiro.au/units/ARER/_layouts/15/start.aspx#/SitePages/Home.aspx

3.1 Provide some basic animal information and any background issues relating to the species to be used on this project.

The animals are cattle, a range of breeds and genders and sizes from 150 kg upwards.

The aim is to cover a range of cattle that are representative of normal commercial slaughter in the southern beef industry

3.2 What are the specific aims of this research project?

To assess the duration of insensibility provided using DTS:Diathermic Syncope® as the stunning method.

To investigate animal and technology factors affecting the duration of insensibility.

3.3 Detail how the research has been designed so that it will achieve the stated aims.

Cattle will be sourced by the abattoir using their normal procurement processes, either direct from farms or from local saleyards. They will be rested in lairage (a place where cattle or sheep may be rested on the way to market or slaughter), or in paddocks adjacent to the lairage, for a period of at least 4 days (the normal procedure at this abattoir). On the day of slaughter, they will be brought individually to the restraint unit, and Diathermic Syncope (DTS) applied according to the normal operating procedures at the abattoir.

We will use stopwatches to time the duration between induction of unconsciousness (collapse) and the first signs of recovering consciousness (return of the blink reflex) in 200 cattle of varying size and class, recording a range of animal parameters (body weight, gender, breed type), and technology parameters (energy and power settings). This will allow us to run a multivariate regression analysis to assess which (if any) of the factors strongly affect duration of insensibility;

and will also allow us to estimate the mean and 95% confidence interval around duration of insensibility, in order to assess the potential suitability of the technique for the Halal market.

3.4 What is the broad context of your research? Within the response to this question, make a scientific case supporting the proposed research including references if necessary.

Billions of cattle are slaughtered each year around the world to produce meat, a valuable source of protein in our diets. Regardless of culture, we all agree that slaughter should be carried out in the best possible manner, however, we disagree on what constitutes the best manner. Modern slaughter often involves stunning of the animal prior to the slaughter cut, rendering the animal unconscious such that it does not feel the pain of the neck cut. However, existing stunning methods are not acceptable to all markets (e.g. Muslim or Jewish), and they are not perfect. The Muslim (Halal) and Jewish (Kosher) laws require that animals are healthy, whole and undamaged at the point of slaughter, slaughter being defined as the cutting of the neck such that the animal dies as a consequence of blood loss.

Mechanical stunning can be carried out by use of the penetrative captive bolt, or the non-penetrating (mushroom) stun. The penetrative captive bolt produces a hole in the skull, so is not acceptable according to the Muslim or Jewish laws. Furthermore, although penetrative captive bolt is considered to be the 'gold standard' stunning method, it is not 100% effective. Where the head of the animal is not fixed in position, it can be difficult for the operator to accurately position the 'shot'. Where no head restraint is used, up to 50% of stuns may be misapplied, and not effective (Gouveia et al., 2009). In Europe, where head restraint is commonly used, 7 to 15% of penetrative captive bolt stuns are estimated to be ineffective (Endres, 2005; Marzin et al., 2008; Von Holleben et al., 2010), whereas in the US, failure rates have been reported to be in the order of 0.6 – 1.2% (Grandin, 1994), where effective head restraint is used. The Australian Export meat industry has set targets of 95% of animals to be effectively stunned on first shot.

Non-penetrative mechanical stunning has been accepted by the Australian, Malaysian and Indonesian Islamic authorities, and is utilised throughout the Australian meat industry. It is estimated that 80% of cattle slaughtered in Australian Export-registered abattoirs are stunned using non-penetrative mechanical stunning. The challenges with non-penetrative mechanical stunning (also called mushroom stun), are that it can produce large cracks and fractures on the skull, visible when the head is skinned. If these cracks are present, the carcase is unacceptable for Halal. To reduce the number of carcases demonstrating skull fractures, attempts are made to minimise the power (kinetic energy) delivered by the instrument. However, where the power is reduced, the incidence of improper (failed) stuns increases, and animals may need two or more 'shots' to be rendered fully unconscious. The back-up method is application of a penetrative captive bolt stun, for welfare reasons, and the carcase is not acceptable as Halal. Reported failure to stun rates with non-penetrative mechanical stunning are high and very variable, e.g. Lambooij et al. (1981) could only produce immediate unconsciousness in 15 out of 19 veal calves of 200 kilograms live weight (i.e. a 21% failure rate); Blackmore (1979) found that 80 percent of 90 calves between one and 2 weeks of age were effectively stunned as determined by behavioural observations (i.e. a 20 % failure rate); Hoffmann (2003) used either cartridge activated or pneumatic concussion stunning devices and found that 12 percent out of 1248 cattle had to be re-stunned (Thesis findings reported in von Holleben et al. (2010)); Endres (2005) examined stunning effectiveness in more than 5500 cattle, using two different pneumatic non-penetrative devices. In total only 83.3 percent of the cattle were stunned by the first blow (16.7% failure rate). Highest re-stun rates of 20 percent were found in young bulls. Sixty percent of the 548 heads examined showed profound injuries of the frontal bone in the impact area of the bolt including inner and outer bone laminae and partly the dura mater. (Thesis findings reported in von Holleben et al. (2010)); Oliveira et al. (2018) reported a need for back-up stunning in 29% of cattle stunned using non-penetrative mechanical stunning; and Gibson et al. (2019) reported a failure rate of 18%, based on EEG data of young bulls, of 550 kg live weight. Thus, for welfare reasons, there is an urgent need to provide the Halal industry with a more reliable method of inducing unconsciousness prior to slaughter.

Electrical head-only stunning has been accepted by the Australian, Malaysian and Indonesian Islamic authorities. The challenges with electrical head-only stunning applied to cattle are that the electrical current sometimes must be applied for 3 minutes or more in order to achieve the required epileptiform activity in the brain, this can lead to burn marks on the head at the point of application, which are considered unacceptable for Halal, and the duration of insensibility can be very short, such that animals may be regaining consciousness during the bleed-out period. Where animals are observed to be regaining consciousness during the bleed-out period, a penetrative captive bolt stun is applied as a back-up, for welfare reasons, and the carcase is not acceptable as Halal.

The DTS:Diathermic Syncope® system (patent PCT/AU2011/000527) uses radiofrequency electromagnetic energy to raise the temperature in the brain by approximately 7° C, to the point that hyperthermic syncope (fainting) occurs, but below the point at which irreversible brain damage and death occurs. Thermal unconsciousness such as that induced by exercise heat stress or fever is reported to occur when core body temperatures reach between 40 and 45°C (Ohshima et al., 1992). Pilot trials were successful, cattle being rendered insensible (based on electroencephalogram (EEG) and behavioural indicators), and the insensibility appeared to last for 3-4 minutes (McLean et al., 2017; Small et al., 2019). Meat quality attributes (pHu 24 h post slaughter; pH, Warner Bratzler Shear Force and Drip loss at 1 or 10 weeks post slaughter) and physiological measures (cortisol, ACTH, β-endorphin, adrenaline and noradrenaline) were similar to that induced by penetrative captive bolt. DTS carcases were slightly yellower at quartering (MINOLTA b* 2.71 ± 0.59 DTS; 1.06 ± 0.44 control); DTS loins were slightly redder (MINOLTA a* 23.22 ± 0.92 DTS; 20.89 ± 0.69 control) and slightly yellower (MINOLTA b* 2.79 ± 0.93 DTS; 0.77 ± 0.70 control); and DTS rounds were slightly lighter (MINOLTA L* 43.32 ± 1.05 DTS; 40.94 ± 0.78 control) at week 1, than control samples ($P<0.05$). There were no differences between DTS and captive bolt meat colour measurements at 10 weeks post slaughter (Manuscript on physiological measures and meat quality attributes is in preparation).

In conclusion, there is preliminary evidence that the DTS system may provide better welfare outcomes and a more consistent induction of insensibility than non-penetrating mechanical stunning, without skull damage, and as such may be suited to the Halal market. The purpose of the proposed project is to gather sufficient data to understand the factors affecting duration of insensibility, and the confidence intervals around the duration of insensibility, such that a decision regarding the use of the Halal neck cut for exsanguination can be made. To ensure good welfare at slaughter, the duration of unconsciousness needs to be at least as long as the time required for bleed-out to ensure death due to loss of blood pressure using the Halal cut procedure. This time period has been previously determined to be 2 minutes from the Halal cut.

The reason the Halal cut procedure is different from non-Halal exsanguination (known as 'thoracic stick') is related to the blood vessels being cut (Von Holleben et al., 2010). In the Halal cut procedure, the slaughterman passes a sharp knife across the tissues of the neck, close to the head, as described in the religious texts. This cuts through the carotid arteries and the jugular veins, leading to rapid loss of blood and then death. However, these vessels often 'spring back' and try to close, so the blood flow can be restricted, and there is also a secondary supply of blood to the brain via an artery that runs up the neck close to the spinal cord, and this secondary supply can allow the animal to remain alive (and conscious if the Halal cut is carried out on an un-stunned animal) for a prolonged period. Using the 'thoracic stick', the cut is made into the subclavian and common carotid arteries low in the neck, close to the chest, before the secondary vertebral supply separates from the arterial supply. This technique allows a very rapid bleed-out, and loss of brain function. However, the 'thoracic stick' method is not described in the religious texts as a means of slaughtering cattle, and as such is not considered appropriate for Halal meat production. Further background detail and references can be provided if required, but the review by von Holleben et al. (2010) is quite comprehensive.

3.5 What are the potential benefits to be derived from this proposed research? (Discuss the potential value of the research in relation to the improvement of human or animal health and welfare)

If we can confirm that the duration of insensibility is reliably 3-4 minutes (as indicated by the pilot work), that gives plenty of time to allow death from loss of blood using the Halal neck cut, and DTS:Diathermic Syncope® can be offered to the Halal certification authorities for evaluation. If accepted by the Halal authorities, DTS offers a better welfare outcome than the current non-penetrative mechanical stun procedure. In Australia, it is estimated that 80% of cattle processed at export-registered abattoirs are stunned using the non- penetrative mechanical stun procedure, with which first-stun failure rates can be high.

3.6 Why are you proposing to use animals in this project? (The AEC is obliged to assess whether or not the use of animals is justified. Will this research allow worthwhile scientific objectives to be met?)

Live cattle are required in order to assess the duration of insensibility.

The cattle are destined to be slaughtered for human consumption, and we will add merely a delay in exsanguination while we time the interval between collapse and return of blink reflex (which returns prior to return of consciousness, and is considered to be the first indication that consciousness is beginning to return)

3.7 List the alternatives you have considered to replace the use of animals in this project and explain why each alternative is unsuitable. (eg. Computer modelling, literature reviews- examples of relevant websites are: Norecopa (Norwegian reference centre for animal alternatives), FRAME (fund for the replacement of animals in medical experiments), AWIC (animal welfare information centre) and HsVma (human society)).

There are as yet no data sets that can replace this work. However, having gathered this data set, we aim to publish the information as soon as practicable, such that the study does not have to be repeated.

3.8 Does the proposed research repeat previously reported experiment(s)? If so, please justify and provide reference(s) for the research to be replicated.

No – it adds to preliminary data.

3.9 Has an application for approval of this project been lodged (previously or simultaneously) with this AEC or any other AEC? What was the outcome of that application?

No

3.10 What statistical analysis has been undertaken to ensure that the use of the proposed number of animals will lead to a significant result? Please provide the details of statistical power calculations to justify the number of animals.

Data from the preliminary work is very limited, but suggested that insensibility lasted for 3-4 minutes. If we assumed a 1.5 minute (90 second) standard deviation around the mean, and assumed that one factor (e.g. gender, bull vs steer/heifer) impacted on the mean duration of insensibility such that bulls had a mean duration of 90 seconds and steers had a mean duration of 180 seconds, a z-test sample size calculation indicates that we would need 26 in each group to have a 95% power at P< 0.05 (output below). Thus, as we are considering a number of factors (gender [three levels: Bull; Cow; Steer/heifer], bodyweight [continuous data], breed type [two levels: Dairy; Beef]; energy [continuous data] and power setting [continuous data]), we estimate that 200 records will allow us to carry out a statistically robust analysis.

I - Sample Size Calculations for Means			
	Anticipated Values		
	Mean	Stan. Dev	
steers	180	90	Difference in m 50 %
bulls	90	90	

The cells in the table below show the estimated number of subjects needed in each group in order to demonstrate a statistically significant difference at "p" values ranging from 0.10 - 0.01and at varying levels of "power".
Power is the probability of finding a statistically significant difference at a given "P" value with the specified number of subjects in each group.

	Sample Size Needed in Each Group			
alpha level	**Power**			
("p" value)	**95%**	**90%**	**80%**	**50%**
0.10	22	17	12	5
0.05	26	21	16	8
0.02	32	26	20	11
0.01	36	30	23	13

Table for (Z1-alpha/2+Z1-beta)squarec				
	beta			
alpha	0.05	0.1	0.2	0.5
0.1	10.8	8.6	6.2	2.7
0.05	13	10.5	7.8	3.8
0.02	15.8	13	10	5.4
0.01	17.8	14.9	11.7	6.6

3.11 What methods have been proposed to reduce or minimise the number of animals required to achieve the objectives?

Our power analysis indicates that 200 animals will be sufficient to carry out a statistically robust analysis. The animals used are destined for slaughter for human consumption through this abattoir.

3.12 What steps have been taken to identify opportunities for the sharing of tissues or animals used in this research?

Note: Otlet (https://otlet.io/) is a tissue sharing website – consider if you would use a platform such as this to make tissues available for other researchers.

We will also use the opportunity to gather a bank of photographs, thermal images and video footage that could be used to present the technology to the Halal authorities, and, if safe, measure heart rate post stun (another valuable criterion for the Halal authorities).

The animals will be processed for human consumption and the product marketed under the normal commercial supply chain.

3.13 Clearly articulate the steps you have taken to consider and apply the 3R's

See https://teams.csiro.au/units/ARER/_layouts/15/start.aspx#/Advice%20on%20the%203Rs/Forms/AllItems.aspx for advice.

Replacement

No data sets are currently available to provide the information required *Reduction* Power analysis based on preliminary and pilot work has been used to assess minimum numbers required to provide a statistically robust analysis *Refinement* We could use electroencephalography (EEG) to monitor the time to return of consciousness, based on time to return to a baseline EEG pattern. However, this would require restraint of each animal in a crush for up to 10 minutes prior to slaughter, application of EEG pads or insertion of electrodes, and recording of a baseline EEG. All of which would add stress. Loss of corneal (blink) reflex is a cardinal sign of unconsciousness – when the blink reflex is absent, the animal is unconscious. The blink reflex is the first indicator that the EEG is starting to normalise, entering a transition stage, and the animal is beginning to return to consciousness. Blink reflex is used in abattoirs to assess each animal is unconscious prior to rolling it out of the box prior to exsanguination (bleeding), and is checked again during bleeding. If the blink reflex returns, a back-up stun is delivered (AMIC, 2009; Verhoeven et al., 2015; Von Holleben et al., 2010).

3.14 Please identify the funding source for this project.

The project is co-funded by Meat & Livestock Australia and Wagstaff Food Services

3.15 Identify any potential or actual conflicts of interest. (eg funding source, other relationships or affiliations).

Wagstaff Food Services is the organisation that has developed the DTS:Diathermic Syncope® system, and we are utilising the technology in their abattoir. We have full control of data analysis.

SECTION 4 THE ANIMALS TO BE USED IN THE RESEARCH

4.1 Please provide details of the species, sex and numbers of animals to be used during the project. Include 'target' and 'non-target' species.

Species	Common Name	Males	Females	TOTAL
Bos Taurus	Cattle	Mixed genders, actual number will not be		200 processed

		known until the animals are presented at the abattoir	using a traditional knocking box
Bos Taurus	Cattle	Mixed genders, actual number will not be known until the animals are presented at the abattoir	50 processed using a rotary knocking box
Combined Total			250

4.2 Provide the reason(s) for the choice of these animal(s).

The animals represent the normal slaughter ages and genders presented at abattoirs in the southern beef industry

4.3 Please state the source of the animals to be used in this project and the details of any Interstate import/export Permit numbers required to obtain the animals

The animals will be sourced from the abattoir's normal cattle supply chain.

4.4 Are you using privately owned animals?

Yes. If yes, go to http://my.csiro.au/Support-Services/Research-Ethics-in-CSIRO/Animal-Research-Ethics-in-CSIRO.aspx, download and complete the use of privately owned animals form and submit with this application.

4.5 Where will the animals be held or housed (location, room or pen number) during the project?

Location of study: Wagstaff Garfield Abattoir, Lovers lane, Garfield VIC 3814

Animals will be held in the paddocks adjacent to the abattoir prior to the study day (except bulls which will be held in the lairage, segregated from other cattle). In the paddocks they have access to water ad libitum, grass and supplementary hay will be given if the feed base is low. A day or two prior to the study, the animals will be moved into the lairage pens, where they will have access to water ad libitum, and hay will be provided until the morning of slaughter.

4.6. Describe the type of housing/caging to be used. Include flooring type, environmental enrichment provided and cage dimensions.

Lairage pens are approximately 100 square meters in area, with steel rail fencing and a grooved concrete non-slip floor. Water is provided ad libitum, and hay will be provided until the morning of slaughter. Cattle will be held in small groups of 6 – 20 animals so that they have social interaction with familiar peers.

4.7 What procedures and assessment will be undertaken to ensure that the animals have adapted to this location/housing prior to the commencement of research?

Animals will be held in paddocks and lairage for at least 4 days prior to slaughter to ensure they are adapted to the environment. The exception is bulls, as research has shown that bulls are adversely affected by changing accommodation, and the presence of unfamiliar animals in nearby pens can lead to fighting, even within stable social groups, and this can impact on the quality of the meat produced. Ideally bulls are slaughtered on the day of arrival at the lairage, or at least with 24 hours.

4.8 Do you expect animals to die or require humane killing as a result of any treatments used in this experiment or any handling involved in this experiment?

Yes

If yes, provide the specifics below:

Species	Number of animals	Likely cause. **Provide information to support this number.**
Cattle	250	Humane slaughter using DTS:Diathermic Syncope® as a pre-slaughter stunning method, with back-up penetrative captive bolt given prior

		to exsanguination using the ‘thoracic stick’ method.

4.9 Do you expect any animals to die or require humane killing as a result of any non-experimental factors during this experiment?

No

If yes, provide the specifics below:

Species	Number of animals	Likely cause. **Provide information to support this number.**

4.10 Acknowledgement of Animal Facilities Manager that appropriate facilities are available for the species and the estimated number of animals.

Signature of Animal Facilities Manager: ……………………………………………………………

SECTION 5. RESEARCH PROCEDURES, IMPACT ON ANIMALS AND MONITORING

(Standard Operating Procedures (SOPs) may be used in this Section. However, SOPs can only be referred to under the conditions set out in 2.2.33 of the Code)

5.1 Have the experimental procedures been approved previously by an Animal Ethics Committee? If yes, please quote name of the AEC, and original application number(s) and titles: *(Note - previous AEC approval of experimental procedures does not imply automatic re-approval. Procedures set out in this proposal will be assessed against the Committee’s current understanding of the procedures, relevant available technologies and public expectations.)*

Yes – CWLA AEC 2015-25 ‘*Pilot study on halal stunning of buffalo*’ and 2019-05 ‘Halal stunning of buffalo – validation study’ VIC DEDJTR WSIAEC 29.13 and WSIAEC 30.16

5.2 Does this activity involve severe compromise to animal well-being for which the 3Rs cannot be fully applied for the project to proceed (Code 2.7.4 (v))? That is:

Unrelieved pain and distress, including where planned endpoints will allow severe adverse effects to occur NO■

Death as an end point NO■

Reuse and repeated use of animals NO■

Prolonged restraint or confinement NO■

The use of non-human primates NO■

Particular justification is needed to warrant a YES response to any of the above. Please provide it below.

5.3 Give <u>details</u> of what will happen to the animals from the time they are sourced until the project is completed. *This must include a description of* ***all*** *procedures including how the animals will be handled, transported, sedated, anaesthetised, any surgical procedures, and the dose and route of all drugs, chemicals and biological agents to be used.* ***A time-line setting out the sequence of events from start to finish for individuals or groups of animals should be included****.*

Animals will be sourced by the abattoir using their normal procurement processes, either direct from farms or from local saleyards. Buyers will be instructed to source a range of animals of varying breed, gender and live weight.

The animals will be rested in lairage, or in paddocks adjacent to the lairage, for a period of at least 4 days, the exception being bulls, which will be slaughtered within 24 hours of arrival at the abattoir.

In this project we will use stopwatches to time the duration between induction of unconsciousness (collapse) and the first signs of recovering consciousness (return of the blink reflex) in 250 cattle of varying size and class, recording a range of animal parameters (body weight, gender, breed type), and technology parameters (energy and power settings). This will allow us to run a multivariate regression analysis to assess which (if any) of the factors strongly affect duration of insensibility; and will also allow us to estimate the mean and 95% confidence interval around duration of insensibility, in order to assess the potential suitability of the technique for the Halal market.

We will also use the opportunity to gather a bank of photographs, thermal images and video footage that could be used to present the technology to the Halal authorities, and, if safe, measure heart rate post stun (another valuable criterion for the Halal authorities).

Thermal imaging has been identified as important, because we have been asked if the skin surface is overheated (this was a feature of the very early work in which excessive energy applications were used, but this is not a feature of the commercial system).

The halal certifier has asked if we can process the animals using the halal exsanguination procedure. The client is in favour of this approach, as it opens the market for sale of the product sooner than anticipated.

Although a purist would argue that use of the halal exsanguination procedure would impact on the results gathered, because it is likely that the animal will die as a result of blood loss prior to the point of potential recovery, the proposed modification is more commercially relevant, as the reason for understanding duration of insensibility is to allay fears that under commercial conditions an animal that has undergone the halal exsanguination procedure might regain consciousness during the bleed phase. By closely monitoring all animals for signs of returning reflexes during the bleed phase, we can gather data that actually reflects the commercial situation, as opposed to generating predictive data. Thereby we ultimately **reduce** the number of animals used in the research, by removing the need for a further commercial validation phase; and also **refine** the protocol for the current project.

In order to ensure that animal welfare is not compromised as a result of using the halal cut (which is a slower bleed-out than using the thoracic stick method), we will process the first 50 animals using thoracic stick, to confirm that the duration of insensibility is greater than 2 minutes (the time required to allow the neck cut bleed-out to ensure death).

Protocol for the first 200 animals:

For each animal:

1. Animal moved to the restraint unit by a familiar stockperson. Stockpersons at the abattoir are trained in livestock handling and use low-stress techniques such as use of a 'flapper' instead of a stick or goad; use of flight-zone pressure and release; quiet, calm behaviour.
2. Restraint in DTS restraint box, identification recorded, pre-stun photograph and thermal image taken
3. Application of DTS, power and energy settings recorded
4. Behavioural monitoring of the stunning method by real-time video, as soon as the animal collapses, the stopwatch is started
5. Reflexes (corneal reflex and somatic withdrawal reflex) checked to confirm unconsciousness
6. Roll-out of unconscious animal, recheck reflexes every 5 seconds
7. Post stun thermal imaging and photograph

8. Heart rate and respiratory rate measurement if safe. This will involve either applying a digital heart rate monitor to the tail, or use of a stethoscope and watch. Operator safety is important – if the animal is convulsing, heart rate will not be measured.
9. As soon as a corneal reflex is noted, apply captive bolt, stop the stopwatch.
10. Exsanguination using thoracic stick
11. Reflexes re-assessed during exsanguination
12. Carcase processed according to commercial practice

Steps 1 to 10 will be completed for each animal, and the animal confirmed dead, prior to beginning work on the subsequent animal.

Protocol for the remaining 50 animals:

For each animal:

1. Animal moved to the restraint unit by a familiar stockperson. Stockpersons at the abattoir are trained in livestock handling and use low-stress techniques such as use of a 'flapper' instead of a stick or goad; use of flight-zone pressure and release; quiet, calm behaviour.
2. Restraint in DTS rotary restraint box, identification recorded, pre-stun photograph and thermal image taken
3. Application of DTS, power and energy settings recorded
4. Behavioural monitoring of the stunning method by real-time video, as soon as the animal collapses, the stopwatch is started
5. Reflexes (corneal reflex and somatic withdrawal reflex) checked to confirm unconsciousness
6. Roll-out of unconscious animal, recheck reflexes **and apply halal (neck) cut**
7. Post stun thermal imaging and photograph
8. **Recheck reflexes every 2 seconds during exsanguination**
9. Heart rate and respiratory rate measurement if safe. This will involve either applying a digital heart rate monitor to the tail, or use of a stethoscope and watch. Operator safety is important – if the animal is convulsing, heart rate will not be measured.
10. As soon as a corneal reflex is noted, apply captive bolt, stop the stopwatch and exsanguinate using thoracic stick
11. Reflexes re-assessed during exsanguination **until animal confirmed dead (dilated pupils)**
12. Carcase processed according to commercial practice

Steps 1 to **11** will be completed for each animal, and the animal confirmed dead, prior to beginning work on the subsequent animal.

5.4 Humane killing of animals.

a) What is the fate of the animals at the end of this research project?

Humane slaughter and processed for human consumption

b) If animals are to be humanely killed, please describe the method.

Animals stunned prior to slaughter using DTS:Diathermic Syncope®, then Penetrative captive bolt followed by thoracic stick exsanguination

c) If animals are to be humanely killed, how many animals will this effect?

250 animals – 100%

5.5 Identify *__all__* known and potential causes of adverse impacts on the wellbeing of animals from the time they are obtained until they are released, killed or no longer being used in the project. Explain how these will be avoided or minimised. This must include both experimental and non-experimental factors. Add more rows as required.

Known and potential causes of adverse impacts	Mechanism(s) to avoid or minimise
Stress due to mixing with unfamiliar animals	Animals will be kept in the social groups in which they are purchased; Bulls will be segregated into lairage pens
Stress due to adverse weather	Animals will be brought into the lairage pens if adverse weather conditions prevail.
Stress due to handling from paddocks to pens and from pens to restraint	Stockpersons at the abattoir are trained in livestock handling and use low-stress techniques such as use of a 'flapper' instead of a stick or goad; use of flight-zone pressure and release; quiet, calm behaviour. Tim Barwick will carry out the majority of animal care, with assistance from Mal Webster. Therefore, the animals will become familiarised to these two individuals.
Stress due to restraint	The restraint unit has been carefully designed to suit DTS application, and minimise animal stress. The animal will be restrained for the minimum period required (estimated 2 minutes maximum) The rotary restraint unit is in commercial use in a number of abattoirs in Australia, and will be modified to suit DTS application, i.e. installation of a shielded chin-lift device.
Recovery of consciousness	Corneal and palpebral reflexes will be monitored every 5 seconds post roll-out, and a

	penetrative captive bolt stun applied as soon as a reflex returns. Thoracic stick exsanguination will be carried out immediately after captive bolt application. When we are confident that the duration of unconsciousness is greater than 2 minutes (the time required to allow the neck cut bleed-out to ensure death), subsequent animals will be exsanguinated using the halal neck cut, and reflexes checked every 2 seconds during exsanguination. A penetrative captive bolt stun applied as soon as a reflex returns.

5.6 Monitoring: Detail how the wellbeing of the animals will be assessed throughout the project.

This _must_ *include the method and frequency of routine monitoring and additional monitoring after specific procedures such as inoculation or surgery. A copy of monitoring sheets must be included. Please add as Appendix 1 to this document. If this is not applicable to your project please note this here.*

Tim Barwick and/or Mal Webster will check the animals twice daily from arrival at the abattoir to the point of slaughter, according to the routine procedures at the abattoir.

During the study, unconsciousness will be assessed by experienced slaughter personnel, and experienced research staff. As the time interval between reflex testing is short (5 seconds), the process will be video recorded and voice recording used instead of monitoring sheets.

An example of the data collection sheets is presented in Appendix 1

5.7 Care: Detail the care provided to the animals to support their well-being. Include the specifics of any animal husbandry required during the project.

Animals will be held in the paddocks adjacent to the abattoir prior to the study day (except bulls which will be held in the lairage, segregated from other cattle). In the paddocks they have access to water ad libitum, grass and supplementary hay will be given if the feed base is low.

A day or two prior to the study, the animals will be moved into the lairage pens, where they will have access to water ad libitum, and hay will be provided until the morning of slaughter.

5.8 Treatment: What are the criteria for intervention, treatment or withdrawal of any animal from the project following identification of a problem? What implications will this have upon the outcome of the project?

In the event that an animal becomes unwell prior to the study, the abattoir has specific procedures involving notification of the supervising DAWR veterinarian, and taking action based on his/her advice. No animal will enter the study unless it has passed ante-mortem inspection within 24 hours of slaughter.

In terms of the project, there will be no impact, as numbers will be made up from the scheduled kill: specific individuals are not assigned to the study until the point of slaughter.

5.9 Emergencies: Identify the possible emergencies involving your research animals and set out your contingency plan for the management of these emergencies including the name of the veterinarian and/or veterinary practice that would be involved in the treatment of injured animals.

Emergency situations (e.g. an animal breaks a limb) are also handled according to the abattoir standard operating procedure, usually involving immediate slaughter and salvage or disposal of the carcase as not fit for human consumption, on the advice of the supervising DAWR veterinarian

5.10 Provide the name(s) of the person(s) who will be responsible for the routine and post-procedural monitoring and for dealing with emergencies.

Tim Barwick is responsible for day-to-day care of animals at the Wagstaff Garfield Abattoir.

5.11 In the case of an unexpected adverse incident resulting in an animal's death or humane killing, identify a source of expert advice regarding which samples need to be collected with a view to identifying the cause. This may be a member of the research team, a researcher at an alternate institute, a pathologist from a pathology service or CSIROs Animal Welfare Officer. Researchers must be aware of the requirements of both the AEC and the Code that a necropsy must be performed on any animals that die unexpectedly.

The supervising DAWR veterinarian is the first contact point. He/she is overseen by Dr Jason Ollington (Technical Field Officer, DAWR)

5.12 Provide acknowledgment that you are aware of, and will adhere to, the requirements of the Code and the AEC to investigate and report the cause of any unexpected deaths, unscheduled humane killing or any unexpected adverse welfare to your research animals. Acknowledge that the requirement of the Code and the AEC to perform post mortem examination by a competent person, on animals which die unexpectedly or are humanely killed for any reason which is not described in this project application will occur.

Requirements of such investigations could include taking photographs of abnormal findings, videos of unusual behaviour, biopsy and histopathology of tissues such as tumours and/or diagnostic sampling of blood, urine, faeces or other bodily fluids for clinical pathology.

I am aware that any deviations in animal welfare or 'expected' deaths described in this application constitute an unexpected adverse event. Such events will be reported to the AEC within 24 hours and a written report submitted within 7 days. I understand that a necropsy must be performed on animals that have died unexpectedly or been humanely killed unexpectedly. I understand that this is a requirement of the AEC and the Code.

Signature of PI - __ __________

5.13 Specify any special risks to other animals or humans arising from this project and the steps that will be taken to minimise these risks.

Bulls may fight – they will be kept in the social groups in which they were obtained, and segregated from other cattle;

Handling large livestock can be dangerous – the abattoir has facilities that are specifically designed to allow animals to be moved without the need for the handler to enter the pen with the animals; and if they do need to enter the pen, there are escape corners. Stockpersons are trained and experienced in handling animals; The DTS energy is potentially dangerous – the unit is fully enclosed in a faraday cage, and safety interlocks prevent the generator from operating if the faraday cage is not sealed

5.14 Is there a risk management plan in place that includes consideration of zoonosis?

Yes Both the CSIRO welfare team and the abattoir risk management plan and induction procedures highlight foodborne pathogens and Q-fever as potential zoonotic hazards. All personnel are vaccinated or certified immune to Q-fever; and all have been trained in good hygiene practices (e.g. washing hands properly on entering and leaving the processing area, before eating, and after visiting the bathroom). The abattoir supplies PPE (white shirt and pants, gumboots, hair/beard nets and gloves)

5.15 List all agents, drugs (generic), tranquillisers, anaesthetics and analgesics to be administered (add additional tables for each). NONE

Agent/drug	
Route of administration	
Dose rate	
Volume	
Needle gauge	
Frequency of administration	
Purpose and expected effect	
Have you used this previously?	
Possible adverse effects	

SECTION 6. JUSTIFICATION

(Note: The Code states [2.4.2] *Investigators must only consider using animals when they are satisfied that a case can be made that the proposed use is ethically acceptable, based on whether such use demonstrates the principles in Clause 1.1, and balancing whether the potential effects on the wellbeing of the animals involved is justified by the potential benefits [1.3].*

6.1 Provide information to support the case for the ethical acceptability of the proposed use of animals.

An estimated 80% of cattle slaughtered in Australian export abattoirs are slaughtered using the currently accepted Halal procedure, namely non-penetrating captive bolt stun followed by Halal neck cut. The non-penetrating captive bolt method is known to have a fairly high first-stun failure rate (up to 29% failure has been reported in literature), meaning that animals are not rendered insensible on first shot, and need to be re-stunned. This is a welfare concern.

Furthermore, there are increasing questions being asked by Halal authorities around the world as to the Halal-acceptability of non-penetrating captive bolt stunning, given the degree of brain haemorrhage, bruising and skull damage produced (one of the main tenets of the Halal slaughtering procedure being that the animas is whole and undamaged at the point of exsanguination).

Thus there is an urgent need to provide an alternative means of rendering the animal insensible prior to exsanguination (to ensure good animal welfare at slaughter), that is also Halal-acceptable.

DTS:Diathermic Syncope® may provide just such a method, but data on duration of insensibility and 'recoverability' is required to allow assessment of the method by Halal certification authorities.

The animals to be used in the proposed study will be processed for human consumption using DTS whether or not the study proceeds. We merely wish to add a delay to exsanguination (followed by immediate re-stun and rapid exsanguination) in order to measure the duration of insensibility.

6.2 Justify the impact on the animals to be used in this research project (as identified in response to Question 5.5), in terms of the predicted scientific value of the project.

The only impact on the animals over and above the normal slaughter process at the abattoir is a prolonged 'stun-to-stick' (stun to exsanguination) interval. This brings in the risk that animals will begin to recover from the DTS insensibility. However, this risk is mitigated by close monitoring of reflexes, and a captive bolt stun applied immediately a reflex returns (the reflexes return prior to consciousness returning). We believe, that with this mitigation step in place, animal welfare can be maintained while gathering vital data to present to the Halal certification authorities. Back-up stunning if a reflex is observed is a routine operation in abattoirs.

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Appendix 1

Animal monitoring sheets must be included here:

If this is not applicable to your project please note it here.

Animal Information sheet

Animal number	Breed/Type	Gender	Liveweight	Carcase Weight	Comments

Monitoring sheet

Animal number	DTS settings	Animal unconscious (Y/N)*	Time of collapse	Signs of recovery time (bolt applied)	Energy delivered	Reflectance	Comments

***Animal Unconscious**

A PASS score (Y) is gained when the animal is rendered unconscious, based on: absence of corneal (blink) reflex; absence of somatic (flinch) reflex; initial loss of posture; tonic (stiff) then clonic (kicking) epileptiform movements.
A FAIL score (N) occurs when the animal displays corneal (blink) and somatic (flinch) reflexes

Appendix 2

SCIENTIFIC PURPOSE FOR WHICH THE ANIMALS ARE USED

1. The Understanding of Human or Animal Biology: Using animals for activities that aim to increase the basic understanding of the structure, function and behaviour of animals and humans, and processes involved in physiology, biochemistry and pathology.

Examples:

- Molecular biology studies
- Studies of hormone levels for reproductive physiology

2. The Maintenance and Improvement of Human or Animal Health and Welfare: Activities that aim to produce improvements in the health and welfare of animals, including humans.

Examples:

- Animals used to develop a new diagnostic test for a disease
- Development of a painless method of spaying cattle
- Developing a new vaccine for animals or humans
- Production of biological products such as anti-sera, hormones and antibodies

3. The Improvement of Animal Management or Production: Activities that aim to produce improvements in domestic or captive animal management or production.

Examples:

- Developing an improved molasses/urea based supplement for cattle
- Determining optimum stocking rate for a pasture
- Evaluation of a calcium supplement for layer hens

4. The Achievement of Educational Objectives: Activities carried out for the achievement of educational objectives. The purpose of the activity is not to acquire new knowledge, rather to

pass on established knowledge to others. This would include interactive or demonstration classes in methods of animal husbandry, management, examination and treatment.

Examples:

- Animals used by veterinary schools to teach examination procedures such as pregnancy diagnosis or artificial insemination
- Sheep used in shearing demonstration classes for students; Dogs used to teach animal care to TAFE students;
- Animals used at pre-, primary or secondary schools or colleges; Rats and toads used in schools for dissection classes
- Animals used in agricultural colleges or schools to teach routine husbandry procedures

5. Environmental Study: Activities that aim to increase the understanding of the animal's environment or its role in it, or aim to manage wild or feral populations. These will include studies to determine population levels and diversity and may involve techniques such as collection of voucher specimens, radio tracking or capture and release.

Examples:

- Fauna surveys for environmental impact studies
- Research into methods to control feral animals

Appendix 3

CATEGORY OF PROCEDURE

Select a category from the list below. If animals are subjected to different categories of procedure simultaneously, you must record the highest impact category for the combined activity.

1. Observational Studies Involving Minor Interference

Animals are not interacted with or, where there is interaction, it would not be expected to compromise the animal's welfare any more than normal handling, feeding, etc. there is no pain or suffering involved.

Examples:

- Observational study only such as photographing whales at close quarters
- Pasture studies using grazing animals
- Breeding or reproductive study with no detriment to the animal
- Feeding trial, such as Digestible Energy determination of feed in a balanced diet.
- Behavioural study with minor environmental manipulation
- Teaching of normal, non-invasive husbandry such as handling, grooming, etc.
- Production of products such as hormones or drugs, in milk or eggs from animals which are subject to normal husbandry procedures only

2. Animal Unconscious Without Recovery

Animal is rendered unconscious under controlled circumstances with as little pain or distress as possible. Capture methods are not required. Any pain is minor and brief and does not require analgesia. Procedures are carried out on the unconscious animal that is then killed without regaining consciousness.

Examples:

- No experimentation on living animals e.g. animals killed painlessly for dissection, biochemical analysis, in vitro cell culture, tissue or organ studies

- Teaching surgical techniques on live, anaesthetised animals which are not allowed to recover following the procedure
- Live animals humanely killed for later scientific use, e.g. rats and toads for dissection
- Collecting blood or plasma from anaesthetised dogs prior to humane killing
- Animals killed for museum specimens

3. Minor Conscious Intervention Without Anaesthesia

Animal is subjected to minor procedures that would normally not require anaesthesia or analgesia. Any pain is minor and analgesia usually unnecessary, although some distress may occur as a result of trapping or handling.

Examples:

- Injections (not vaccination trials), blood sampling in conscious animal
- Minor dietary or environmental deprivation or manipulation, such as feeding nutrient-deficient diets for short periods
- Trapping and release as used in species impact studies, etc.
- Trapping and humane killing for collection of specimens
- Stomach tubing, branding, dehorning young animals, shearing, etc.

4. Minor Operative Procedures With Recovery

Animal may be rendered unconscious with as little pain or distress as possible. A minor procedure such as cannulation or skin biopsy is carried out and the animal allowed to recover. Depending on the procedure, pain may be minor or moderate and post-operative analgesia may be appropriate. Field capture using chemical restraint methods is also included here.

Examples:

- Biopsies
- Cannulations
- Sedation/anaesthesia for relocation, examination or injections/blood sampling

5. Surgery With Recovery

Animal may be rendered unconscious with as little pain or distress as possible. A major procedure such as abdominal or orthopaedic surgery is carried out and the animal allowed to recover. Postoperative pain is usually considerable and at a level requiring analgesia.

Examples:

- Orthopaedic surgery
- Abdominal or thoracic surgery
- Transplant surgery
- Mulesing, castration without anaesthesia

6. Minor Physiological Challenge

Animal remains conscious for some or all of the procedure. There is interference with the animal's physiological or psychological processes. The challenge may cause only a small degree of pain/distress or any pain/distress is quickly and effectively alleviated.

Examples:

- Minor infection, minor or moderate phenotypic modification, early oncogenesis
- Arthritis studies with pain alleviation
- Prolonged deficient diets, induction of metabolic disease
- Polyclonal antibody production
- Antiserum production
- Vaccination trials

7. Major Physiological Challenge

Animal remains conscious for some or all of the procedure. There is interference with the animal's physiological or psychological processes. The challenge causes a moderate or large degree of pain/distress that is not quickly or effectively alleviated.

Examples:

- Major infection, major phenotypic modification, oncogenesis without pain alleviation
- Arthritis studies with no pain alleviation, uncontrolled metabolic disease
- Isolation or environmental deprivation for extended periods
- Monoclonal antibody raising in mice

8. Death As An Endpoint (not humane killing)

This category only applies in those rare cases where the death (rather than humane killing) of the animal is a deliberate measure of the data collection phase of the activity. Where the investigator or teacher will not intervene to kill the animal humanely before death occurs in the course of the scientific activity.

Death as an end-point does include:

- Lethality testing (LD50, LC50);
- Toxicity testing with death as a planned end-point without humane killing;
- Dose rate studies for feral animal control; or
- Disease studies in which it is planned that animals will die.

Death as an end-point does not include:

- death by natural causes (incidental to the scientific use);
- animals which are humanely killed on completion of the project;
- animals which are humanely killed as a result of an unexpected adverse event;
- animals humanely killed for dissection or for use as museum voucher specimens; or
- accidental deaths.

Where predictive signs of death have been determined and humane killing is carried out before significant suffering occurs, they may be placed in category 6 or 7.

8.3Appendix 3 - Preliminary confirmation of previous science outcomes

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DTS: Diathermic Syncope – commercial validation trials

Prototype 4 validation report

Alison Small, Leisha Hewitt, Dominic Niemeyer, Sue Belson, Troy Kalinowski and James Lea

19th April 2018

Wagstaff Food Services

Contents

Executive Summary

A total of 20 cattle were processed using Prototype 4 of the DTS: Diathermic Syncope system, under Victorian State Government Wildlife Animal Ethics Approval 30.16. Energy applications ranged from 200 kJ to 360 kJ. Seventeen were assessed as insensible following DTS application. These animals demonstrated behavioural and EEG signs consistent with an electrical stun, loss of posture occurring between 1 and 8 seconds after onset of energy delivery. In 4 animals, which received 300-360 kJ, this insensibility progressed to death. In the remaining 13 animals, absence of corneal reflex persisted for between 100 and 170, at which point captive bolt was administered in case of returning consciousness. When these timeframes are compared with those reported for head-only electrical stunning in cattle (application of 4 to >20 sec, duration 31 to 90 seconds), it is evident that DTS provides a better welfare outcome.

For each of the three animals that were not deemed insensible, there had been problems with maintaining contact between the waveguide and the forehead, resulting in leakage of energy into the environment, rather than penetration into the brain. All three appeared partially conscious, with loss of eye focus and no visual following of movement, and a slow response to a touch on the cornea. All three were stunned using captive bolt immediately following assessment of consciousness.

Of the 20 animals processed, 11 EEG recordings were generated: the post-DTS recording was not collected from animal 2 due to accidental shut-down of the laptop running LabChart, and in animal 13, corneal reflexes were present and the animal was stunned using the back-up captive bolt shortly after opening of the faraday cage, prior to EEG recording. For animals 14 to 20, baseline EEG was not recorded, and post-DTS EEG was recorded on the bleed-conveyor during exsanguination. Thus, baseline EEG for normalisation was not present, and the post-DTS EEG could only be used qualitatively. In the event, once movement artefact was removed, there were very little EEG data available for animals 15-20 prior to the return of the corneal reflex and captive bolt application. All the animals for which usable EEG recordings were collected showed changes from baseline, particularly in terms of Ptot, while for animal 14, for which EEG data was collected post-DTS only and captive bolt was not applied, high amplitude spiking of Ptot was observed. These patterns on EEG are considered to be incompatible with sensibility.

Thermal imaging indicated that the average surface temperature on the forehead at the point of loss of posture would be between 26 and 49 °C, although some animals (animals 6 and 7) that showed a delayed loss of posture also demonstrated hot-spots of up to 109 °C. In context, Australian cattle may experience ambient air temperatures of 45 °C in summer, and could experience higher surface temperatures when standing in in direct sunlight. Surface temperatures above 50 °C may be uncomfortable to cattle, although no literature on thermal contact sensitivity in cattle could be located to confirm or refute this supposition, while temperatures above 60 °C will result in skin tissue damage, as seen in hot branding which is commonly used for identification of cattle.

During the course of the trial, a number of modifications to the waveguide positioning apparatus were made, with the aim of improving contact and reducing leakage. Some potential adjustments to the restraint system, to improve animal handling and reduce the risk of leakage were also identified.

The results gathered were confounded by problems with waveguide to forehead contact, resulting in loss of energy to the environment instead of being transmitted into the brain. This variability in energy delivery may account for the variability in latency to loss of consciousness, using loss of posture as the proxy indicator, and problems with waveguide to forehead contact may have contributed to the variability in surface temperatures and the presence of hot spots on the forehead.

In conclusion:

- DTS Prototype 4 successfully rendered cattle insensible based on physical (reflex) and EEG data;
- Prolonged duration of insensibility suggests that DTS may be better than the existing commercially available head-only electrical stunning method for cattle;
- Some engineering modifications are required to ensure consistent delivery of energy to the brain and minimise leakage.

We recommend that the next stage of development is to carry out engineering modifications, such that consistent delivery of energy is achieved prior to attempting statistically representative studies. This development activity will require use of live animals to test the modifications for evidence of leakage, and the data collected during this development will contribute to the body of evidence required to bring the technology to a commercially viable form.

1 Introduction

Wagstaff Food Services Pty Ltd and Advanced Microwave technologies have designed a system for rendering an animal unconscious prior to slaughter, using radiofrequency electromagnetic energy (PCT/AU2011/000527). The mechanism of action is by selectively increasing the temperature in the brain to the point that hyperthermic syncope (fainting) occurs. Thermal unconsciousness such as that induced by exercise heat stress or fever is reported to occur when core body temperatures reach between 40 and 45°C (McDaniel, Jenkins et al. 1991; Ohshima, Maeda et al. 1992; Mohanty, Gomez et al. 1997; Roccatto, Modenese et al. 2010; Lerman, Bruchim et al. 2014; Yoshizawa, Omori et al. 2016). A pilot study carried out in 2014 indicated that DTS provides a humane method of inducing insensibility prior to exsanguination, of sufficient duration to allow exsanguination prior to recovery, and produces comparable post slaughter meat quality and physiological responses in treated cattle to those stunned using penetrative captive bolt (Small, McLean et al. 2015). However, energy leakage occurred when the animal convulsed on entering the insensible state, leading to activation of the generator safety cut-out, and incomplete delivery of energy to the brain. Subsequent to the pilot study, the restraint, head capture and waveguide set-up have been re-engineered to improve animal handling and restraint, and to limit energy leakage and automatic cut-out of the generator. The trial described in this report aims to validate the efficacy of the re-engineered system, termed 'Prototype 4', prior to the conduct of more extensive commercial scale studies and statistically robust assessments. The protocol and conduct of the experiment was approved by the DEDJTR Wildlife and Small Institutions Animal Ethics Committee under the VIC Prevention of Cruelty to Animals Act, 1986 (Animal Research Authority 30.16).

2 Method

2.1 Data collection

DTS Prototype 4 was installed and commissioned at Wagstaff Garfield Abattoir, VIC. Twenty cattle were randomly selected from a group of cattle held at the abattoir premises, at pasture adjacent to the abattoir, with access to the lairage pens for shelter. Selection of animals was based merely on drafting a smaller group from the main mob, by allowing five or 6 animals to walk into the lairage pens, and then turning the remainder back into the pasture. Subsequently, the order in which animals were processed was the order in which they presented themselves to the race. The cattle were of mixed breed, predominantly dairy crosses, some were aged cull cows, and others were poor-quality dairy cross steers. Age and gender of individuals was not recorded as part of the protocol for this validation trial.

A total of 20 cattle were processed during the period 4th – 6th October 2017, using Prototype 4 of the DTS: Diathermic Syncope system, under Animal Ethics Approval 30.16, Part 1. Energy application began at 360 kJ, delivered using a power setting of 30 kW, and when each energy setting was confirmed to induce insensibility, the energy setting was sequentially reduced by increments of 25 kJ throughout the 20 animals, with a return to higher energy levels at the beginning of each processing day such that the settings applied were: one animal at 360 kJ, five at 300 kJ, five at 275 kJ, two at 250 kJ, five at 225 kJ, and two at 200 kJ. Towards the end of the study, power settings of 20 kW were attempted, with the total energy delivery set at levels (275 kJ and 200 kJ) that had produced insensibility when delivered using a power setting of 30 kW and finally one animal was processed using an energy delivery setting of 200 kJ and power setting of 15 kW.

Cattle were brought individually to the restraint unit. For the first three cattle, Electroencephalogram (EEG) recording pads were applied in the restraint crush in the lairage, and a baseline EEG recording taken. It was subsequently decided that it would be more efficient, and less stressful to the animal if the pads were applied and the baseline EEG recorded when the animal was restrained in the DTS restraint unit, obviating the need for a second handling event. This then was the procedure for animals 4-20. For application of DTS, the cattle were individually restrained in a stun box with neck yoke, head capture and chin lift (Figure 1), all of which was fully enclosed in a faraday cage.

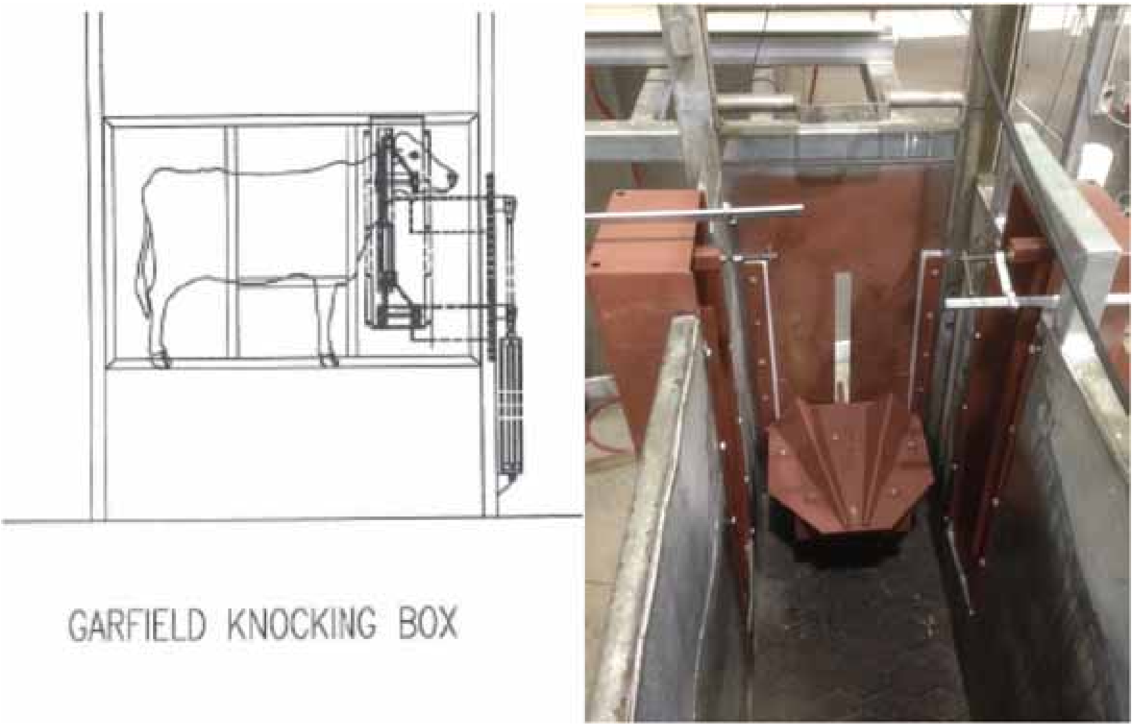

Figure 1: Engineering concept diagram of restraint unit (left), photograph of chin lift unit, taken during installation, prior to enclosure with faraday cage shielding (right).

EEG data was captured using a single channel, bi-hemispheric, four-electrode EEG montage (ground electrode on a bony prominence on one side of the poll; common reference electrode midline on the nose, midway between the nostrils and the eyes; and the inverting electrodes on the each frontal bone, between the eye and the poll). RedDotMini electrode pads (3M Australia, North Ryde, NSW) were applied to the head, with good contact achieved by soaking the hair below the pad in superglue (Loctite 454, Loctite Australia, Caringbah, NSW). The EEG was recorded, using PowerLab and LabChart (ADInstruments, Sydney, Australia) for a period of up to 2 minutes, or until a stable, clear signal lasting for 30 seconds was observed on the screen, whichever occurred first, prior to application of the energy, and again for up to 90 seconds or until a stable, clear signal lasting for 30 seconds was observed on the screen prior to sticking, unless the animal was deemed to be regaining consciousness, in which case a back-up stun was delivered. EEG traces were qualitatively assessed during the course of the trial.

Following collection of baseline EEG data, the EEG leads were removed, because there is a risk of damage to the equipment if excess energy leakage occurs, and a baseline image taken using an infrared thermography (IRT) camera (ThermaCam T640, FLIR Systems AB, Danderyd, Sweden). The waveguide was then positioned on the fore head at the application point (Figure 2), checked for fit, the faraday cage shielding fastened, and personnel evacuated the immediate area in case of shielding failure.

Figure 2: Waveguide positioned on forehead of animal. White disks are EEG recording pads, blue container is a water reservoir for absorption of stray energy, nose 'comforter' has a cushioned contact area.

DTS was then applied to a pre-determined total energy delivery, at a defined power setting, which varied for each individual. Immediately the generator deactivated following energy application, the faraday cage was opened, the waveguide removed and the animal visually assessed for signs of distress (and in the event that distress was evident, which did not occur, the instructions were to immediately apply captive bolt). Corneal reflex was tested, a post-application thermal image taken, and the EEG leads reattached. Post-application EEG was recorded, with corneal or somatic withdrawal reflex tested at 30-second intervals. When 90 seconds (or 30 seconds of stable, clear signal was observed on the screen) of EEG had been recorded, the leads were again removed, and the body released from the restraint unit onto the bleed conveyor, where it was rodded (the oesophagus sealed), and exsanguinated using the 'thoracic stick method' which severs the common carotid artery close to the thoracic inlet. If signs of returning consciousness were detected, namely the return of corneal reflex or eye focus and following movements, the animal was stunned using captive bolt, regardless of where in the process it was – i.e. if it was still in the restraint unit, it was stunned, then rolled out before exsanguination; if it was on the bleed conveyor, it was stunned there and exsanguinated immediately if this had not previously begun. Once the body was exsanguinated, it was processed and inspected for human consumption according to the normal practices at this abattoir.

During application of DTS, the animals were monitored using real-time video capture through a security camera system (Dahua HCVR4108HS-S3/8, Zhejiang Dahua Technology Co. Ltd, China) with one camera positioned over the animal's head within the head capture unit (feeding two channels in case one failed), one over the body within the restraint box unit (feeding two channels in case one failed), one over the control panel, and two over the bleed conveyor (Figure 3). Observations on animal reaction were recorded from the video footage in real time, then subsequently footage was played back at reduced speed (up to 16 times reduction) in order to prepare a detailed event log for each individual.

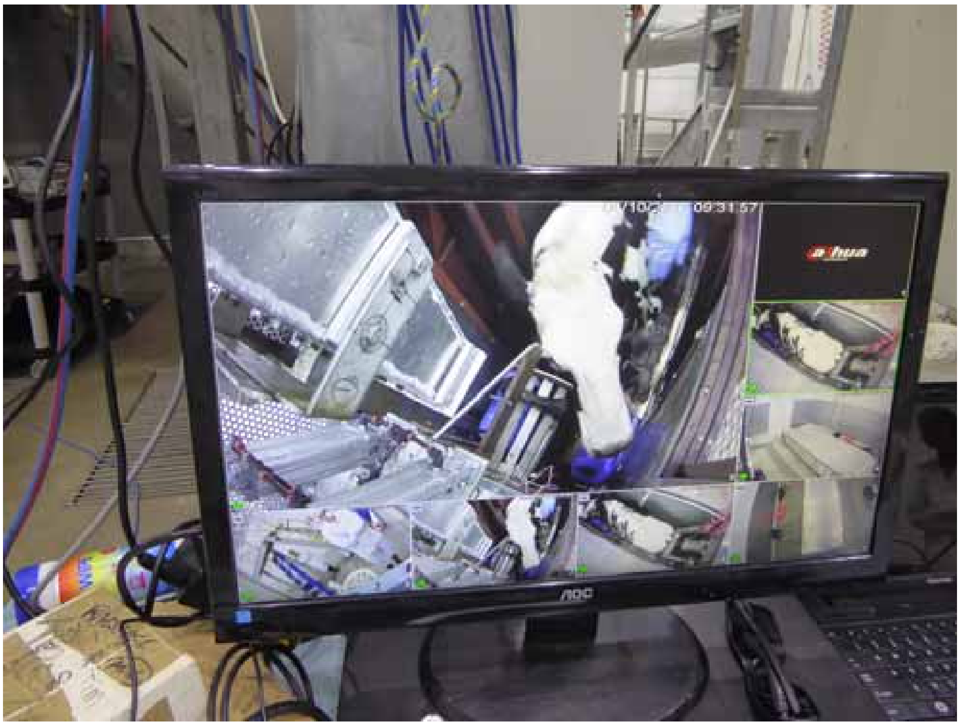

Figure 3: A screen shot of the real-time video footage.

2.2 EEG data analysis

The EEG data were analysed offline using LabChart 8 (ADInstruments, Sydney, Australia). Artefacts were identified and rejected manually, with reference to video footage to identify event-related artefact (e.g. animal movements, eye/ear movements, personnel movement or movement of leads), and the first and last two seconds of each recording were removed to eliminate edge artefacts. Heavily contaminated recordings, and recordings in which poor electrode contact was present were discarded in entirety. A total of 11 usable recordings were generated: the post-DTS recording was not collected from animal 2 due to accidental shut-down of the laptop running LabChart, and in animal 13, corneal reflexes were present and the animal was stunned using the back-up captive bolt shortly after opening of the faraday cage. For animals 14 to 20, baseline EEG was not recorded, and post-DTS EEG was recorded on the bleed-conveyor during exsanguination. This change to protocol allowed an assessment of the time required to remove the unconscious animal from the restraint and exsanguinate, an important aspect for fit to the commercial environment. However, it meant that a baseline EEG for normalisation was not present, and the post-DTS EEG could only be used qualitatively. For illustrative purposes, Total EEG power was converted to decibel change from lowest recorded value to bring it into a form that could be visually compared with traces from the other animals processed. In the event, the post-DTS EEG was heavily contaminated by artefact from the conveyor mechanism and from running water in a nearby drain, and once movement artefact was removed, there were very little EEG data available for animals 15-20 prior to the return of the corneal reflex and captive bolt application.

A band-pass filter of 0.1 to 30 Hz was applied to the raw data, and the Spectral Analysis Package within LabChart 8 was used to apply Fast Fourier Transformation (FFT), with multiplication using a Hann window in 1-second epochs with a 25% overlap. Total power (Ptot), median frequency (SEF50) and 95% Spectral Edge frequency (SEF95) were extracted. The median value of Ptot in the pre-DTS recording was calculated and this was used as the baseline value. Baseline normalization was then carried out by transforming data for each 1-s epoch into decibel change from baseline according to the formula: *dB= 10*log10(value/baseline)*, to bring all data sets into a comparable format. These data, and data for F95 and F50 were charted and inspected for EEG suppression and epileptiform activity, and where possible time to resolution of EEG suppression was recorded.

2.3 Thermal image analysis

Image data were imported into ResearchIR (FLIR Systems AB, Danderyd, Sweden) for analysis. On each image, the forehead area was delineated to exclude the ears, eyes and muzzle area (Figure 4). The minimum, maximum and average temperatures within this area and the number of pixels represented by the delineated area was returned from the software. For post-DTS images, the image was then manipulated to show only areas where the surface temperature was greater than 45 °C (Figure 5); greater than 50 °C (Figure 6) or greater than 60 °C (Figure 7) and the number of pixels represented by each of these subsets returned from the software, and these were expressed as a proportion of the number of pixels in the delineated forehead area. Pre and post-DTS minimum, maximum and mean temperatures were used along with the total energy delivered as recorded by the DTS control software to generate a 'temperature change by energy delivered' chart for each individual (example in Figure 8). Comparing 'total energy delivered by time' from the DTS control software with video annotation of behaviours allowed an estimation of the energy delivered at the point of loss of posture, and the associated surface temperature at that point could then be estimated from the 'temperature change by energy delivered' chart.

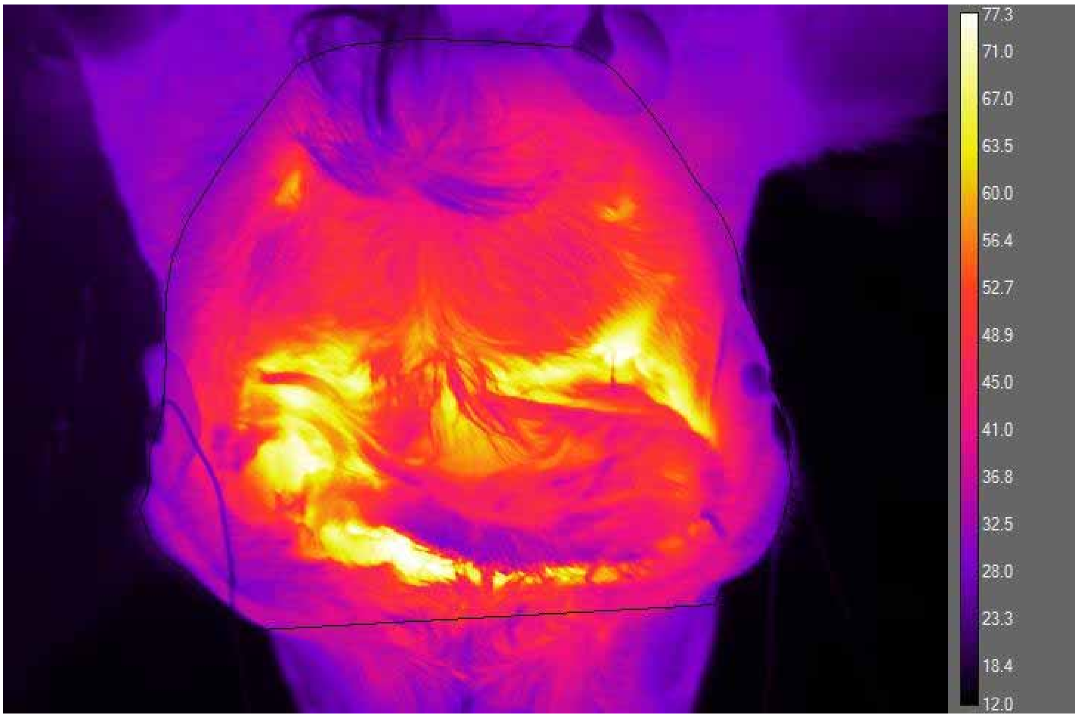

Figure 4: Example of delineation of forehead on thermography image: Animal 15 post DTS, having received 310.4 kJ in total.

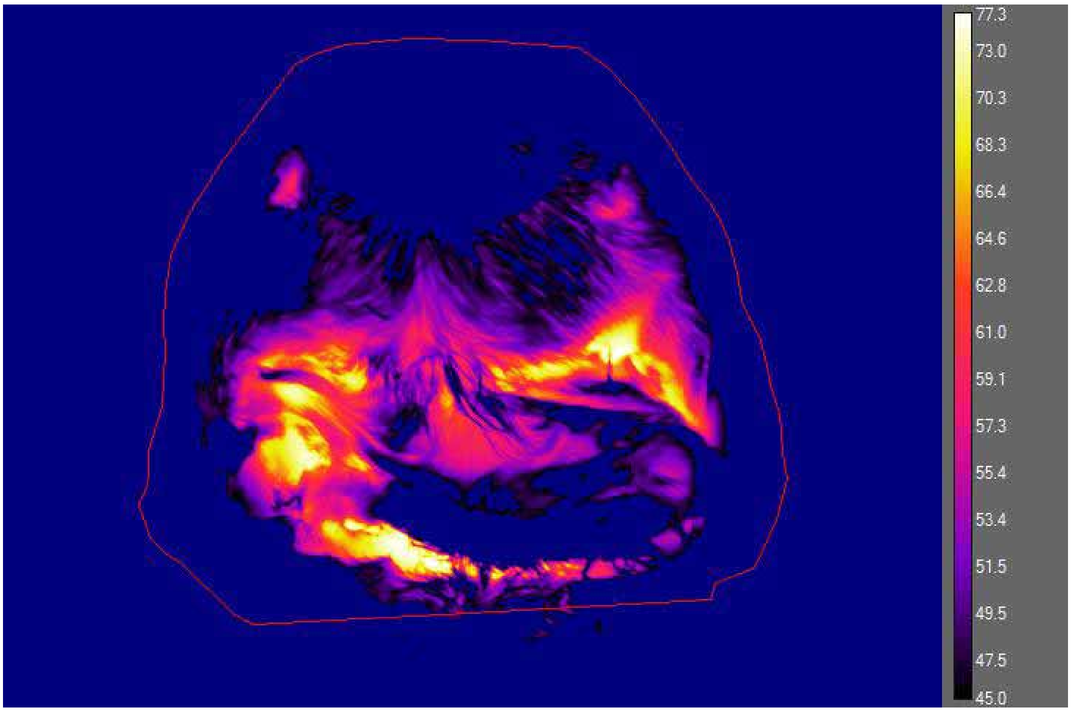

Figure 5: Animal 15 post DTS, thermal image showing areas with a surface temperature of > 45 °C.

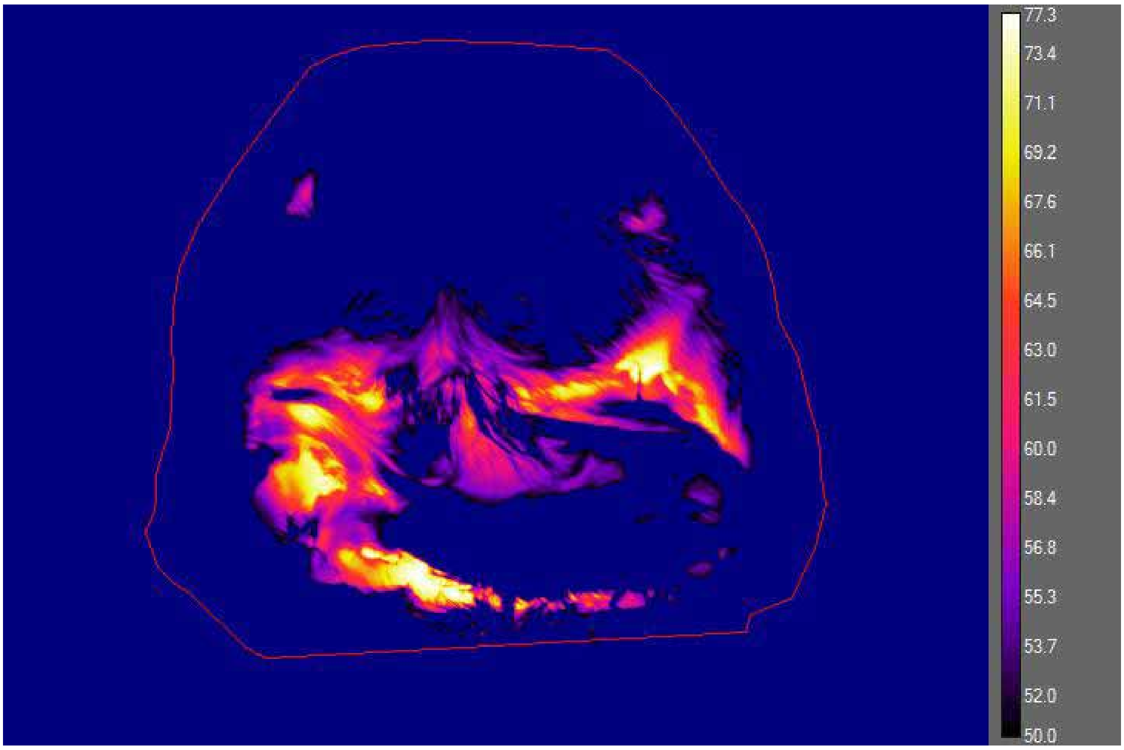

Figure 6: Animal 15 post DTS, thermal image showing areas with a surface temperature of > 50 °C.

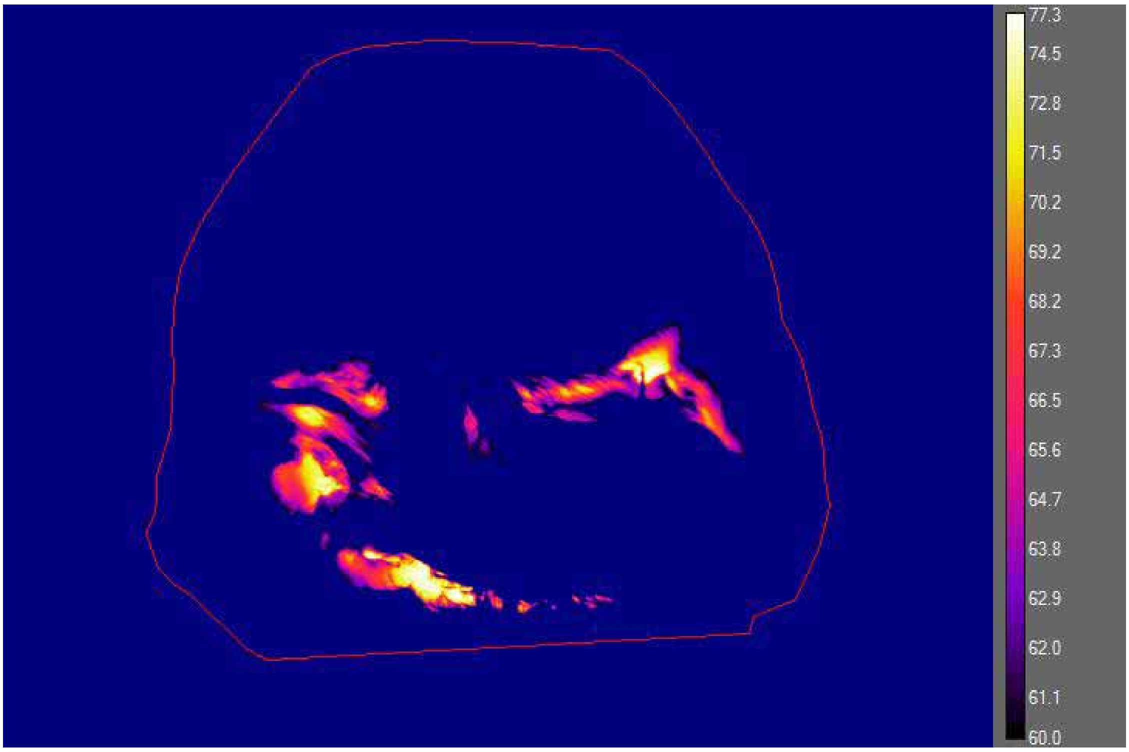

Figure 7: Animal 15 post DTS, thermal image showing areas with a surface temperature of > 60 °C.

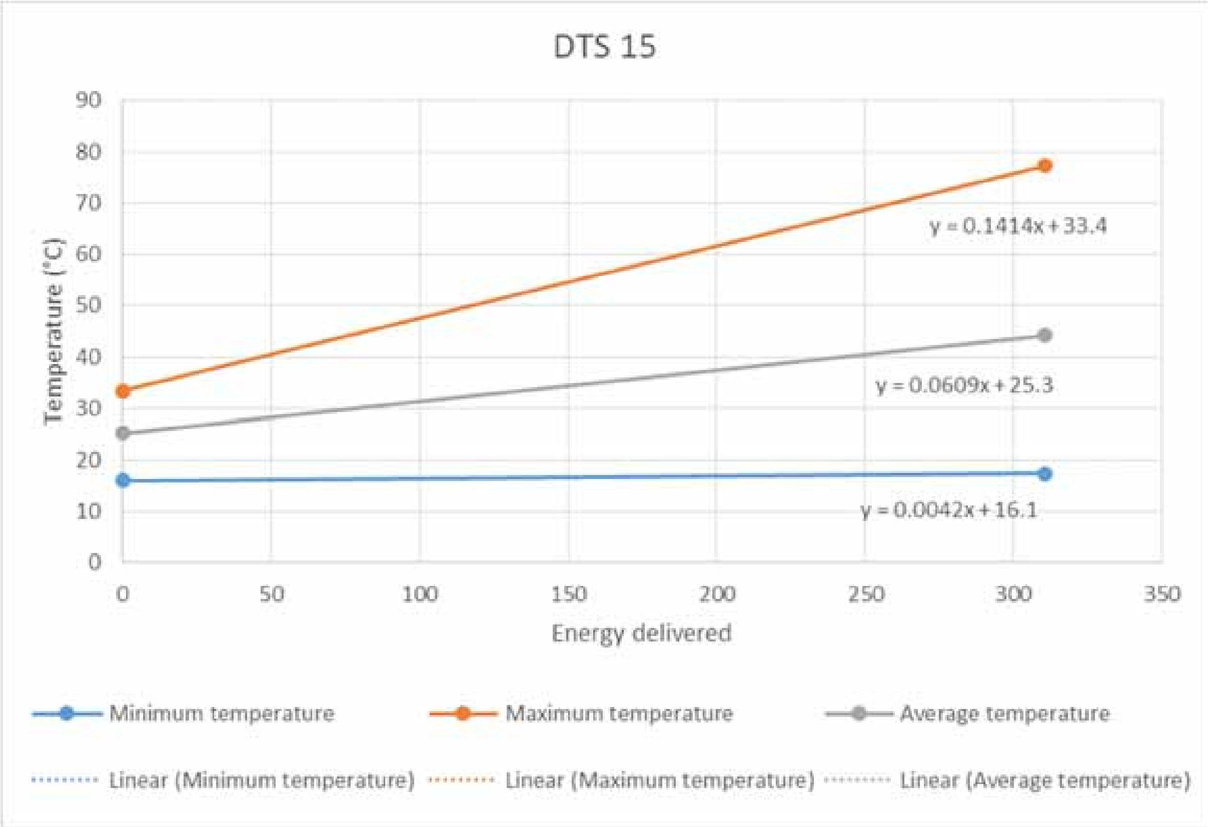

Figure 8: Animal 15 estimated temperature change on the forehead by energy delivered.

3 Results and Discussion

Detailed information on each animal is presented in the Appendix and a summary of data collected is shown in Table 4.

3.1 Real-time observations

Notes recorded for each animal at the time of DTS application are presented in the Appendix.

Of the 20 cattle processed, 17 were assessed as insensible following DTS application, based on loss of posture and loss of corneal and somatic withdrawal reflexes (Von Holleben, Von Wenzlawowicz et al. 2010). These animals demonstrated behavioural signs consistent with an electrical stun – rapid blinking or flicking of the eyelids including the membrane nictitans (third eyelid), loss of posture, tonic (stiff) and clonic (convulsive) phases. Loss of posture occurred between 1 and 8 seconds after onset of energy delivery. In 4 animals, which received 300-360 kJ, this insensibility progressed to death. In the remaining 13 animals, absence of corneal reflex persisted for between 100 and 170 seconds, at which point captive bolt was administered in case of returning consciousness. For each of the three animals that were not deemed insensible, there had been problems with maintaining contact between the waveguide and the forehead, resulting in leakage of energy into the environment, rather than penetration into the brain. All three appeared partially conscious, with loss of eye focus and no visual following of movement, and a slow response to a touch on the cornea. All three were stunned using captive bolt immediately following assessment of consciousness.

As the behavioural signs obtained using DTS are similar to those produced by electrical stunning (Table 1), it is of value to compare the outcome of DTS against those reported for electrical stunning. In cattle, electrical stunning can be applied as head-only, which affects the brain, resulting in an epileptiform seizure, from which the animal can regain consciousness; or as head-to-back (or head-to-chest), which incorporates a current passed through the heart, resulting in cardiac arrest, and the death of the animal. As DTS does not directly affect cardiac function, the comparison should be against head-only electrical stunning.

The biggest problem with head-only stunning of cattle is a very short duration of epilepsy, followed by strong convulsions (EFSA 2004). The electrical current must be applied across the brain for sufficient duration to overcome the inherent impedance (resistance) across the head, and applications ranging from 4 to greater than 20 seconds (Devine, Tavener et al. 1986; Vonmickwitz, Heer et al. 1989; Schatzmann and Jaggin-Schmucker 2000). The duration of unconsciousness in these studies was reported as 31 - 90 seconds. In contrast, DTS induces loss of posture within 8 seconds of onset of energy application, and the duration of unconsciousness recorded during the current study was between 100 and 170 seconds, based on absence of corneal reflex.

Table 1: Behavioural indicators of unconsciousness following DTS application, as compared with electrical stun.

Behavioural sign	*DTS*	*EFSA Scientific Opinion 2004*	*AMIC animal welfare standard*
Immediate collapse	☑*	☑*	☑*
Epileptiform seizure (described in detail)	☑†	☑	☑
Lack of normal rhythmic breathing	☑#	☑	☑
No spontaneous eye blinking	☑		☑
Gasping (breathing in without breathing out) sometimes occurs			☑
Upward rotation of eyes		☑	
Dilated pupils		☑	
No response to nose prick (painful stimulus)	☑	☑	

*: With regards to induction of insensibility prior to slaughter, there is much discussion over the 'immediacy' of the lapse into unconsciousness. in electrical stunning, the current must flow for a period of at least 2 (sheep) or 5 (cattle) seconds in order to ensure a sustained loss of consciousness (Warrington 1974; Von Holleben, Von Wenzlawowicz et al. 2010).

†: It was difficult to assess epileptiform seizure in the current study, due to the positioning of the animal in the restraint unit and the need to capture EEG recordings prior to rolling the body onto the bleed conveyor. However, it did appear that a tonic phase would occur following collapse, and convulsive movements were evident in some animals after roll-out.

#: In the current study, total absence of respiration was evident in some animals, but not all. However, when respiration was present it was much slower and deeper than normal.

From the DTS control panel, it appeared that loss of posture occurred at around 140-150 kJ of energy delivered, in those animal in which there was minimal leakage. Unfortunately, there was a glitch in the software that resulted in the cumulative energy curves not being saved in real time, so we could not interrogate the data offline, in true alignment with the video footage. However, the cumulative energy delivered could be re-charted from reflected and forward energy data stored by the DTS control software and post-hoc estimations of energy delivered at the time of loss of posture could be estimated using data from video footage (section 3.2). The work on anesthetised cattle, carried out by Rault et al. (2013), indicated that shorter durations of applications resulted in more rapid onset of EEG suppression, while greater power settings resulted in a longer duration of insensibility. This is difficult to interpret fully, based on the small numbers processed in their study, but there is a relationship between power setting and rate of heating. The units of power (kW) is shorthand for 'kJ per second', so a higher power setting will reduce the time required to reach an energy delivery of 150 kJ than a lower power setting. However, the higher power setting may predispose to focal overheating. Animal 20, which was not deemed unconscious, was processed using a total energy setting of 200 kJ, at 15 kW. Unfortunately, there was energy leakage during application, so it is difficult to ascertain whether the brain did receive 150 kJ (which would be expected to induce unconsciousness), but if it did receive the required energy dose, perhaps 15 kW was heating too slowly, so that the brain circulation could cool the brain sufficiently to overcome the heating effects. Indeed, this was the only animal for which a withdrawal response was observed, suggesting that it was aware of DTS application.

Unfortunately, as a result of leakage issues, we were unable to test the hypothesised 150 kJ 'critical value' in the current study. When contact issues have been resolved, it will be important to test this energy application, but in the interim period, while engineering modifications are underway, we would recommend continuing with energy deliveries of 200-250 kJ. Another aspect that is important to test is use of variable power profiles during energy application – for example starting with a high power to achieve the rapid loss of posture, but then completing the cycle using a lower power to ensure even heat distribution throughout the brain, minimising focal overheating and ensuring prolonged insensibility may be optimal. Conversely, it may be more appropriate to 'prime' the brain temperature with a short period at lower power, then complete the cycle at higher power. Again, testing of alternative power profiles should occur after the contact issues have been corrected.

During the course of the trial, a number of modifications to the waveguide positioning apparatus were made, with the aim of improving contact and reducing leakage. After the pre-trial testing using cadaver heads, the waveguide was rotated through 90 degrees, such that the widest part of the aperture lay across the forehead rather than along the length of the forehead. This was because, when the head were opened and the brain inspected, it was felt that the energy application was focused a little too far forward, affecting the frontal lobes and perhaps the ethmoid apparatus (the sensory part of the nose), rather than being focused into the bulk of the brain tissue. Excessive energy leakage and movement of the waveguide relative to the forehead was identified on animal 5, so the attachment that located the waveguide with respect to the poll was adjusted to improve the fit. On animals 12 and 13, a waveguide with additional tuning baffles to increase efficiency was fitted, but excessive leakage occurred, so the original waveguide was re-installed for animals 14 to 20.

Some potential adjustments to the restraint system, to improve animal handling and reduce the risk of leakage were also identified. It was noticed that the animal could move around excessively in the restraint box, and the flooring, although checker-plate steel, became slippery during processing. Suggestions were aired with regard to having a 'squeeze' type box, which would clasp and support the body more closely, without applying undue pressure; or a belly sling. A belly sling had been utilised in the assessment of Prototype 2, in the anaesthetised animal trial carried out by Rault et al. (2013), but this had been removed when conscious animal testing began. The absence of the belly sling allowed easy identification of loss of posture, but the changing neck-head angle resulted in energy leakage and arcing. During the course of the current study, a metal plate was attached to the chin lift in an attempt to support the neck and reduce the movement on loss of posture. However, the plate may have resulted in compression of the larynx in some animals, as two were observed to produce noise on exhalation when in the collapsed position.

3.2 Video annotation

Video footage was played back at reduced speed (up to 16 times reduction) in order to prepare a detailed event log for each individual. The event log for each individual is presented in the Appendix. A summary of the key time intervals associated with indicators of loss of consciousness and duration of insensibility identified are shown in Table 2 below. Loss of posture, which is considered to be a definitive indicator of unconsciousness (EFSA 2004; Muir 2007; Von Holleben, Von Wenzlawowicz et al. 2010; EFSA 2013), occurred between less than 1 second from DTS application to 19 seconds after the onset of DTS application. It is unclear from the current data set whether leakage of energy contributed to the more prolonged intervals from onset of DTS application to loss of posture, but it could be expected that leakage of energy resulted in slower heating of the brain, and thus a delay in loss of posture. Furthermore it was also unclear if the early loss of posture seen in some animals (1-2 seconds post DTS application) was indeed loss of consciousness, or merely loss of footing on the slippery floor of the restraint box. A number of animals (12 in total, all within the first 13 animals) returned to standing after initial loss of posture. This occurred between 5 and 144 seconds after loss of posture (mean 50.75; median 25.5 seconds), often prior to roll-out as the animals remained in the restraint for post-DTS EEG capture. The animals were considered unconscious due to lack of reflex responses, and the EEG data confirms this. Thus, it is likely that the 'return to standing' is actually the body entering the tonic (stiff) phase of the stun, and because it is still within the restraint box, when the legs extend firmly, the only position that can be assumed is apparent standing.

Return of reflexes are considered to be the earliest indication that consciousness is returning, and was the point at which captive bolt was applied for animal welfare reasons. This occurred between 2 minutes 5 seconds and 3 minutes 37 seconds from loss of posture, which would allow ample time to exsanguinate the animal prior to recovery. In animal 20, captive bolt was applied at 1 minute 42 seconds from loss of posture – this animal was considered to be not truly insensible when assessed at the end of DTS application. The DTS application had used 15 kW, where all other animals had been treated using 20 or 30 kW, and it is possible that the rate of heating generated at this lower power setting was insufficient to overcome the body's ability to cool the brain via the cerebral circulation.

Table 2: Time intervals from onset of DTS application to behavioural changes and interval from loss of posture to return of reflexes.

Animal number	Time from onset of energy application to onset of physical response (and response character)	Time from onset of energy application to loss of posture	Time from onset of onset of physical response to loss of posture	Time from loss of posture to return of reflexes (last reflex tested before (or) captive bolt applied)
1	< 1 sec (rapid blinking)	7 sec	<7 sec	Indefinite, animal died
2	< 1 sec (eye fixed open) 5 sec (rapid blinking)	5 sec	< 5 sec	2 min 5 sec
3	1 sec (loss of posture) 4 sec (rapid blinking)	1 sec	<1 sec	Indefinite, animal died
4	<1 sec (rapid blinking, loss of posture)	1 sec, regained feet then fell again at 6 sec	<1 sec	3 min 4 sec
5	2 sec (rapid blinking)	6 sec	4 sec	Indefinite, animal died
6	2 sec (hind limb movement) 19 sec (rapid blinking, loss of posture)	19 sec	17 sec	Indefinite, animal died
7	1 sec (eyes open wide) 2 sec (body movements)	15 sec	14 sec	3 min 36 sec
8	1 sec (eyes open wide)	9 sec	8 sec	3 min 37 sec
9	4 sec (eyes close tight then rapid blinking)	5 sec	1 sec	2 min 47 sec
10	2 sec (eyes closed) 5 sec (loss of posture)	5 sec	3 sec	2 min 52 sec
11	1 sec (eyes close tight then open wide) 6 sec (body movements and blinking)	9 sec	8 sec	2 min 52 sec
12	1 sec (rapid blinking, loss of posture)	<1 sec	1 sec	2 min 12 sec
13	<1 sec (blinks hard)	4 sec	3 sec	2 min 25 sec
14	2 sec (rapid blinking) 4 sec (front legs paddling)	8 sec	6 sec	>3 min 45 sec (exsanguinated without application of bolt)
15	1 sec (blinks)	5 sec	4 sec	3 min 35 sec (back-up stun applied during exsanguination)
16	5 sec (blinks, loss of posture)	5 sec	<1 sec	2 min 14 sec
17	1 sec (blinks) 2 sec (loss of posture)	2 sec	1 sec	>2 min 34 sec (captive bolt applied early for personnel safety reasons as

Animal number	Time from onset of energy application to onset of physical response (and response character)	Time from onset of energy application to loss of posture	Time from onset of onset of physical response to loss of posture	Time from loss of posture to return of reflexes (last reflex tested before (or) captive bolt applied)
				body was trapped in crate)
18	2 sec (blinks, loss of posture)	2 sec	<1 sec	>1 min 22sec (captive bolt applied early for personnel safety reasons as body was trapped in crate)
19	6 sec (eyes closed)	8 sec	2 sec	Estimated 3 min (visibility obscured by person carrying out reflex testing)
20	4 sec (pull back)	8 sec	4 sec	1 min 42 sec

3.3 Thermal images

*Thermal images and temperature*energy curves for each animal are presented in the Appendix.*

A summary of the surface temperatures recorded on the delineated forehead are of cattle pre- and post-DTS, and the percentage area post-DTS in which surface temperature was greater than 45, 50 and 60 °C is presented in Table 3. However, it must be borne in mind that the post DTS images were taken after the entire energy load had been delivered. From live and video observations, loss of posture usually occurred before the entire energy load had been delivered, so the remaining energy can be considered to be excess energy, not required for inducing insensibility. The continued application of energy would continue to heat tissues, resulting in the thermal maps produced. By comparing the data prior to energy application against the post-application data, it was possible to estimate a heating rate by energy delivered, and utilising this heating rate, estimate the surface temperature at the point of loss of posture. These data for each animal are shown in Table 4 but to summarise, the average surface temperature on the forehead at the point of loss of posture was estimated to be between 26 and 49 °C, although some animals (animals 6 and 7) that showed a delayed loss of posture also demonstrated hot-spots of up to 109 °C. In context, Australian cattle may experience ambient air temperatures of 45 °C in summer, and could experience higher surface temperatures when standing in in direct sunlight. Surface temperatures above 50 °C may be uncomfortable to cattle, although no literature on thermal contact sensitivity in cattle could be located to confirm or refute this supposition, while temperatures above 60 °C will result in skin tissue damage, as seen in hot branding which is commonly used for identification of cattle. Hot branding uses an iron heated to over 500 °C, held onto the skin of the shoulder or hip for 5-8 seconds (Tucker, Mintline et al. 2014).

Table 3: Surface temperatures recorded on the forehead of cattle pre- and post-DTS, and the percentage area post-DTS in which surface temperature was greater than 45, 50 and 60 °C.

Animal number	Minimum temperature (°C)	Maximum temperature (°C)	Average temperature (°C)	Area > 45 °C (%)	Area > 50 °C (%)	Area > 60 °C (%)
1 pre-DTS	20.4	35.7	27.8			
1 post-DTS	23.9	83.1	47.8	54	45	18
2 pre-DTS	21.8	38.1	29.6			
2 post-DTS	24.0	106.8	49.9	58	49	27
3 pre-DTS	19.8	35.5	25.0			
3 post-DTS	22.1	95.5	43.6	45	38	18
4 pre-DTS	17.1	34.4	24.4			
4 post-DTS	20.9	82.8	39.4	25	18	7
5 pre-DTS	18.2	34.6	24.1			
5 post-DTS	20.5	91.8	45.5	49	38	10
6 pre-DTS	20.8	36.4	27.9			
6 post-DTS	24.0	99.6	49.7	57	44	17
7 pre-DTS	20.0	37.5	26.9			
7 post-DTS	23.1	109.2	46.6	46	41	27
8 pre-DTS	23.2	34.4	29.4			
8 post-DTS	24.0	95.5	51.4	60	50	32
9 pre-DTS	21.9	35.5	28.5			
9 post-DTS	24.0	92.3	49.3	52	44	26
10 pre-DTS	21.9	34.9	28.1			
10 post-DTS	21.8	98.5	46.4	51	43	21
11 pre-DTS	20.4	39.4	27.9			
11 post-DTS	23.8	89.2	51.9	62	53	37
12 pre-DTS	18.4	34.3	24.7			
12 post-DTS	21.4	77.6	31.6	10	7	3
14 pre-DTS	12.6	34.4	20.2			
14 post-DTS	15.2	91.1	41.4	32	23	13
15 pre-DTS	16.1	33.4	25.3			
15 post-DTS	17.4	77.3	44.2	46	27	9
16 pre-DTS	14.8	31.6	21.8			
16 post-DTS	17.0	90.1	43.1	41	33	11
18 pre-DTS	17.1	35.1	25.2			
18 post-DTS	15.5	62.9	39.1	23	08	0
19 pre-DTS	17.2	36.2	26.1			
19 post-DTS	16.6	80.3	43.3	45	32	7
20 pre-DTS	15.9	36.2	24.7			
20 post-DTS	17.7	79.9	42.9	36	25	13

3.4 EEG

Images of the baselined EEG traces for each animal f are presented in the Appendix.

Of the 20 animals processed, 11 EEG recordings were generated: the post-DTS recording was not collected from animal 2 due to accidental shut-down of the laptop running LabChart, and in animal 13, corneal reflexes were present and the animal was stunned using the back-up captive bolt shortly after opening of the faraday cage, prior to EEG recording. For animals 14 to 20, baseline EEG was not recorded, and post-DTS EEG was recorded on the bleed-conveyor during exsanguination. Thus, baseline EEG for normalisation was not present, and the post-DTS EEG could only be used qualitatively. In the event, once movement artefact was removed, there were very little EEG data available for animals 15-20 prior to the return of the corneal reflex and captive bolt application.

Figure 9 shows an example of the EEG data collected, compared against a similar chart reproduced from Rault et al. (2013; 2014), both showing a period of 2.5 – 3 minutes post DTS application, scaled to similar length. Although the software and detail of smoothing methods used to generate these charts differ, clear similarities are evident: total EEG power (Ptot, green line) is dramatically increased and shows high amplitude spiking in the two-minute period post DTS application; 95% spectral edge frequency (SEF95, blue line) and 50% spectral edge frequency (SEF50, red line) drop following DTS application, and then begin to return towards baseline. In the current study, movement artefact posed a significant challenge to EEG recording, as the animals began to twitch and convulse, similar to the movements seen during the phases of an electric stun. This movement artefact was not present in the Rault study, as the animals were anaesthetised. Furthermore, in the current study, background electrical noise from the abattoir infrastructure tended to interfere with the EEG signal, so interpretation of the data, particularly the spectral edge frequencies, is challenging

All the animals for which usable EEG recordings were collected showed changes from baseline, particularly in terms of Ptot, while for animal 14, for which EEG data was collected post-DTS only and captive bolt was not applied, high amplitude spiking of Ptot was observed. These patterns on EEG are considered to be incompatible with sensibility (EFSA 2004).

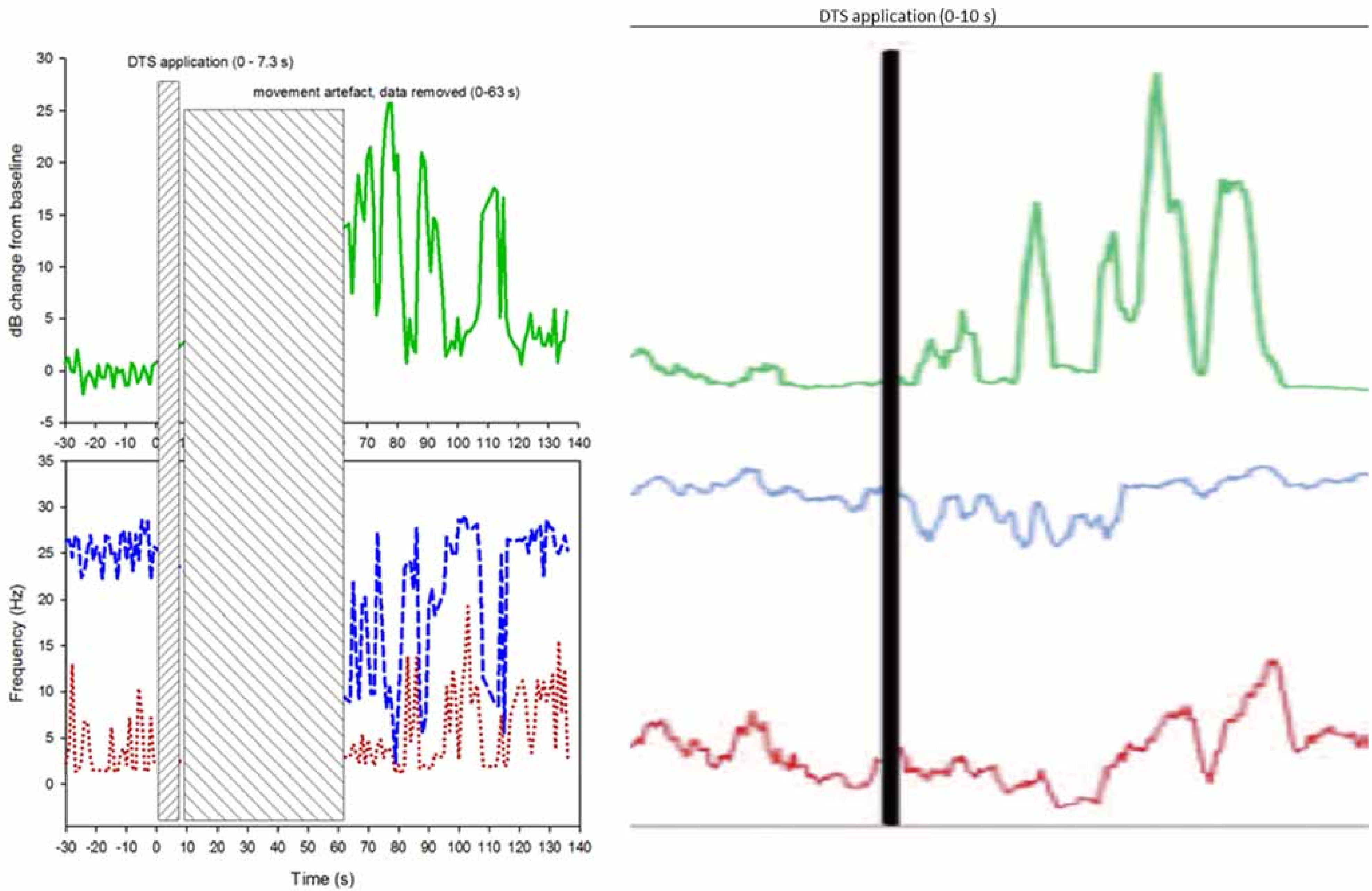

Figure 9: Examples of EEG data. Left: Animal 10 in the current study, Right: Animal 6 from Rault et al. 2013 and 2014 (reproduction of an approximate 3-minute block). Green indicates total EEG power (Ptot) as change from baseline, blue indicates 95% spectral edge frequency (SEF95) and red indicates 50% spectral edge frequency (SEF50).

Table 4: Summary of data collected.

Animal	setting (kJ, kW)	energy delivered (kJ)	latency to react (s)	latency to loss of posture (s)	loss of posture to stand (s)	loss of posture to reflex return (s)	energy at loss of posture (kJ)	mean surface temp at loss of posture (°C)	min (°C)	max (°C)	unconscious	leakage	EEG DB change from baseline	Change in SEF95	Change in SEF50	time EEG trending towards baseline (s)
1	360, 30	421	1	7	never	never	170	35.88	21.81	54.83	yes, then death	not observed	-10	+12	+5	> 90
2	300, 30	350	1	5	88	125	104	35.63	22.46	58.5	yes	evident on live observation	not recorded	not recorded	not recorded	not recorded
3	300, 30	351	1	1	69	never	135	32.16	20.69	58.57	yes, then death	evident on live observation	+10	-10	-8	> 140
4	300, 30	171	1	6	80	184	143	36.88	20.26	74.7	yes	evident on live observation	+4	0	-5	100
5	300, 30	338	2	6	17	never	138	32.82	19.13	57.9	yes, then death	visible on thermal image	-5	0	-5	> 110
6	275, 30	317	2	19	5	never	317	49.71	24	99.64	yes, then death	visible on thermal image	+30	+3	+2	160
7	250, 30	292	1	15	144	216	292	46.55	23.1	109.07	yes	not observed	-7	-20	-12	> 190
8	250, 30	284	1	9	17	217	233	47.39	23.85	84.38	yes	visible on thermal image	+8	-10	-5	> 140
9	225, 30	267	4	5	5	167	114	37.37	22.8	59.7	yes	evident on live observation	+3	-10	-8	> 130
10	225, 30	258	2	5	14	172	112	36.04	21.94	62.51	yes	evident on live observation	+15	-12	-2	130

Animal	setting (kJ, kW)	energy delivered (kJ)	latency to react (s)	latency to loss of posture (s)	loss of posture to stand (s)	loss of posture to reflex return (s)	energy at loss of posture (kJ)	mean surface temp at loss of posture (°C)	min (°C)	max (°C)	unconscious	leakage	EEG DB change from baseline	Change in SEF95	Change in SEF50	time EEG trending towards baseline (s)
11	225, 30	261	1	9	10	172	215	47.59	23.2	80.27	yes	evident on live observation	+3	+2	0	110
12	225, 30	125	1	1	34	132	6	25.02	18.54	36.29	partial	evident on live observation	+12	-18	-5	> 80
13	225, 20	247	1	4	126	145	58	not recorded			partial	evident on live observation	baseline EEG not recorded			
14	300, 30	351	2	8	never	> 225	196	32.04	14.05	66.03	yes	not observed	baseline EEG not recorded			
15	275, 30	310	1	5	never	215	110	32	16.23	48.95	yes	visible on thermal image	baseline EEG not recorded			
16	275, 20	310	5	5	never	134	80	27.28	15.37	46.66	yes	evident on live observation	baseline EEG not recorded			
17	275, 20	312	1	2	never	> 154	23	not recorded			yes	evident on live observation	baseline EEG not recorded			
18	275, 20	297	2	2	never	> 82	23	26.27	16.98	37.24	partial	evident on live observation	baseline EEG not recorded			
19	200, 20	226	6	8	never	180	135	36.36	17.56	62.51	yes	evident on live observation	baseline EEG not recorded			
20	200, 15	223	4	8	43	102	101	32.92	16.71	55.94	yes	visible on thermal image	baseline EEG not recorded			

4 Conclusion and Recommendation

Overall, DTS rendered cattle insensible, based on physical (reflex) and EEG data. The results gathered were confounded by problems with waveguide to forehead contact, resulting in loss of energy to the environment instead of being transmitted into the brain. This variability in energy delivery may account for the variability in latency to loss of consciousness, using loss of posture as the proxy indicator (Muir 2007), and problems with waveguide to forehead contact may have contributed to the variability in surface temperatures and the presence of hot spots on the forehead.

To summarise:

- DTS Prototype 4 successfully rendered cattle insensible based on physical (reflex) and EEG data;
- Prolonged duration of insensibility suggests that DTS may be better than the existing commercially available head-only electrical stunning method for cattle;
- Some engineering modifications to the restraint unit and to the waveguide positioning apparatus are required to ensure consistent delivery of energy to the brain and minimise leakage.

We recommend that the next stage of development is to carry out the engineering modifications, such that consistent delivery of energy is achieved prior to attempting statistically representative studies. This development activity will require use of live animals to test the modifications for evidence of leakage, and the data collected during this development will contribute to the body of evidence required to bring the technology to a commercially viable from.

4.1 Protocol for next phase of development, as submitted to the Animal Ethics Committee

The objective of the next phase of development is to carry out modifications to the restraint unit, the waveguide and the waveguide positioning apparatus in order to improve contact with the forehead and minimise leakage. Adjustments to the DTS control software will also be made to correct the glitch in storing the cumulative energy graphs, and to allow variable power profiles for energy delivery. Following each iteration of modifications, a small number of animals (maximum 20) will be processed in order to assess the improvements made. The procedure for testing will be as per the efficacy validation trial described in this report, without collection of EEG data. Animals will be individually restrained, and energy applications within the range of 200-250 kJ applied. Animals will be assessed for consciousness based on the behavioural indicators:

- Loss of posture;
- Loss of corneal and palpebral reflexes;
- Absence of focusing of eye and ability to follow movement.

If at any point an animal is deemed to be regaining consciousness, either before exsanguination or during exsanguination, captive bolt will be applied.

If leakage occurs, or an animal is not rendered unconscious as a result of energy application, the components of the waveguide will be assessed for arcing and goodness of fit to the forehead assessed. Any issues will be corrected before any further animals are processed.

When restraint and contact issues are corrected, a further more detailed assessment will be planned and executed, with EEG recording, to explore the efficacy of lower energy applications (stepwise reduction to 150 kJ, in a manner as per the efficacy validation trial described in this report); and the efficacy of altering power profile during energy application.

Acknowledgements

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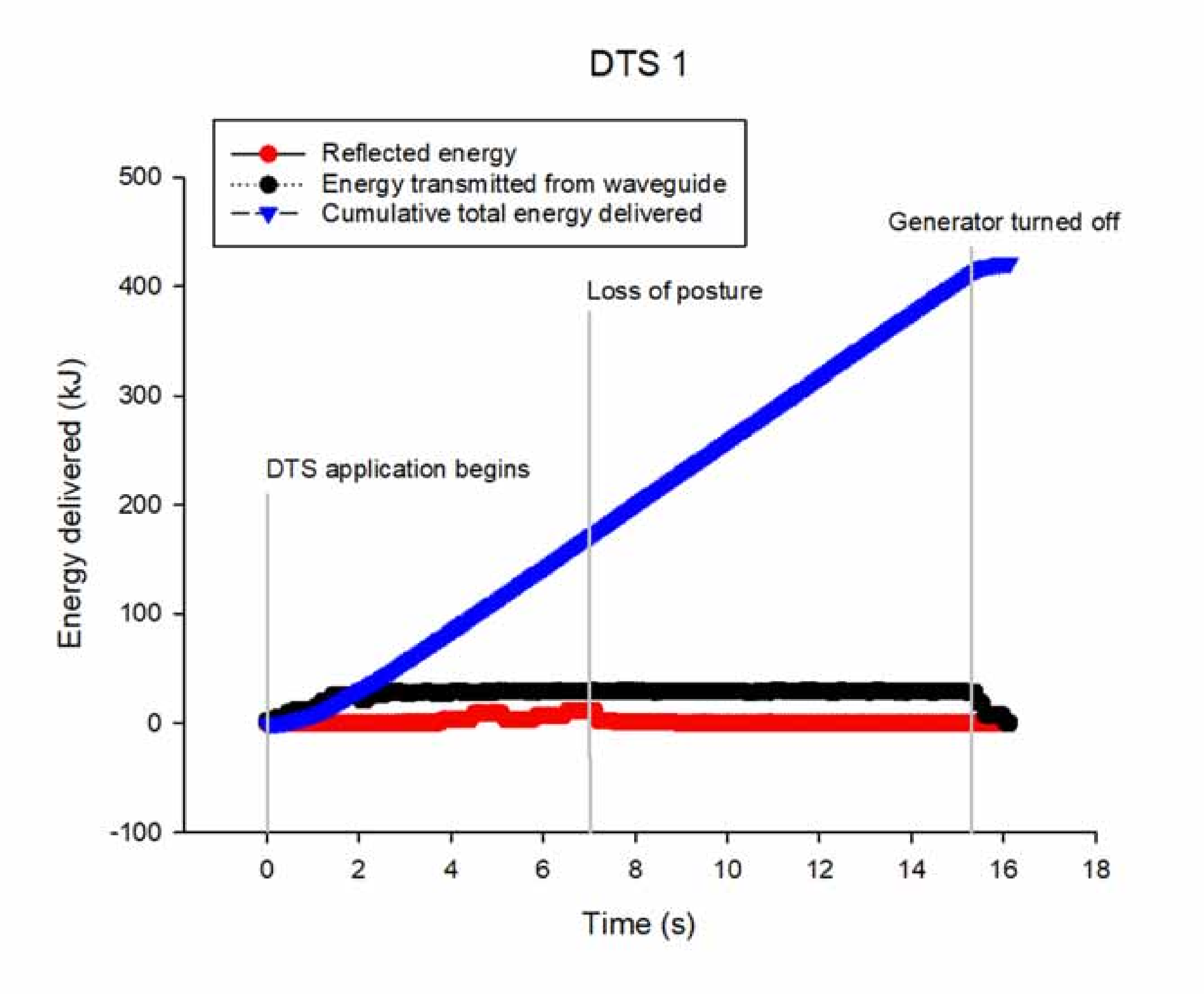
Appendix 1

Animal 1

DTS settings

360 kJ, 30 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 7 seconds post onset of DTS application, which is equivalent to approximately 170 kJ of energy delivered.



Notes from real-time observations

Loss of posture, flaccid body, no corneal reflex, fixed staring eye.

Animal appears dead, rolled out following EEG recording, then exsanguinated.

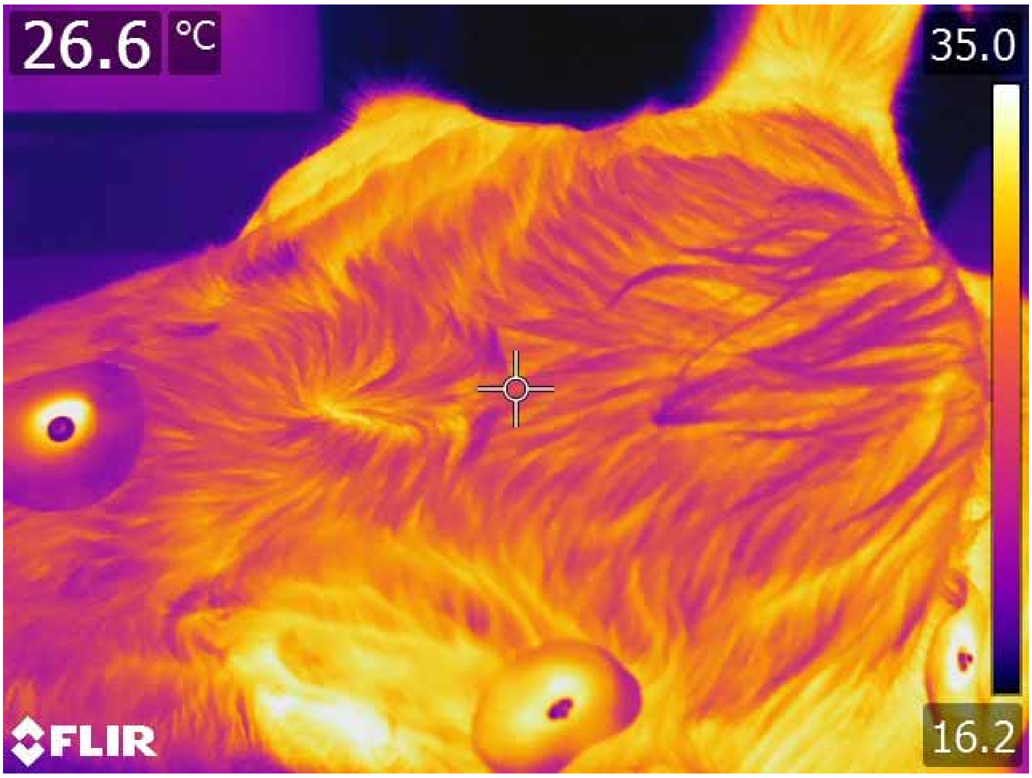
Heart rate by auscultation, immediately prior to exsanguination: 180 beats/minute.

Detailed event log from video footage

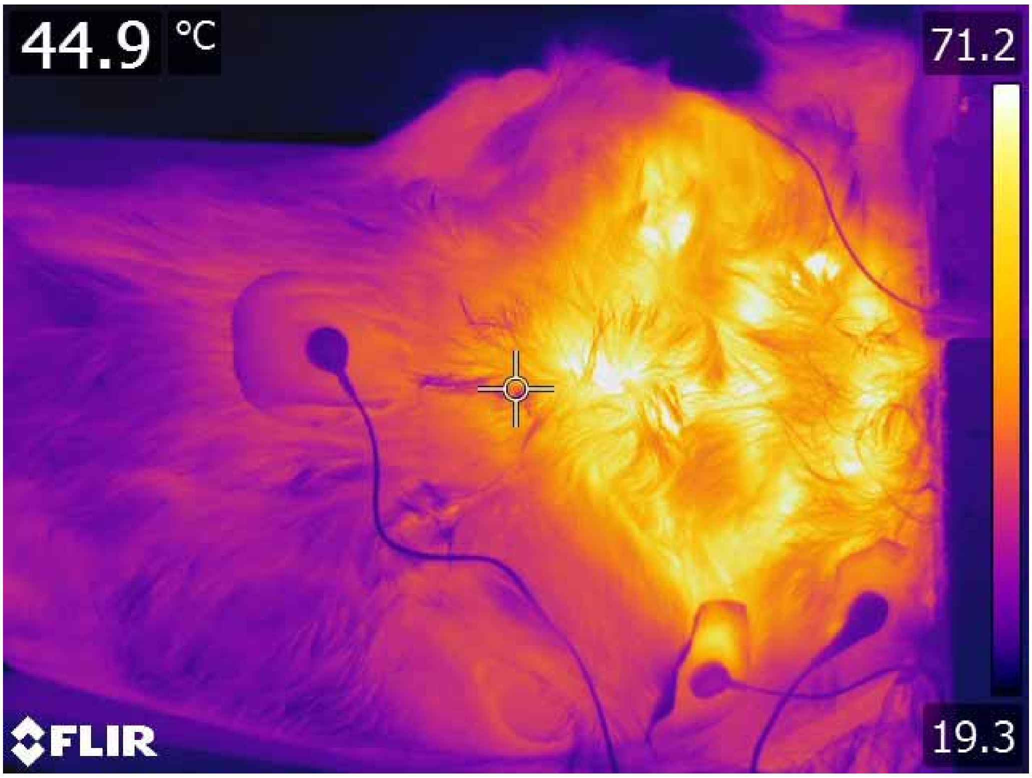
Animal number	Date	time	action
1	4/10/2017	14:29:34	DTS starts
1	4/10/2017	14:29:34	blinking, then shuts eyes tight
1	4/10/2017	14:29:37	legs under body
1	4/10/2017	14:29:39	tail swishing
1	4/10/2017	14:29:41	rear end collapses (legs underneath)
1	4/10/2017	14:29:45	blinking, steam obscures head
1	4/10/2017	14:29:56	eyes fixed open
1	4/10/2017	14:29:59	no obvious breathing
1	4/10/2017	14:30:16	wand removed
1	4/10/2017	14:30:24	corneal reflex
1	4/10/2017	14:30:46	leads on, difficult, extra button applied
1	4/10/2017	14:31:46	thermal camera head
1	4/10/2017	14:32:15	nose prick
1	4/10/2017	14:32:21	corneal reflex
1	4/10/2017	14:32:56	leads removed
1	4/10/2017	14:33:30	some movement rear legs
1	4/10/2017	14:33:43	chin lift dropped
1	4/10/2017	14:33:47	head restraint opened
1	4/10/2017	14:33:54	side opened
1	4/10/2017	14:34:18	animal pulled onto table
1	4/10/2017	14:34:27	video ends

Thermal images

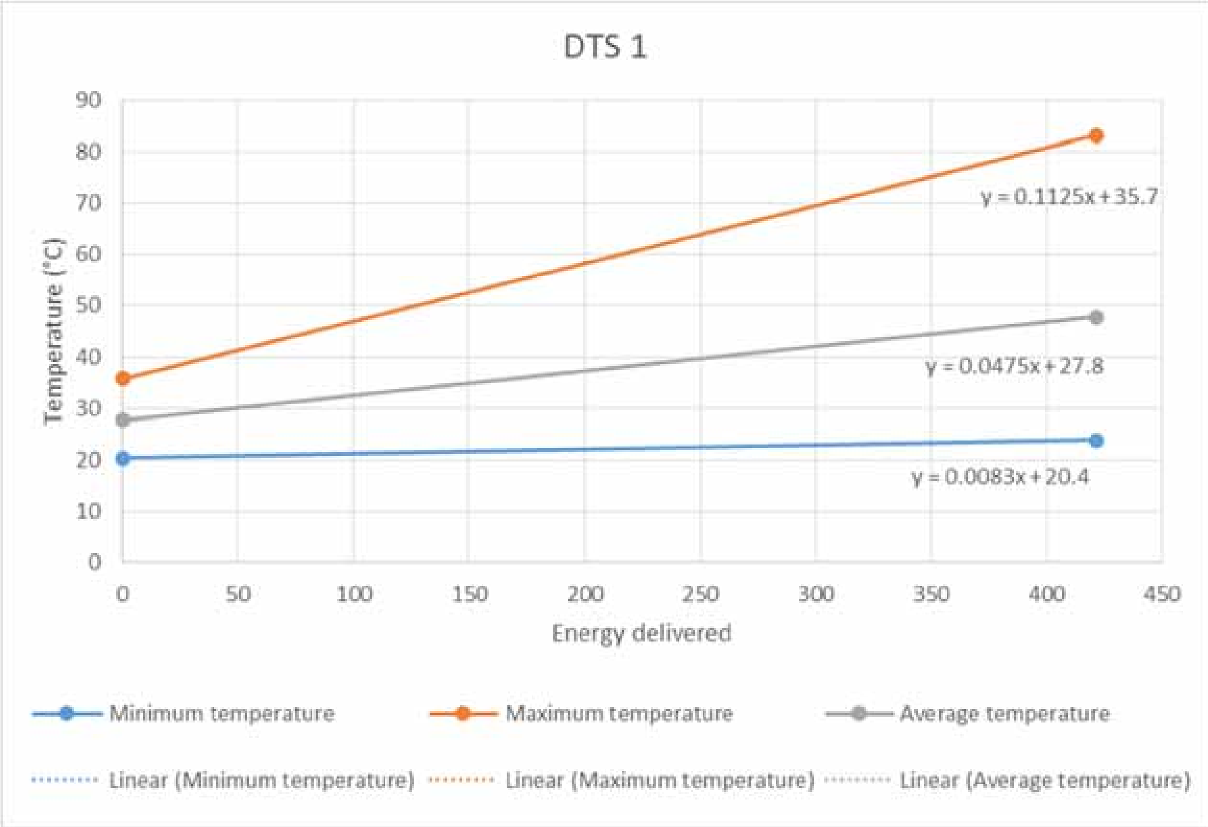
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 1 lost posture at 7 seconds post onset of DTS application, equivalent to approximately 170 kJ of energy delivered. At 170 kJ energy delivered the estimated average forehead temperature is 35.88 °C (21.81 – 54.83).



Animal 1 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 26.6 °C; maximum temperature in image (white): 35.0 °C; minimum temperature in image (black): 16.2 °C. Disk shapes with cool centres are EEG recording electrode pads.



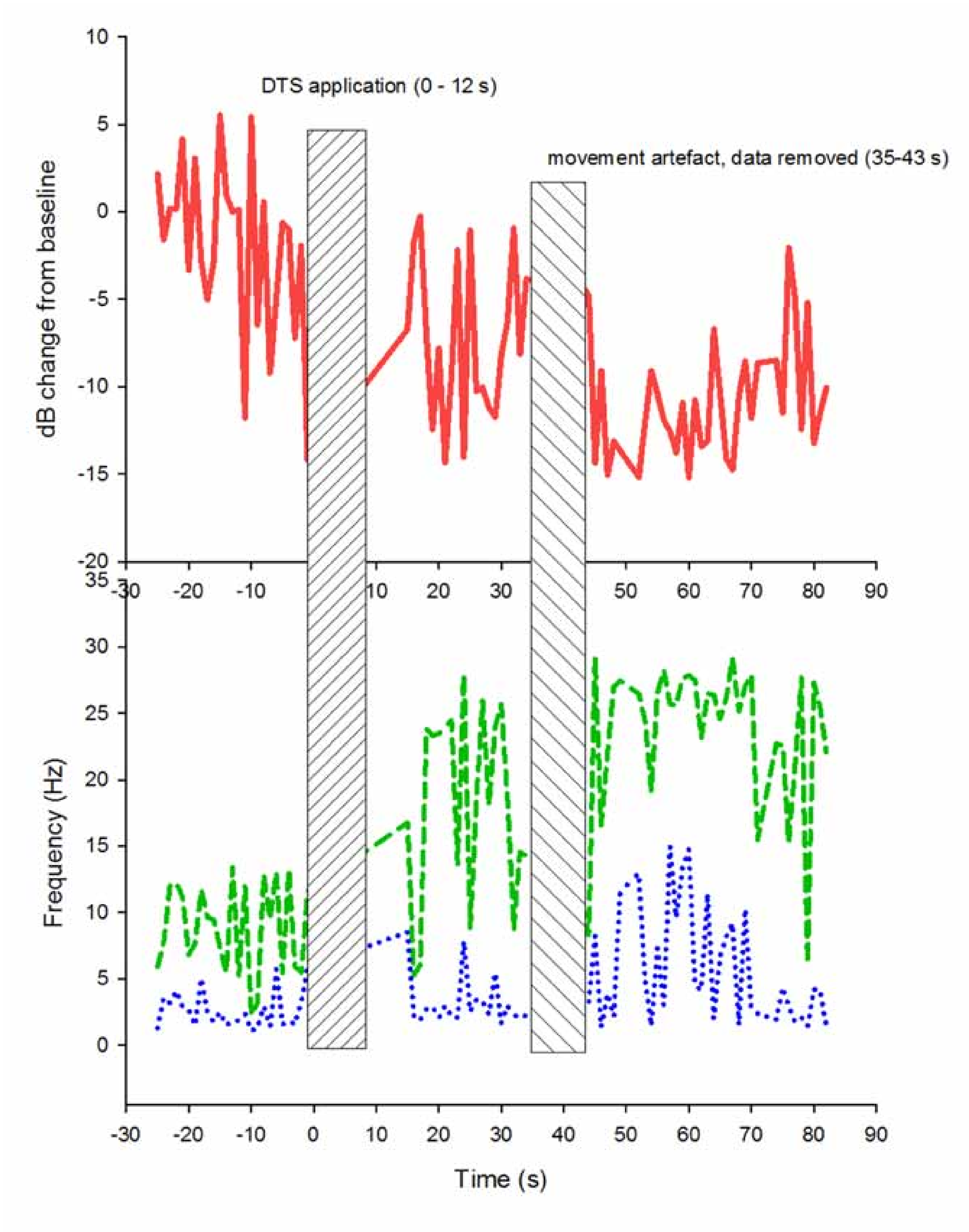
Animal 1 thermal map of forehead immediately after DTS application. Temperature at cursor: 44.9 °C; maximum temperature in image (white): 71.2 °C; minimum temperature in image (black): 19.3 °C. Disk shapes with cool centres and cables are EEG recording electrode pads and leads.



Animal 1 estimated temperature change on the forehead by energy delivered.

EEG traces

A chart showing changes in EEG parameters following DTS application (narrow hatching) in Animal 1 is shown below. Total EEG power (Ptot, red line) is decreased post DTS application; 95% spectral edge frequency (SEF95, green line) and 50% spectral edge frequency (SEF50, blue line) increased following DTS application. It is difficult to interpret the spectral edge frequency data, as the EEG recordings are heavily contaminated by electrical noise from the abattoir infrastructure, however, a decrease in Ptot could indicate progression toward death.

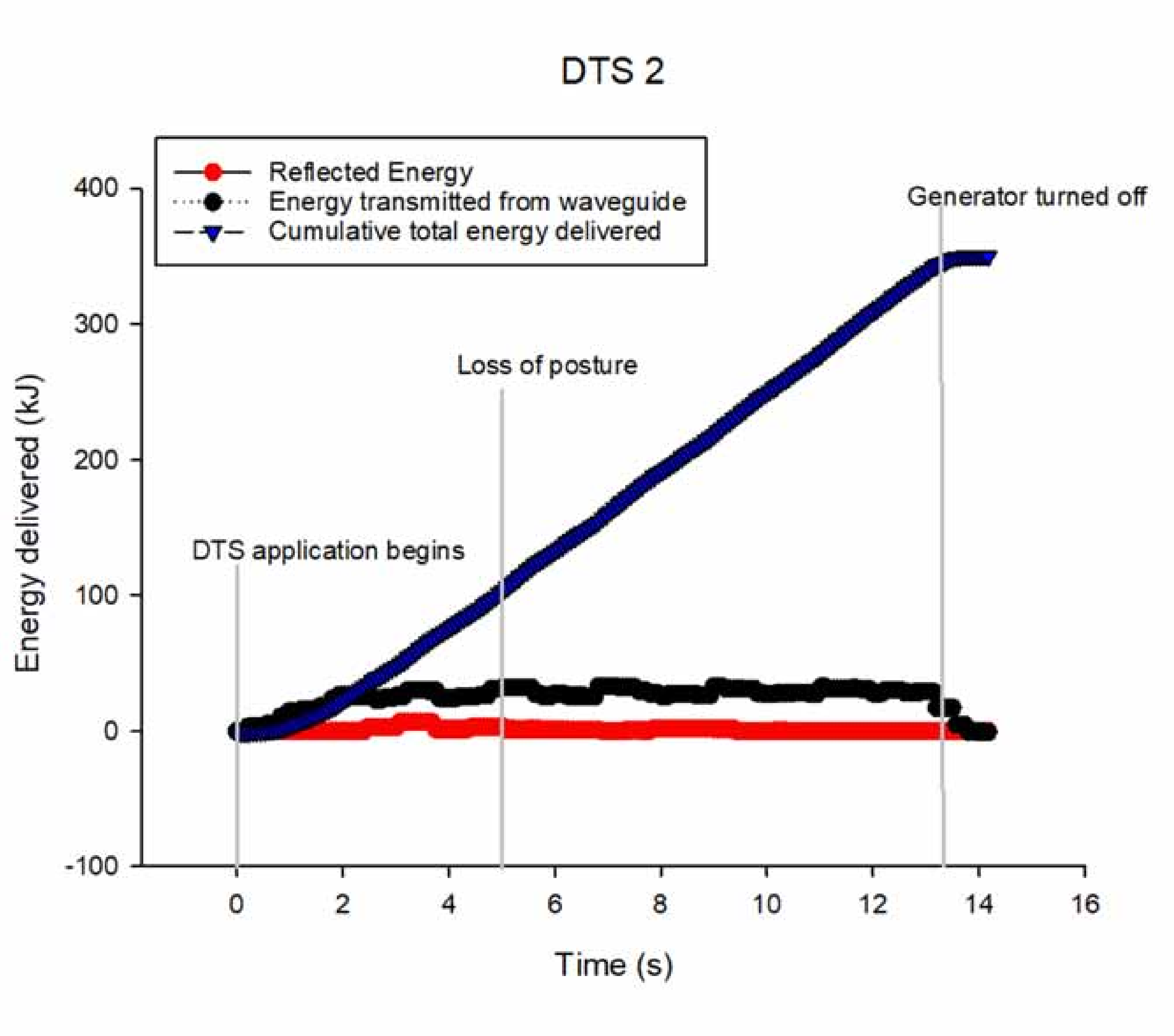


Animal 2

DTS settings

300 kJ, 30 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 5 seconds post onset of DTS application, which is equivalent to approximately 104 kJ of energy delivered.



Notes from real-time observations

Some leakage occurred (arcing observed on screen), so entire energy load was not delivered to the brain.

Loss of posture, corneal reflex absent for three test events, reflex returning on fourth test, righting reflex returned. Captive bolt applied then body rolled out for exsanguination.

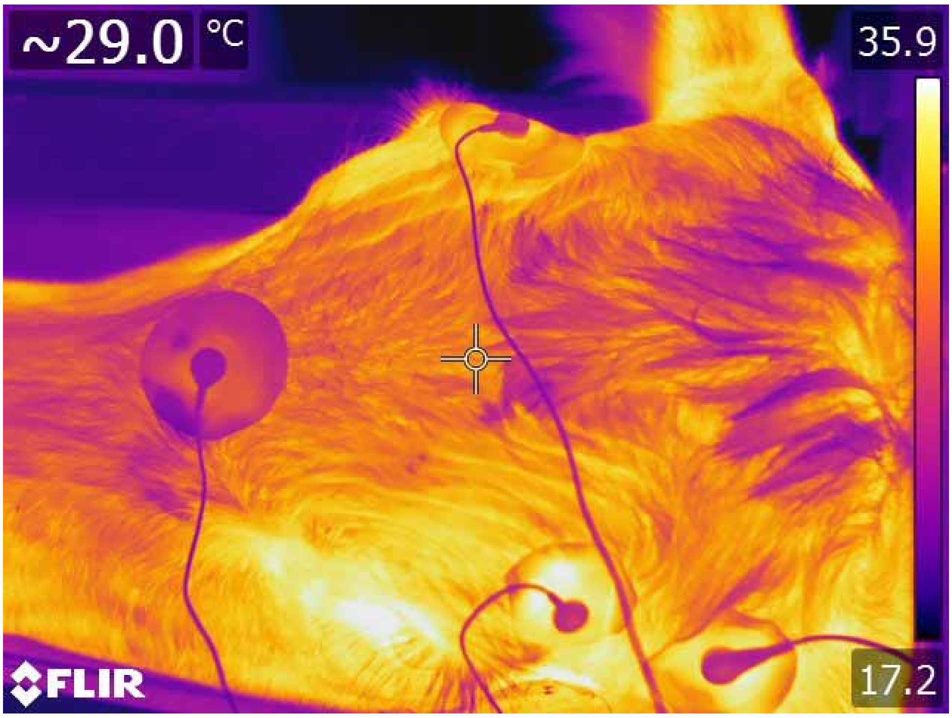
Review of video footage immediately after exsanguination showed that the arcing occurred as the animal lost posture, and the waveguide slipped up the forehead toward the poll. This heating of the poll is evident in the thermal image (below).

Detailed event log from video footage

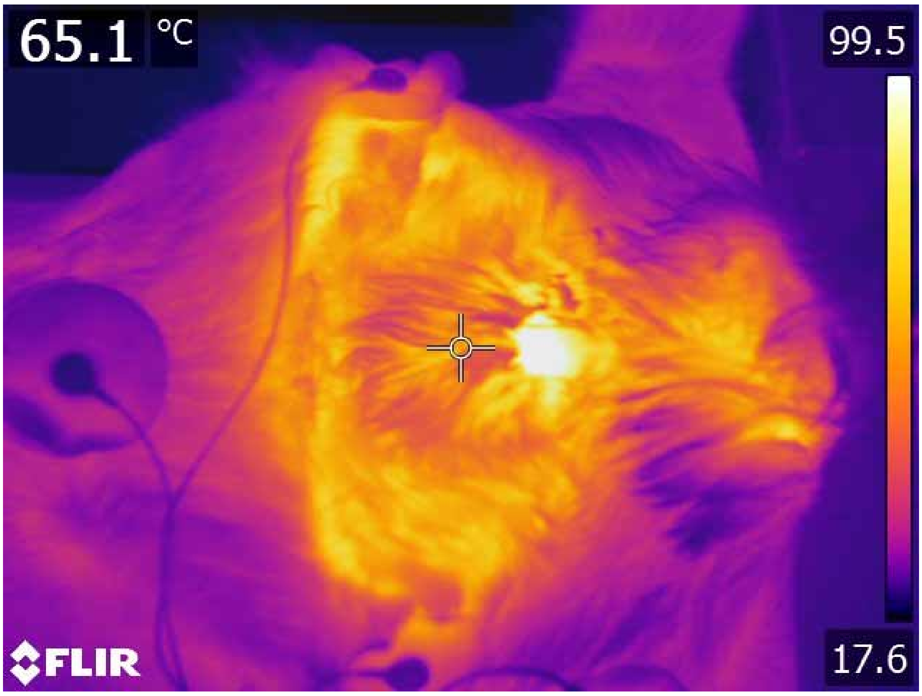
Animal number	Date	time	action
2	4/10/2017	14:58:20	in head bale
2	4/10/2017	14:58:36	DTS starts,
2	4/10/2017	14:58:36	eyes fixed open
2	4/10/2017	14:58:41	rapid blink
2	4/10/2017	14:58:41	rear end collapses (legs underneath)
2	4/10/2017	14:59:02	breathing, rapid blinks
2	4/10/2017	14:59:06	wand removed
2	4/10/2017	14:59:15	ears twitching
2	4/10/2017	14:59:16	eyes fixed open
2	4/10/2017	14:59:39	leads attached
2	4/10/2017	14:59:42	corneal reflex
2	4/10/2017	15:00:09	animal stands
2	4/10/2017	15:00:12	tail swishing
2	4/10/2017	15:00:46	corneal reflex
2	4/10/2017	15:00:50	leads removed
2	4/10/2017	15:00:54	moving around in crate
2	4/10/2017	15:01:00	captive bolt
2	4/10/2017	15:01:02	rear end collapses (legs underneath)
2	4/10/2017	15:02:12	chin lift dropped
2	4/10/2017	15:01:21	head restraint opened
2	4/10/2017	15:01:37	side opened
2	4/10/2017	15:01:47	breathing, rapid blinks
2	4/10/2017	15:01:50	on table
2	4/10/2017	15:02:22	not breathing
2	4/10/2017	15:02:39	skin cut
2	4/10/2017	15:02:44	rodded, rear legs paddle
2	4/10/2017	15:03:12	clip and cut oesophagus
2	4/10/2017	15:03:31	exsanguinated

Thermal images

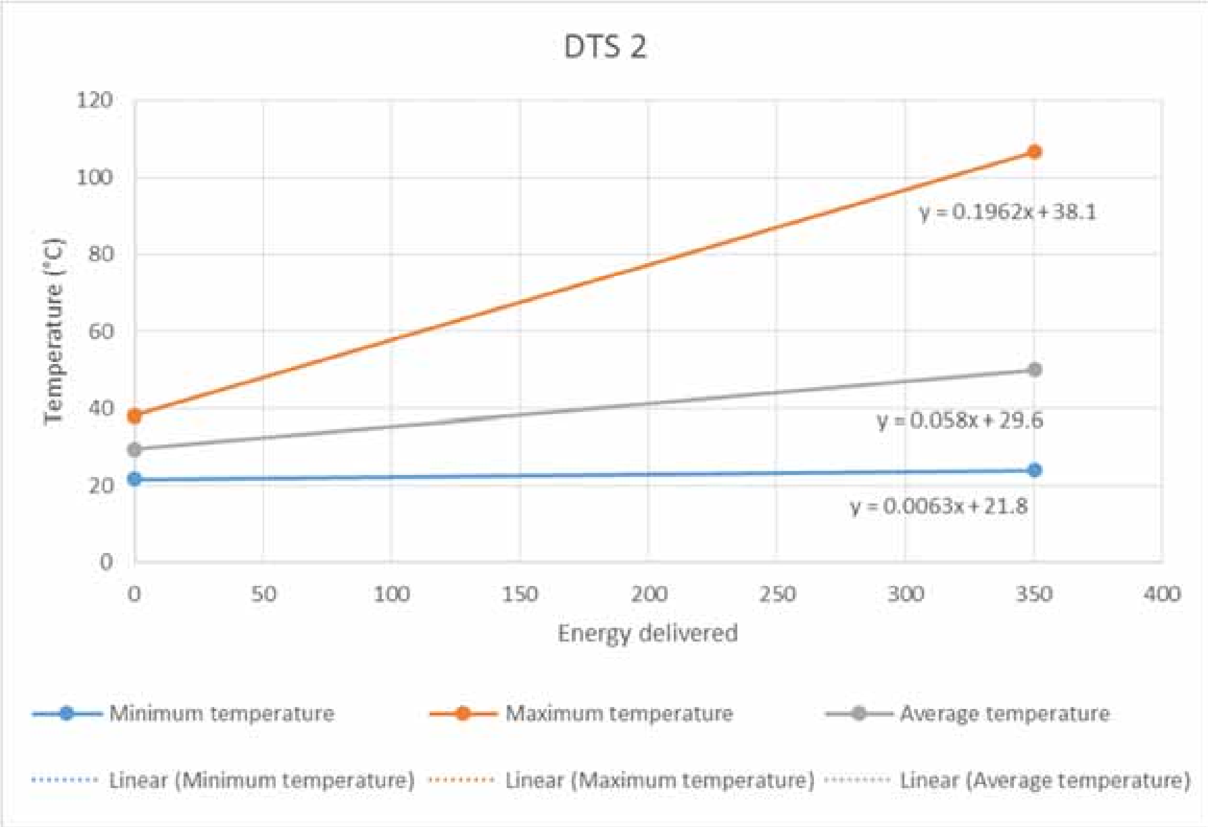
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 2 lost posture at approximately 5 seconds post onset of DTS application, which is equivalent to approximately 104 kJ of energy delivered. At 104 kJ energy delivered the estimated average forehead temperature is 35.63 °C (22.46 – 58.50).



Animal 2 thermal map of forehead immediately prior to DTS application. Temperature at cursor: ~29.0 °C; maximum temperature in image (white): 35.9 °C; minimum temperature in image (black): 17.2 °C. Disk shapes with cool centres and cables are EEG recording electrode pads and leads.



Animal 2 thermal map of forehead immediately after DTS application. Temperature at cursor: 65.1 °C; maximum temperature in image (white): 99.5 °C; minimum temperature in image (black): 17.6 °C. Heating of poll area after slippage of waveguide is evident. Disk shapes with cool centres and cables are EEG recording electrode pads and leads.



Animal 2 estimated temperature change on the forehead by energy delivered.

EEG traces

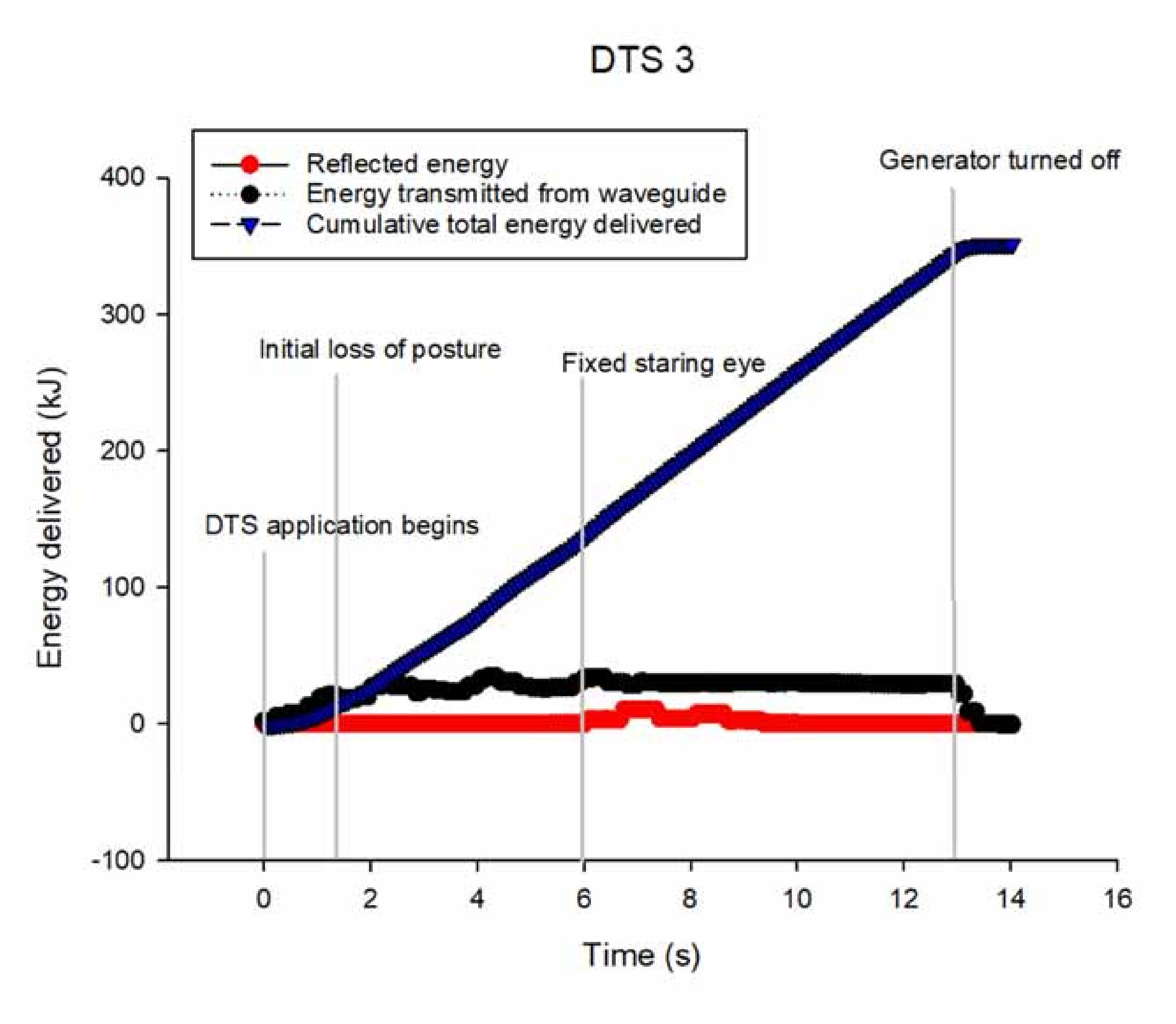
No EEG available – the computer was accidentally shut down prior to DTS application, losing the pre-application data, and by the time it was re-started and the EEG program loaded, the animal was showing signs of recovery.

Animal 3

DTS settings

300 kJ, 30 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, the time a fixed staring eye was evident, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 1 seconds post onset of DTS application, which is equivalent to approximately 6 kJ of energy delivered, while fixed, staring eye (also associated with unconsciousness) occurred at 6 seconds post onset of DTS application, which is equivalent to approximately 135 kJ of energy delivered.



Notes from real-time observations

Possible leakage occurred, based on data from the DTS operating software.

Loss of posture, corneal reflex absent for two tests, noisy exhalation, which may be associated with body position – the head remains in the head capture, while the body has collapsed, so the larynx may be compressed.

Animal appeared dead, so rolled out and exsanguinated. No heart beat detected on auscultation. Jugular blood flow temperature at thoracic inlet 40.2 °C.

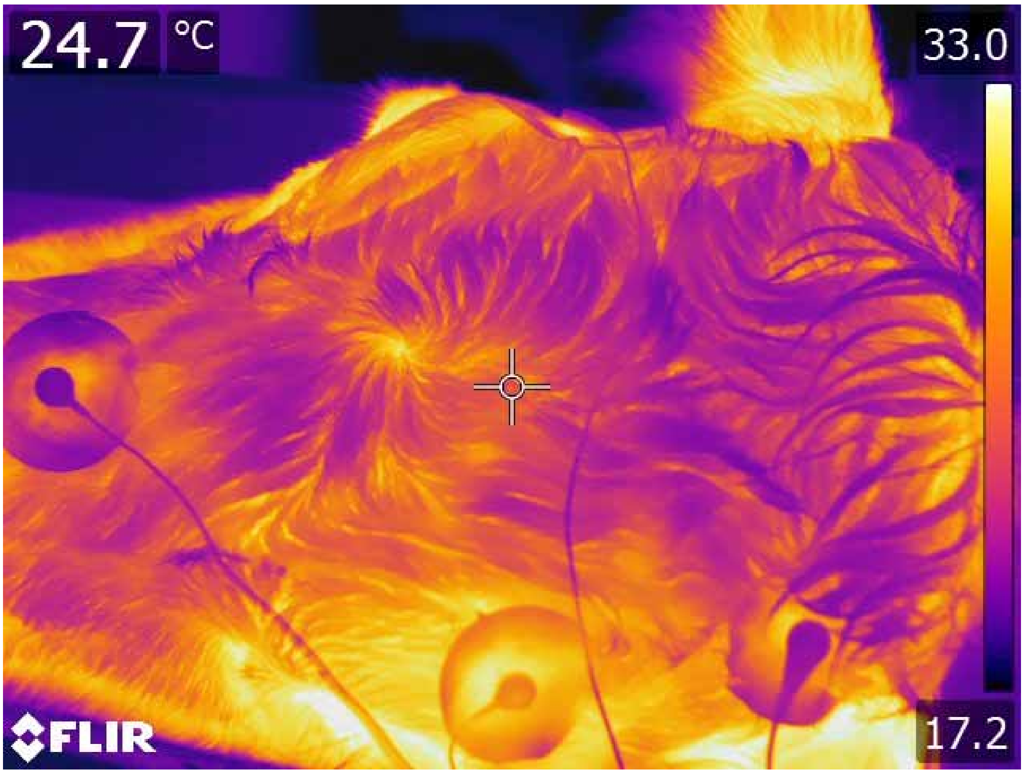
Skin split on forehead.

Detailed event log from video footage

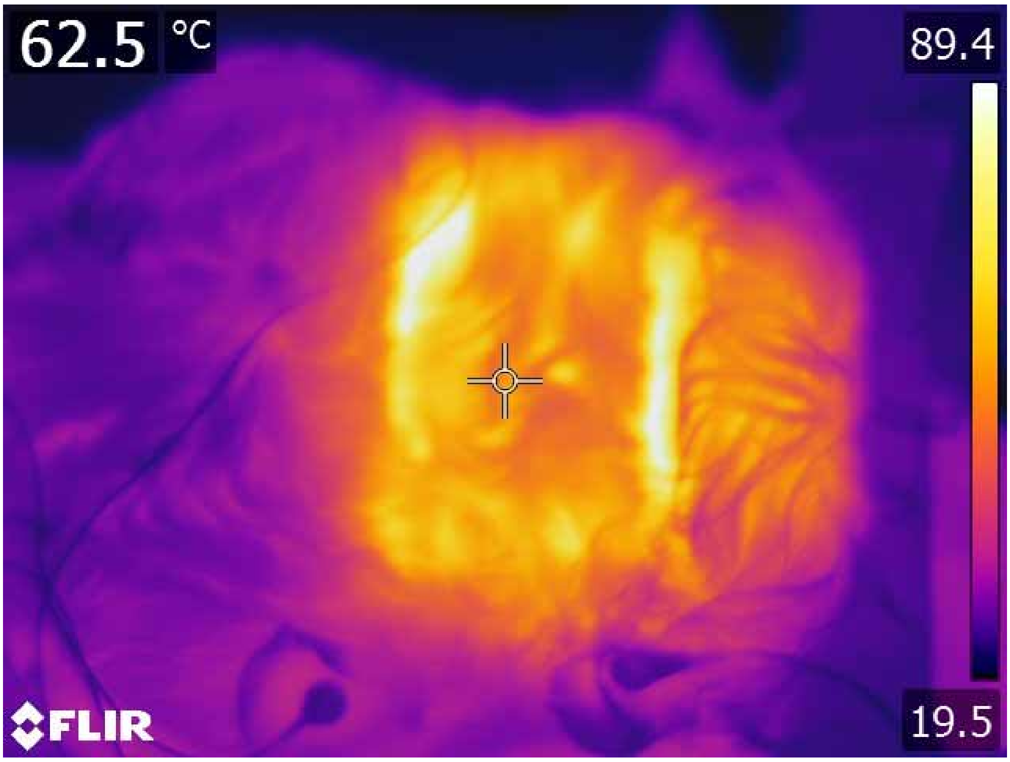
Animal number	Date	time	action
3	4/10/2017	15:49:54	in crate
3	4/10/2017	15:51:03	DTS starts
3	4/10/2017	15:51:04	rear end collapses (legs underneath)
3	4/10/2017	15:51:07	rapid blink
3	4/10/2017	15:51:07	rear legs paddling
3	4/10/2017	15:51:09	top knot lifting (?)
3	4/10/2017	15:51:11	eyes fixed open
3	4/10/2017	15:51:12	steam obscures
3	4/10/2017	15:51:23	collapses on side
3	4/10/2017	15:51:24	breathing
3	4/10/2017	15:51:38	wand removed
3	4/10/2017	15:51:58	leads attached
3	4/10/2017	15:52:13	legs kicking, assumes upright stance
3	4/10/2017	15:52:13	tail swishing
3	4/10/2017	15:53:07	corneal reflex
3	4/10/2017	15:53:17	corneal reflex
3	4/10/2017	15:53:37	corneal reflex
3	4/10/2017	15:53:48	leads removed
3	4/10/2017	15:53:49	not breathing
3	4/10/2017	15:54:57	chin lift dropped
3	4/10/2017	15:55:04	head restraint opened
3	4/10/2017	15:55:17	side opened
3	4/10/2017	15:55:17	video ends

Thermal images

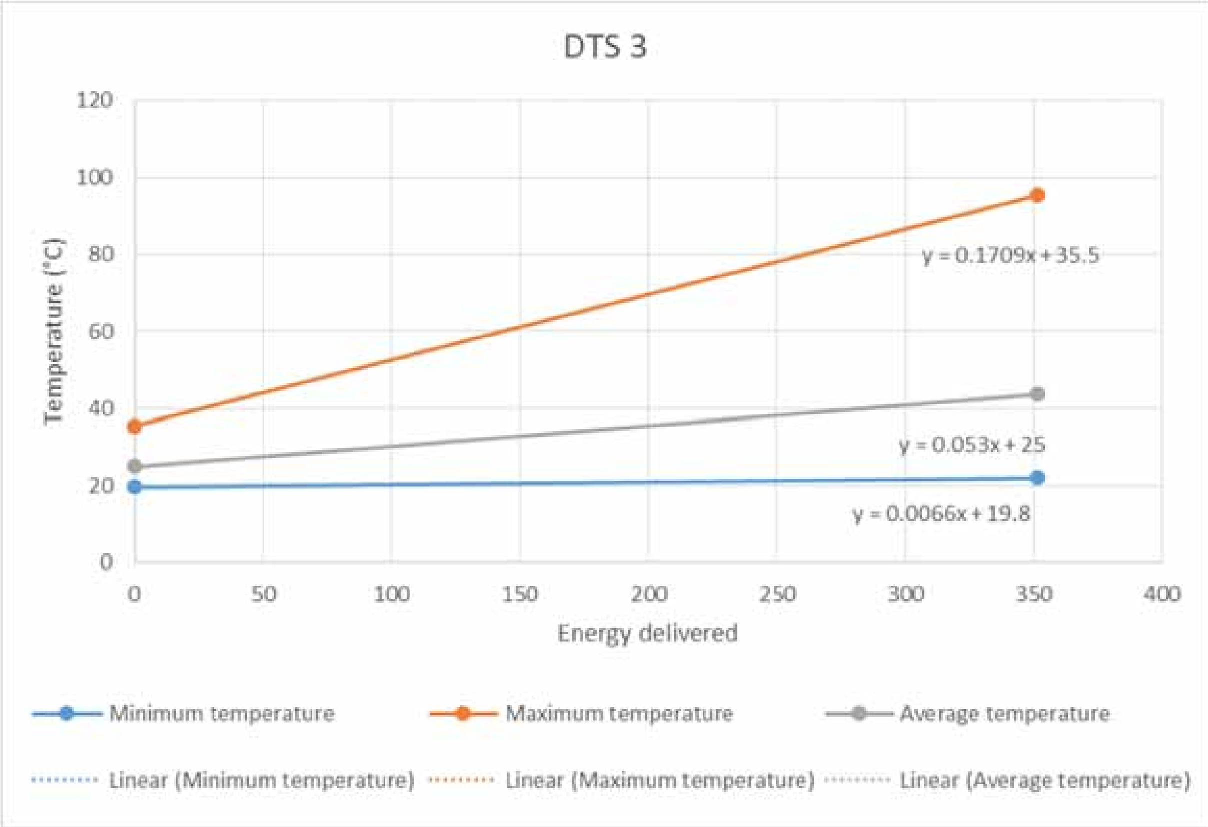
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 3 lost posture at approximately 1 seconds post onset of DTS application, which is equivalent to approximately 6 kJ of energy delivered, while fixed, staring eye (also associated with unconsciousness) occurred at 6 seconds post onset of DTS application, which is equivalent to approximately 135 kJ. At 6 kJ energy delivered the estimated average forehead temperature is 25.31 °C (19.84 – 36.53), while at 135 kJ, the estimated average forehead temperature is 32.16 °C (20.69 – 58.57).



Animal 3 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 24.7 °C; maximum temperature in image (white): 33.0 °C; minimum temperature in image (black): 17.2 °C. Disk shapes with cool centres and cables are EEG recording electrode pads and leads.



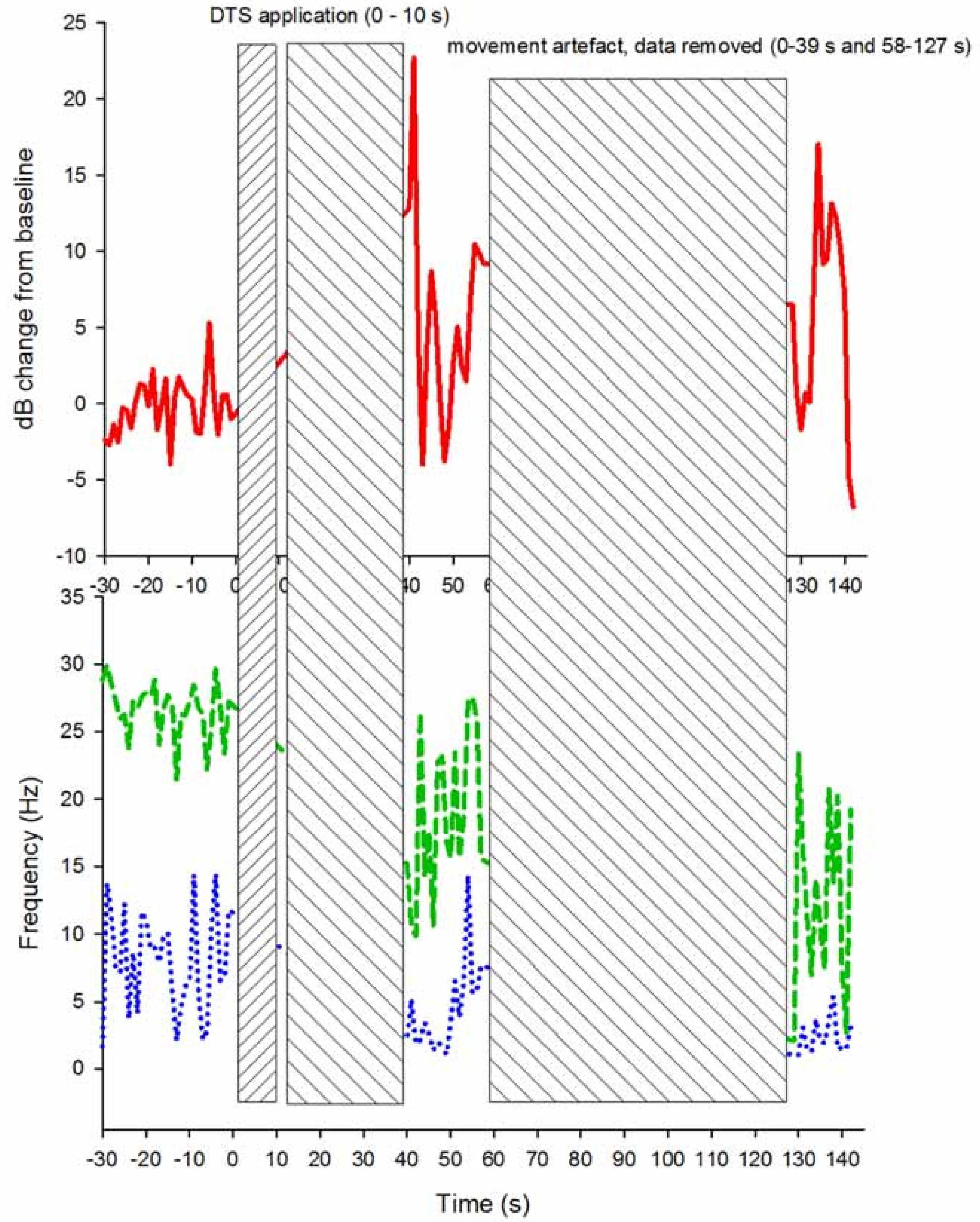
Animal 3 thermal map of forehead immediately after DTS application. Temperature at cursor: 62.5 °C; maximum temperature in image (white): 89.4 °C; minimum temperature in image (black): 19.5 °C. Heating at edges of waveguide is evident. Disk shapes with cool centres and cables are EEG recording electrode pads and leads.



Animal 3 estimated temperature change on the forehead by energy delivered.

EEG traces

A chart showing changes in EEG parameters following DTS application (narrow hatching) in Animal 3 is shown below. Total EEG power (Ptot, red line) is dramatically increased and shows high amplitude spiking post DTS application; 95% spectral edge frequency (SEF95, green line) and 50% spectral edge frequency (SEF50, blue line) both dropped following DTS application. It is difficult to interpret the spectral edge frequency data, as the EEG recordings are heavily contaminated by electrical noise from the abattoir infrastructure, however, high amplitude spiking in Ptot and low frequency activity are considered to be incompatible with consciousness.

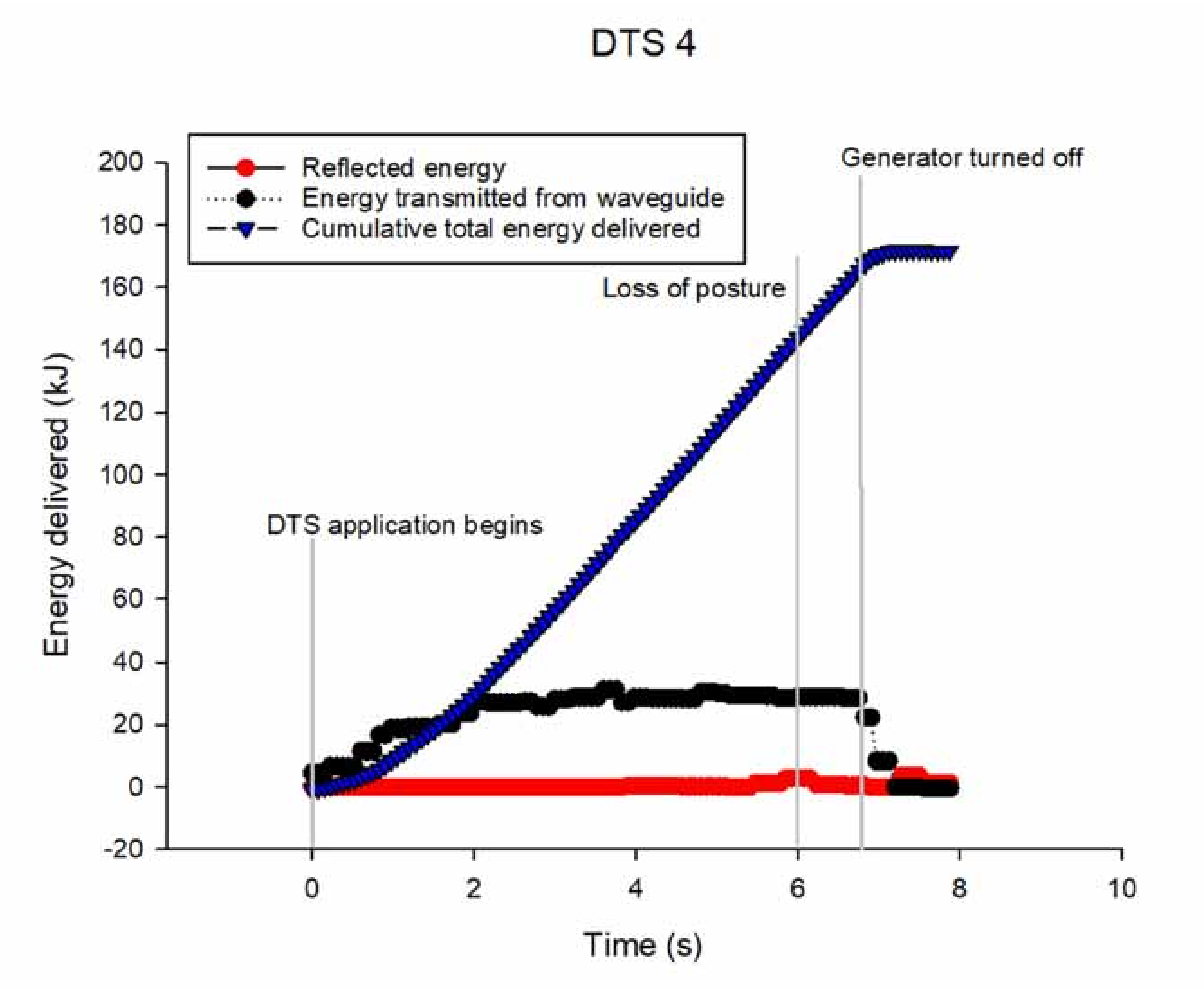

DTS application (0 - 10 s)
movement artefact, data removed (0-39 s and 58-127 s)
dB change from baseline
25
20
15
10
5
0
-5
-10
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
Frequency (Hz)
35
30
25
20
15
10
5
0
Time (s)

Animal 4

DTS settings

300 kJ, 30 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 6 seconds post onset of DTS application (although there was an initial loss of posture and return to standing at 1 second), which is equivalent to approximately 143 kJ of energy delivered.



Notes from real-time observations

The animal has an extremely contoured head, with a deep channel midline, so contact with the waveguide was not good.

At the point of loss of posture (140 kJ delivered), excessive arcing was evident.

Corneal reflex was absent for two tests, then began to return. Righting reflex returned, so captive bolt was applied before roll-out and exsanguination.

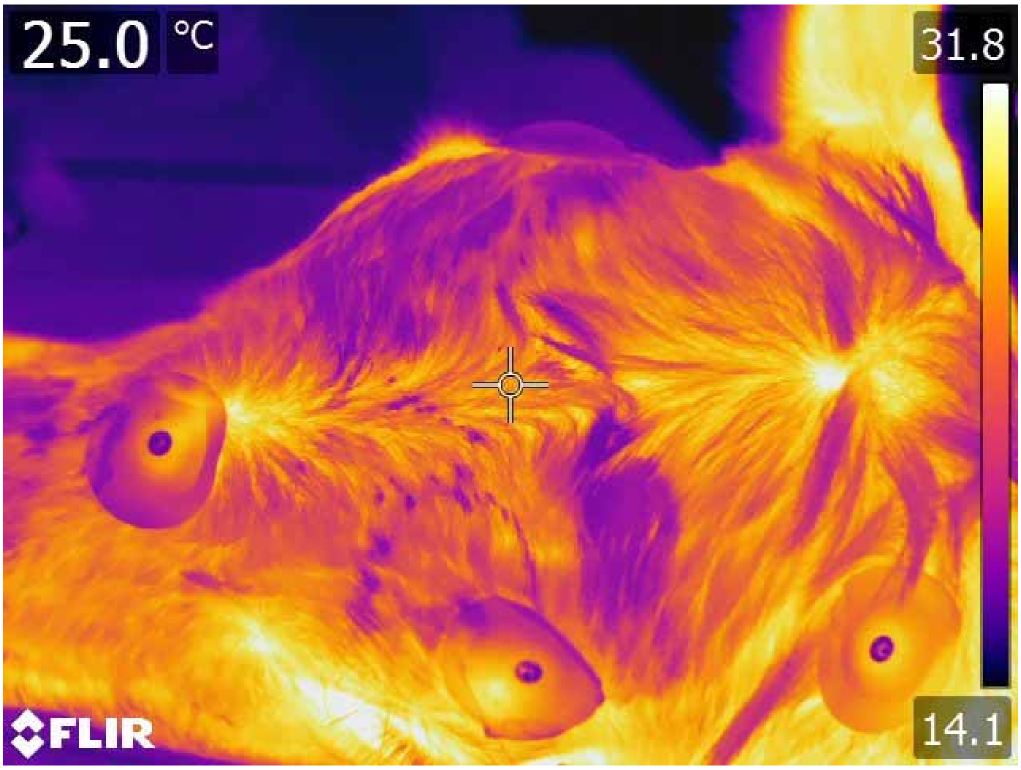
Examination of the waveguide indicated that the arcing occurred between the two prongs that were designed to slip over the poll and reduce the risk of waveguide movement. A break in proceedings was taken at this stage so that this positioning device could be re-designed to widen the prongs and improve fit over the poll, and also to insulate the prongs using duct tape.

Detailed event log from video footage

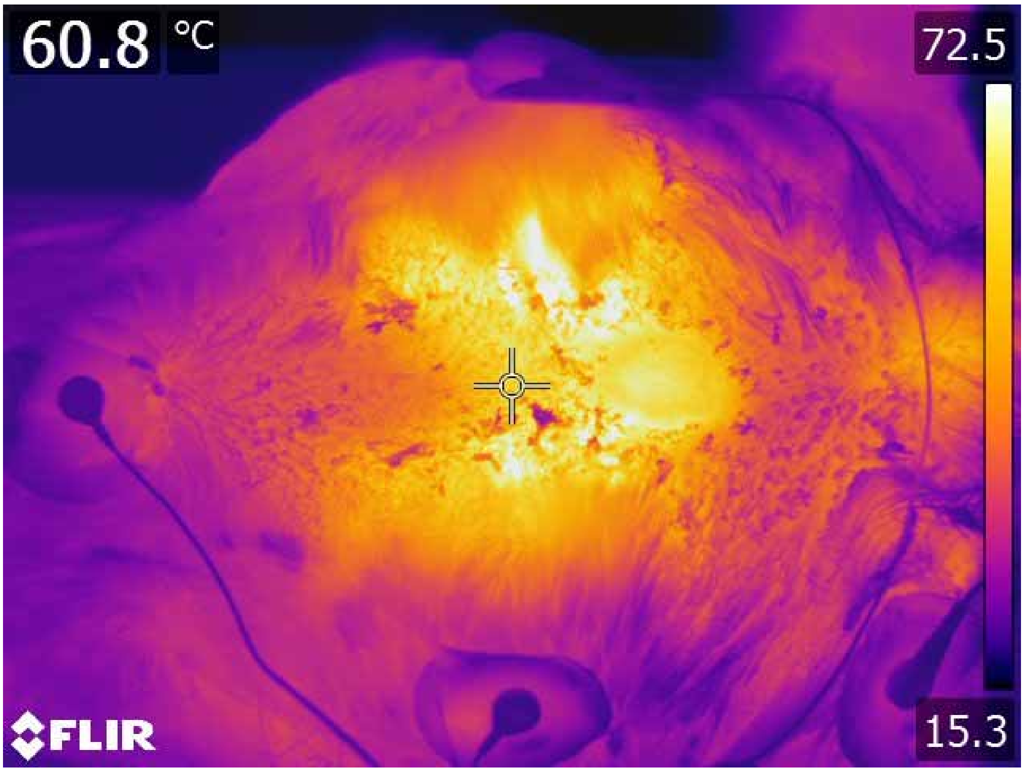
4	5/10/2017	8:58:02	animal in chin lift up wand in place
4	5/10/2017	8:58:22	DTS starts
4	5/10/2017	8:58:28	big plasma leakage (pyrotechnics!) rapid blinking, rear legs collapse
4	5/10/2017	8:59:11	leads on
4	5/10/2017	8:59:36	breathing
4	5/10/2017	8:59:44	stands
4	5/10/2017	8:59:59	tail swishing
4	5/10/2017	9:00:03	wand removed
4	5/10/2017	9:00:03	scorching to top of head
4	5/10/2017	9:00:26	ears moving
4	5/10/2017	9:00:30	urinates and blinks
4	5/10/2017	9:00:40	blinks
4	5/10/2017	9:00:48	blinks
4	5/10/2017	9:01:00	corneal reflex
4	5/10/2017	9:01:05	leads removed
4	5/10/2017	9:01:16	blinks
4	5/10/2017	9:01:23	blinks
4	5/10/2017	9:01:32	captive bolt, rear end collapses
4	5/10/2017	9:01:40	chin lift dropped
4	5/10/2017	9:01:53	head bale opens, left front leg stuck
4	5/10/2017	9:02:25	side opens, still stuck
4	5/10/2017	9:02:28	on table
4	5/10/2017	9:03:32	skin cut
4	5/10/2017	9:04:03	clip and cut oesophagus
4	5/10/2017	9:04:15	stuck.
4	5/10/2017	9:05:18	hung

Thermal images

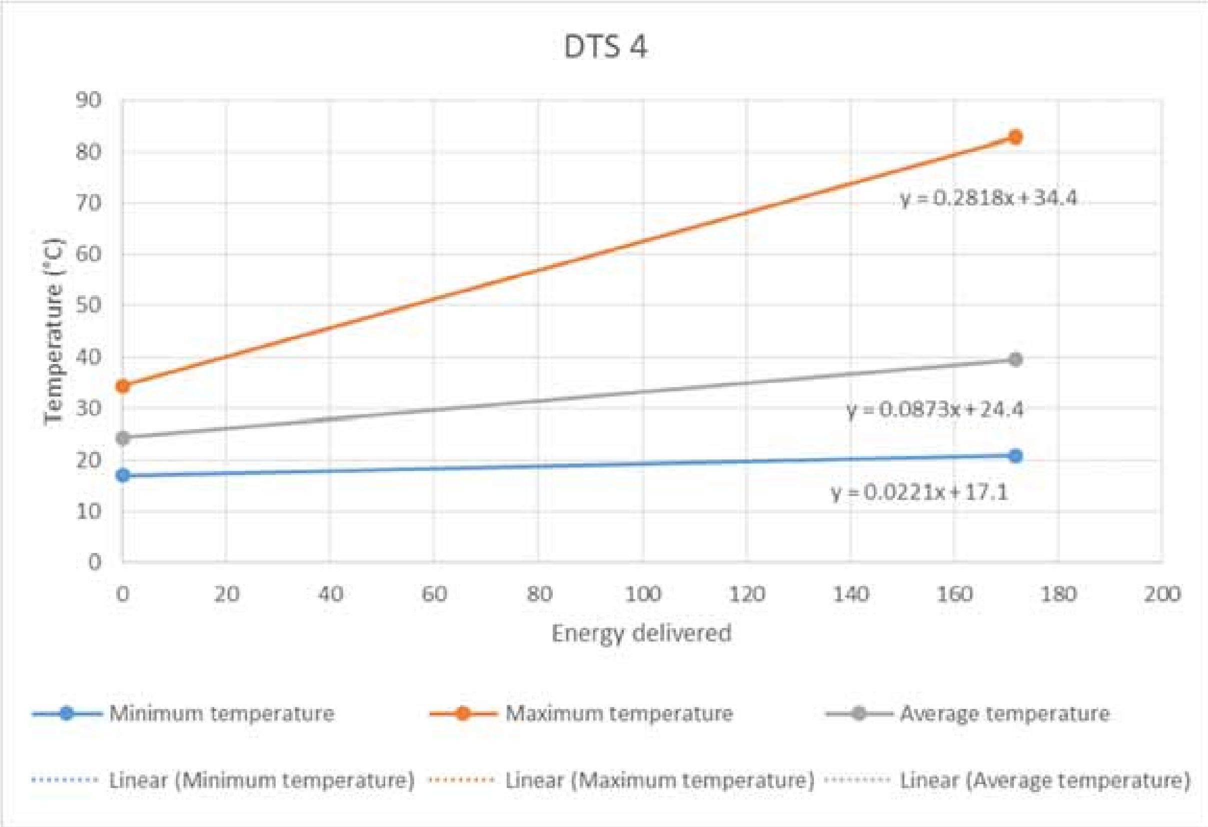
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 4 lost posture at approximately 6 seconds post onset of DTS application, which is equivalent to approximately 143 kJ of energy delivered. At 143 kJ energy delivered the estimated average forehead temperature is 36.88 °C (20.26 – 74.70).



Animal 4 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 25.0 °C; maximum temperature in image (white): 31.8 °C; minimum temperature in image (black): 14.1 °C. Disk shapes with cool centres are EEG recording electrode pads.



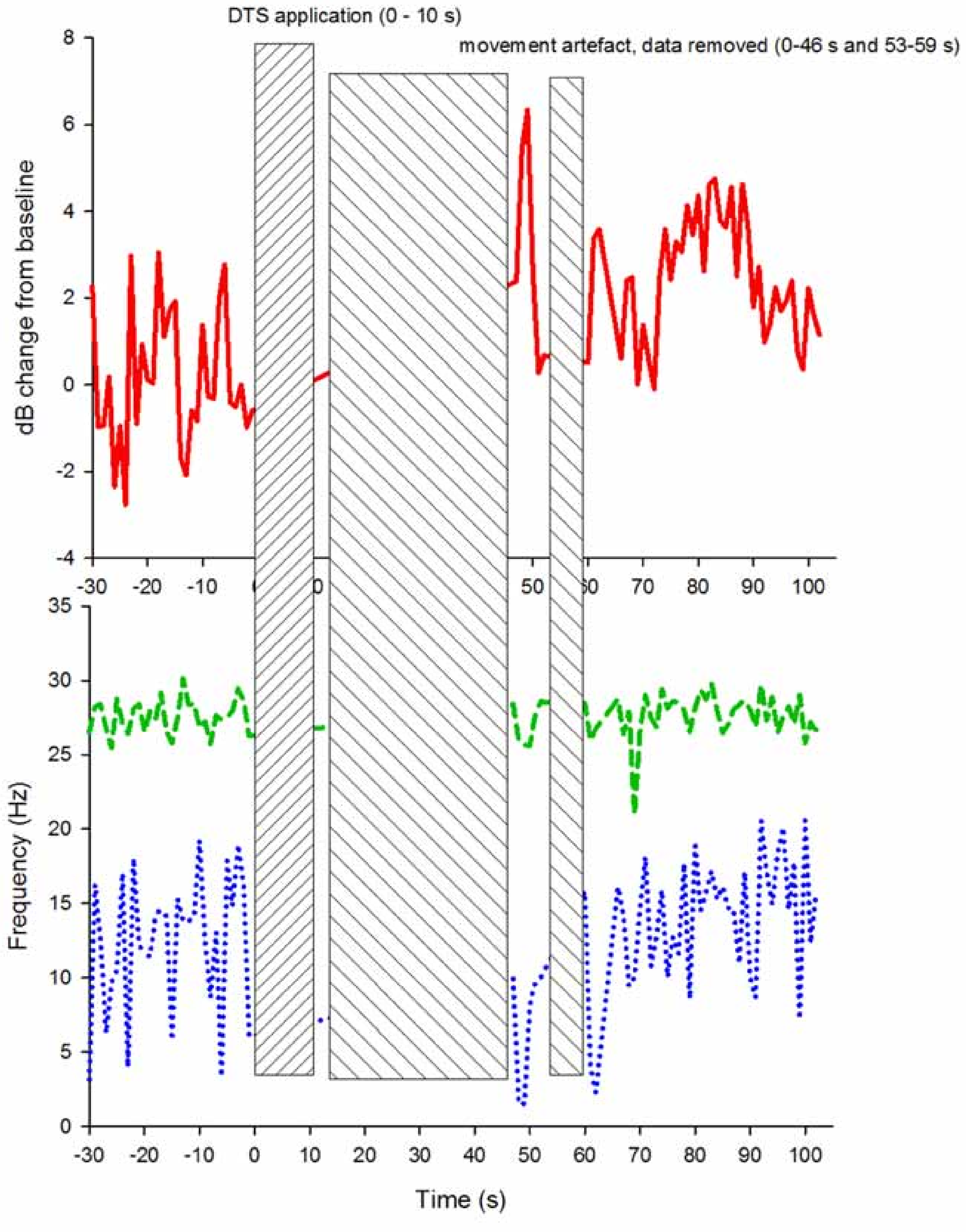
Animal 4 thermal map of forehead immediately after DTS application. Temperature at cursor: 60.8 °C; maximum temperature in image (white): 72.5 °C; minimum temperature in image (black): 15.3 °C. Singed hair as a result of arcing and plasma formation, and heating towards the poll as a result of leakage are evident. Disk shapes with cool centres and cables are EEG recording electrode pads and leads.



Animal 4 estimated temperature change on the forehead by energy delivered.

EEG traces

A chart showing changes in EEG parameters following DTS application (narrow hatching) in Animal 4 is shown below. Total EEG power (Ptot, red line) is dramatically increased and shows high amplitude spiking post DTS application; 50% spectral edge frequency (SEF50, blue line) dropped following DTS application. It is difficult to interpret the spectral edge frequency data, as the EEG recordings are heavily contaminated by electrical noise from the abattoir infrastructure, however, high amplitude spiking in Ptot is considered to be incompatible with consciousness.

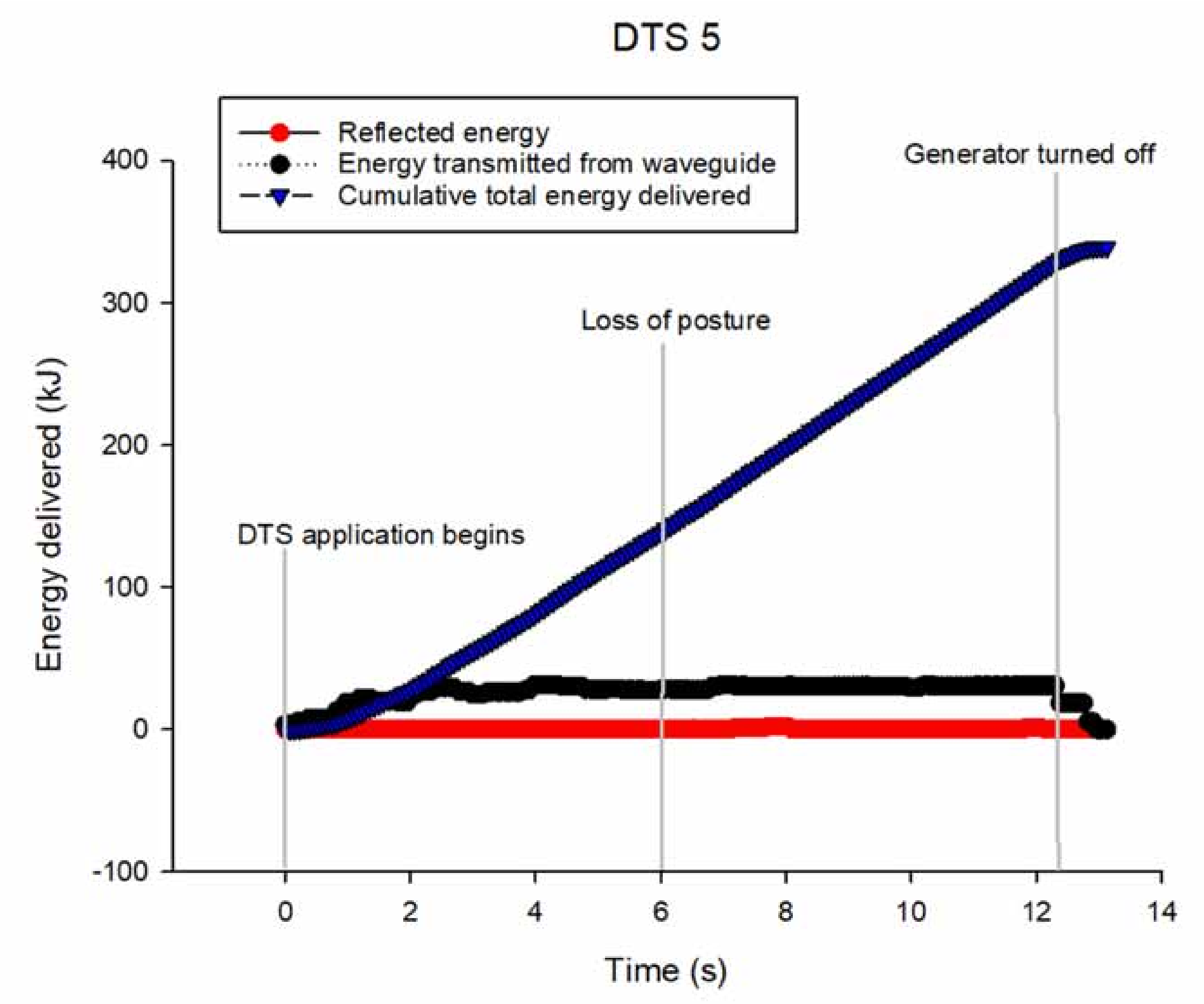

DTS application (0 - 10 s)
movement artefact, data removed (0-46 s and 53-59 s)
dB change from baseline
Frequency (Hz)
Time (s)

Animal 5

DTS settings

300 kJ, 30 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 6 seconds post onset of DTS application, which is equivalent to approximately 138 kJ of energy delivered.



Notes from real-time observations

Loss of posture, flickering eyelids, similar to electric stun.

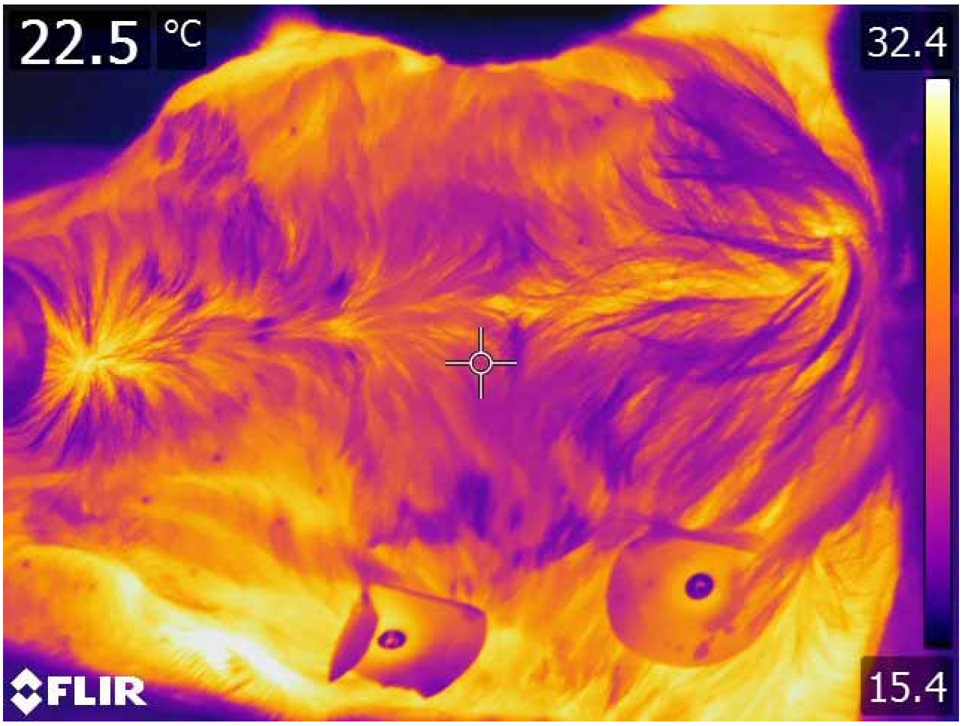
Corneal reflex absent, but slow ocular withdrawal present. Transitioned to loss of withdrawal, then appeared dead. Rolled out and exsanguinated.

Detailed event log from video footage

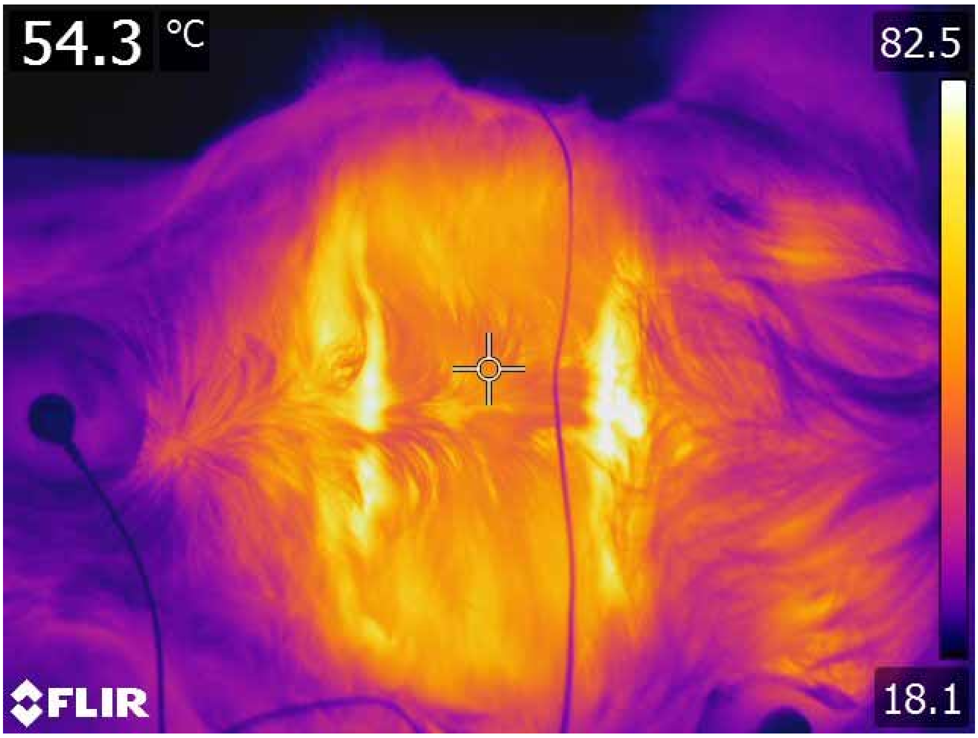
5	5/10/2017	10:43:03	animal in chin lift up wand in place
5	5/10/2017	10:43:20	body movements in crate
5	5/10/2017	10:43:29	DTS starts
5	5/10/2017	10:43:31	eyes blinking rapidly
5	5/10/2017	10:43:35	body movements, rear end collapses
5	5/10/2017	10:43:36	lots of steam, obscures head
5	5/10/2017	10:43:51	rapid blinking
5	5/10/2017	10:43:52	stands up, breathing
5	5/10/2017	10:43:58	body movements in crate
5	5/10/2017	10:43:58	head camera moved, view obscured.
5	5/10/2017	10:44:46	wand removed
5	5/10/2017	10:44:50	corneal reflex, blinking
5	5/10/2017	10:45:06	leads on
5	5/10/2017	10:45:45	rear end collapses
5	5/10/2017	10:45:56	corneal reflex
5	5/10/2017	10:46:23	corneal reflex
5	5/10/2017	10:46:41	leads removed
5	5/10/2017	10:46:53	corneal reflex
5	5/10/2017	10:47:04	chin lift dropped
5	5/10/2017	10:47:10	head bale open
5	5/10/2017	10:47:26	side door opened, on table
5	5/10/2017	10:47:44	rolls off table and onto floor
5	5/10/2017	10:47:58	skin cut
5	5/10/2017	10:48:27	clip and cut oesophagus
5	5/10/2017	10:47:33	stuck.
5	5/10/2017	10:50:02	hung then dropped back on table?
5	5/10/2017	10:56:46	rehung. Video stops

Thermal images

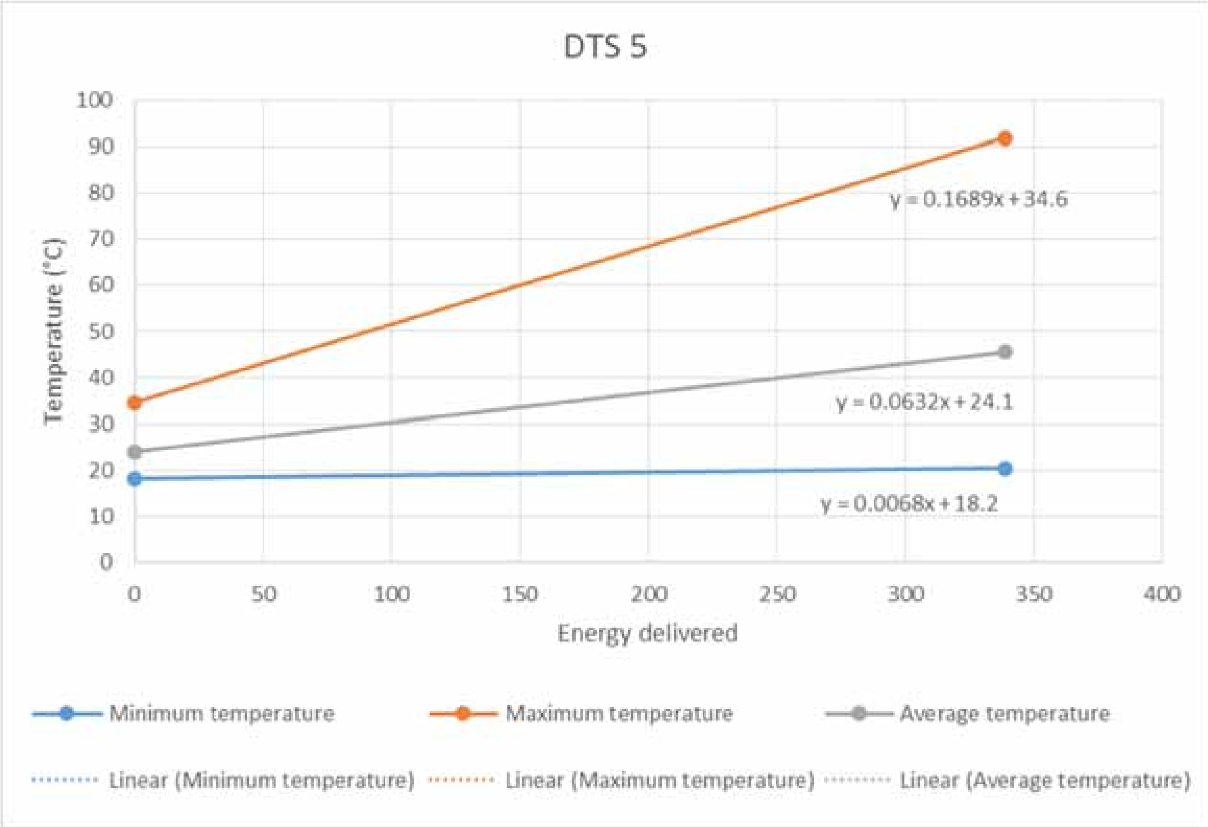
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 5 lost posture at approximately 6 seconds post onset of DTS application, which is equivalent to approximately 138 kJ of energy delivered. At 138 kJ energy delivered the estimated average forehead temperature is 32.82 °C (19.13 – 57.90).



Animal 5 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 22.5 °C; maximum temperature in image (white): 32.4 °C; minimum temperature in image (black): 15.4 °C. Disk shapes with cool centres are EEG recording electrode pads.



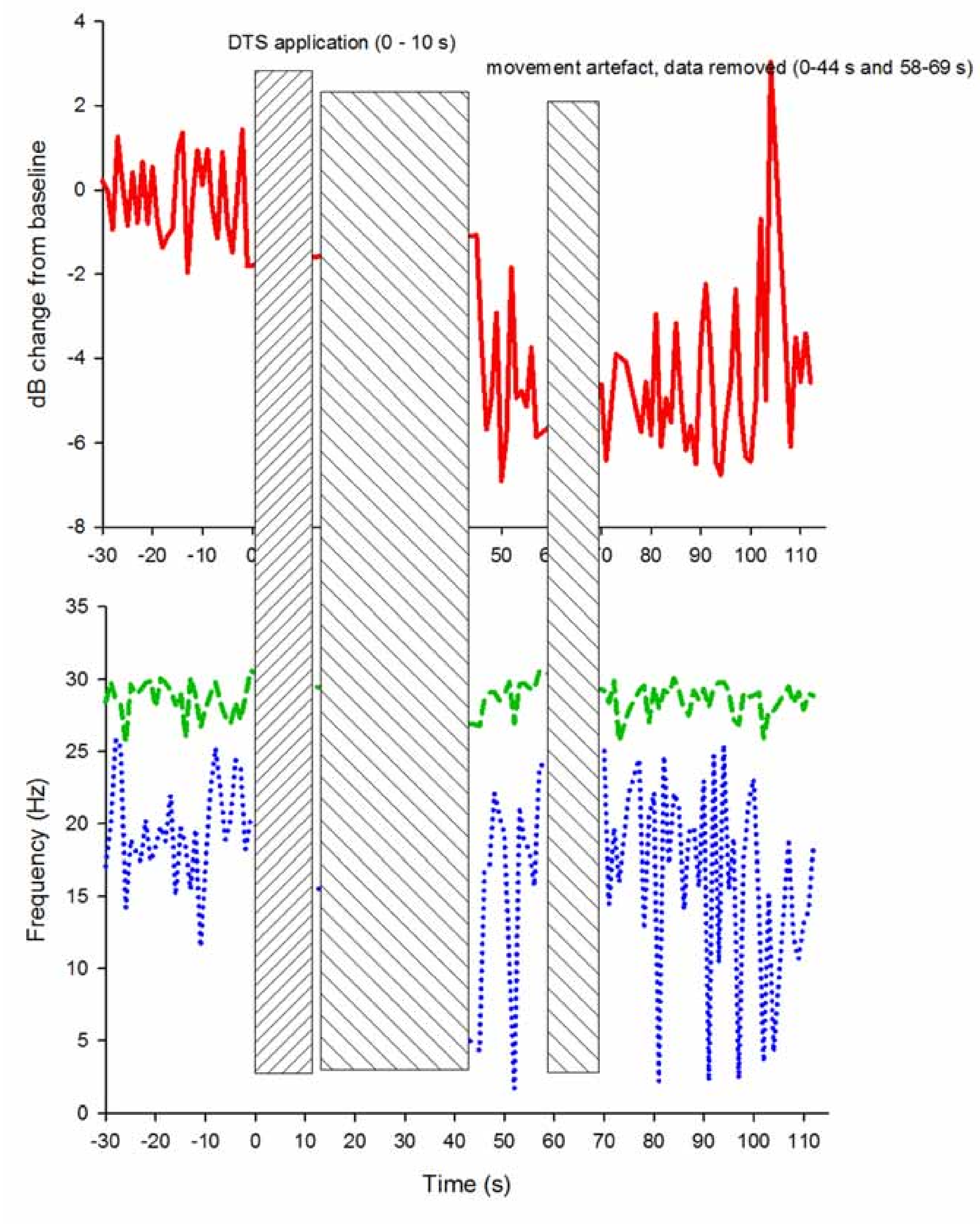
Animal 5 thermal map of forehead immediately after DTS application. Temperature at cursor: 54.3 °C; maximum temperature in image (white): 82.5 °C; minimum temperature in image (black): 18.1 °C. Heating at edges of waveguide is evident. Disk shapes with cool centres and cables are EEG recording electrode pads and leads.



Animal 5 estimated temperature change on the forehead by energy delivered.

EEG traces

A chart showing changes in EEG parameters following DTS application (narrow hatching) in Animal 5 is shown below. Total EEG power (Ptot, red line) is dramatically decreased post DTS application; 95% spectral edge frequency (SEF95, green line) and 50% spectral edge frequency (SEF50, blue line) both dropped slightly following DTS application, with SEF50 showing high amplitude variability. It is difficult to interpret these data, as the EEG recordings are heavily contaminated by electrical noise from the abattoir infrastructure.

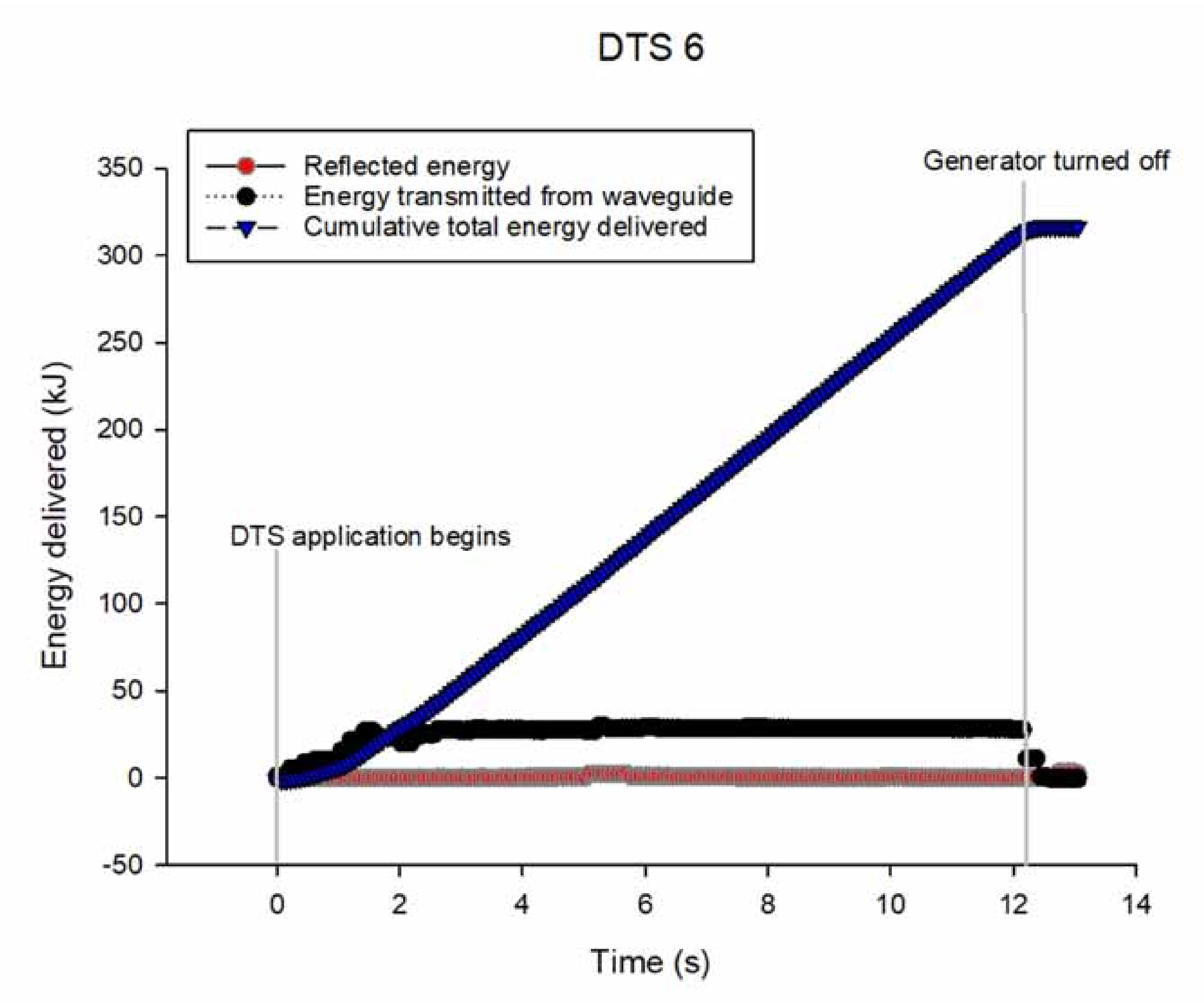

DTS application (0 - 10 s)
movement artefact, data removed (0-44 s and 58-69 s)
dB change from baseline
Frequency (Hz)
Time (s)

Animal 6

DTS settings

275 kJ, 30 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 19 seconds post onset of DTS application, which was after completion of DTS application and total energy delivered of 317 kJ.



Notes from real-time observations

Loss of posture, flickering eyelids, similar to electric stun.

Slow corneal reflex or ocular withdrawal, deep rhythmic breathing.

Regained standing posture, but ataxic, then convulsive movements, collapsed again, EEG trace transitioned towards isoelectric.

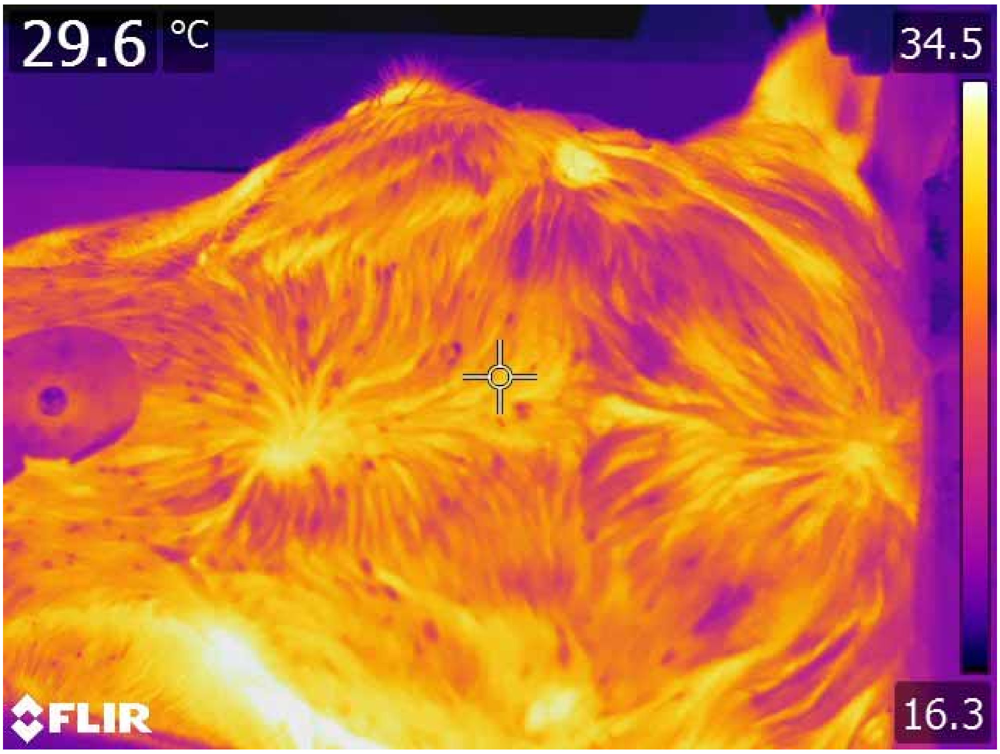
Animal appeared dead, rolled out and exsanguinated.

Detailed event log from video footage

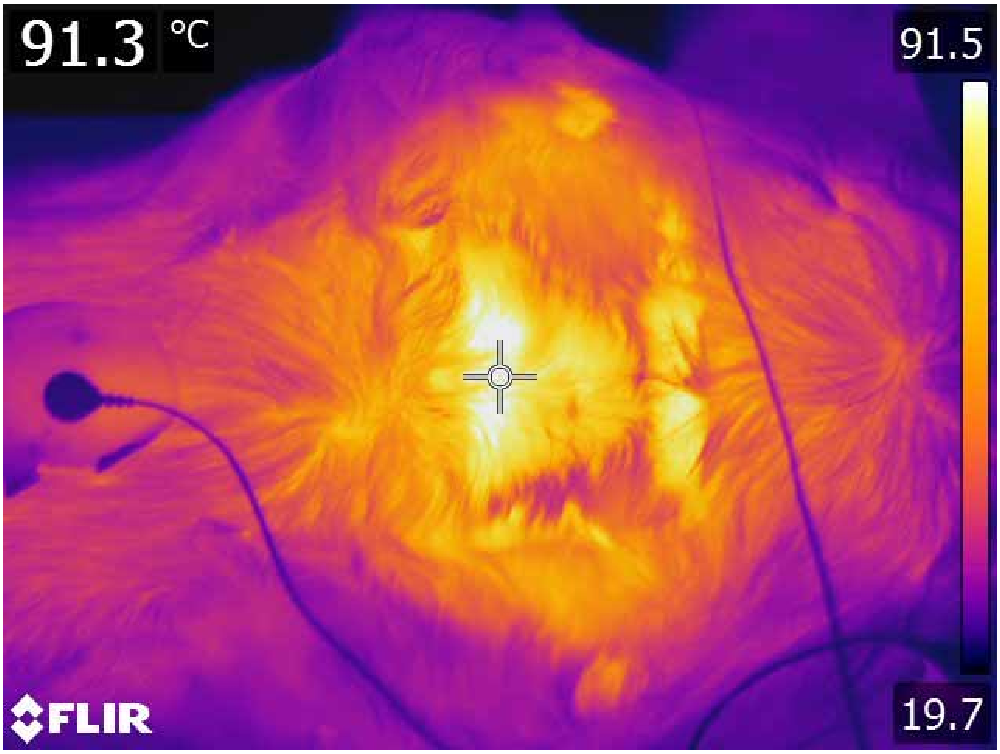
6	5/10/2017	11:05:01	animal in chin lift up wand in place
6	5/10/2017	11:05:29	DTS starts
6	5/10/2017	11:05:31	moves rear legs
6	5/10/2017	11:05:33	steam rises
6	5/10/2017	11:05:48	rapid blinking, rear end collapses
6	5/10/2017	11:05:53	stands, tail swishing
6	5/10/2017	11:06:13	wand removed
6	5/10/2017	11:06:17	corneal reflex
6	5/10/2017	11:06:35	leads on
6	5/10/2017	11:06:47	body movements in crate
6	5/10/2017	11:06:49	thermal image
6	5/10/2017	11:07:01	body movement continues
6	5/10/2017	11:07:30	body movement continues
6	5/10/2017	11:07:39	corneal reflex
6	5/10/2017	11:08:17	corneal reflex
6	5/10/2017	11:08:34	corneal reflex
6	5/10/2017	11:08:57	rear legs collapse
6	5/10/2017	11:09:17	remove leads
6	5/10/2017	11:09:33	chin lift dropped
6	5/10/2017	11:09:38	head bale opened
6	5/10/2017	11:09:52	side door opens, falls onto table
6	5/10/2017	11:10:13	skin cut
6	5/10/2017	11:10:48	clip and cut oesophagus
6	5/10/2017	11:11:00	stuck.
6	5/10/2017	11:11:55	hung

Thermal images

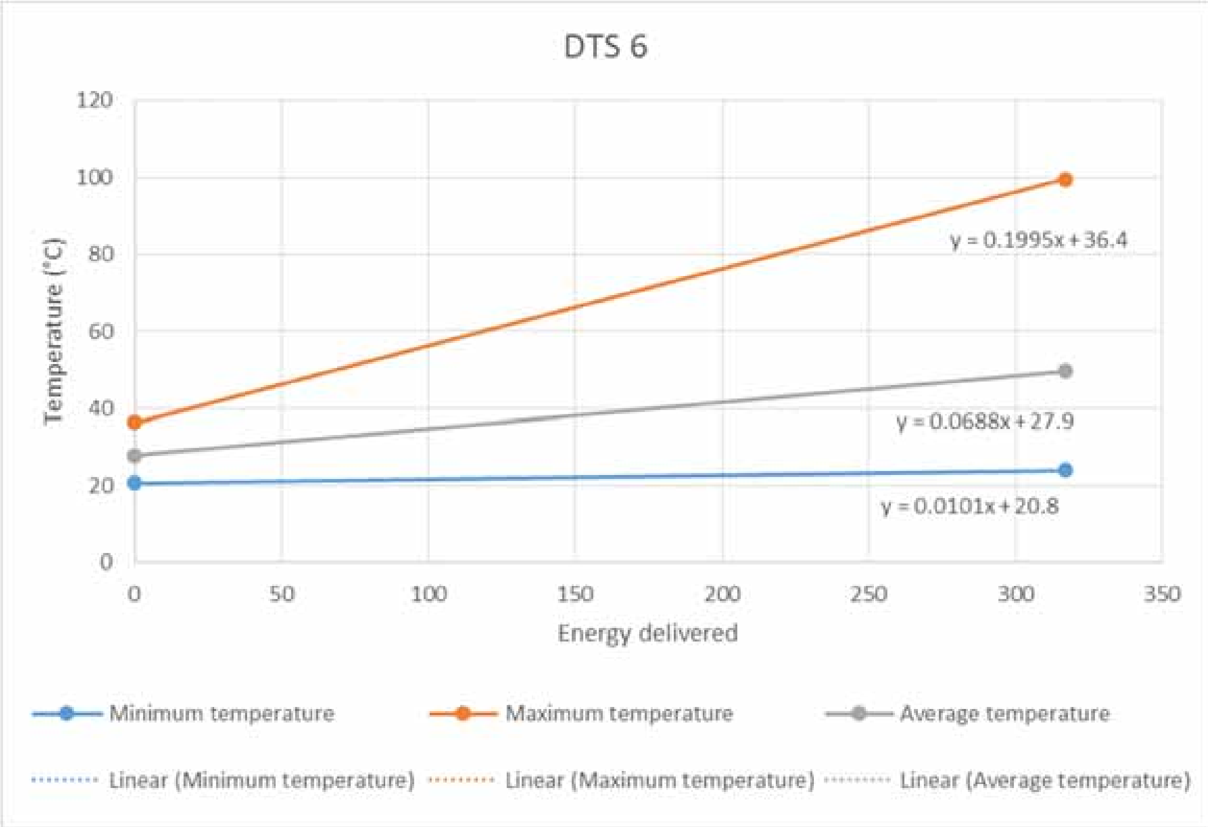
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 6 lost posture at approximately 19 seconds post onset of DTS application, after the entire energy application had completed, delivering a total of 317 kJ. At 317 kJ energy delivered the estimated average forehead temperature is 49.71 °C (24.00 – 99.64).



Animal 6 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 29.6 °C; maximum temperature in image (white): 34.5 °C; minimum temperature in image (black): 16.3 °C. Disk shapes with cool centres are EEG recording electrode pads.



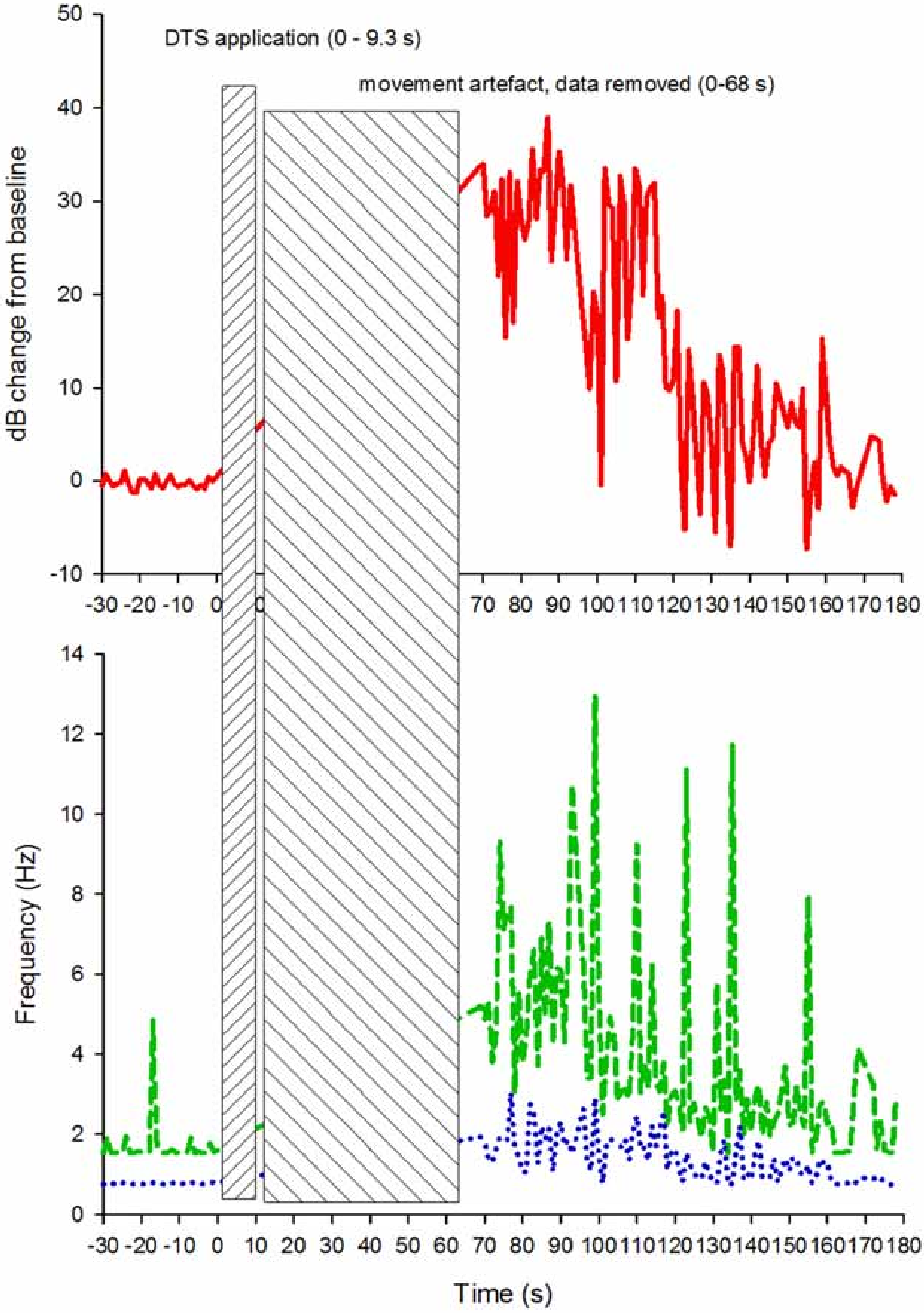
Animal 6 thermal map of forehead immediately after DTS application. Temperature at cursor: 91.3 °C; maximum temperature in image (white): 91.5 °C; minimum temperature in image (black): 19.7 °C. Heating at edges of waveguide is evident, and also heating around the waveguide aperture, as a result of leakage. Disk shapes with cool centres and cables are EEG recording electrode pads and leads.



Animal 6 estimated temperature change on the forehead by energy delivered.

EEG traces

A chart showing changes in EEG parameters following DTS application (narrow hatching) in Animal 6 is shown below. Total EEG power (Ptot, red line) is dramatically increased and shows high amplitude spiking post DTS application; 95% spectral edge frequency (SEF95, green line) increased, and shows high amplitude variability following DST application and 50% spectral edge frequency (SEF50, blue line) increased following DTS application. It is difficult to interpret the spectral edge frequency data, as the EEG recordings are heavily contaminated by electrical noise from the abattoir infrastructure, however, high amplitude spiking in Ptot is considered to be incompatible with consciousness.



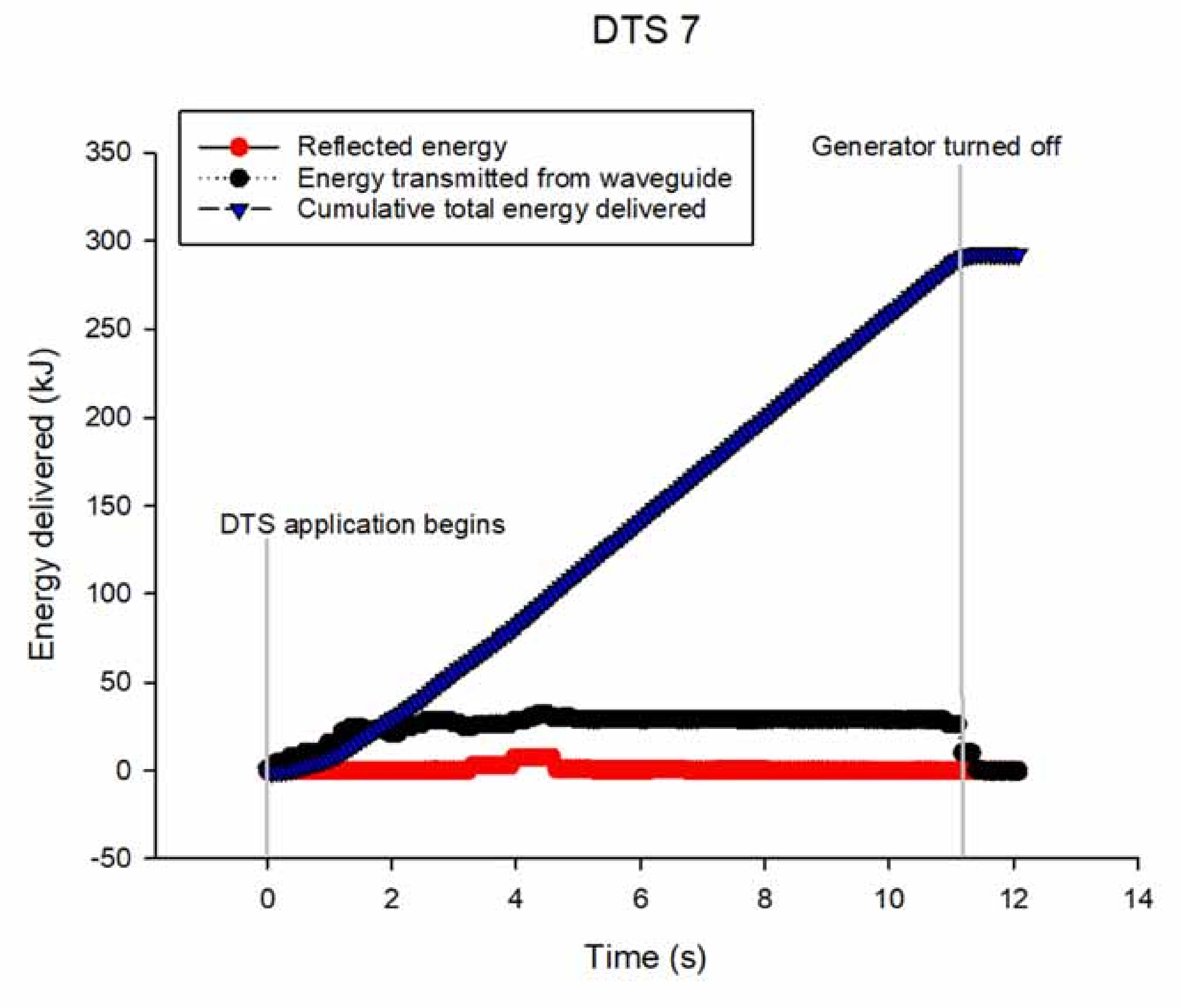
DTS application (0 - 9.3 s)
movement artefact, data removed (0-68 s)
dB change from baseline
50
40
30
20
10
0
-10
-30 -20 -10 0 70 80 90 100 110 120 130 140 150 160 170 180
Frequency (Hz)
14
12
10
8
6
4
2
0
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180
Time (s)

Animal 7

DTS settings

250 kJ, 30 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 15 seconds post onset of DTS application, which was after completion of the DTS application and total energy delivered of 292 kJ.



Notes from real-time observations

Loss of posture, flickering eyelids.

Eye rolled down, and deep breathing, similar to deep sedation. Corneal reflex initially absent, then slow response. Then corneal reflex returned, righting reflex returned and the animal regained standing posture in an almost deliberate manner. The eye then rolled back to normal position, and consciousness appeared to return.

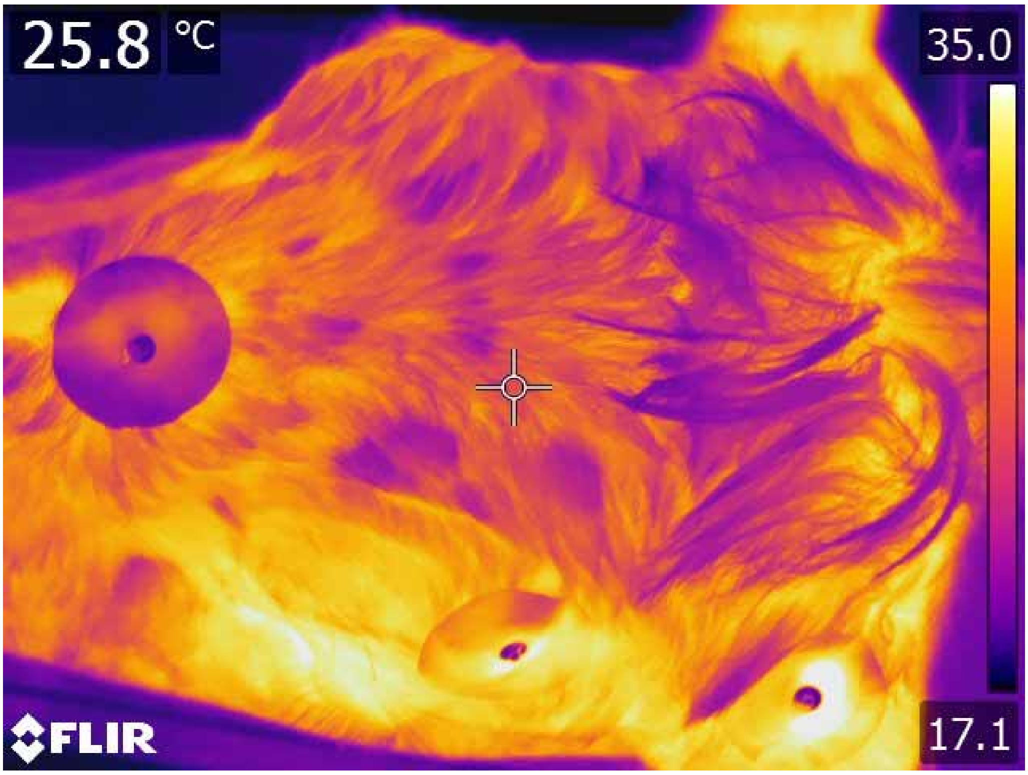
Captive bolt was applied, and the body rolled out for exsanguination.

Detailed event log from video footage

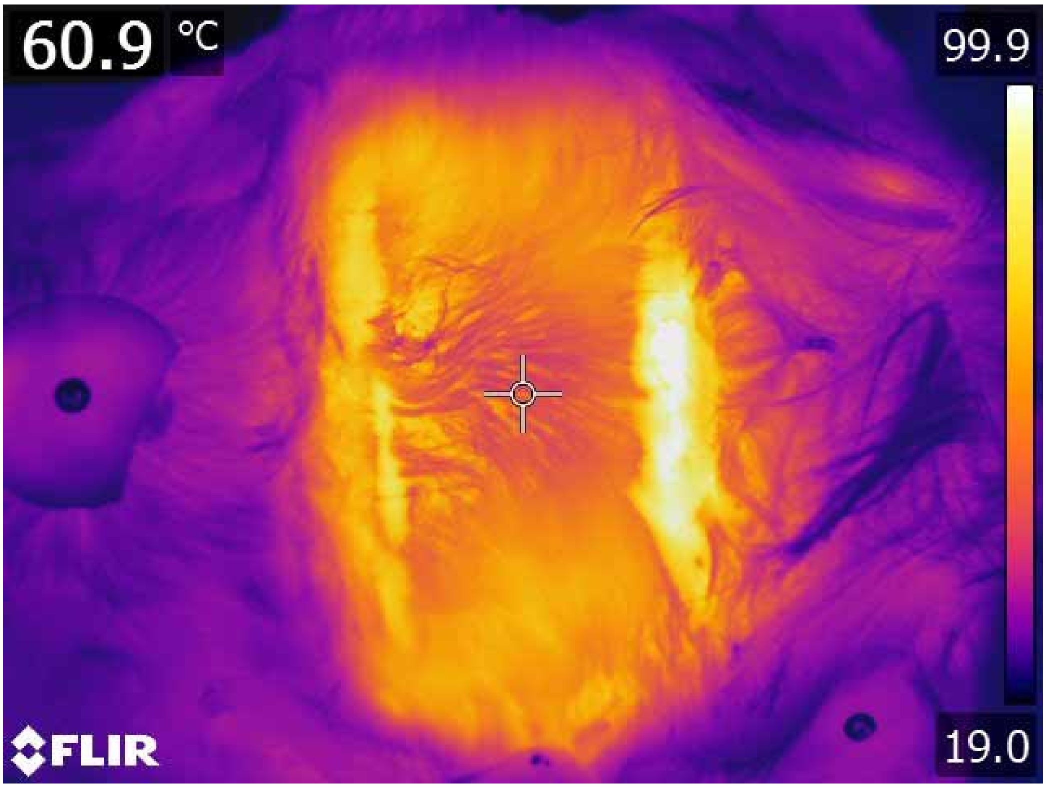
7	5/10/2017	11:33:25	animal in chin lift up wand in place
7	5/10/2017	11:34:04	DTS starts
7	5/10/2017	11:34:05	eyes open wide
7	5/10/2017	11:34:06	body movements in crate
7	5/10/2017	11:34:11	steam obscures
7	5/10/2017	11:34:19	rear legs collapse
7	5/10/2017	11:34:32	rear legs paddling
7	5/10/2017	11:34:44	wand removed, skull cap lifted
7	5/10/2017	11:34:48	stops paddling
7	5/10/2017	11:34:53	blinks
7	5/10/2017	11:35:05	thermal image, eyes wide
7	5/10/2017	11:35:18	leads on
7	5/10/2017	11:35:21	corneal reflex
7	5/10/2017	11:35:36	deep breathing
7	5/10/2017	11:35:49	corneal reflex
7	5/10/2017	11:36:33	continues deep breathing
7	5/10/2017	11:36:51	corneal reflex
7	5/10/2017	11:36:53	stands
7	5/10/2017	11:37:10	corneal reflex
7	5/10/2017	11:37:19	corneal reflex
7	5/10/2017	11:37:28	body movements in crate
7	5/10/2017	11:37:34	leads removed
7	5/10/2017	11:37:55	captive bolt
7	5/10/2017	11:37:57	rear legs collapse
7	5/10/2017	11:38:03	chin lift dropped
7	5/10/2017	11:38:07	head bale opened
7	5/10/2017	11:38:20	side door opened, on table
7	5/10/2017	11:38:32	on table
7	5/10/2017	11:39:13	skin cut
7	5/10/2017	11:39:27	clip and cut oesophagus
7	5/10/2017	11:39:33	stuck.

Thermal images

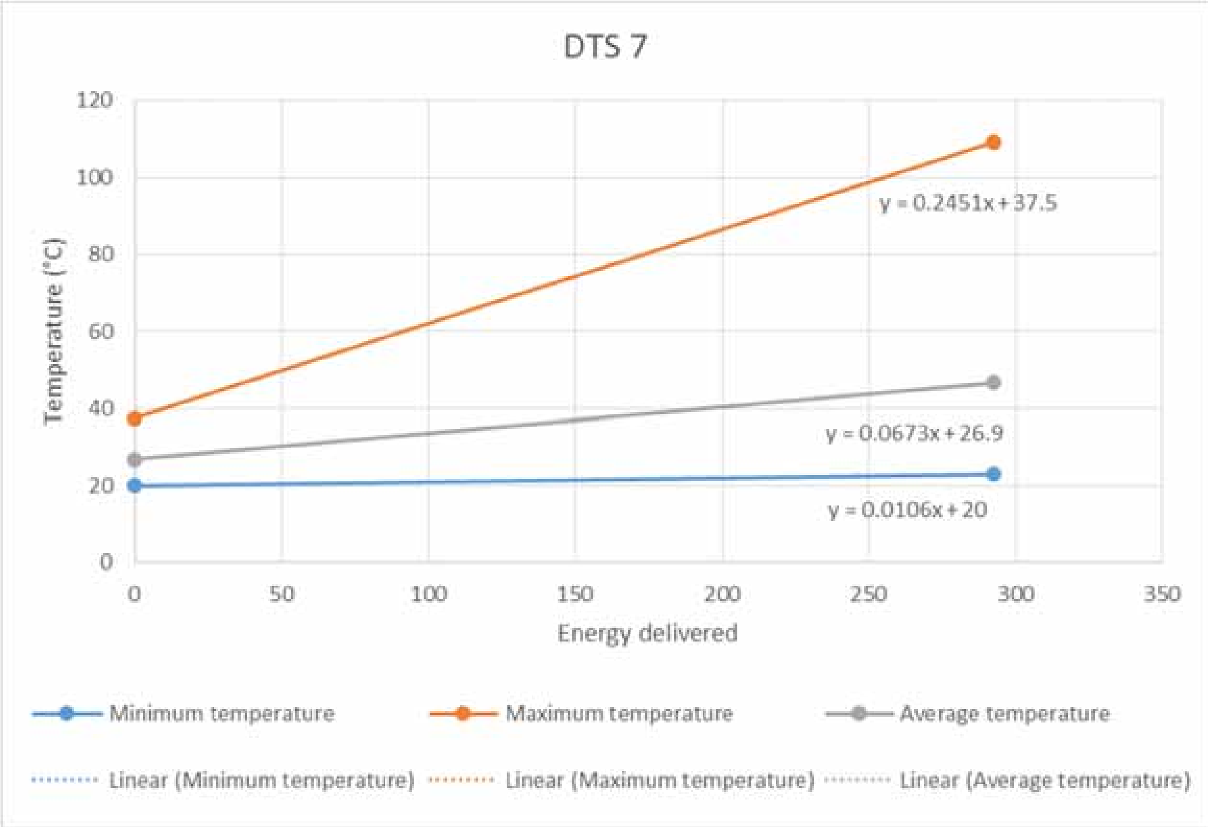
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 7 lost posture at approximately 15 seconds post onset of DTS application, after completion of energy delivery at 292 kJ of energy delivered. At 292 kJ energy delivered the estimated average forehead temperature is 46.55 °C (23.10 – 109.07).



Animal 7 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 25.8 °C; maximum temperature in image (white): 35.0 °C; minimum temperature in image (black): 17.1 °C. Disk shapes with cool centres are EEG recording electrode pads.



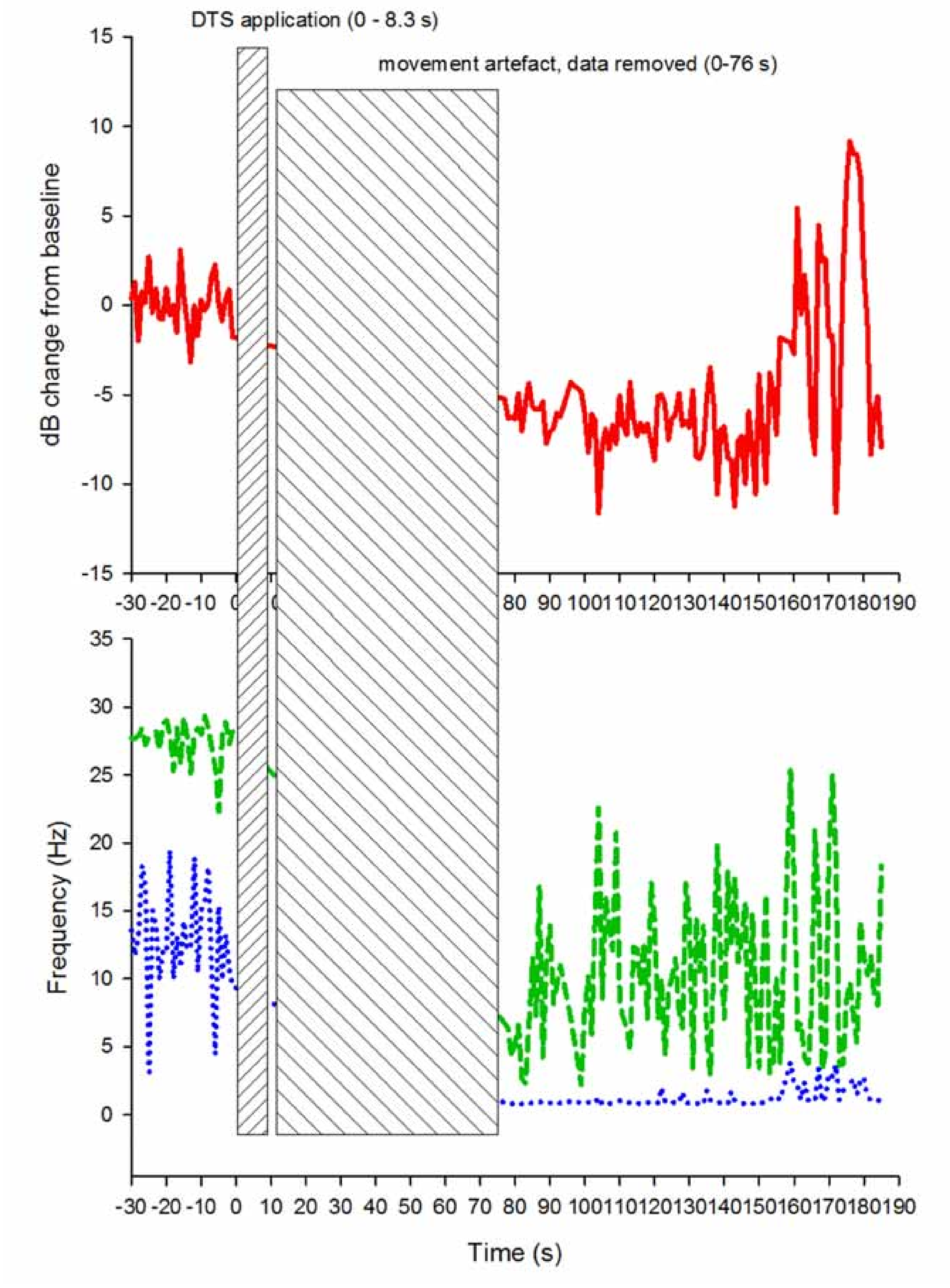
Animal 7 thermal map of forehead immediately after DTS application. Temperature at cursor: 60.9 °C; maximum temperature in image (white): 99.9 °C; minimum temperature in image (black): 19.0 °C. Heating at edges of waveguide is evident. Disk shapes with cool centres are EEG recording electrode pads.



Animal 7 estimated temperature change on the forehead by energy delivered.

EEG traces

A chart showing changes in EEG parameters following DTS application (narrow hatching) in Animal 7 is shown below. Total EEG power (Ptot, red line) dropped post DTS application; 95% spectral edge frequency (SEF95, green line) and 50% spectral edge frequency (SEF50, blue line) both dropped dramatically following DTS application. It is difficult to interpret these data, as the EEG recordings are heavily contaminated by electrical noise from the abattoir infrastructure, however, low frequency activity is considered to be incompatible with consciousness.

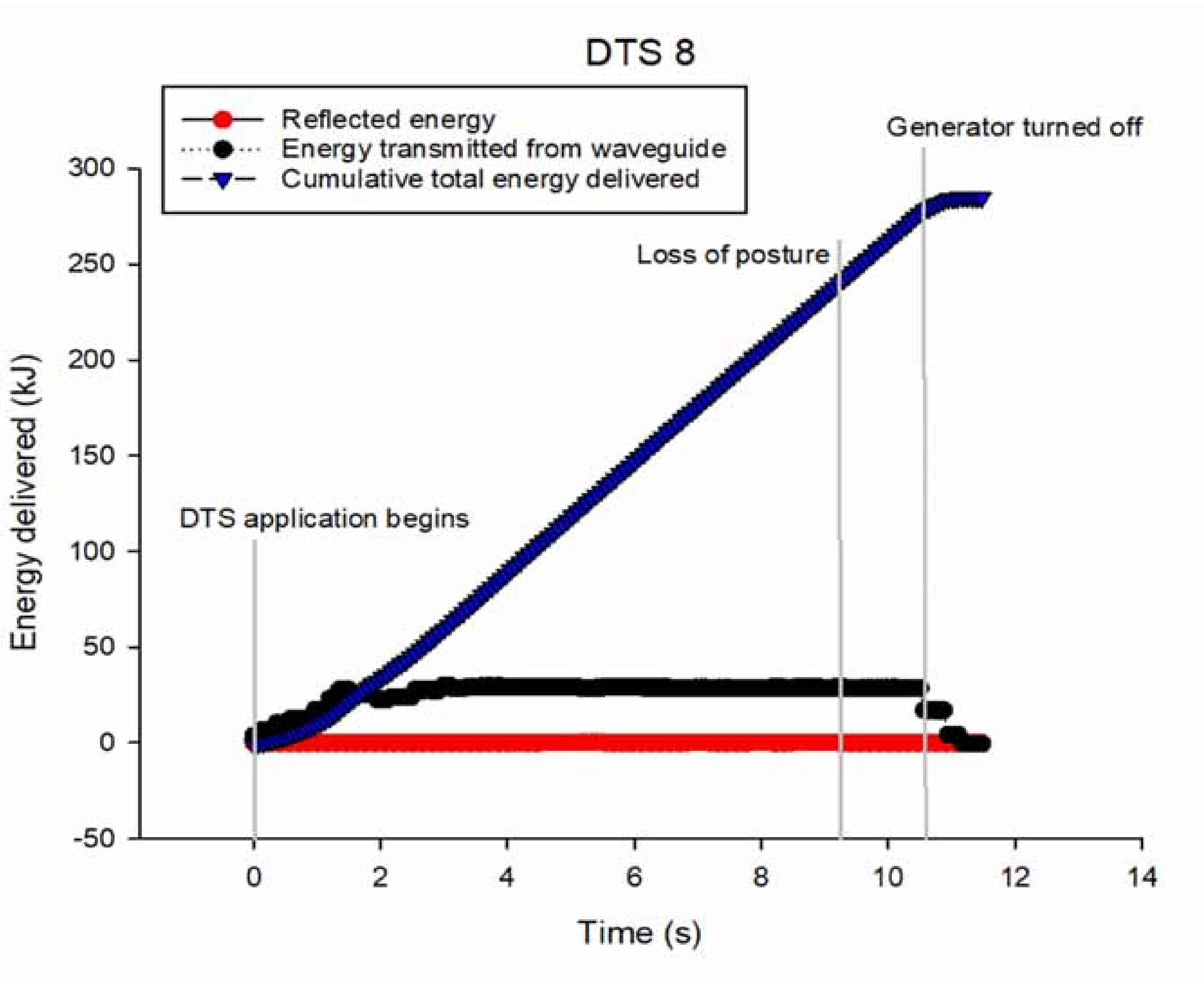

DTS application (0 - 8.3 s)
movement artefact, data removed (0-76 s)
dB change from baseline
Frequency (Hz)
Time (s)
15
10
5
0
-5
-10
-15
35
30
25
20
15
10
5
0
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190

Animal 8

DTS settings

250 kJ, 30 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 9 seconds post onset of DTS application, which is equivalent to approximately 233 kJ of energy delivered.



Notes from real-time observations

Animal has a pronounced ridge on the poll, so positioning of the waveguide was difficult, and took some time. This may pre-stress the animal, but it did not seem to be objecting to the process.

Estimated interval between onset of physical response to DTS (rapid eye blinking) to collapse was 3 sec.

Loss of posture, some flickering of eyelids and ear, similar to an electric stun.

No corneal reflex; deep, slow respiration, eye in normal position.

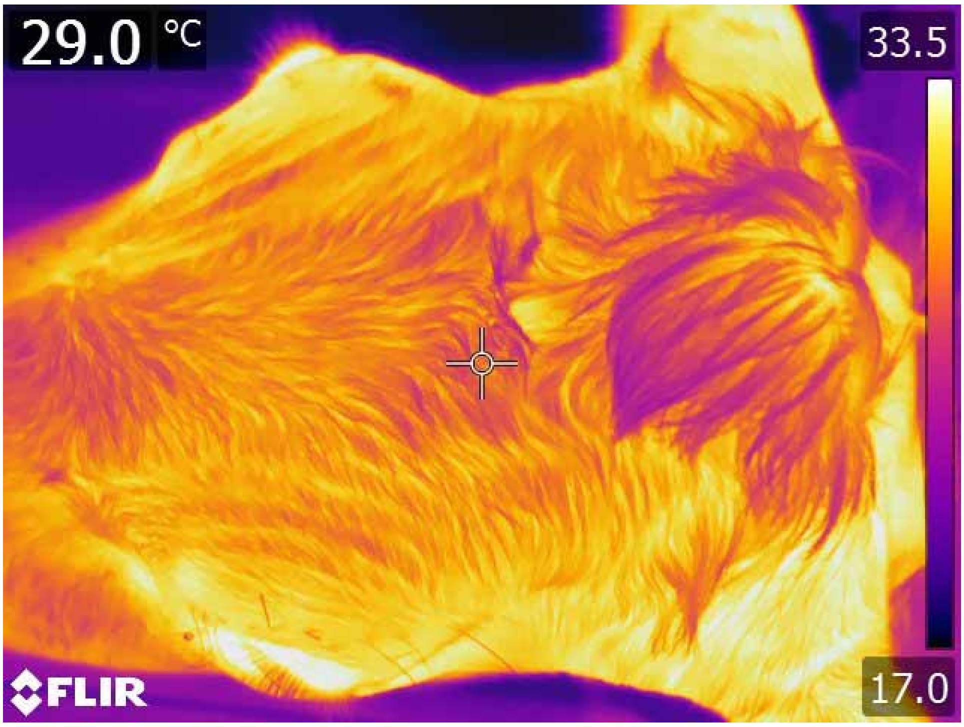
Palpebral reflexes returning at 3 min 10 s from loss of posture (measured using stopwatch). Captive bolt applied and rolled out for exsanguination.

Detailed event log from video footage

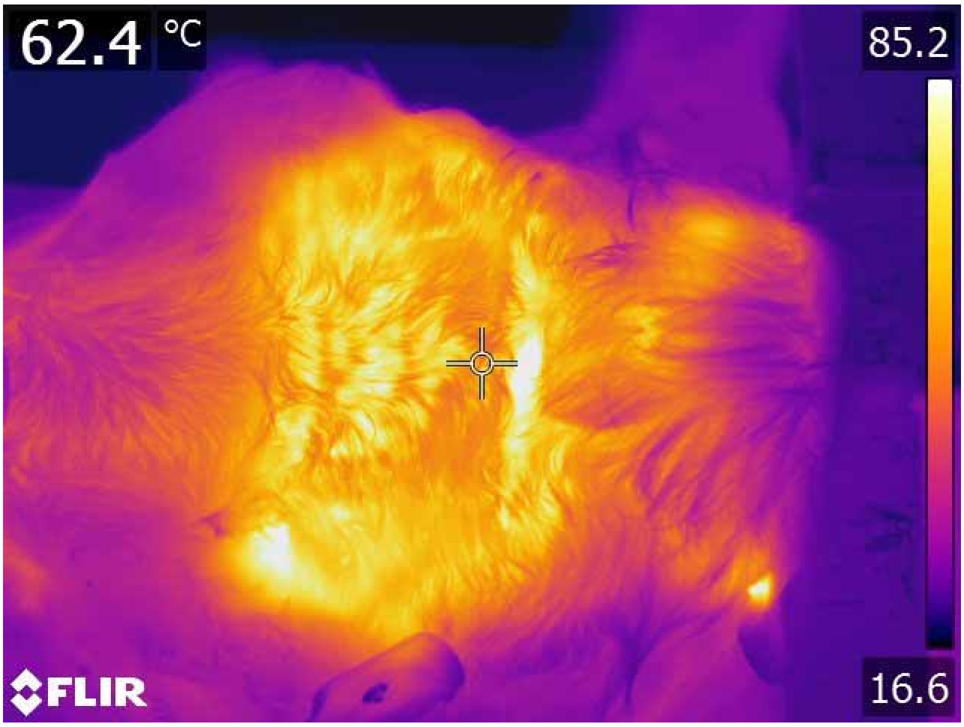
8	5/10/2017	12:03:33	animal in chin lift up wand in place
8	5/10/2017	12:03:46	body movements in crate
8	5/10/2017	12:03:48	DTS starts
8	5/10/2017	12:03:49	eyes open wide, body movements
8	5/10/2017	12:03:53	eyes closed
8	5/10/2017	12:03:57	rear legs collapse
8	5/10/2017	12:03:59	eyes rolling
8	5/10/2017	12:04:01	steam rises
8	5/10/2017	12:04:10	eyes wide, rolling back
8	5/10/2017	12:04:14	stands
8	5/10/2017	12:04:22	wand removed, some damage to skull cap
8	5/10/2017	12:04:29	corneal reflex
8	5/10/2017	12:04:31	thermal image
8	5/10/2017	12:04:48	body movements
8	5/10/2017	12:04:50	corneal reflex
8	5/10/2017	12:05:05	leads on
8	5/10/2017	12:05:24	corneal reflex, body movements
8	5/10/2017	12:05:43	eyes wide/breathing
8	5/10/2017	12:05:47	corneal reflex
8	5/10/2017	12:06:06	corneal reflex/body movements
8	5/10/2017	12:06:26	corneal reflex
8	5/10/2017	12:06:34	corneal reflex
8	5/10/2017	12:06:47	corneal reflex
8	5/10/2017	12:07:03	corneal reflex
8	5/10/2017	12:07:15	leads removed
8	5/10/2017	12:07:35	captive bolt, rear end collapses
8	5/10/2017	12:07:43	chin lift dropped
8	5/10/2017	12:07:46	head bale opened
8	5/10/2017	12:08:03	side door opens and onto table
8	5/10/2017	12:08:41	skin cut
8	5/10/2017	12:09:08	clip and cut oesophagus
8	5/10/2017	12:09:12	stuck.
8	5/10/2017	12:10:09	hung

Thermal images

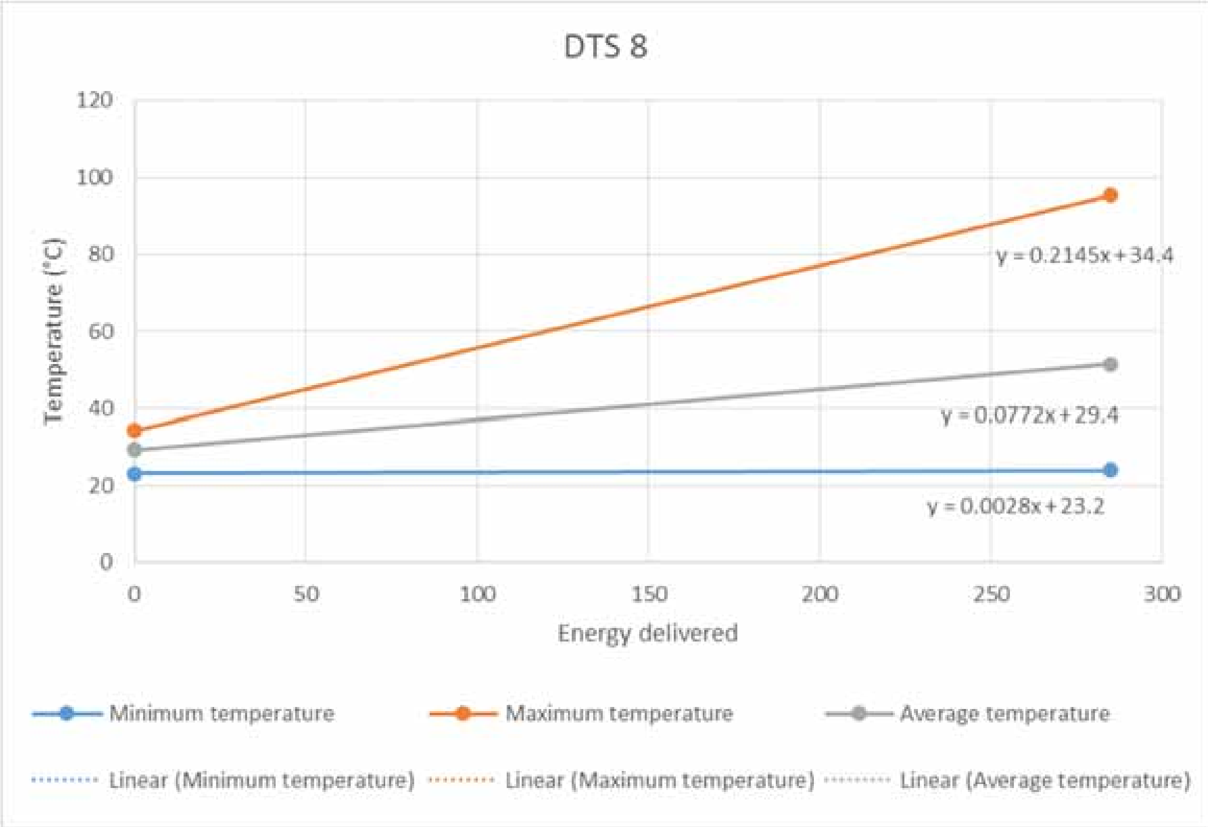
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 8 lost posture at approximately 9 seconds post onset of DTS application, which is equivalent to approximately 233 kJ of energy delivered. At 233 kJ energy delivered the estimated average forehead temperature is 47.39 °C (23.85 – 84.38).



Animal 8 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 29.0 °C; maximum temperature in image (white): 33.5 °C; minimum temperature in image (black): 17.0 °C.



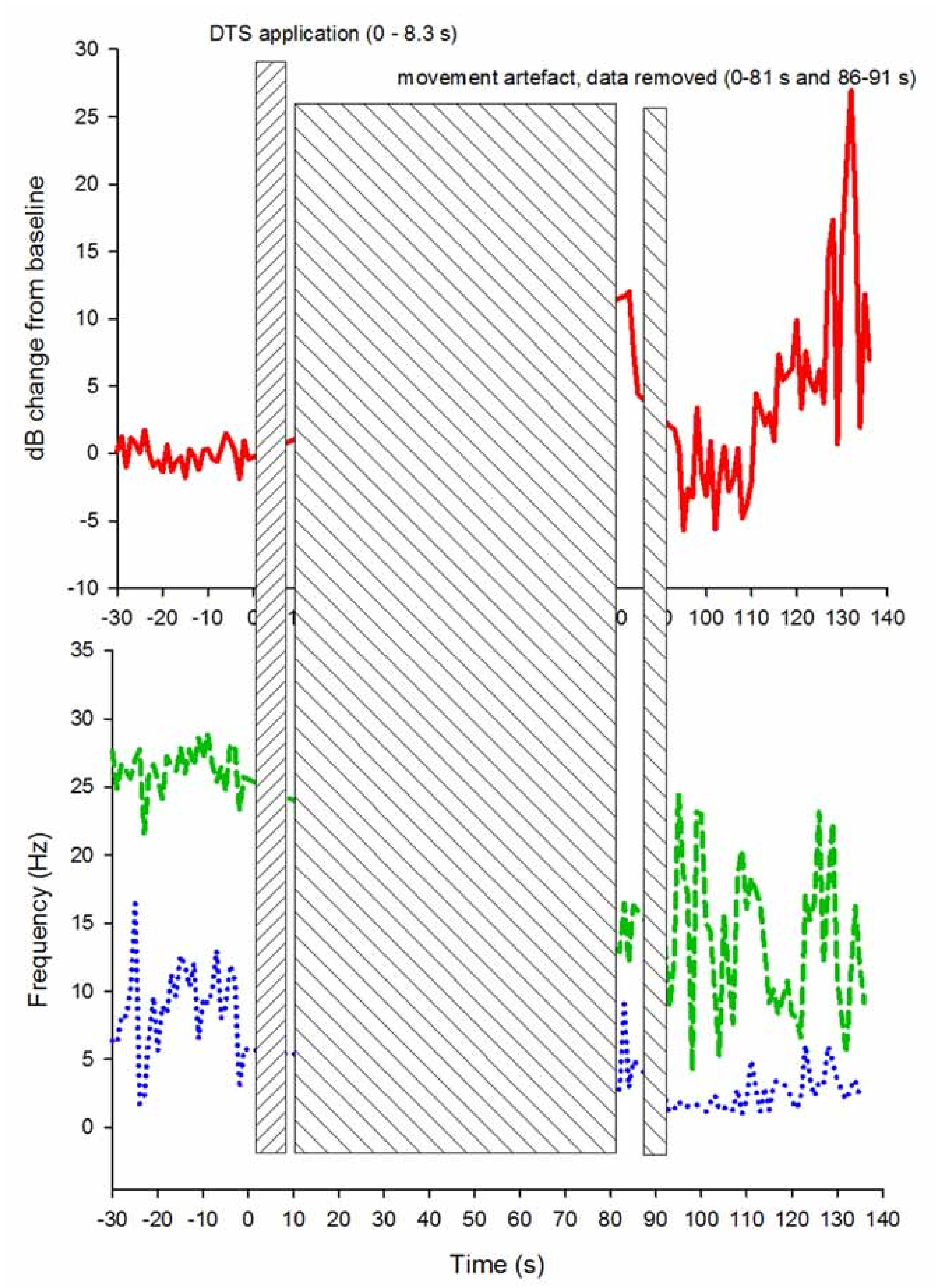
Animal 8 thermal map of forehead immediately after DTS application. Temperature at cursor: 62.4 °C; maximum temperature in image (white): 85.2 °C; minimum temperature in image (black): 16.6 °C. Heating at edges of waveguide, and leakage towards the poll is evident. Disk shapes with cool centres are EEG recording electrode pads.



Animal 8 estimated temperature change on the forehead by energy delivered.

EEG traces

A chart showing changes in EEG parameters following DTS application (narrow hatching) in Animal 8 is shown below. Total EEG power (Ptot, red line) is increased and shows high amplitude spiking post DTS application; 95% spectral edge frequency (SEF95, green line) and 50% spectral edge frequency (SEF50, blue line) both dropped following DTS application. It is difficult to interpret the spectral edge frequency data, as the EEG recordings are heavily contaminated by electrical noise from the abattoir infrastructure, however, high amplitude spiking in Ptot, and low frequency activity are considered to be incompatible with consciousness.



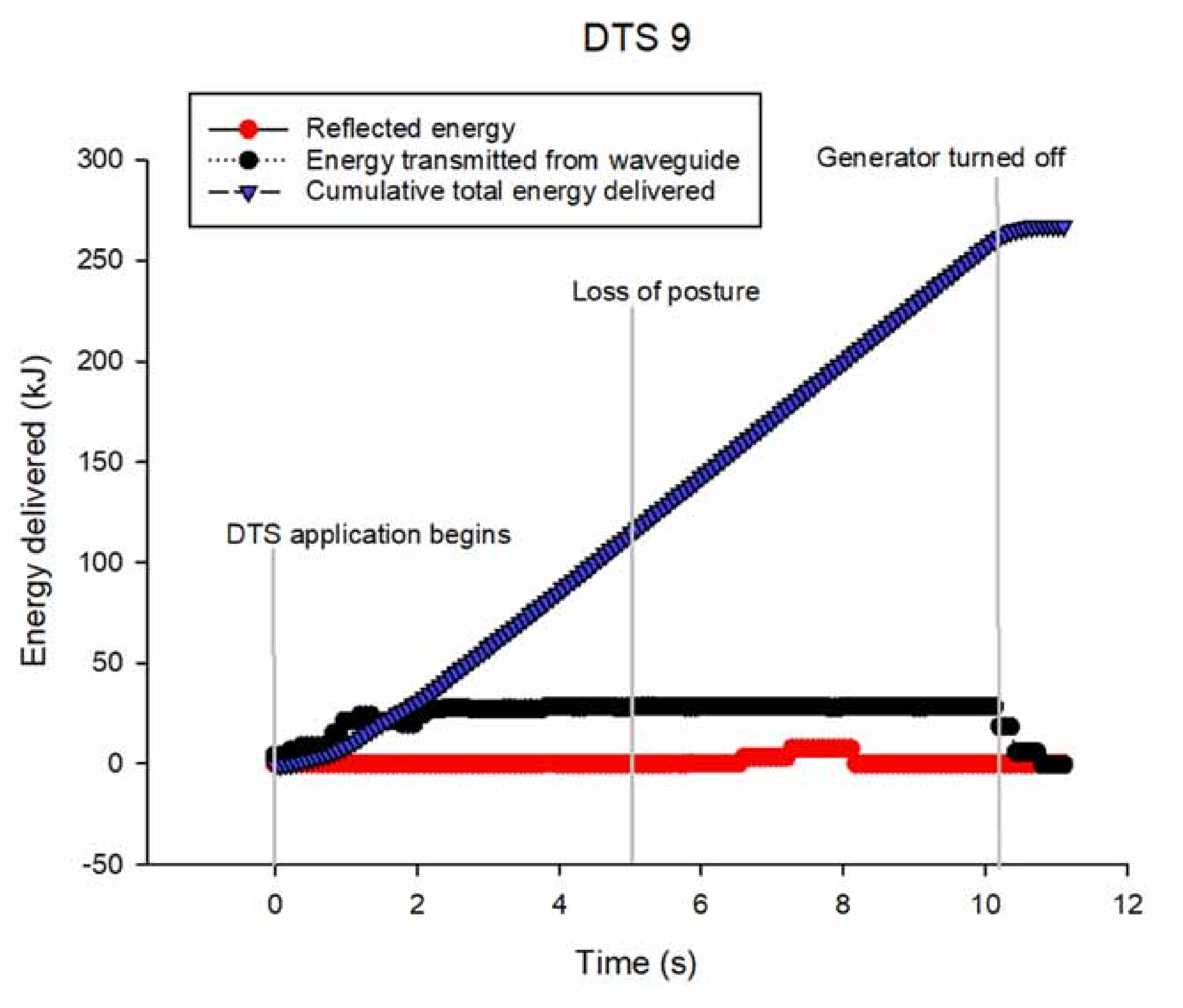
DTS application (0 - 8.3 s)
movement artefact, data removed (0-81 s and 86-91 s)
dB change from baseline
Frequency (Hz)
Time (s)

Animal 9

DTS settings

225 kJ, 30 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 5 seconds post onset of DTS application, which is equivalent to approximately 114 kJ of energy delivered.



Notes from real-time observations

Poor waveguide contact. The animal slipped in the box and sat down before DTS was applied, so there was probably some leakage.

However, still evidence of electrical stun type response: flickering eyelids, convulsions or ataxia.

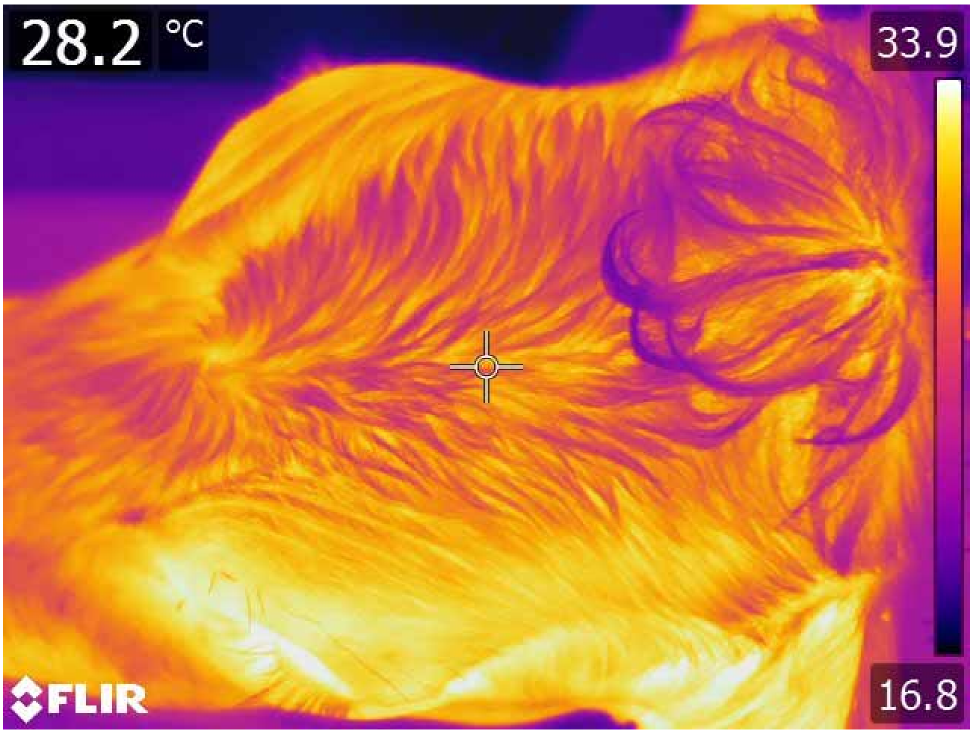
Palpebral response returned at around 2 min 10 sec after onset of rapid blinking during DTS application. Captive bolt applied, and exanguinated.

Detailed event log from video footage

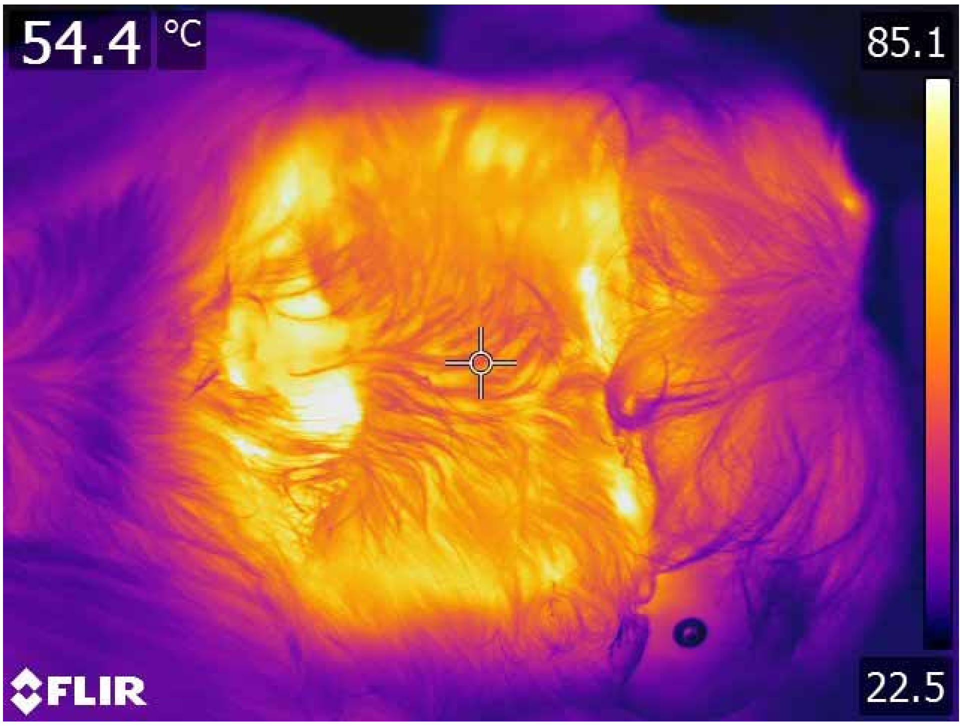
9	5/10/2017	12:25:06	animal in chin lift up wand in place
9	5/10/2017	12:25:26	DTS starts
9	5/10/2017	12:25:30	eyes close tight then rapid blinking
9	5/10/2017	12:25:31	rear legs collapse
9	5/10/2017	12:25:36	stands
9	5/10/2017	12:25:54	rear legs collapse then stands again
9	5/10/2017	12:25:56	door open wand removed
9	5/10/2017	12:26:10	eyes wide, body movements to stand
9	5/10/2017	12:26:18	thermal image
9	5/10/2017	12:26:34	leads on, body movements
9	5/10/2017	12:26:51	corneal reflex
9	5/10/2017	12:27:12	corneal reflex
9	5/10/2017	12:27:27	tail swishing, continued body movements
9	5/10/2017	12:27:38	corneal reflex
9	5/10/2017	12:28:00	leads off
9	5/10/2017	12:28:14	corneal reflex
9	5/10/2017	12:28:18	captive bolt and rear end collapses
9	5/10/2017	12:28:31	chin lift dropped
9	5/10/2017	12:28:56	side door opens
9	5/10/2017	12:29:11	on table
9	5/10/2017	12:29:43	skin cut
9	5/10/2017	12:30:16	clip and cut oesophagus
9	5/10/2017	12:30:30	stuck.
9	5/10/2017	12:30:56	thermal image of blood
9	5/10/2017	12:32:29	hung

Thermal images

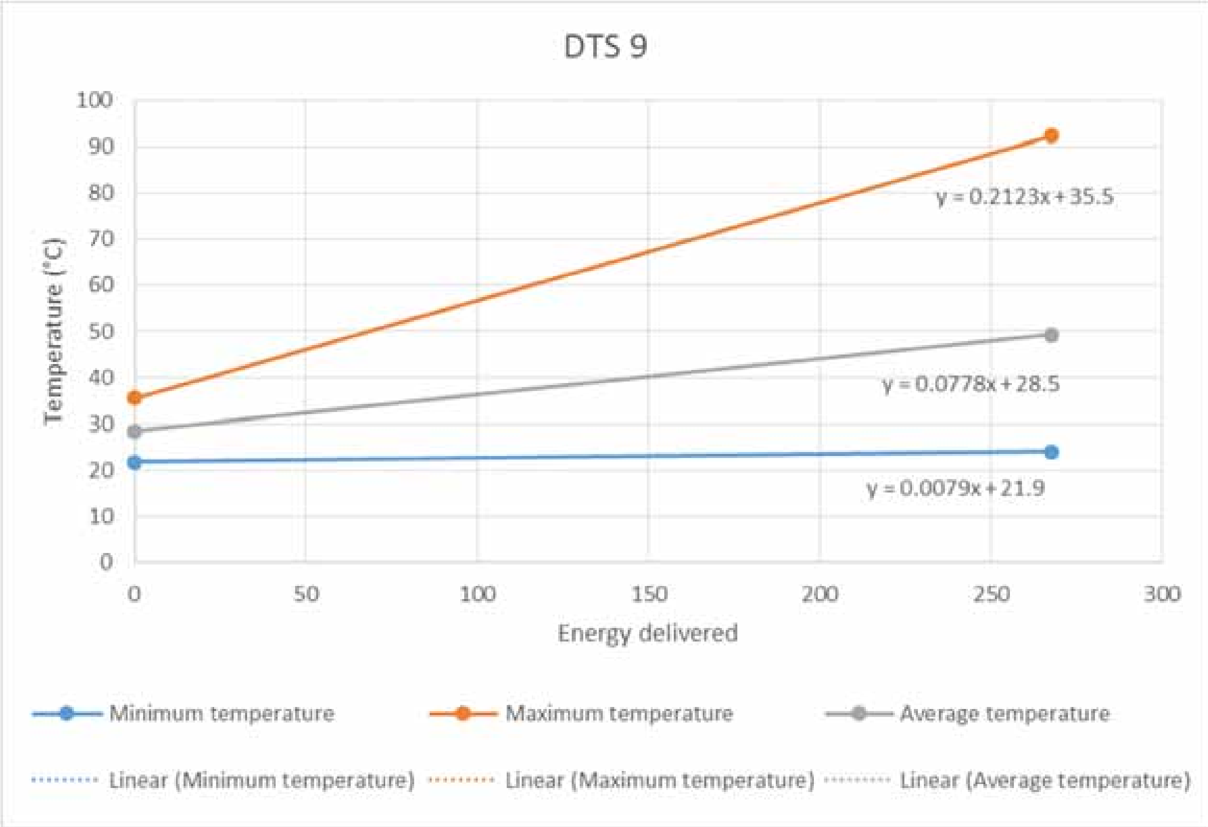
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 9 lost posture at approximately 5 seconds post onset of DTS application, which is equivalent to approximately 114 kJ of energy delivered. At 114 kJ energy delivered the estimated average forehead temperature is 37.37 °C (22.80 – 59.70).



Animal 9 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 28.2 °C; maximum temperature in image (white): 33.9 °C; minimum temperature in image (black): 16.8 °C.



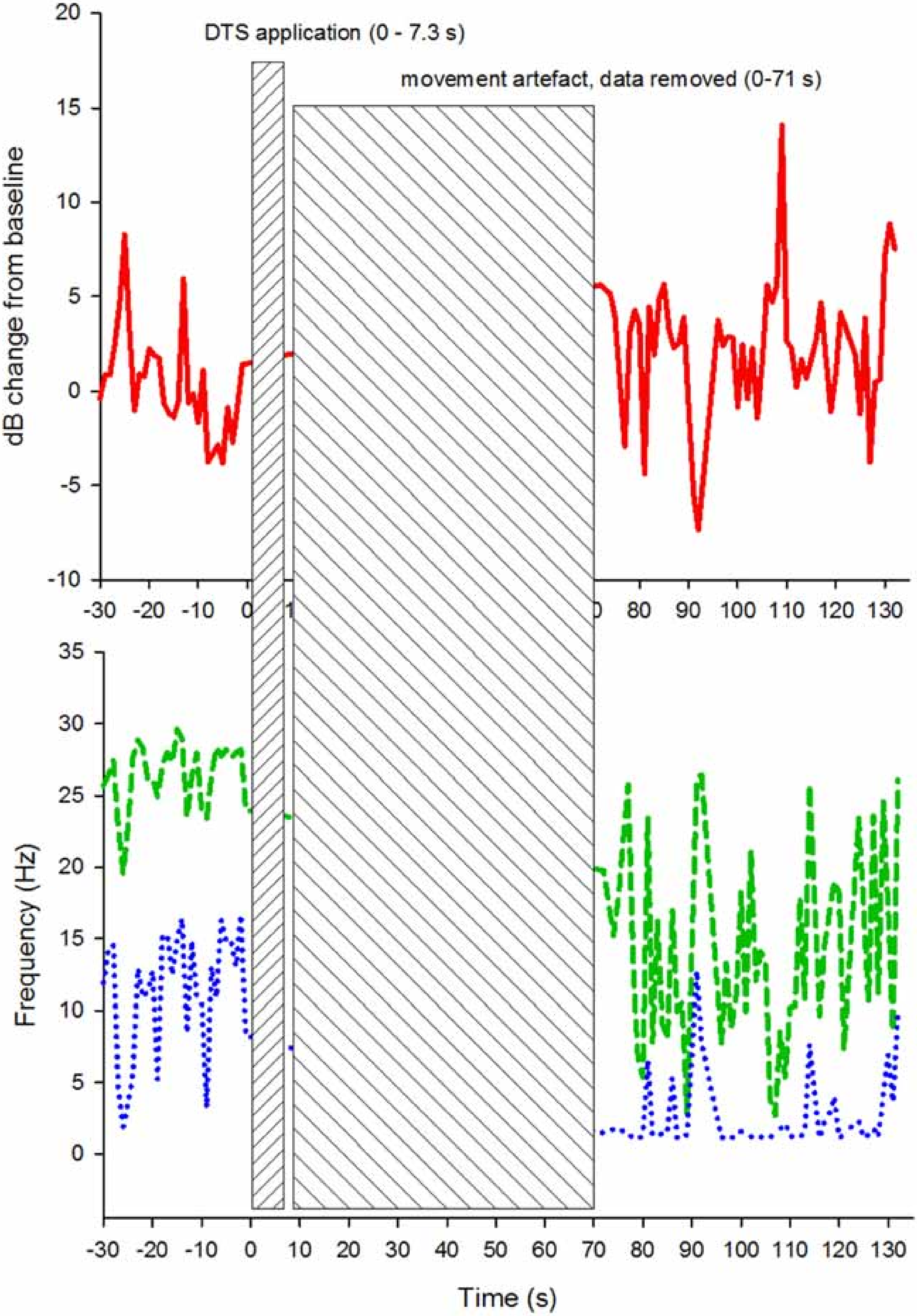
Animal 9 thermal map of forehead immediately after DTS application. Temperature at cursor: 54.4 °C; maximum temperature in image (white): 85.1 °C; minimum temperature in image (black): 22.5 °C. Heating at edges of waveguide is evident. Disk shapes with cool centres are EEG recording electrode pads.



Animal 9 estimated temperature change on the forehead by energy delivered.

EEG traces

A chart showing changes in EEG parameters following DTS application (narrow hatching) in Animal 9 is shown below. Changes in total EEG power (Ptot, red line) are inconclusive, but high amplitude spiking is evident post DTS application; 95% spectral edge frequency (SEF95, green line) and 50% spectral edge frequency (SEF50, blue line) both dropped following DTS application. It is difficult to interpret these data, as the EEG recordings are heavily contaminated by electrical noise from the abattoir infrastructure, however, high amplitude spiking in Ptot, and low frequency activity are considered to be incompatible with consciousness.

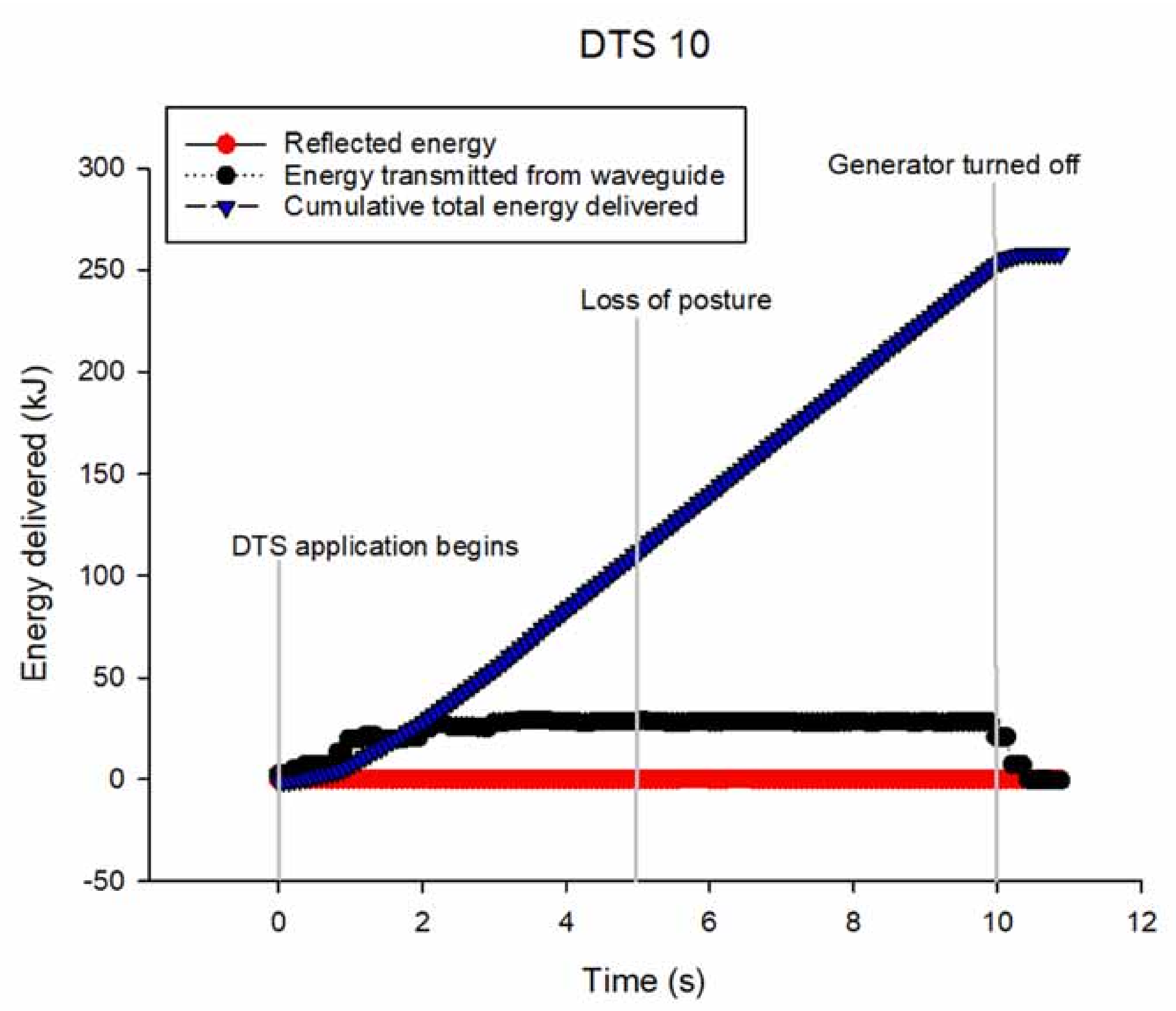

DTS application (0 - 7.3 s)
movement artefact, data removed (0-71 s)
dB change from baseline
20
15
10
5
0
-5
-10
-30 -20 -10 0 80 90 100 110 120 130
Frequency (Hz)
35
30
25
20
15
10
5
0
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100 110 120 130
Time (s)

Animal 10

DTS settings

225 kJ, 30 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 5 seconds post onset of DTS application, which is equivalent to approximately 112 kJ of energy delivered.



Notes from real-time observations

Waveguide contact poor.

Loss of posture, apparent tonic then clonic responses.

Clonic responses appearing around 1 minute post collapse.

No corneal reflex at 1 min 30 sec.

Slow/inconclusive corneal response at 2 minutes

Positive corneal reflex at 2 min 22 sec.

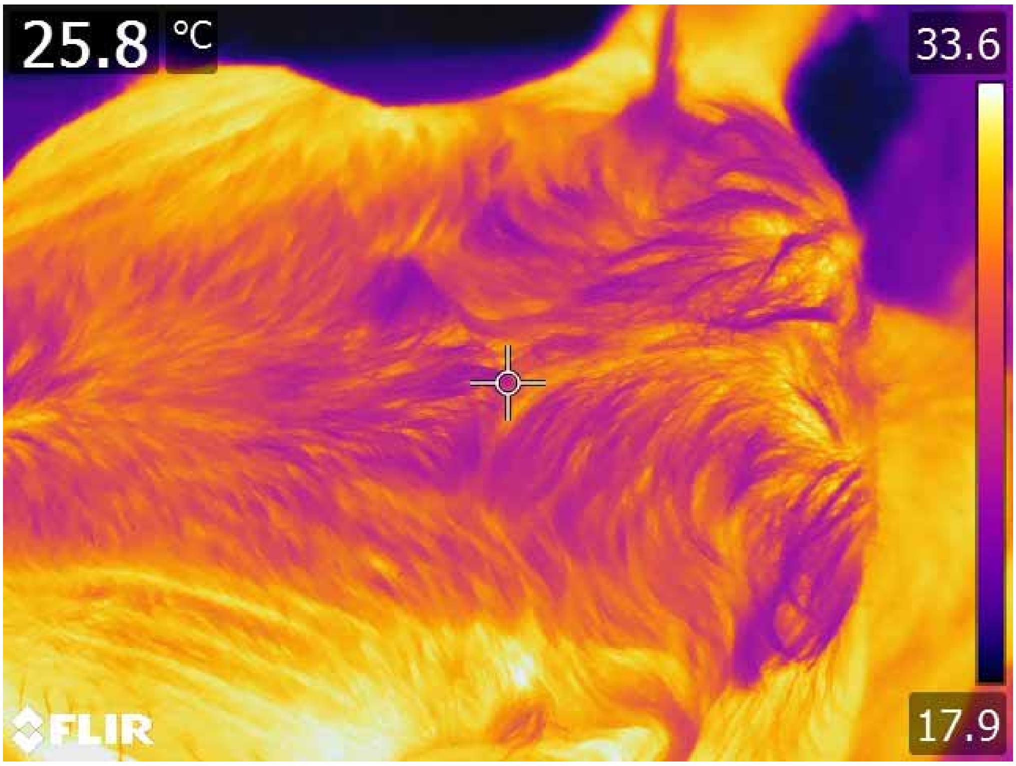
Captive bolt applied and exsanguinated.

Detailed event log from video footage

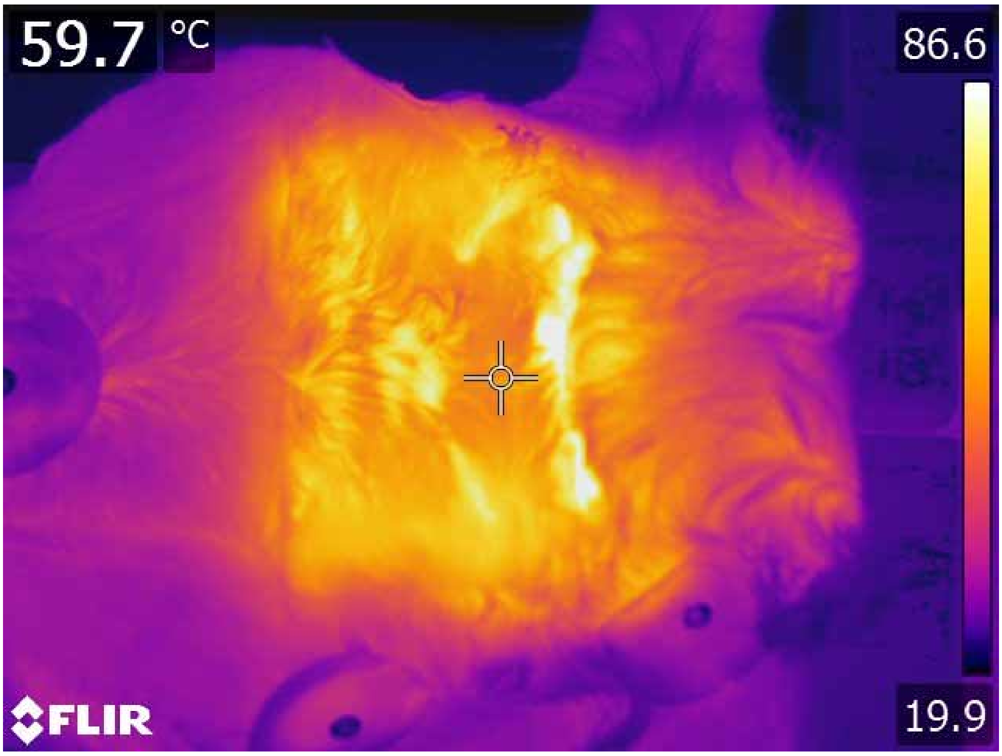
10	5/10/2017	12:43:32	animal in chin lift up wand in place
10	5/10/2017	12:43:41	DTS starts
10	5/10/2017	12:43:43	eyes appear closed
10	5/10/2017	12:43:46	rear end collapses
10	5/10/2017	12:43:47	steam rises
10	5/10/2017	12:43:50	eyes rolling
10	5/10/2017	12:44:00	stands
10	5/10/2017	12:44:01	eyes are open
10	5/10/2017	12:44:02	eyes blinking
10	5/10/2017	12:44:06	door open wand removed some cap damage
10	5/10/2017	12:44:23	blinking/ears twitching
10	5/10/2017	12:44:42	leads on
10	5/10/2017	12:44:56	body movements in crate
10	5/10/2017	12:45:15	corneal reflex
10	5/10/2017	12:45:29	corneal reflex
10	5/10/2017	12:45:36	corneal reflex
10	5/10/2017	12:45:53	corneal reflex, stops body movements
10	5/10/2017	12:46:03	cornel reflex, heavy breathing
10	5/10/2017	12:46:16	body movements in crate
10	5/10/2017	12:46:20	leads removed
10	5/10/2017	12:46:38	captive bolt, rear end collapses
10	5/10/2017	12:46:40	eyes wide open
10	5/10/2017	12:46:47	chin lift dropped
10	5/10/2017	12:47:14	side door opened
10	5/10/2017	12:47:44	on table
10	5/10/2017	12:48:23	skin cut
10	5/10/2017	12:49:09	clip and cut oesophagus
10	5/10/2017	12:49:41	stuck.

Thermal images

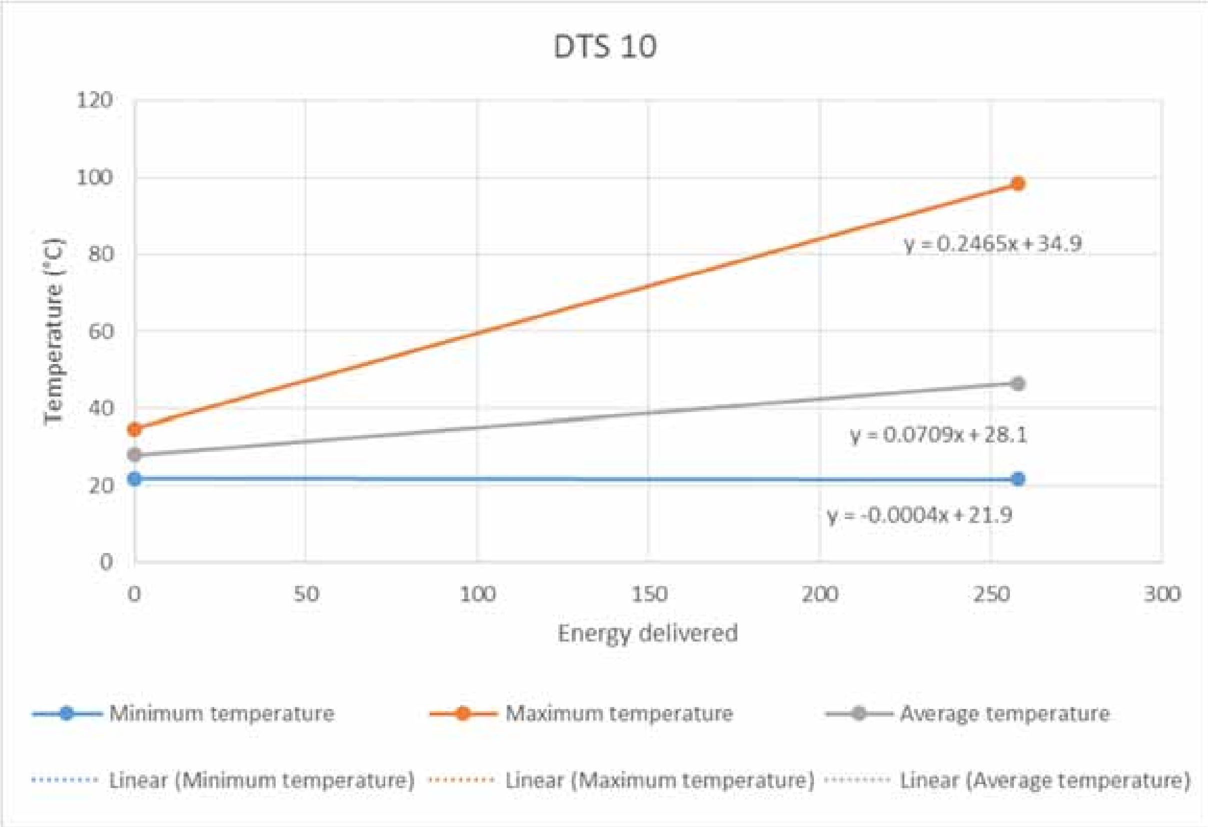
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 10 lost posture at approximately 5 seconds post onset of DTS application, which is equivalent to approximately 112 kJ of energy delivered. At 112 kJ energy delivered the estimated average forehead temperature is 36.04 °C (21.94 – 62.51).



Animal 10 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 25.8°C; maximum temperature in image (white): 33.6 °C; minimum temperature in image (black): 17.9 °C.



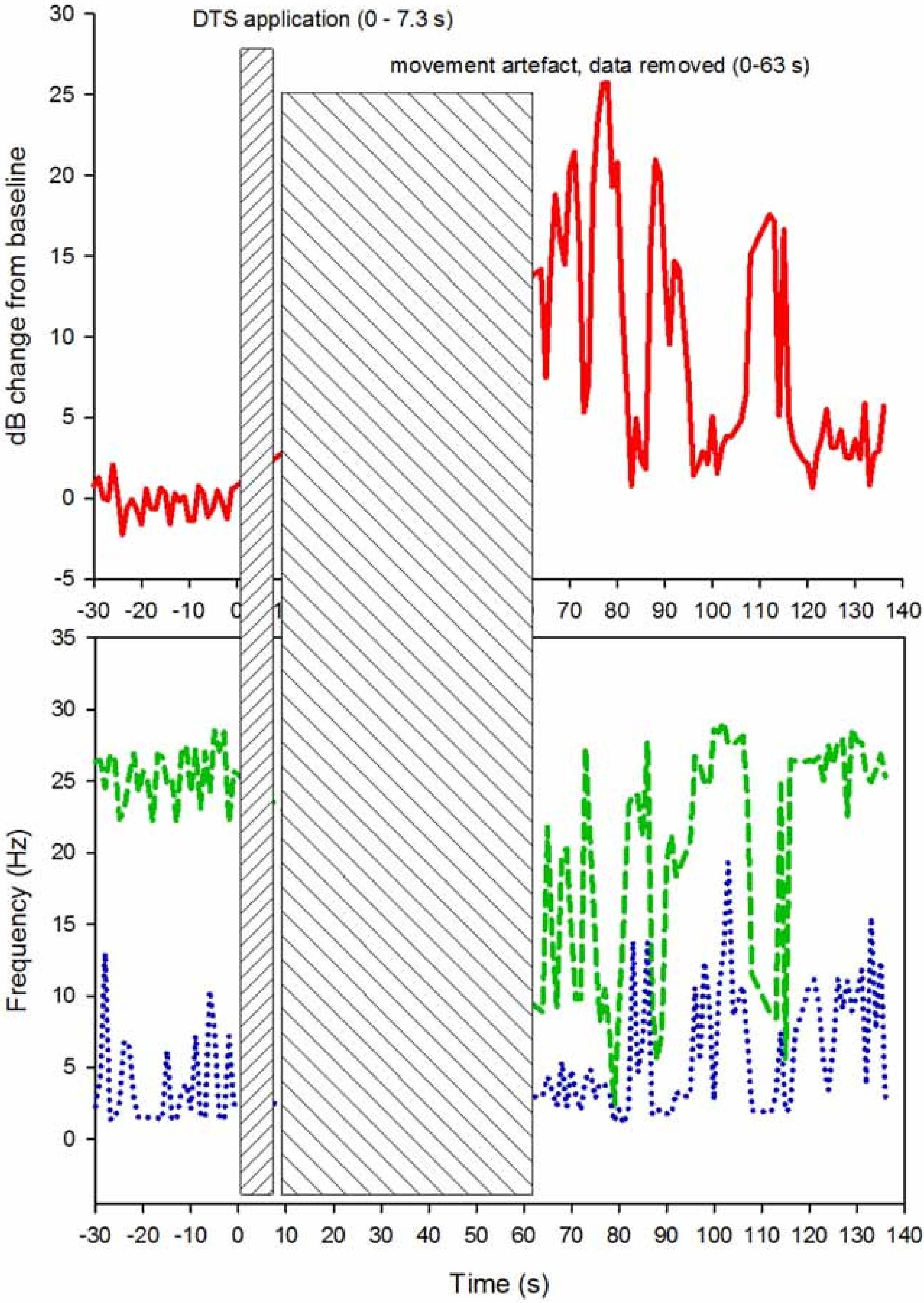
Animal 10 thermal map of forehead immediately after DTS application. Temperature at cursor: 59.7 °C; maximum temperature in image (white): 86.6 °C; minimum temperature in image (black): 19.9 °C. Heating at edges of waveguide, and heating of the poll associated with energy leakage is evident. Disk shapes with cool centres are EEG recording electrode pads.



Animal 10 estimated temperature change on the forehead by energy delivered.

EEG traces

A chart showing changes in EEG parameters following DTS application (narrow hatching) in Animal 10 is shown below. Total EEG power (Ptot, red line) is dramatically increased and shows high amplitude spiking post DTS application; 95% spectral edge frequency (SEF95, green line) and 50% spectral edge frequency (SEF50, blue line) both dropped following DTS application. It is difficult to interpret these data, as the EEG recordings are heavily contaminated by electrical noise from the abattoir infrastructure, however, high amplitude spiking in Ptot, and low frequency activity are considered to be incompatible with consciousness.



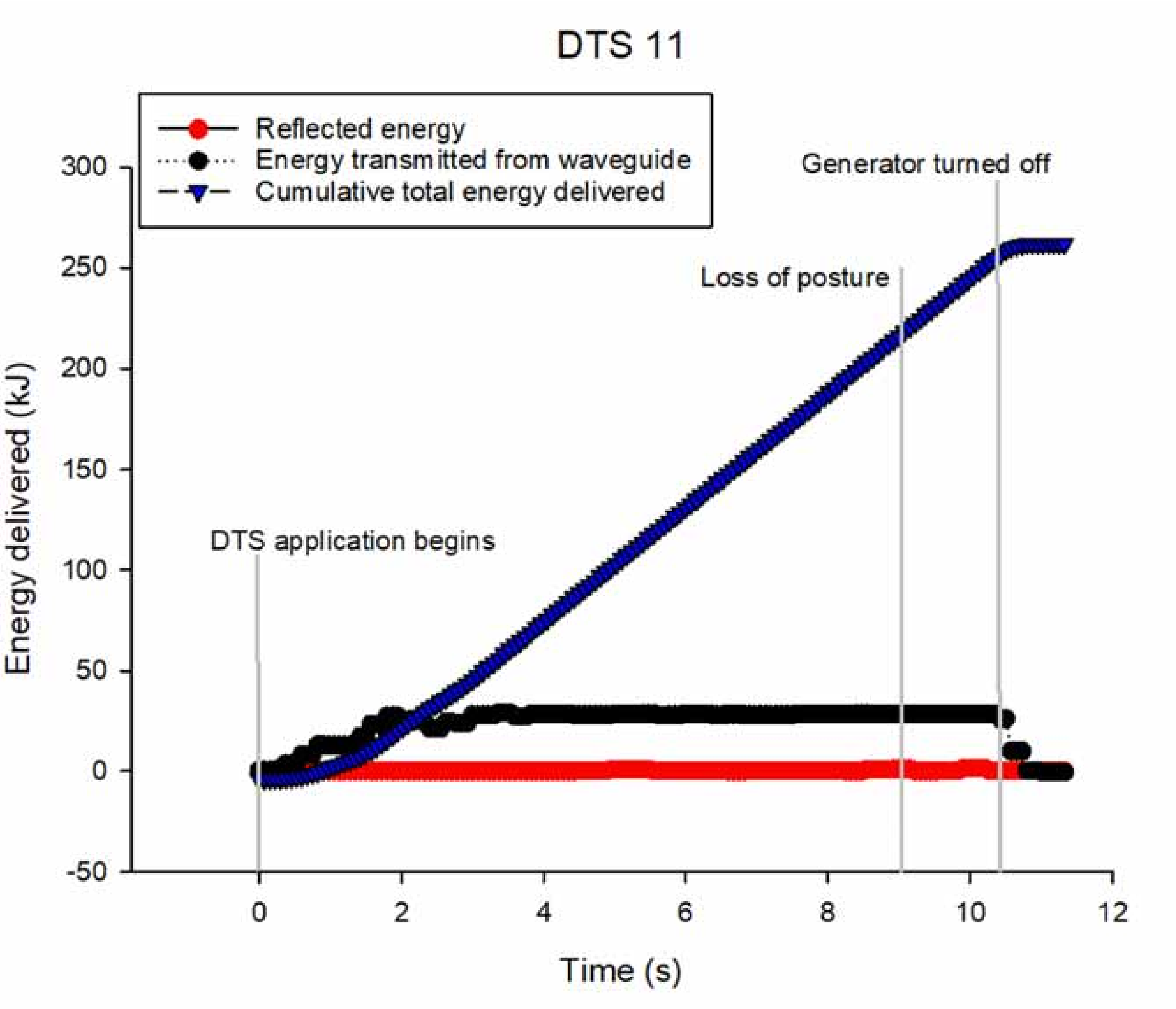
DTS application (0 - 7.3 s)
movement artefact, data removed (0-63 s)
dB change from baseline
Frequency (Hz)
Time (s)

Animal 11

DTS settings

225 kJ, 30 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 9 seconds post onset of DTS application, which is equivalent to approximately 215 kJ of energy delivered.



Notes from real-time observations

Better waveguide contact than previous two animals, but still some leakage occurred, so video footage was lost. Video footage returned by the end of energy application.

Animal had lost posture, eyelids flickering.

Animal returned to standing posture, but no corneal reflex. Deep slow breathing. Little evidence of clonic reaction.

Corneal and palpebral reflexes returned at around 2 min 25 sec post energy application. Captive bolt applied and rolled out for exsanguination.

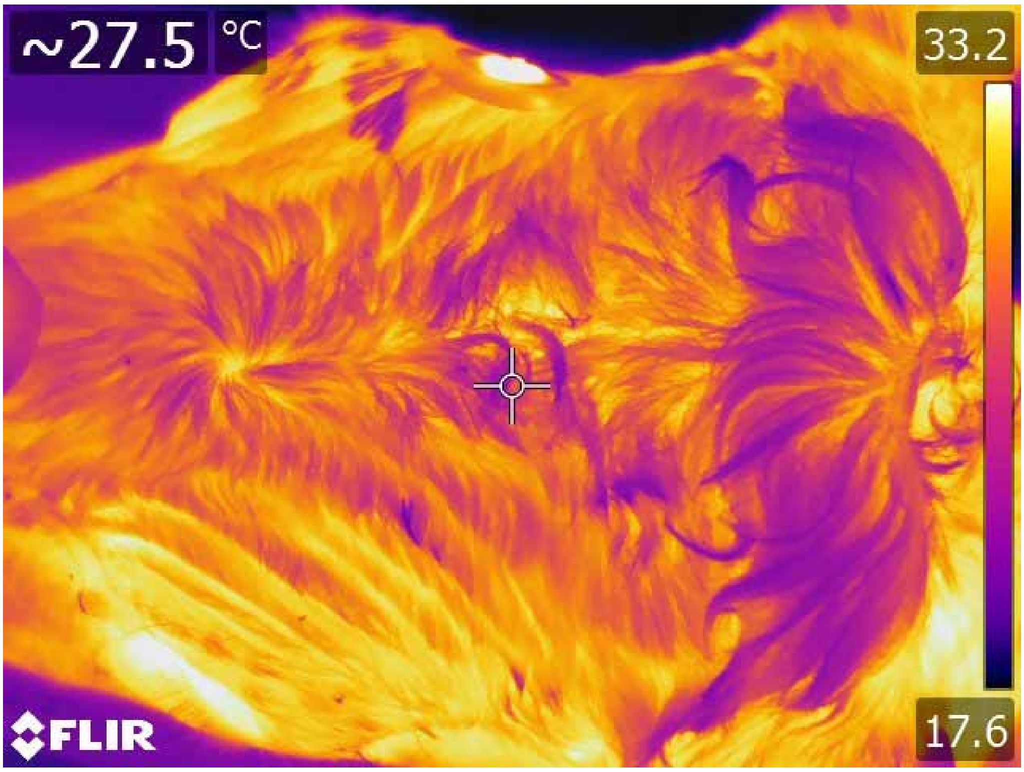
Inspection of the head suggested that the waveguide had slipped down the nose – perhaps when the animal lost posture.

Detailed event log from video footage

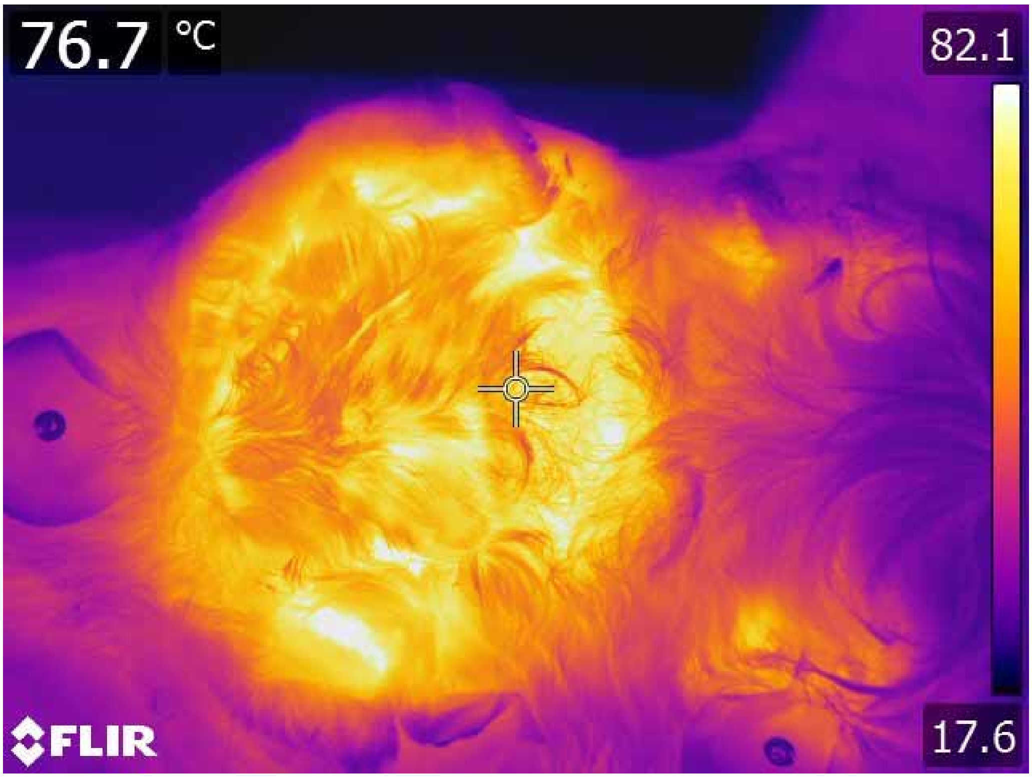
11	5/10/2017	12:59:32	animal in chin lift up wand in place
11	5/10/2017	13:00:05	DTS starts
11	5/10/2017	13:00:06	eyes close tight then open wide
11	5/10/2017	13:00:11	body movements and blinks
11	5/10/2017	13:00:14	rear end collapses
11	5/10/2017	13:00:24	stands blinks
11	5/10/2017	13:00:30	wand removed, body movements
11	5/10/2017	13:00:59	eyes roll back
11	5/10/2017	13:01:11	leads attached
11	5/10/2017	13:01:35	corneal reflex
11	5/10/2017	13:01:51	corneal reflex
11	5/10/2017	13:02:09	corneal reflex
11	5/10/2017	13:02:27	corneal reflex
11	5/10/2017	13:02:36	corneal reflex
11	5/10/2017	13:02:56	leads removed
11	5/10/2017	13:03:06	captive bolt, rear end collapses
11	5/10/2017	13:03:12	chin lift dropped
11	5/10/2017	13:03:22	head bale opened
11	5/10/2017	13:03:43	side door opened
11	5/10/2017	13:03:47	on table
11	5/10/2017	13:04:44	skin cut
11	5/10/2017	13:05:18	clip and cut oesophagus
11	5/10/2017	13:05:24	stuck.

Thermal images

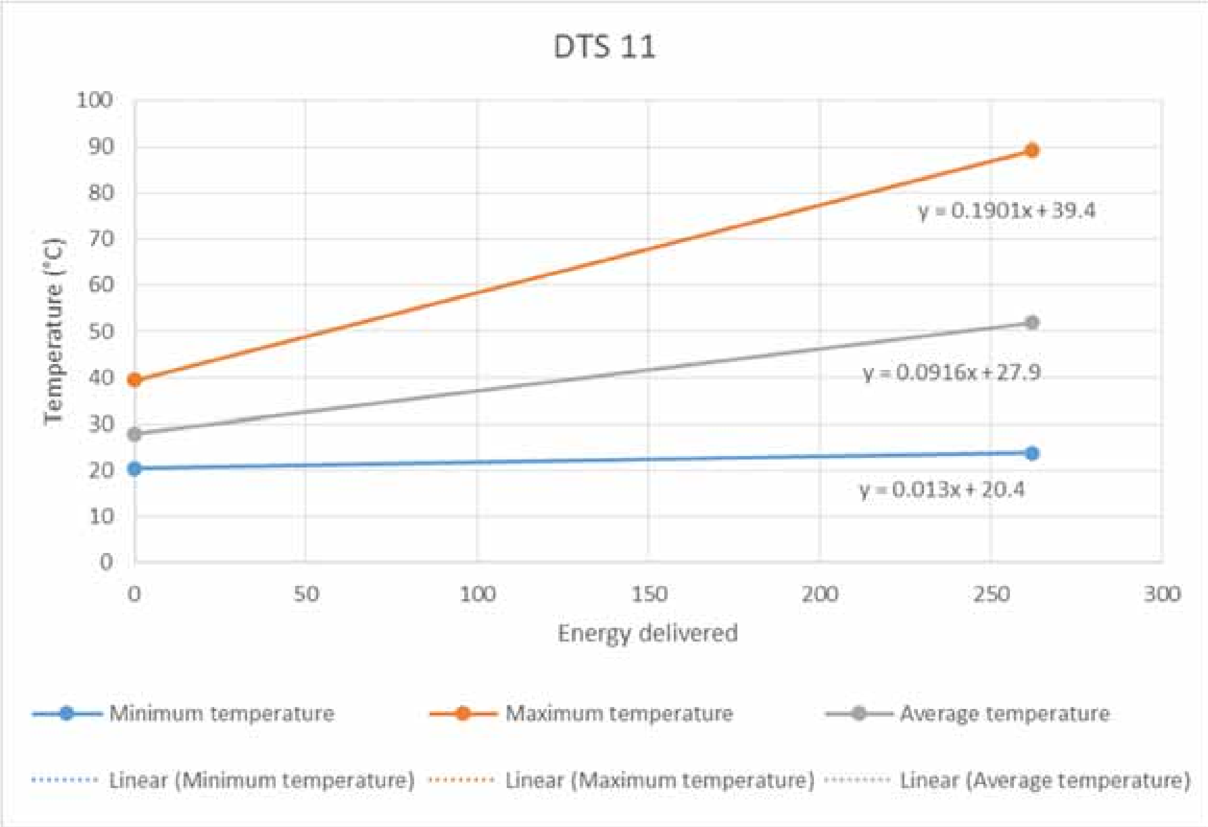
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 11 lost posture at approximately 9 seconds post onset of DTS application, which is equivalent to approximately 215 kJ of energy delivered. At 215 kJ energy delivered the estimated average forehead temperature is 47.59 °C (23.20 – 80.27).



Animal 11 thermal map of forehead immediately prior to DTS application. Temperature at cursor: ~27.5 °C; maximum temperature in image (white): 33.2 °C; minimum temperature in image (black): 17.6 °C. Disk shapes with hot centre is an EEG recording electrode pad.



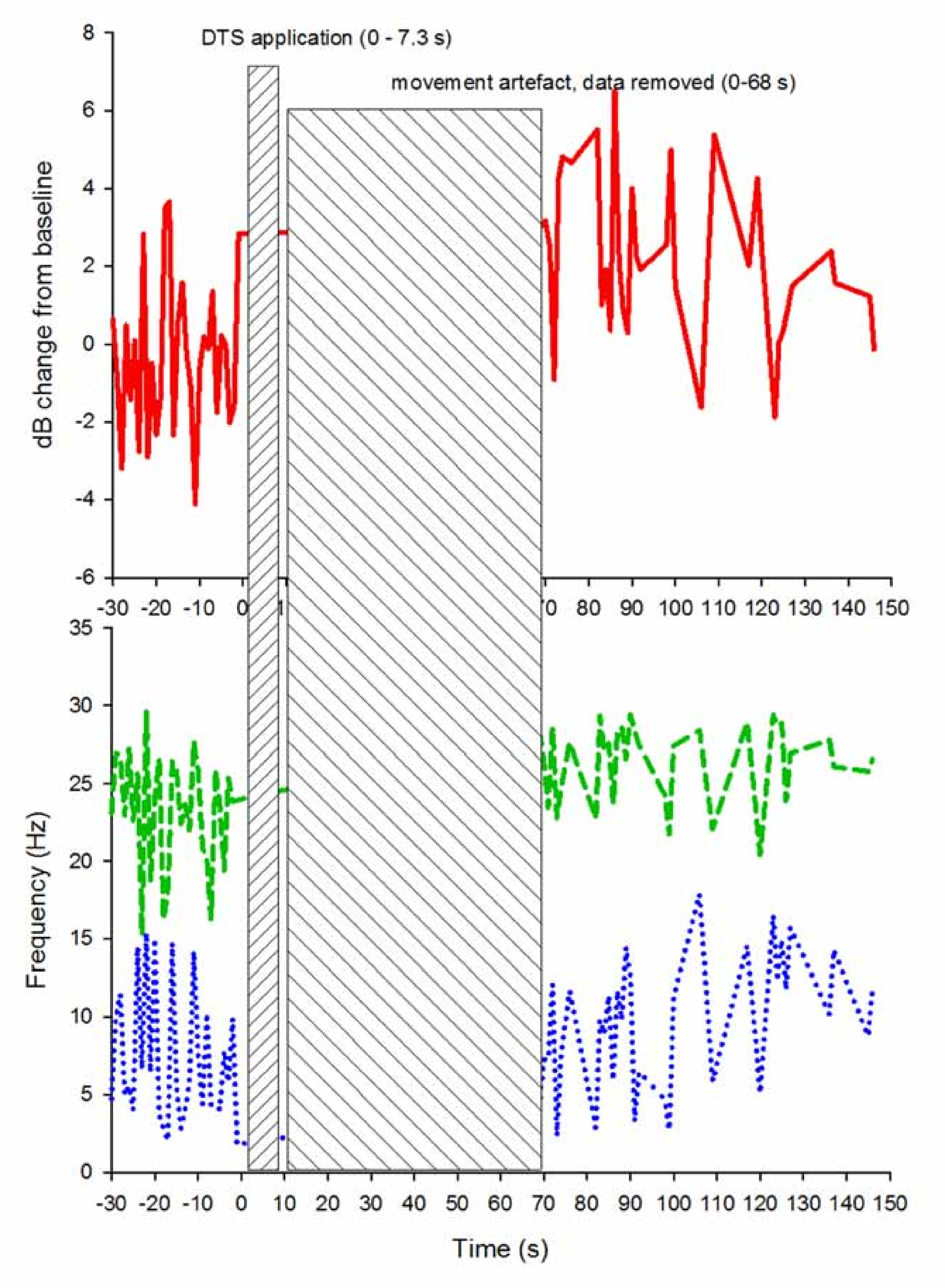
Animal 11 thermal map of forehead immediately after DTS application. Temperature at cursor: 76.7 °C; maximum temperature in image (white): 82.1 °C; minimum temperature in image (black): 17.6 °C. Heating around the waveguide as a result of leakage is evident. Disk shapes with cool centres are EEG recording electrode pads.



Animal 11 estimated temperature change on the forehead by energy delivered.

EEG traces

A chart showing changes in EEG parameters following DTS application (narrow hatching) in Animal 11 is shown below. Total EEG power (Ptot, red line) is increased and shows high amplitude spiking post DTS application; clear changes are not evident in 95% spectral edge frequency (SEF95, green line) and 50% spectral edge frequency (SEF50, blue line). It is difficult to interpret the spectral edge frequency data, as the EEG recordings are heavily contaminated by electrical noise from the abattoir infrastructure, however, high amplitude spiking in Ptot is considered to be incompatible with consciousness.

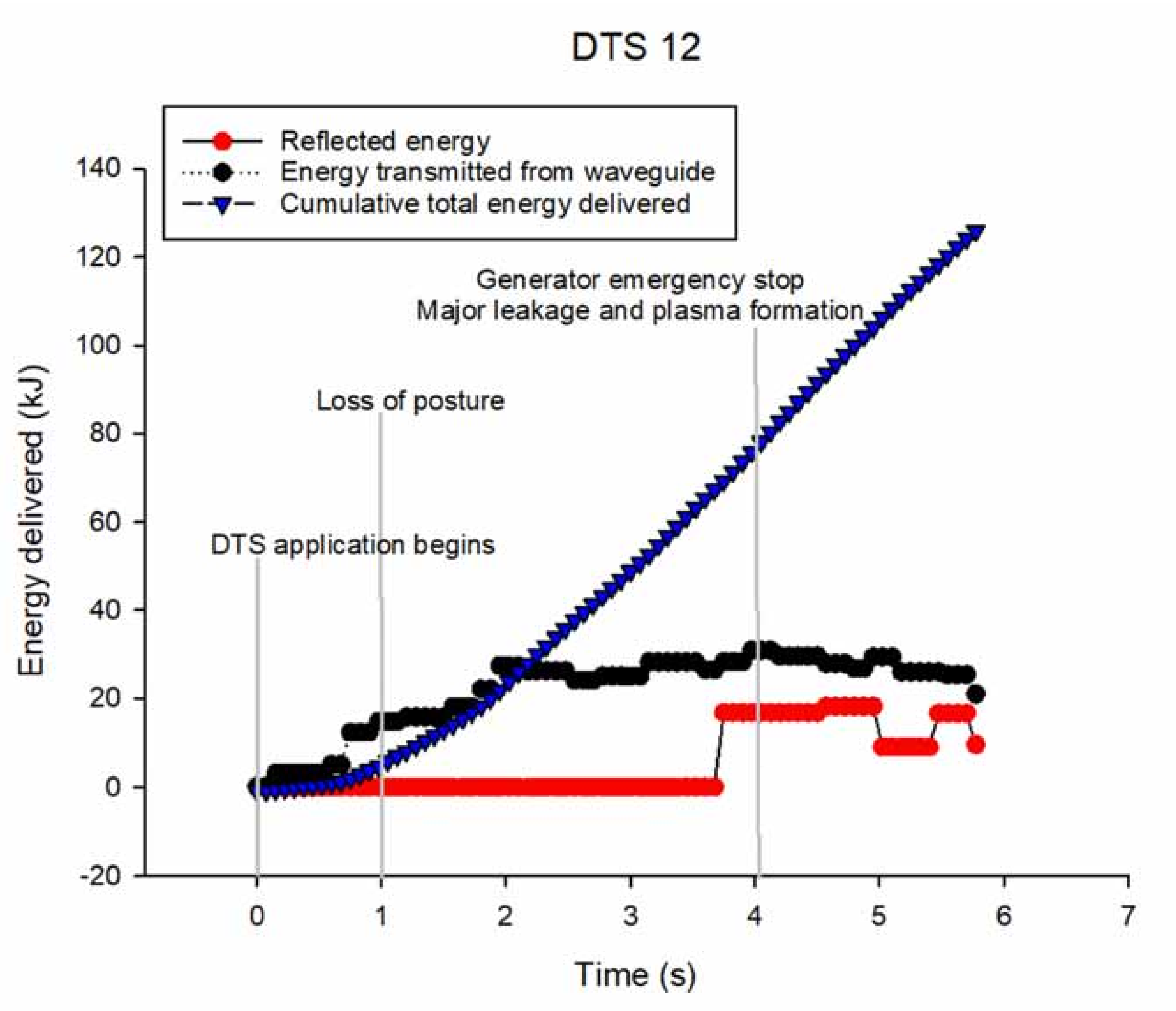

DTS application (0 - 7.3 s)
movement artefact, data removed (0-68 s)
dB change from baseline
8
6
4
2
0
-2
-4
-6
-30 -20 -10 0 10 70 80 90 100 110 120 130 140 150
35
30
25
20
15
10
5
0
Frequency (Hz)
-30 -20 -10 0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
Time (s)

Animal 12

DTS settings

225 kJ, 30 kW. Alternative waveguide fitted, with different tuning baffles.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 1 seconds post onset of DTS application, which is equivalent to approximately 6 kJ of energy delivered.



Notes from real-time observations

Major energy leakage with plasma formation. DTS terminated after 80 kJ delivered (inclusive of that lost to the environment). Unclear if loss of posture is merely loss of footing, or loss of consciousness.

Animal appears dazed, but not unconscious. Captive bolt applied and rolled out for exsanguination.

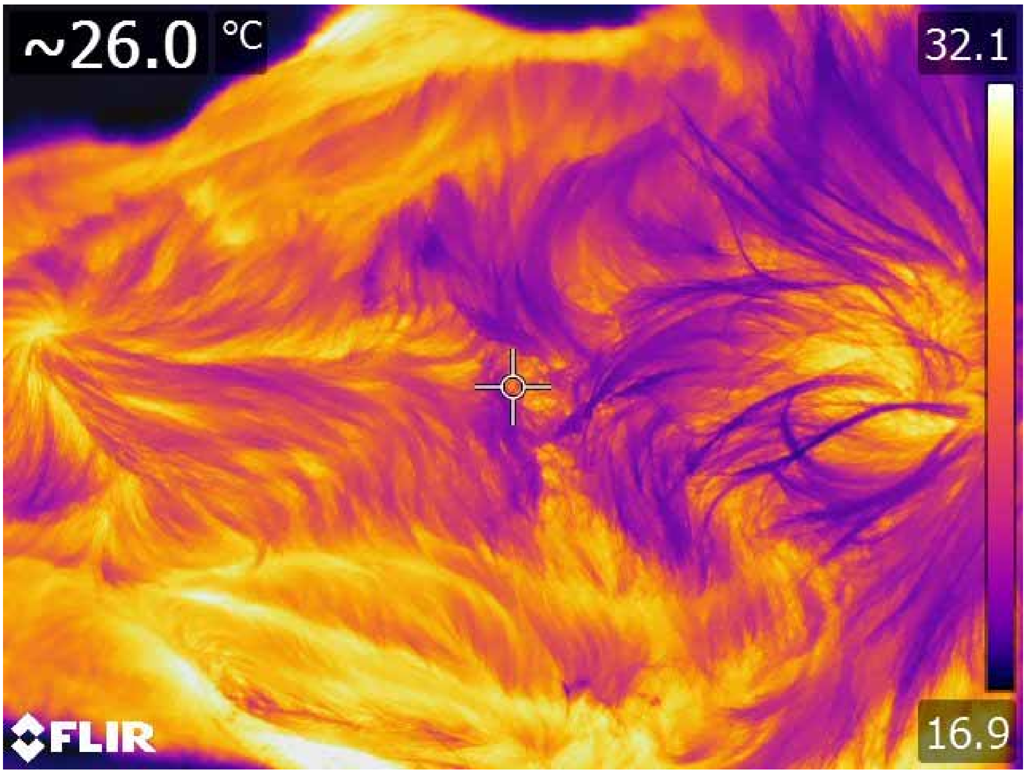
Examination of the waveguide indicated that the arcing and plasma formation was between the grub screws holding the Teflon window to the waveguide aperture. Attempts were made to insulate the grub screws using duct tape.

Detailed event log from video footage

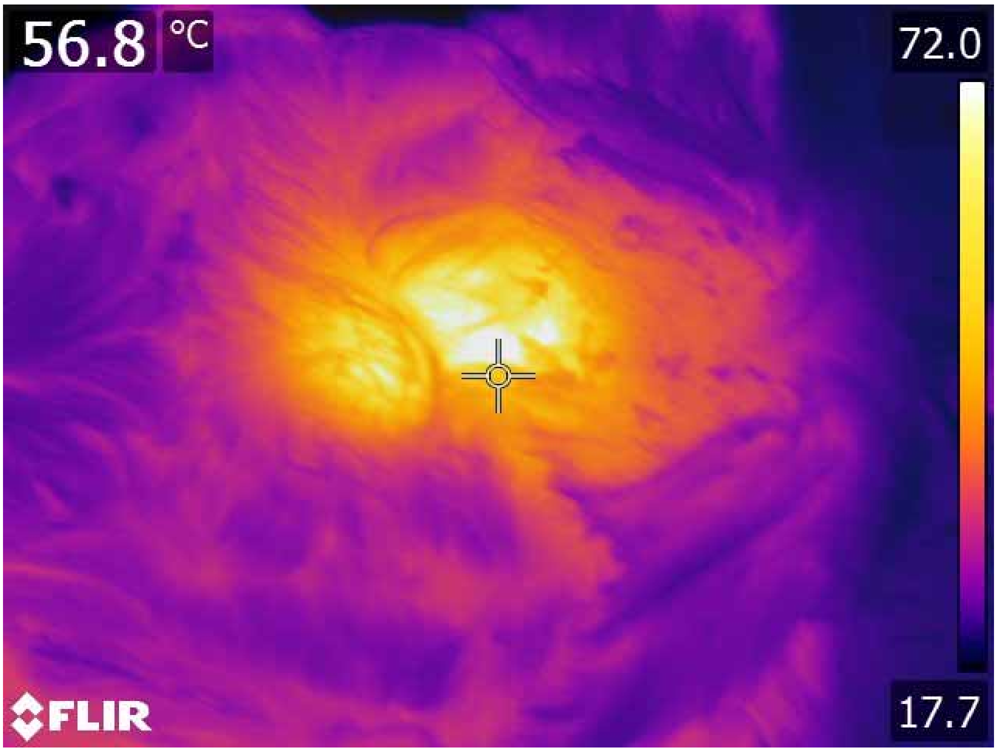
12	5/10/2017	15:35:00	animal in chin lift up wand in place
12	5/10/2017	15:35:02	suspect DTS started, signal not visible
12	5/10/2017	15:35:03	rapid blinking, rear end collapses, plasma leakage
12	5/10/2017	15:35:22	side opened
12	5/10/2017	15:35:28	wand removed
12	5/10/2017	15:35:36	body movements
12	5/10/2017	15:35:37	stands
12	5/10/2017	15:35:44	body movements, tail swishing
12	5/10/2017	15:35:50	leads attached
12	5/10/2017	15:36:15	corneal reflex, deep breathing
12	5/10/2017	15:36:28	tail swishing, pushes forward, then rear end collapses
12	5/10/2017	15:36:30	stands
12	5/10/2017	15:36:40	body movements
12	5/10/2017	15:36:49	leads removed
12	5/10/2017	15:36:52	corneal reflex
12	5/10/2017	15:37:07	body movements
12	5/10/2017	15:37:15	captive bolt
12	5/10/2017	15:37:20	chin lift dropped
12	5/10/2017	15:37:25	head bale opens, collapses
12	5/10/2017	15:37:40	side opens
12	5/10/2017	15:38:29	on table
12	5/10/2017	15:38:59	skin cut
12	5/10/2017	15:39:19	clip and cut oesophagus
12	5/10/2017	15:39:24	stuck.
12	5/10/2017	15:40:50	hung

Thermal images

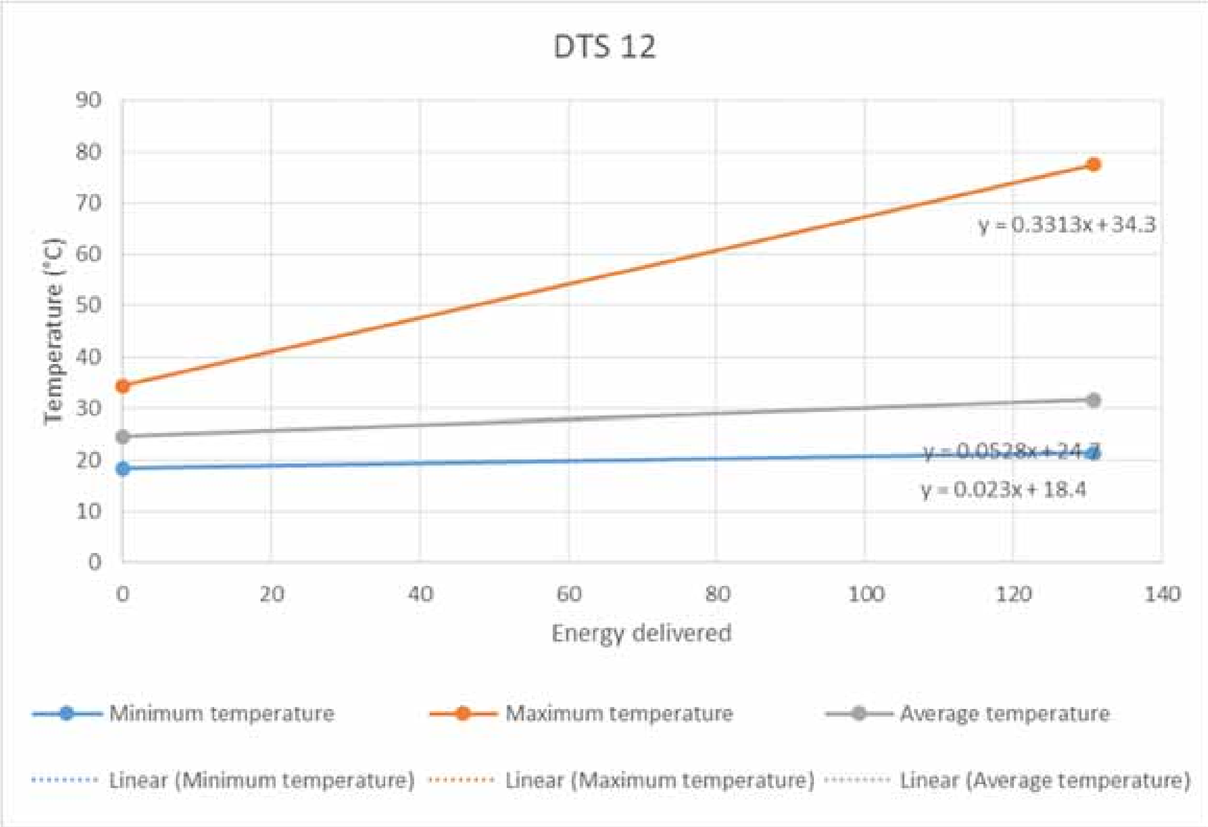
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 12 lost posture at approximately 1 second post onset of DTS application, which is equivalent to approximately 6 kJ of energy delivered. At 6 kJ energy delivered the estimated average forehead temperature is 25.02 °C (18.54 – 36.29).



Animal 12 thermal map of forehead immediately prior to DTS application. Temperature at cursor: ~26.0 °C; maximum temperature in image (white): 32.1 °C; minimum temperature in image (black): 16.9 °C.



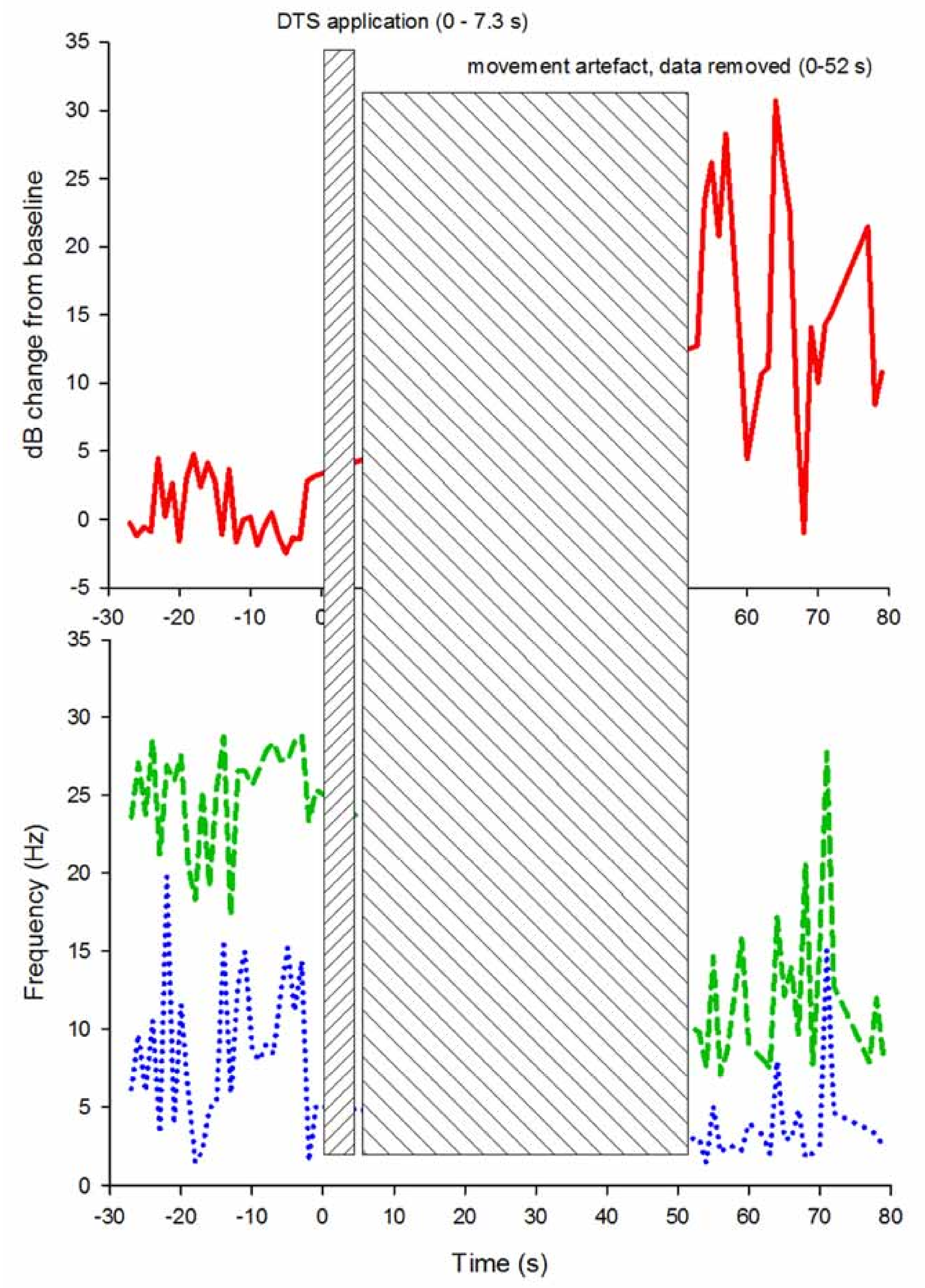
Animal 12 thermal map of forehead immediately after DTS application. Temperature at cursor: 56.8 °C; maximum temperature in image (white): 72.0°C; minimum temperature in image (black): 17.7 °C. Smaller aperture on this waveguide is evident as compared with previous animals.



Animal 12 estimated temperature change on the forehead by energy delivered.

EEG traces

A chart showing changes in EEG parameters following DTS application (narrow hatching) in Animal 12 is shown below. Total EEG power (Ptot, red line) is dramatically increased and shows high amplitude spiking post DTS application; 95% spectral edge frequency (SEF95, green line) and 50% spectral edge frequency (SEF50, blue line) both dropped following DTS application. It is difficult to interpret these data, as the EEG recordings are heavily contaminated by electrical noise from the abattoir infrastructure, however, high amplitude spiking in Ptot, and low frequency activity are considered to be incompatible with consciousness.

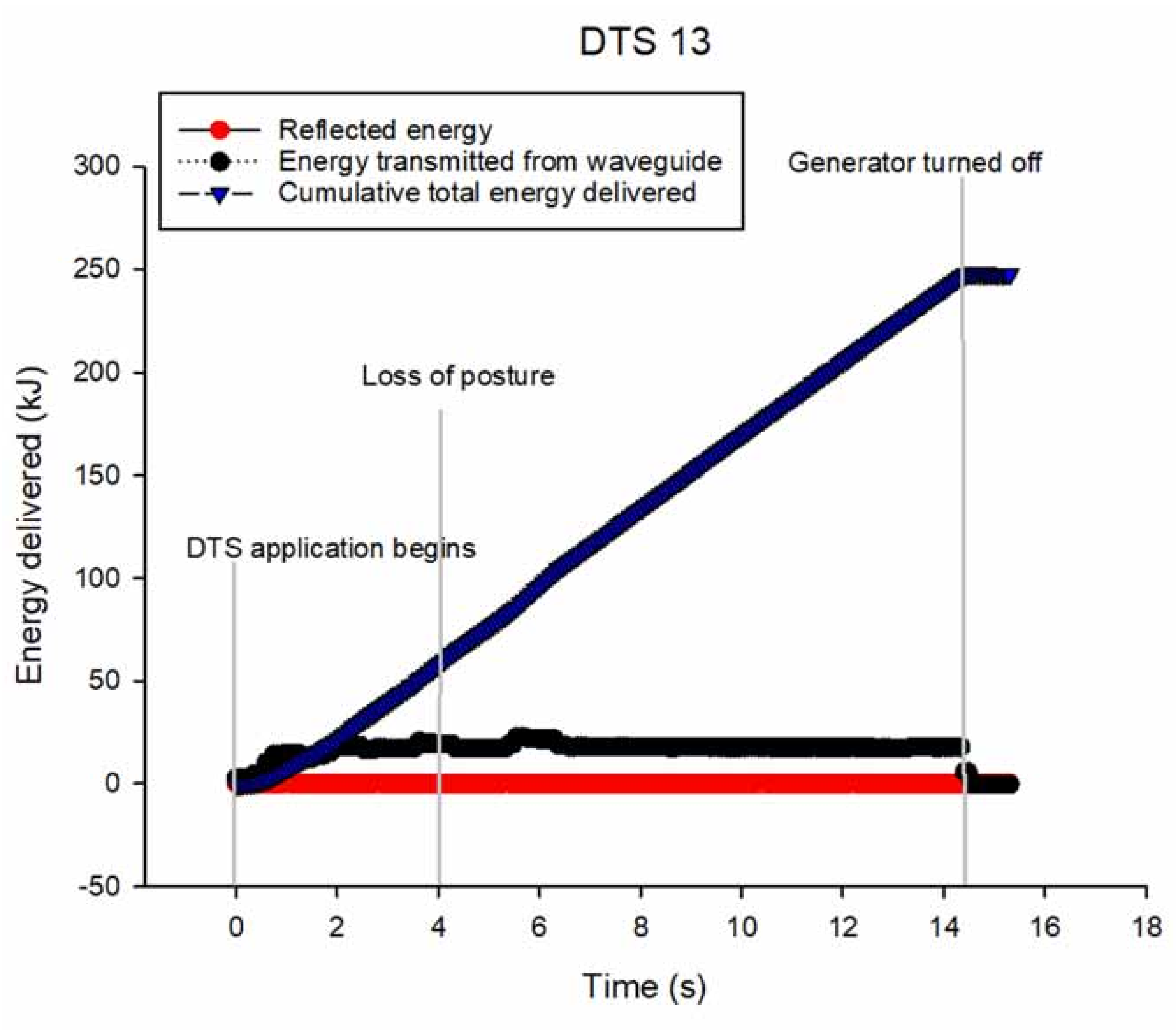

DTS application (0 - 7.3 s)
movement artefact, data removed (0-52 s)
dB change from baseline
35
30
25
20
15
10
5
0
-5
-30
-20
-10
60
70
80
Frequency (Hz)
35
30
25
20
15
10
5
0
-30
-20
-10
0
10
20
30
40
50
60
70
80
Time (s)

Animal 13

DTS settings

225 kJ, 20 kW. Again using the alternative waveguide.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 4 seconds post onset of DTS application, which is equivalent to approximately 58 kJ of energy delivered.



Notes from real-time observations

Reduction on power is expected to reduce the rate of energy delivery, i.e. heating will be slower.

Generator cut out after 1 sec and was restarted suggesting major energy leakage.

Animal again seemed partially unconscious, or dazed. It was ataxic but not fully unconscious.

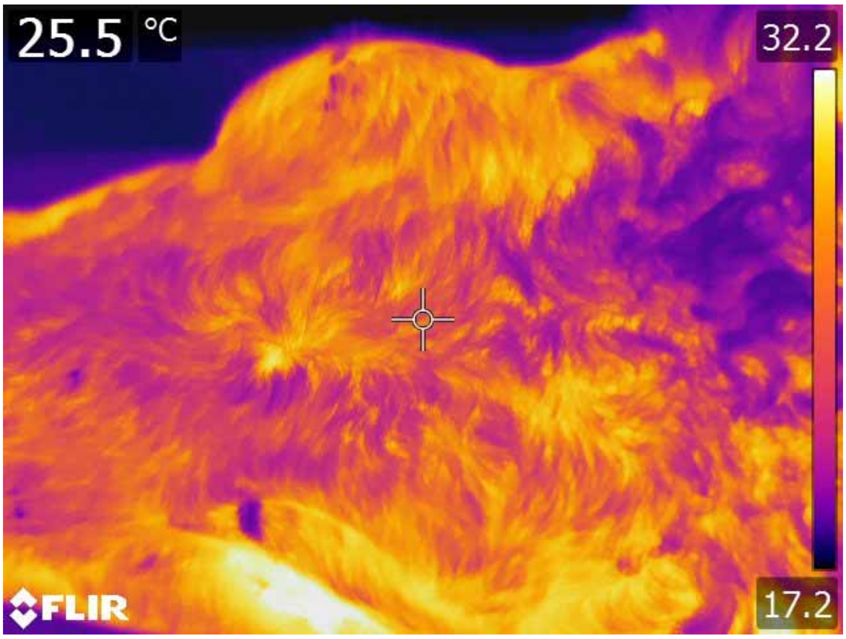
Captive bolt applied and rolled out for exsanguination.

Detailed event log from video footage

13	5/10/2017	15:52:06	animal in chin lift up wand in place
13	5/10/2017	15:52:20	DTS starts
13	5/10/2017	15:52:20	blinks hard
13	5/10/2017	15:52:24	eyes closed, rear end collapses
13	5/10/2017	15:52:34	closed eyes, blinking !
13	5/10/2017	15:52:38	eyes open
13	5/10/2017	15:52:44	rapid blinking
13	5/10/2017	15:53:05	body movements
13	5/10/2017	15:53:17	continues rapid blinking
13	5/10/2017	15:53:45	continued body movements
13	5/10/2017	15:53:46	wand removed
13	5/10/2017	15:54:21	body movements
13	5/10/2017	15:54:24	corneal reflex
13	5/10/2017	15:54:30	standing
13	5/10/2017	15:54:49	captive bolt, rear end collapses
13	5/10/2017	15:54:55	chin lift dropped
13	5/10/2017	15:55:03	head bale opens
13	5/10/2017	15:55:21	on table
13	5/10/2017	15:55:47	skin cut
13	5/10/2017	15:56:26	clip and cut oesophagus
13	5/10/2017	15:57:11	stuck.
13	5/10/2017	15:57:20	hung

Thermal images

Due to generator cut-out, and partial conscious animal, animal was euthanased prior to post-application thermal image capture. Pre DTS thermal image is shown below.



Animal 13 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 25.5 °C; maximum temperature in image (white): 32.2 °C; minimum temperature in image (black): 17.2 °C.

EEG traces

Due to generator cut-out, and partial conscious animal, animal was euthanased prior to post-application EEG capture.

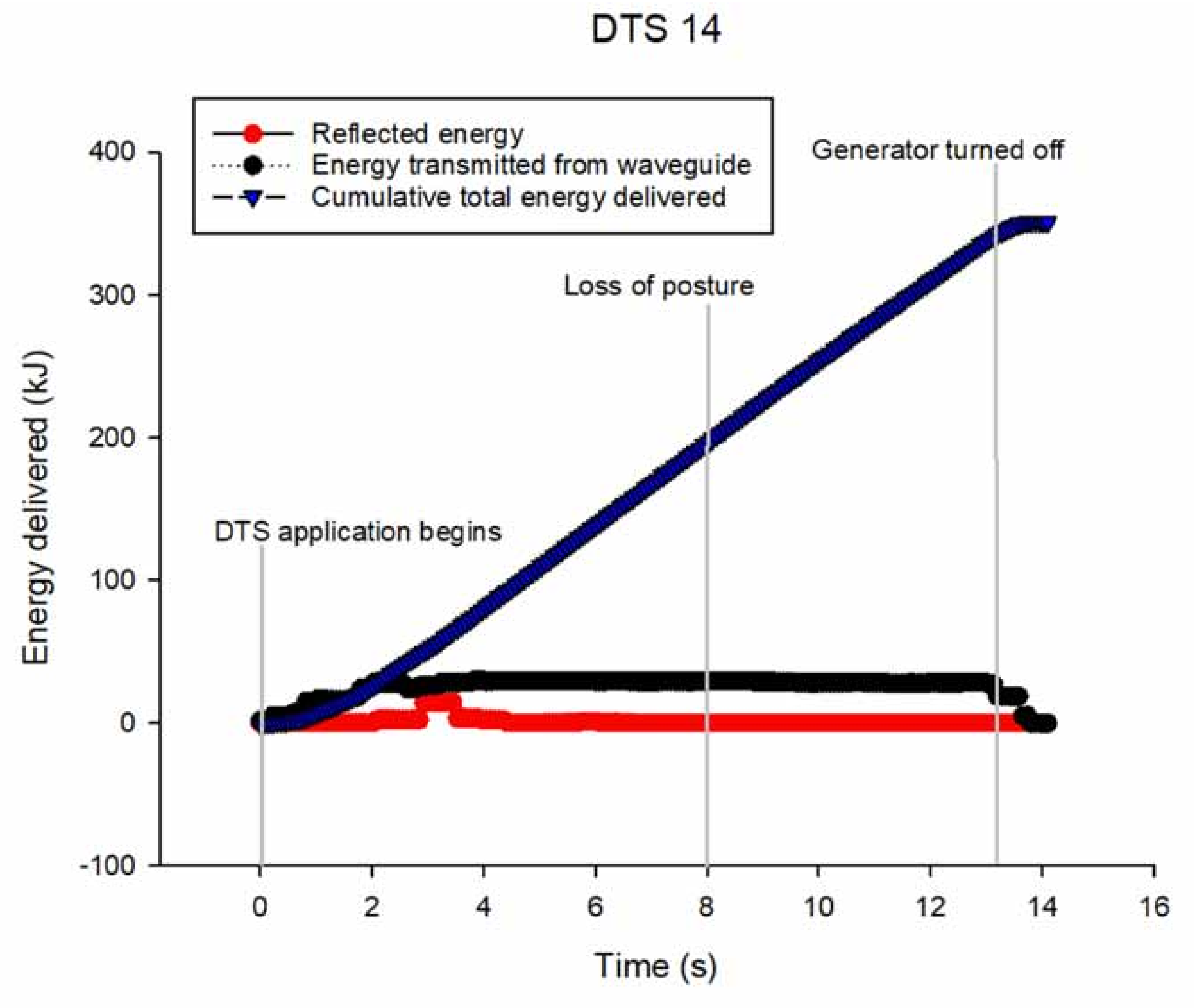
Animal 14

DTS settings

300 kJ, 30 kW.

Return to high energy settings at the beginning of the next processing day.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 8 seconds post onset of DTS application, which is equivalent to approximately 196 kJ of energy delivered.



Notes from real-time observations

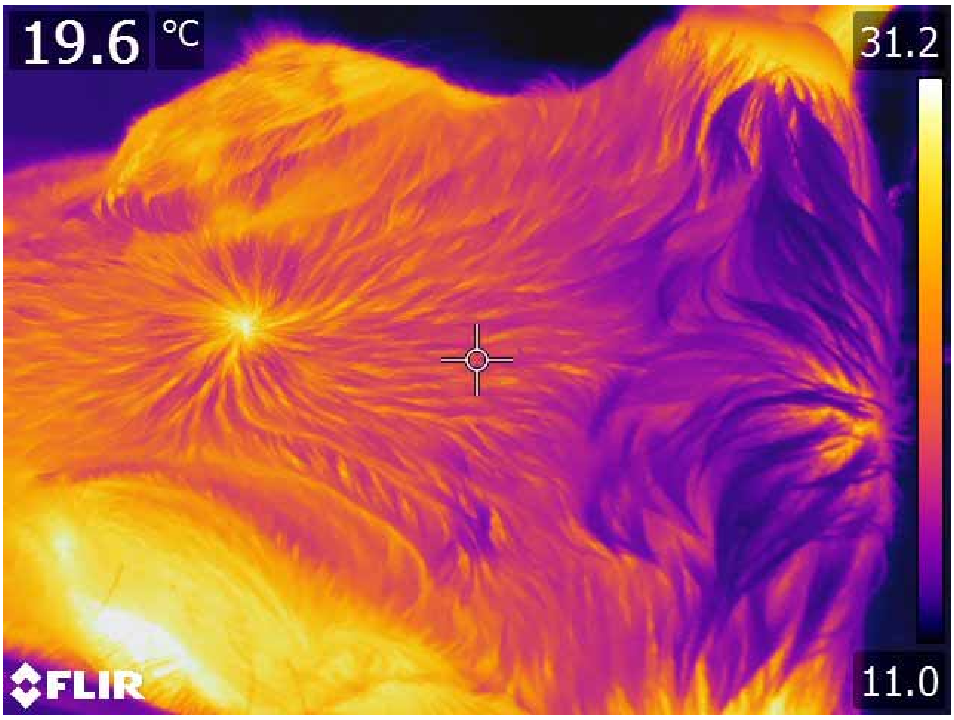
No notes were recorded in relation to this animal – this is likely to be because the attention of the researcher was focused on discussion of the DTS system, modifications and findings to date with the observers on this day.

Detailed event log from video footage

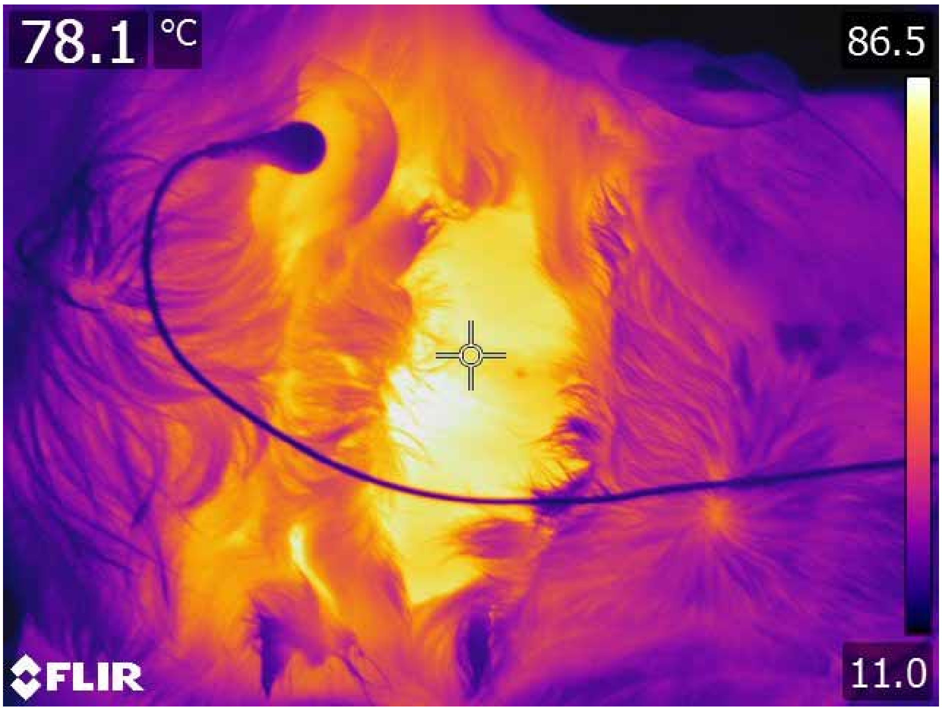
14	6/10/2017	8:26:47	animal in chin lift up wand in place
14	6/10/2017	8:27:01	DTS starts
14	6/10/2017	8:27:03	rapid blink
14	6/10/2017	8:27:05	front legs pawing
14	6/10/2017	8:27:09	rear legs collapse
14	6/10/2017	8:27:13	eyes wide open
14	6/10/2017	8:27:20	breathing
14	6/10/2017	8:27:37	wand removed
14	6/10/2017	8:27:40	eyes wide open
14	6/10/2017	8:27:40	corneal reflex
14	6/10/2017	8:27:45	corneal reflex
14	6/10/2017	8:27:57	chin lift dropped
14	6/10/2017	8:27:57	breathing
14	6/10/2017	8:28:03	crush opened
14	6/10/2017	8:28:12	chin lift raised to clear head
14	6/10/2017	8:28:19	side opened
14	6/10/2017	8:28:38	animal on table
14	6/10/2017	8:28:50	leads attached
14	6/10/2017	8:29:09	thermal image
14	6/10/2017	8:29:09	breathing
14	6/10/2017	8:29:44	corneal reflex
14	6/10/2017	8:29:44	eyes wide open
14	6/10/2017	8:29:50	corneal reflex
14	6/10/2017	8:29:59	corneal reflex
14	6/10/2017	8:30:24	corneal reflex
14	6/10/2017	8:30:31	skin cut
14	6/10/2017	8:30:54	clip and cut oesophagus
14	6/10/2017	8:30:56	stick
14	6/10/2017	8:31:23	leads removed
14	6/10/2017	8:31:35	agonal convulsions
14	6/10/2017	8:33:29	animal hung

Thermal images

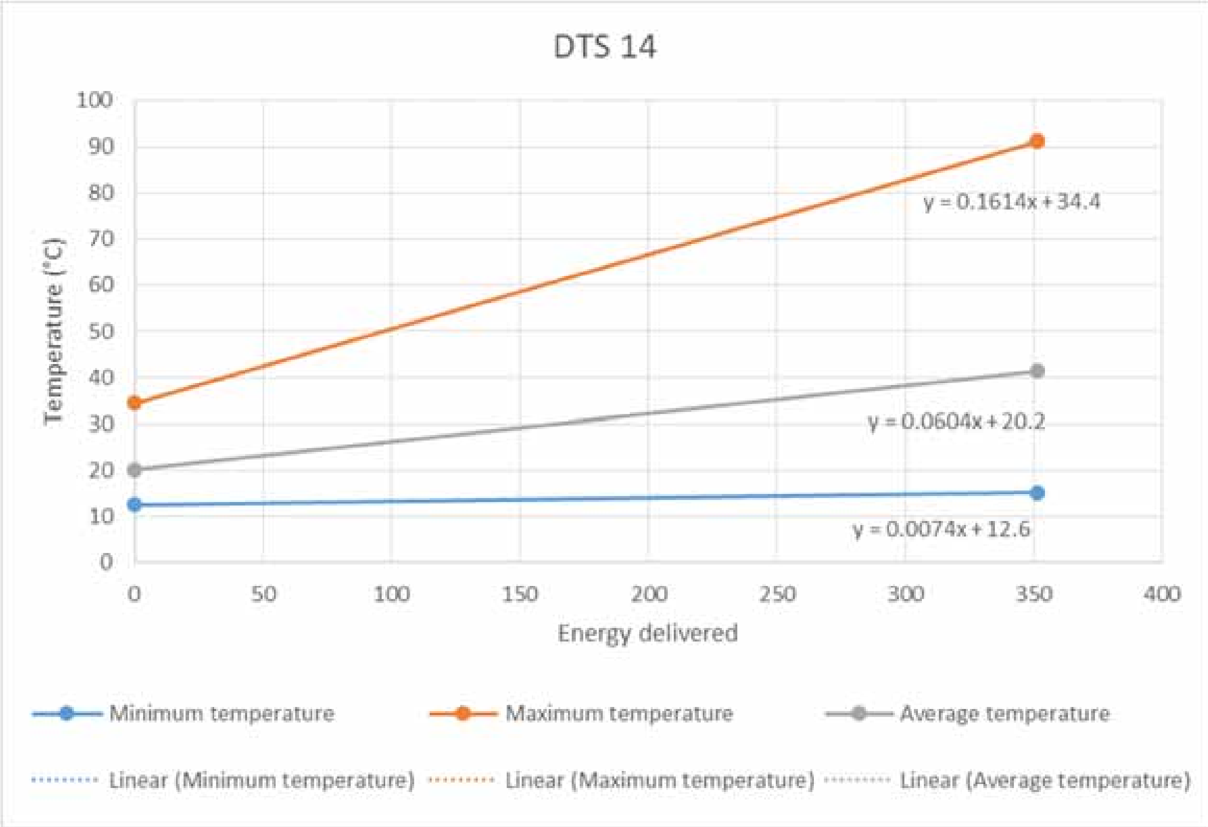
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 14 lost posture at approximately 4 seconds post onset of DTS application, which is equivalent to approximately 196 kJ of energy delivered. At 196 kJ energy delivered the estimated average forehead temperature is 32.04 °C (14.05 – 66.03).



Animal 14 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 19.6 °C; maximum temperature in image (white): 31.2 °C; minimum temperature in image (black): 11.0 °C.



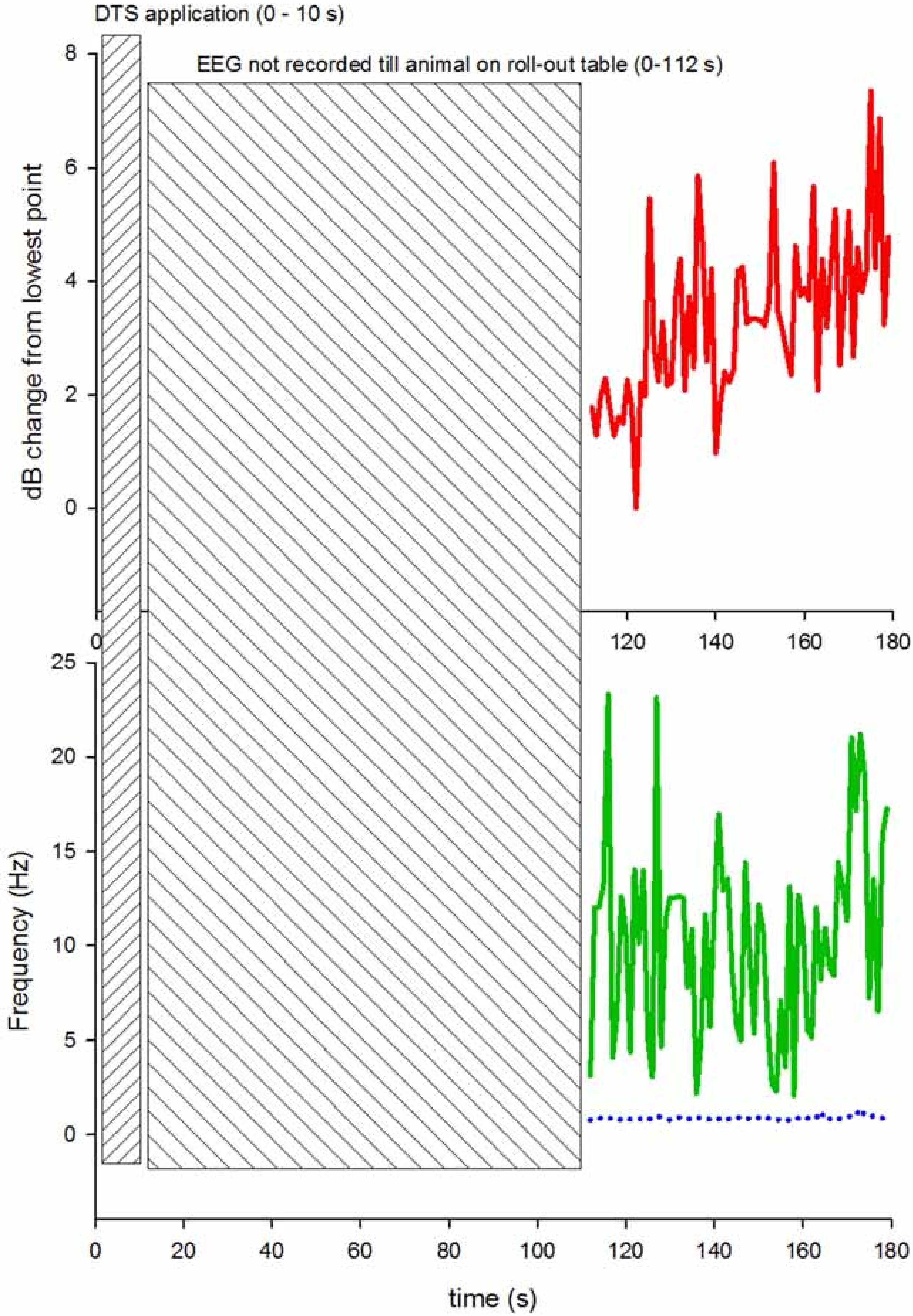
Animal 14 thermal map of forehead immediately after DTS application. Temperature at cursor: 78.1 °C; maximum temperature in image (white): 86.5 °C; minimum temperature in image (black): 11.0 °C. Disk shapes with cool centres and cables are EEG recording electrode pads and leads.



Animal 14 estimated temperature change on the forehead by energy delivered.

EEG traces

Baseline EEG was not recorded, and post-DTS EEG was recorded after the animal was rolled out onto the conveyor for exsanguination. Total EEG power was converted to dB change from lowest recorded value to bring it into a form that could be visually compared with traces from the other animals processed, and the chart. Total EEG power (Ptot, red line) is increasing and shows high amplitude spiking post DTS application; 95% spectral edge frequency (SEF95, green line) shows high amplitude spiking and 50% spectral edge frequency (SEF50, blue line) shows a flat line following DTS application. It is difficult to interpret thses data, as the EEG recordings are heavily contaminated by electrical noise from the abattoir infrastructure, and a baseline was not collected. However, high amplitude spiking in Ptot, and low frequency activity are considered to be incompatible with consciousness.

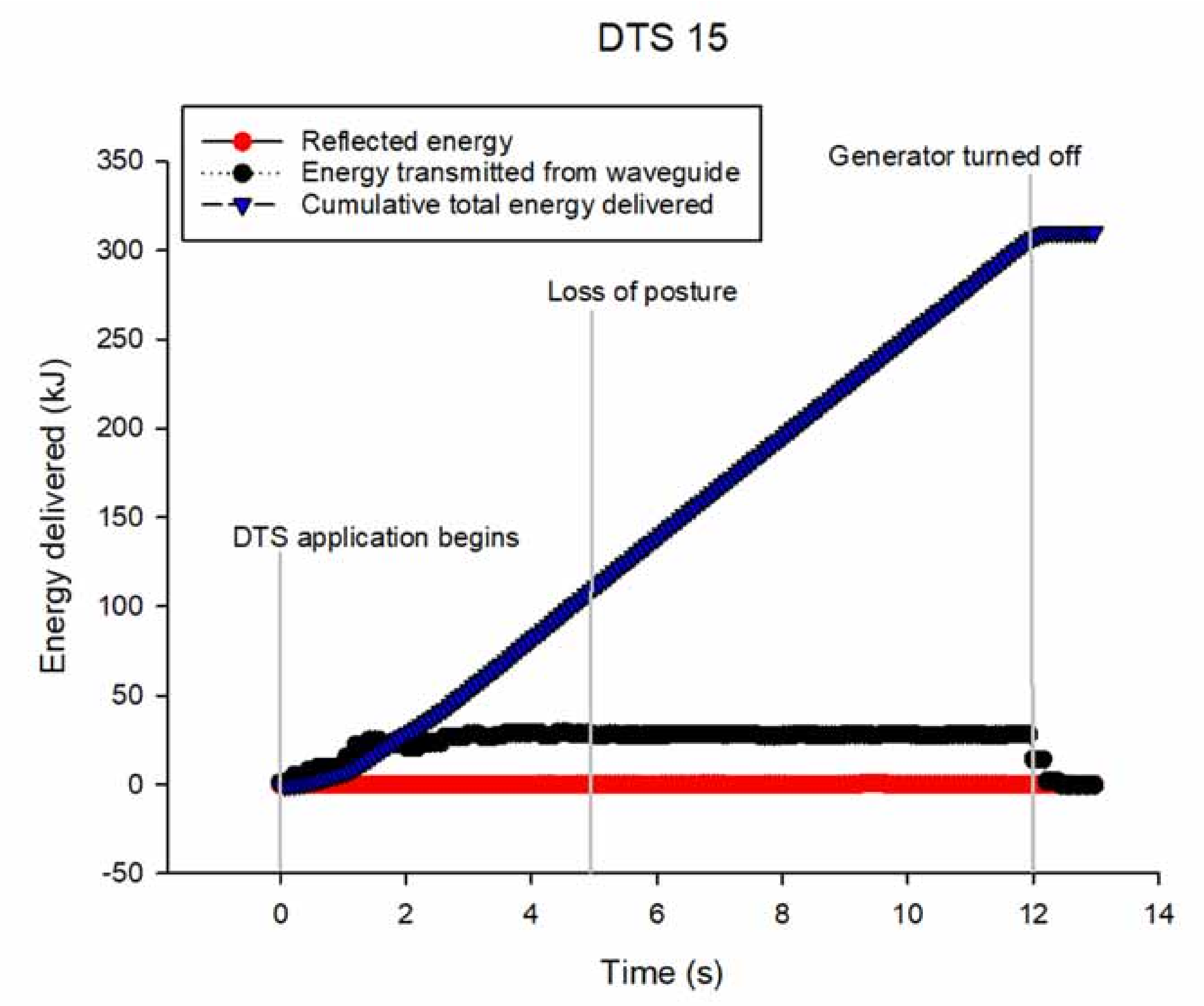

DTS application (0 - 10 s)
EEG not recorded till animal on roll-out table (0-112 s)
dB change from lowest point
Frequency (Hz)
time (s)

Animal 15

DTS settings

275 kJ, 30 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 5 seconds post onset of DTS application, which is equivalent to approximately 110 kJ of energy delivered.



Notes from real-time observations

Corneal reflex returning at around 1 min 45 sec from loss of posture.

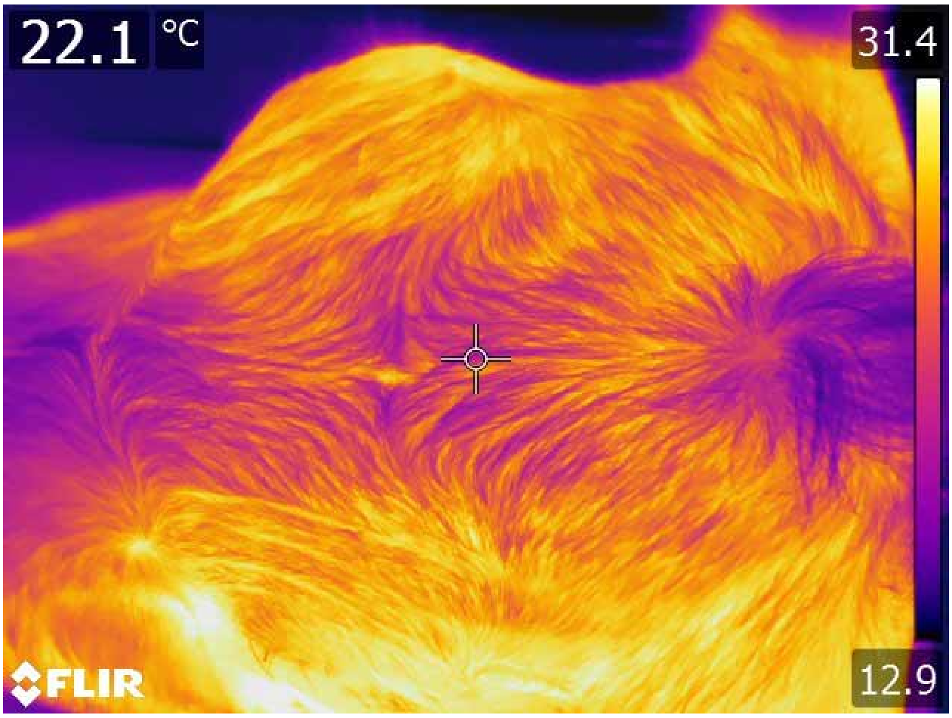
Captive bolt applied.

Detailed event log from video footage

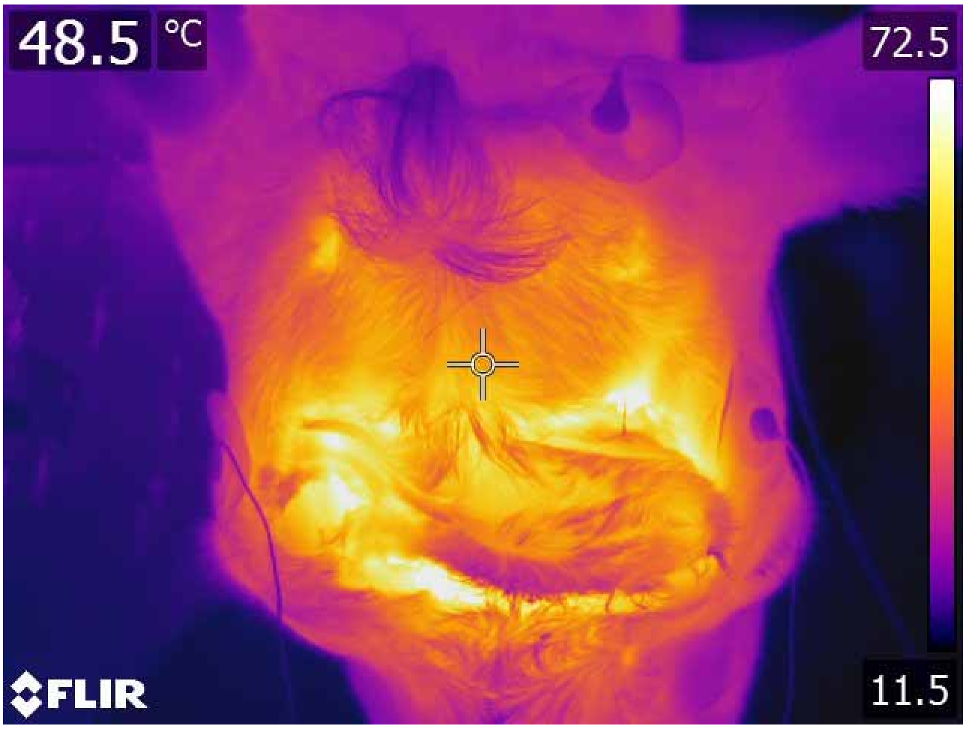
15	6/10/2017	8:50:01	animal in chin lift up wand in place
15	6/10/2017	8:50:24	body movements in crate
15	6/10/2017	8:50:34	DTS starts
15	6/10/2017	8:50:35	blinks
15	6/10/2017	8:50:38	steam obscures
15	6/10/2017	8:50:39	rear legs collapse
15	6/10/2017	8:50:41	eyes appear closed (steam obscures)
15	6/10/2017	8:50:56	wand removed
15	6/10/2017	8:51:10	front legs pawing
15	6/10/2017	8:51:17	shallow breathing
15	6/10/2017	8:51:27	corneal reflex
15	6/10/2017	8:51:40	corneal reflex
15	6/10/2017	8:51:47	chin lift dropped
15	6/10/2017	8:51:58	crush opened
15	6/10/2017	8:52:12	side opened
15	6/10/2017	8:52:20	on table
15	6/10/2017	8:52:36	leads on? Out of frame
15	6/10/2017	8:52:43	thermal image
15	6/10/2017	8:53:39	skin cut
15	6/10/2017	8:53:58	clip and cut oesophagus
15	6/10/2017	8:54:06	stick
15	6/10/2017	8:54:14	captive bolt
15	6/10/2017	8:55:00	leads off
15	6/10/2017	8:57:08	animal hung

Thermal images

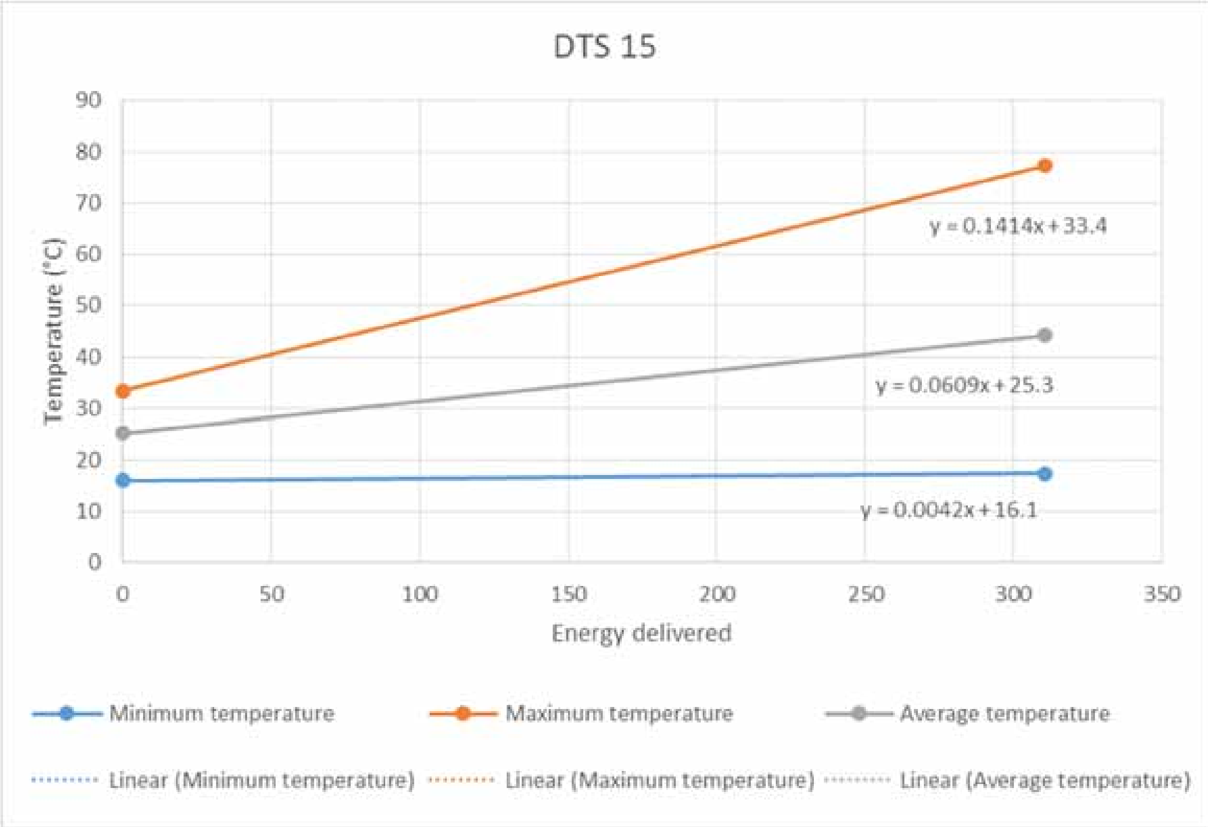
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 15 lost posture at approximately 5 seconds post onset of DTS application, which is equivalent to approximately 110 kJ of energy delivered. At 110 kJ energy delivered the estimated average forehead temperature is 32.00 °C (16.23 – 48.95).



Animal 15 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 22.1 °C; maximum temperature in image (white): 31.4 °C; minimum temperature in image (black): 12.9 °C.



Animal 15 thermal map of forehead immediately after DTS application. Temperature at cursor: 48.5 °C; maximum temperature in image (white): 72.5 °C; minimum temperature in image (black): 11.5 °C. Heating extending towards the poll is indicative of energy leakage. Disk shapes with cool centres and cables are EEG recording electrode pads and leads.



Animal 15 estimated temperature change on the forehead by energy delivered.

EEG traces

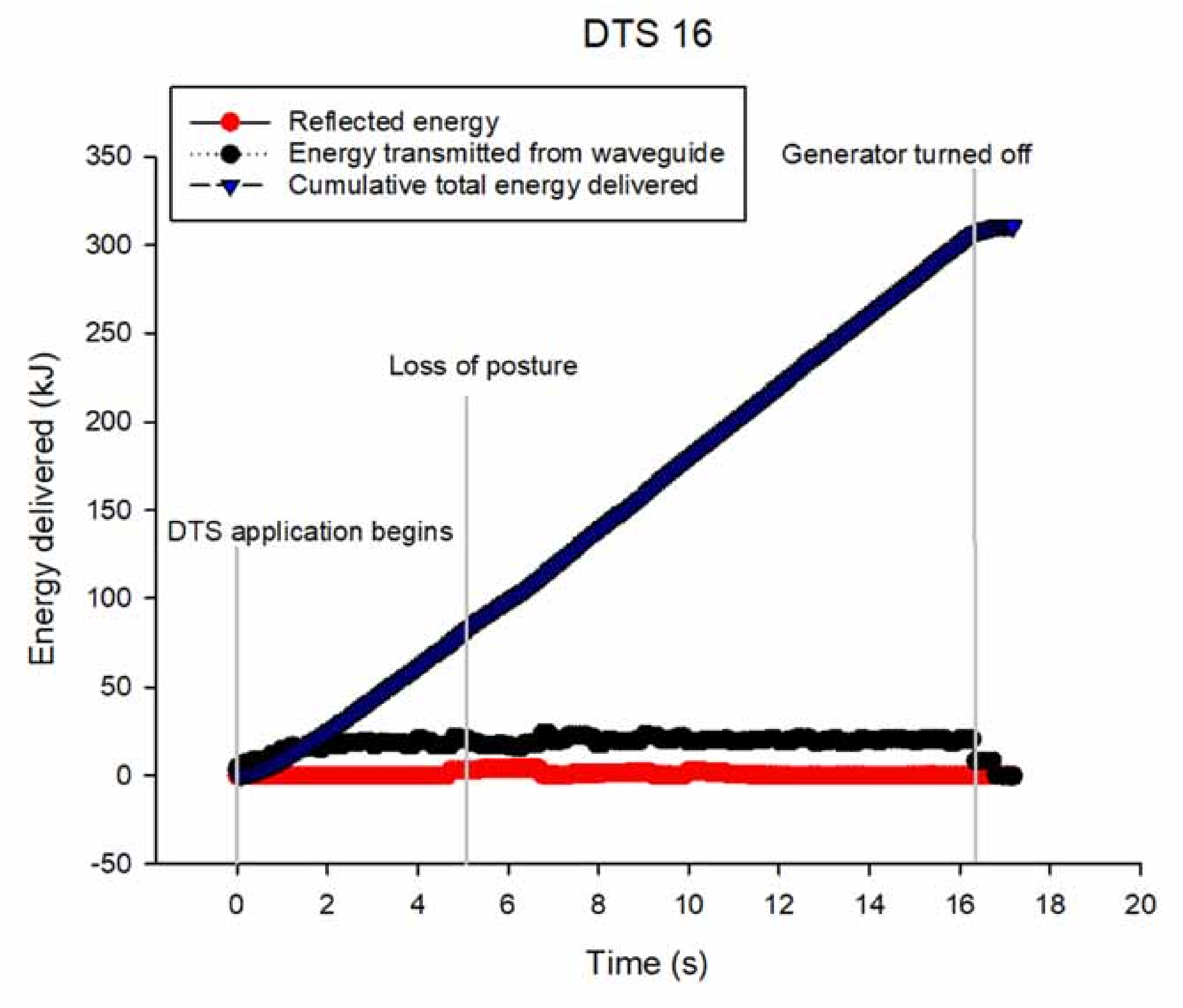
EEG collected on bleed conveyor post roll-out: once movement artefact was removed, there were too little EEG data available prior to the return of the corneal reflex and captive bolt application to generate a meaningful chart.

Animal 16

DTS settings

275 kJ, 20 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 5 seconds post onset of DTS application, which is equivalent to approximately 80 kJ of energy delivered.



Notes from real-time observations

Some leakage, may not have received full energy dose.

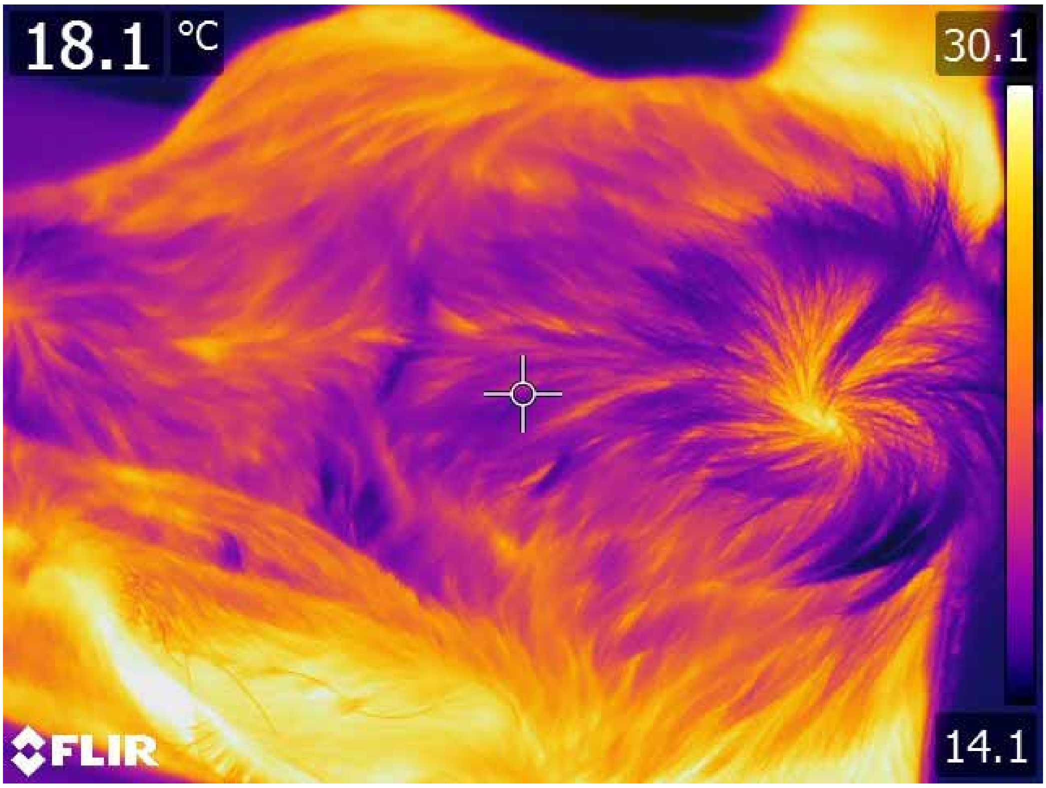
No corneal reflex. Reflex returning at around 1 min 40 sec from loss of posture.

Detailed event log from video footage

16	6/10/2017	9:04:27	animal in chin lift up wand in place
16	6/10/2017	9:04:44	body movements in crate
16	6/10/2017	9:04:54	DTS starts
16	6/10/2017	9:04:59	blinks
16	6/10/2017	9:04:59	plasma leakage
16	6/10/2017	9:04:59	rear legs collapse
16	6/10/2017	9:04:59	steam obscures view
16	6/10/2017	9:05:20	deep breathing
16	6/10/2017	9:05:31	wand removed
16	6/10/2017	9:05:39	corneal reflex
16	6/10/2017	9:05:39	eyes wide open
16	6/10/2017	9:05:48	corneal reflex
16	6/10/2017	9:05:48	torch reflex
16	6/10/2017	9:05:57	chin lift dropped
16	6/10/2017	9:06:00	crush opened
16	6/10/2017	9:06:20	side opened
16	6/10/2017	9:06:26	on table
16	6/10/2017	9:06:40	leads on
16	6/10/2017	9:06:52	corneal reflex
16	6/10/2017	9:06:53	thermal image
16	6/10/2017	9:07:13	captive bolt
16	6/10/2017	9:07:38	leads off
16	6/10/2017	9:07:40	skin cut
16	6/10/2017	9:08:01	clip and cut oesophagus
16	6/10/2017	9:08:08	stick
16	6/10/2017	9:10:08	animal hung

Thermal images

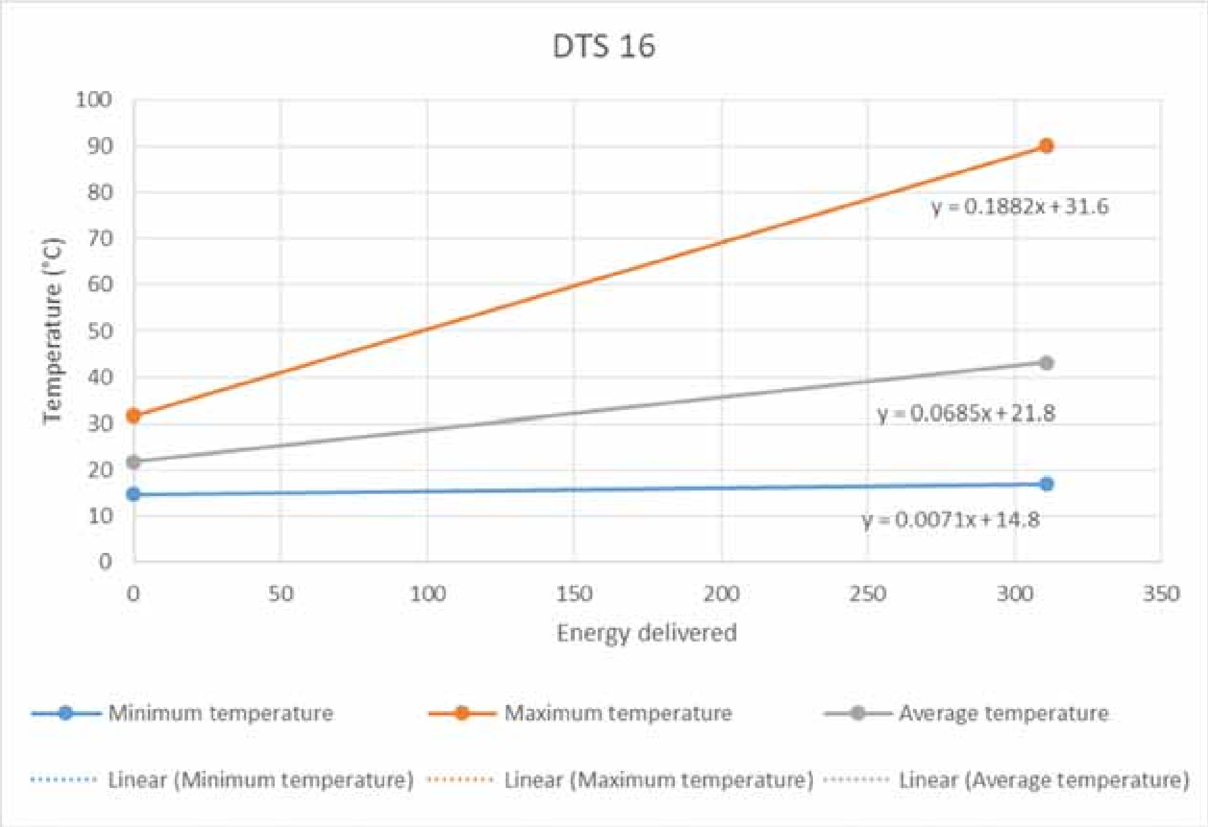
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 16 lost posture at approximately 5 seconds post onset of DTS application, which is equivalent to approximately 80 kJ of energy delivered. At 80 kJ energy delivered the estimated average forehead temperature is 27.28 °C (15.37 – 46.66).



Animal 16 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 18.1 °C; maximum temperature in image (white): 30.1 °C; minimum temperature in image (black): 14.1 °C.

Animal 16 thermal map of forehead immediately after DTS application. Temperature at cursor: 53.2 °C; maximum temperature in image (white): 79.2 °C; minimum temperature in image (black): 12.3 °C. Heating extending towards

the poll is indicative of energy leakage. Disk shapes with cool centres and cables are EEG recording electrode pads and leads.



Animal 16 estimated temperature change on the forehead by energy delivered.

EEG traces

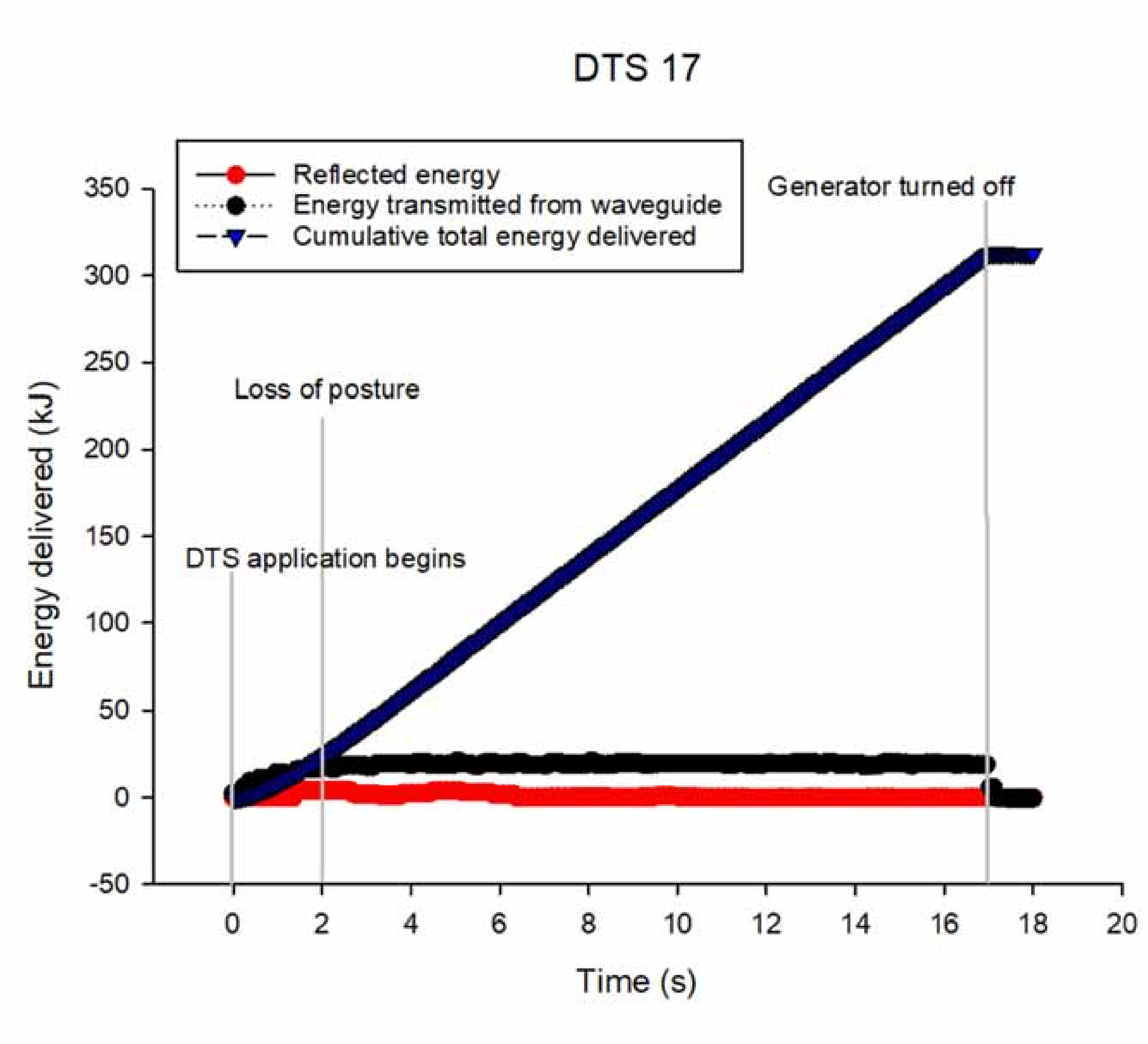
EEG collected on bleed conveyor post roll-out: once movement artefact was removed, there were too little EEG data available prior to the return of the corneal reflex and captive bolt application to generate a meaningful chart.

Animal 17

DTS settings

275 kJ, 20 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 2 seconds post onset of DTS application, which is equivalent to approximately 23 kJ of energy delivered.



Notes from real-time observations

Good stun, no corneal reflex.

Forelimb stuck in neck yoke, so unable to roll out.

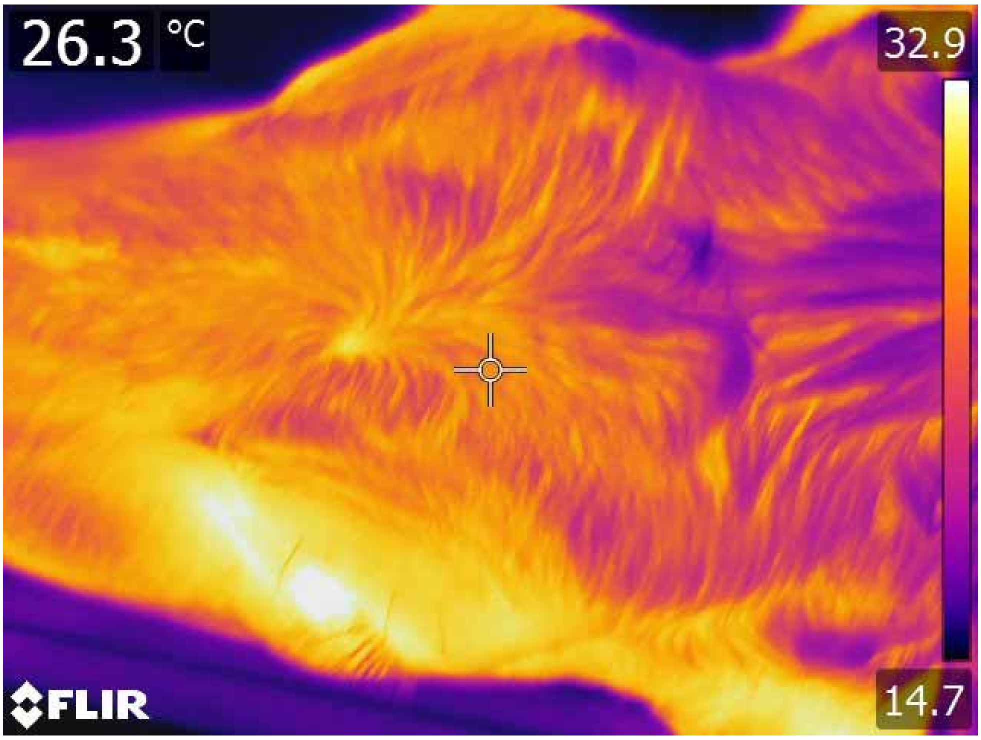
Captive bolt applied at 2 min post loss of posture, so that neck yoke could be safely dismantled in order to extract body. Still no corneal reflex present immediately prior to captive bolt.

Detailed event log from video footage

17	6/10/2017	9:25:45	animal in chin lift up wand in place
17	6/10/2017	9:25:57	DTS starts
17	6/10/2017	9:25:58	blinks, some sparking!
17	6/10/2017	9:25:59	rear legs collapse
17	6/10/2017	9:26:05	eyes wide open
17	6/10/2017	9:26:07	blinks
17	6/10/2017	9:26:10	all legs paddling
17	6/10/2017	9:26:11	eyes roll outwards
17	6/10/2017	9:26:20	legs continue to paddle and tail swishes
17	6/10/2017	9:26:25	breathing
17	6/10/2017	9:26:26	wand removed
17	6/10/2017	9:26:36	eyes wide open
17	6/10/2017	9:26:43	corneal reflex
17	6/10/2017	9:26:48	corneal reflex
17	6/10/2017	9:26:48	torch reflex
17	6/10/2017	9:27:00	chin lift dropped
17	6/10/2017	9:27:05	head restraint opened
17	6/10/2017	9:27:05	right front leg stuck
17	6/10/2017	9:27:56	animal caught in head bale, lots of mucking around, attempting to release
17	6/10/2017	9:28:33	captive bolt ,(operator in crush!)
17	6/10/2017	9:29:20	agonal convulsions
17	6/10/2017	9:30:10	convulsions continue
17	6/10/2017	9:32:46	still stuck in crush, video stops.

Thermal images

Pre DTS thermal image is shown below. No post-DTS image was captured.



Animal 17 thermal map of forehead prior to DTS application. Temperature at cursor: 26.3 °C; maximum temperature in image (white): 32.9 °C; minimum temperature in image (black): 14.7 °C.

EEG traces

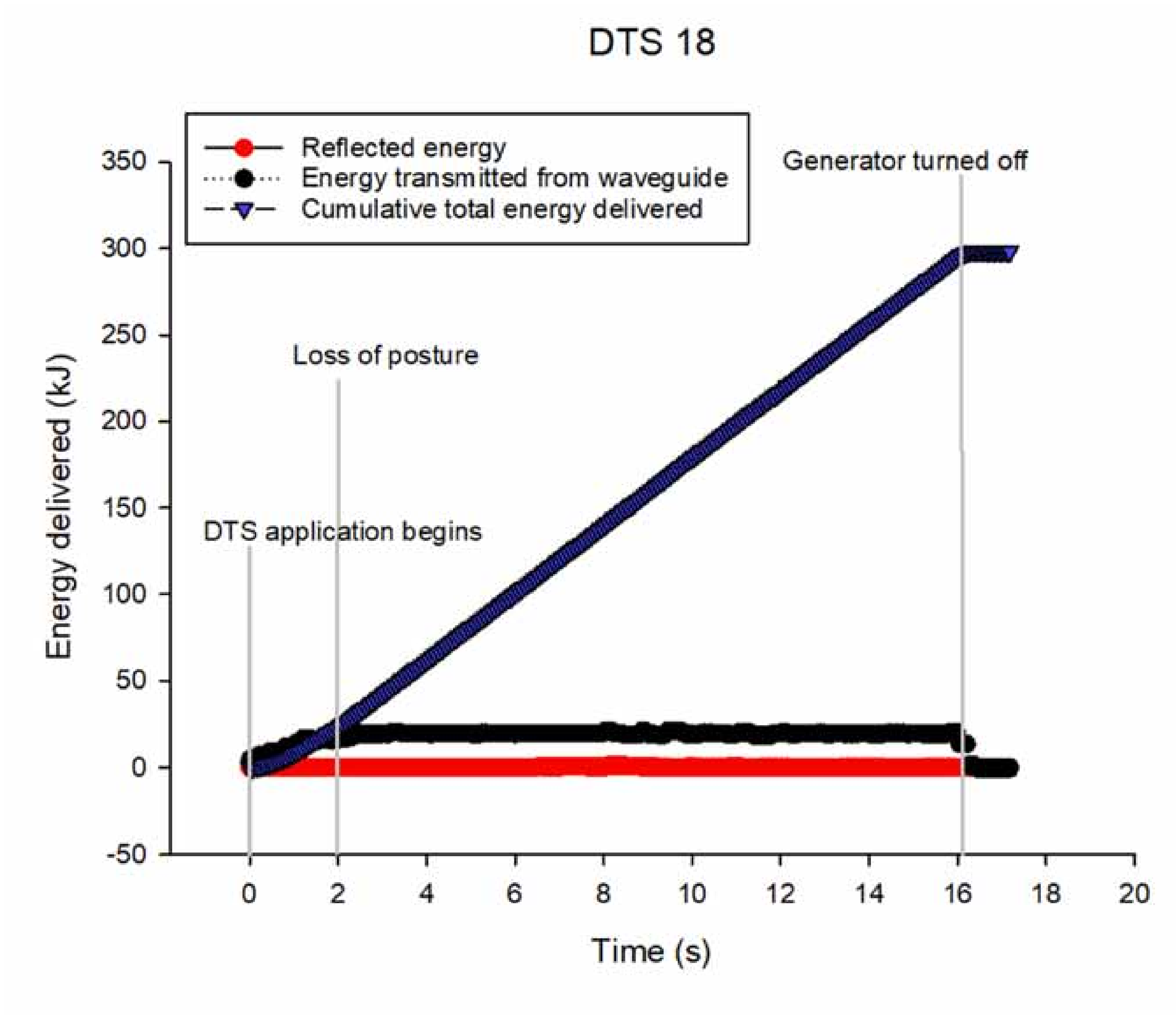
EEG collected on bleed conveyor post roll-out: once movement artefact was removed, there were too little EEG data available prior to the return of the corneal reflex and captive bolt application to generate a meaningful chart.

Animal 18

DTS settings

275 kJ, 20 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 2 seconds post onset of DTS application, which is equivalent to approximately 23 kJ of energy delivered.



Notes from real-time observations

Poor contact between waveguide and head, some arcing evident.

Corneal reflex slow/inconclusive.

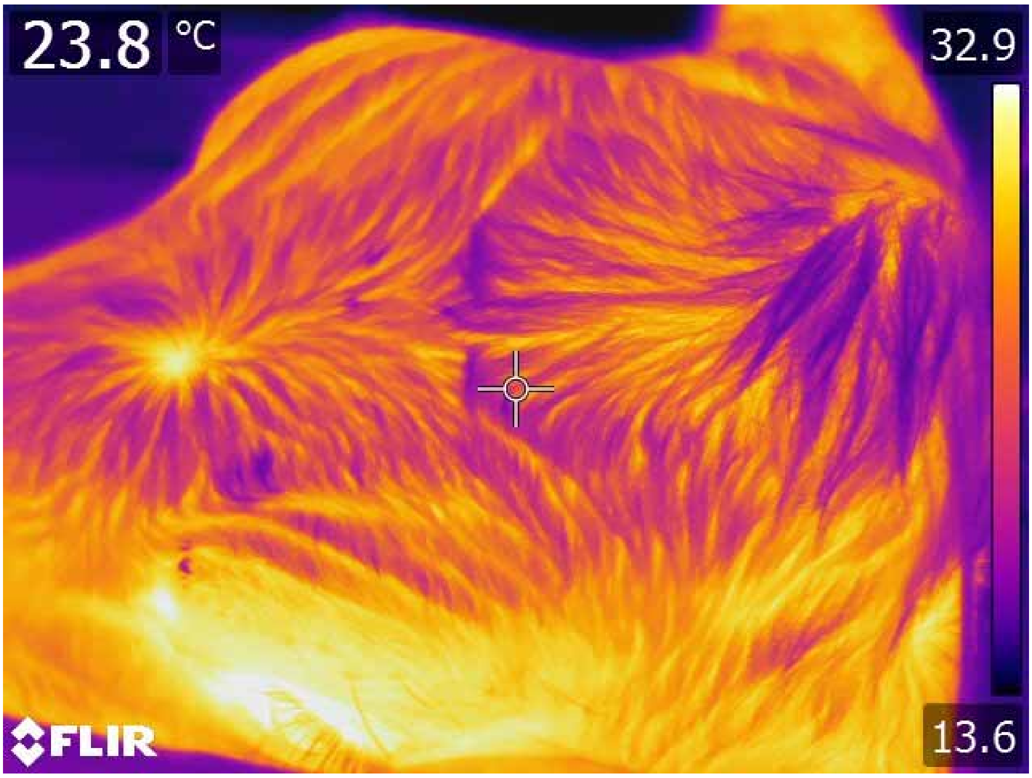
Forelimb stuck in neck yoke, so unable to roll out. Captive bolt applied so that neck yoke could be safely dismantled in order to extract body.

Detailed event log from video footage

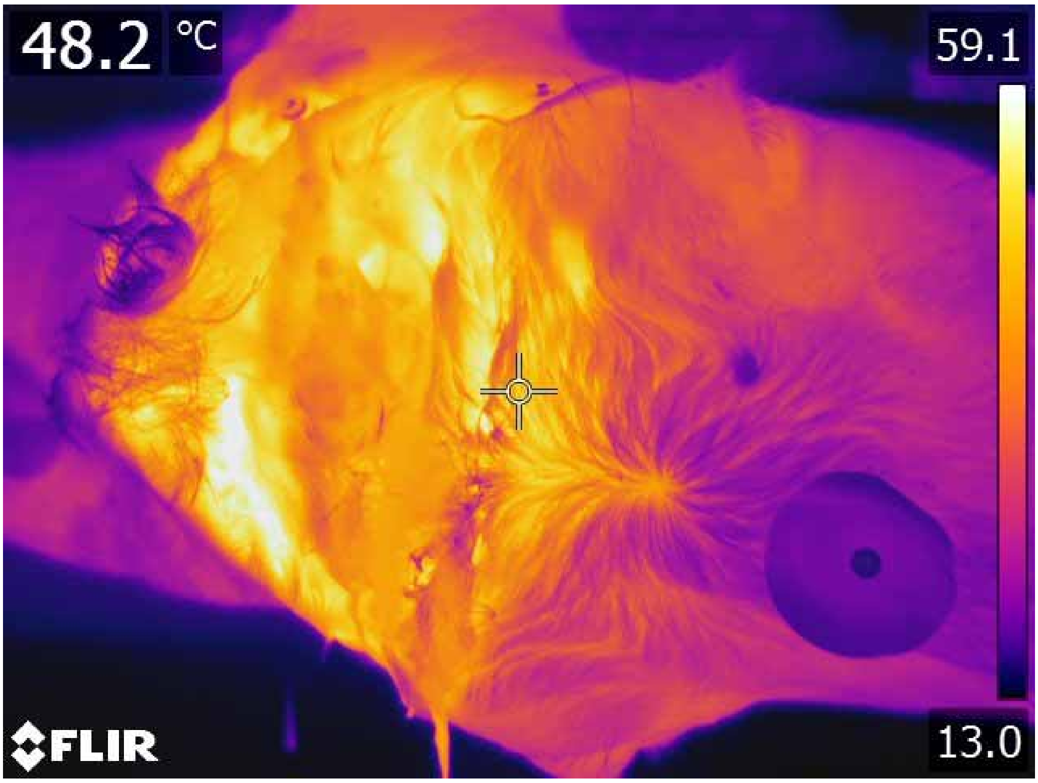
18	6/10/2017	9:52:00	In crush, chin up
18	6/10/2017	9:52:17	wand in place
18	6/10/2017	9:52:40	DTS starts
18	6/10/2017	9:52:42	blink, some sparking
18	6/10/2017	9:52:42	rear legs collapse
18	6/10/2017	9:52:46	legs paddling
18	6/10/2017	9:52:56	steam obscures head
18	6/10/2017	9:53:04	legs paddling, trying to stand
18	6/10/2017	9:53:10	rear legs stretch backwards
18	6/10/2017	9:53:10	wand removed
18	6/10/2017	9:53:22	corneal reflex
18	6/10/2017	9:53:28	corneal reflex
18	6/10/2017	9:53:34	torch reflex
18	6/10/2017	9:54:05	torch and corneal reflex
18	6/10/2017	9:54:05	captive bolt
18	6/10/2017	9:54:20	chin lift dropped
18	6/10/2017	9:54:28	head bale opens, left front leg stuck
18	6/10/2017	9:54:45	side opens, still stuck
18	6/10/2017	9:55:30	side closes
18	6/10/2017	9:56:30	modifications to crush
18	6/10/2017	9:57:43	Agonal convulsions in crush, still stuck
18	6/10/2017	9:58:28	front leg freed
18	6/10/2017	9:58:56	side door opens
18	6/10/2017	9:59:07	on table
18	6/10/2017	9:59:16	skin cut
18	6/10/2017	9:59:58	clip and cut oesophagus
18	6/10/2017	9:59:59	vid stops

Thermal images

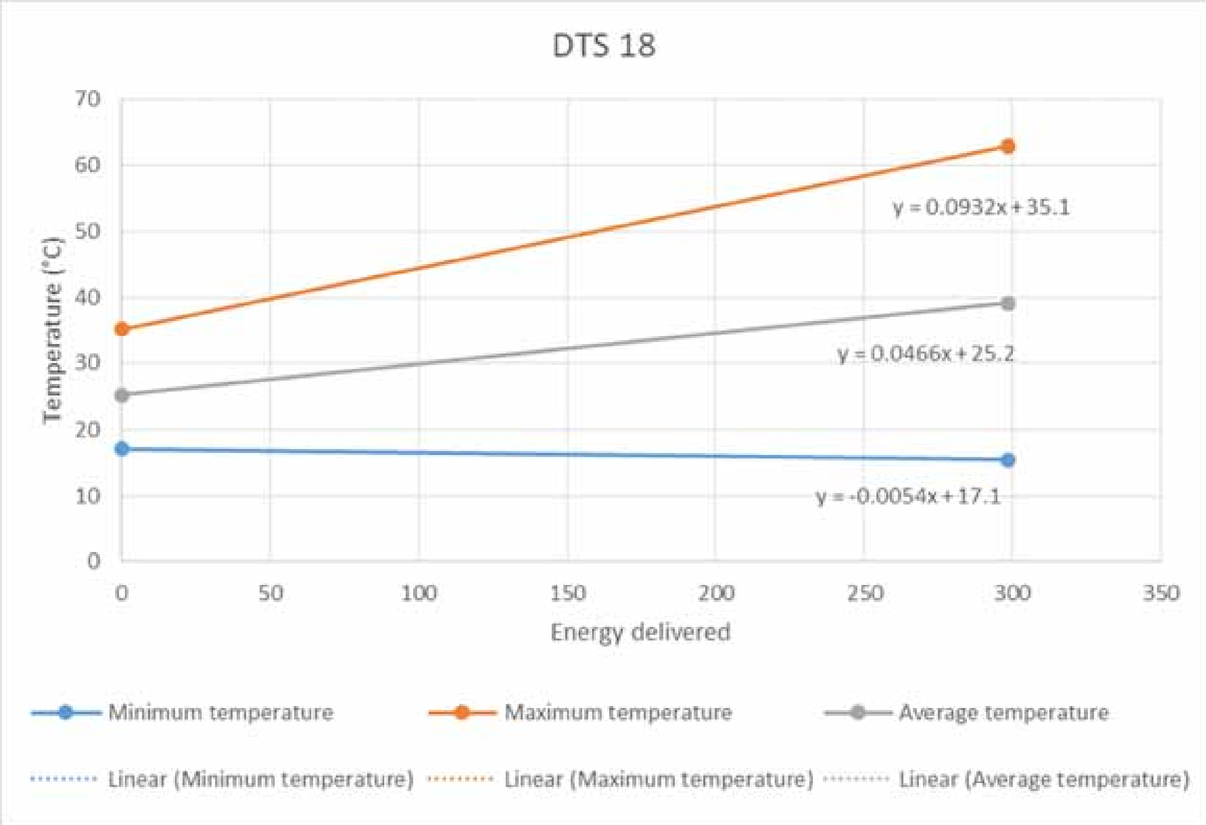
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 18 lost posture at approximately 2 seconds post onset of DTS application, which is equivalent to approximately 23 kJ of energy delivered. At 23 kJ energy delivered the estimated average forehead temperature is 26.27 °C (16.98 – 37.24).



Animal 18 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 23.8 °C; maximum temperature in image (white): 32.9 °C; minimum temperature in image (black): 13.6 °C.



Animal 18 thermal map of forehead immediately after DTS application. Temperature at cursor: 48.2 °C; maximum temperature in image (white): 59.1 °C; minimum temperature in image (black): 13.0 °C. Heating extending towards the poll is indicative of energy leakage. Disk shapes with cool centres and EEG recording electrode pads.



Animal 18 estimated temperature change on the forehead by energy delivered.

EEG traces

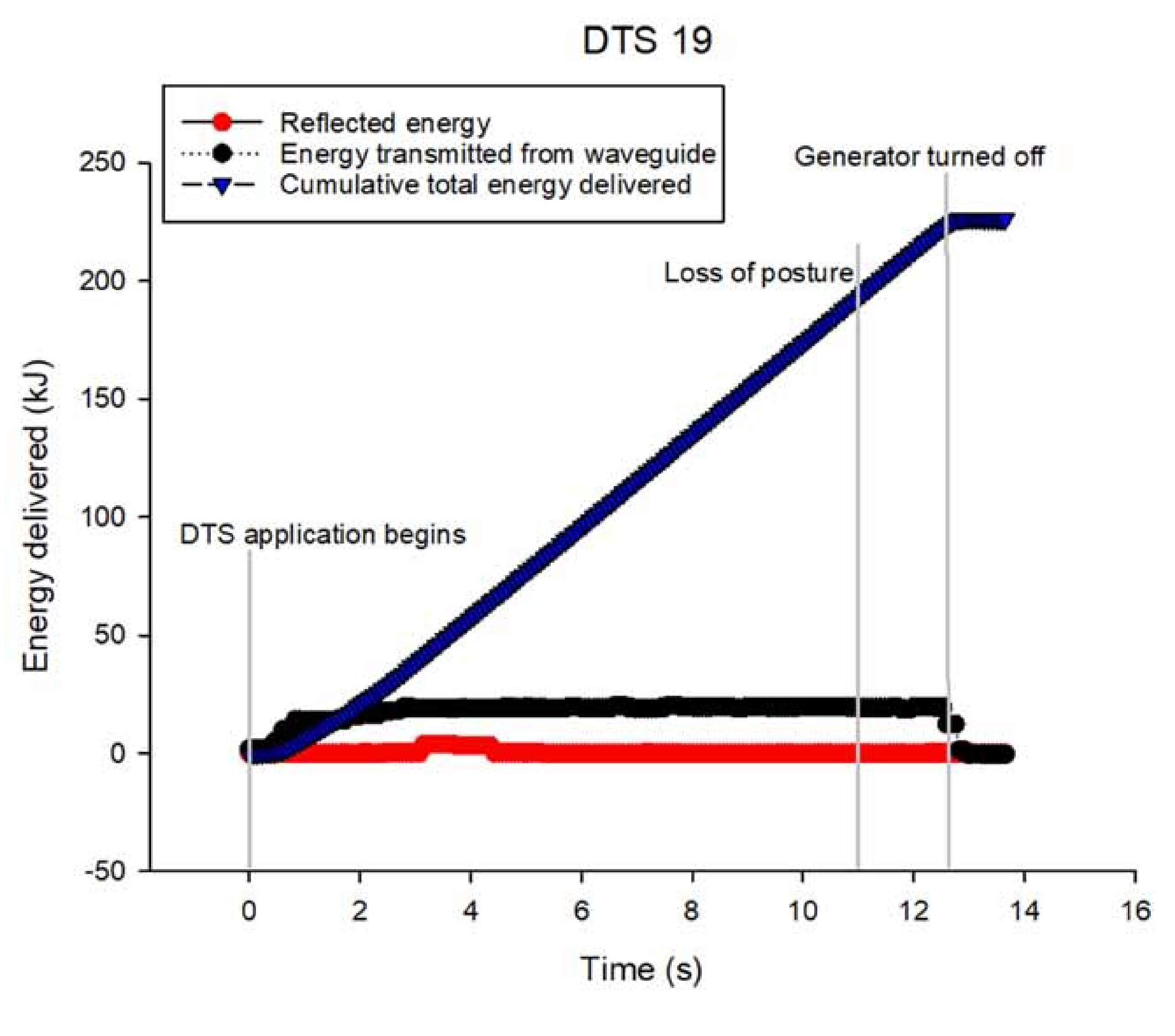
EEG collected on bleed conveyor post roll-out: once movement artefact was removed, there were too little EEG data available prior to the return of the corneal reflex and captive bolt application to generate a meaningful chart.

Animal 19

DTS settings

200 kJ, 20 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 8 seconds post onset of DTS application, which is equivalent to approximately 135 kJ of energy delivered.



Notes from real-time observations

No corneal reflex. Body got stuck in the box.

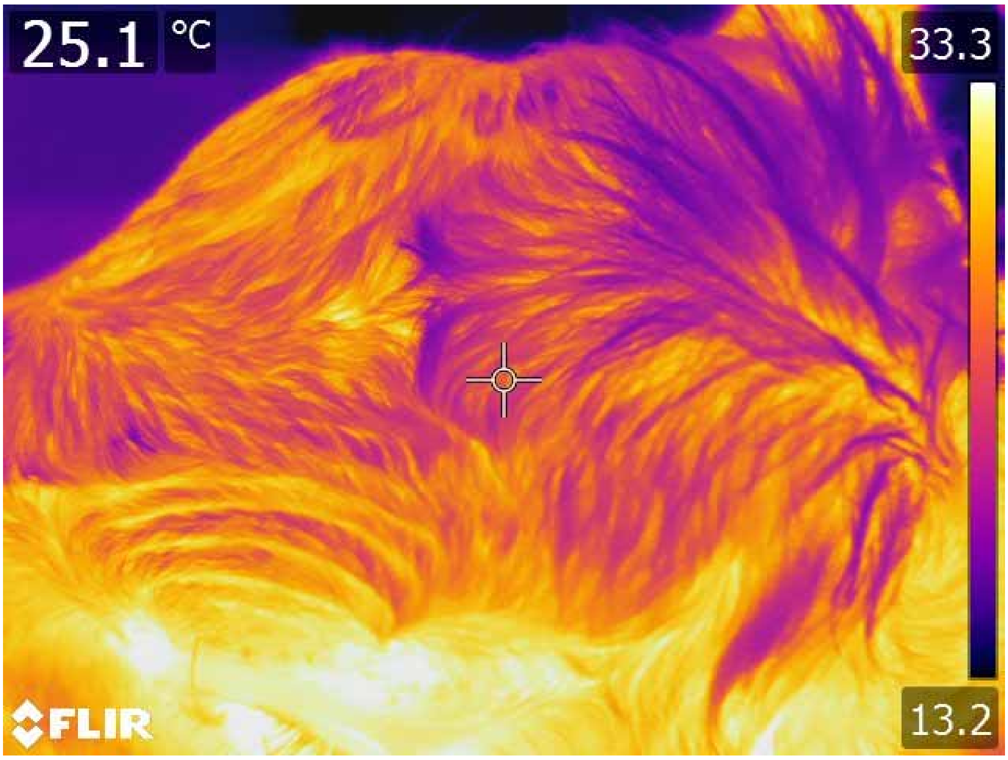
Corneal reflex returning at 2 min 34 sec after loss of posture, so captive bolt applied.

Detailed event log from video footage

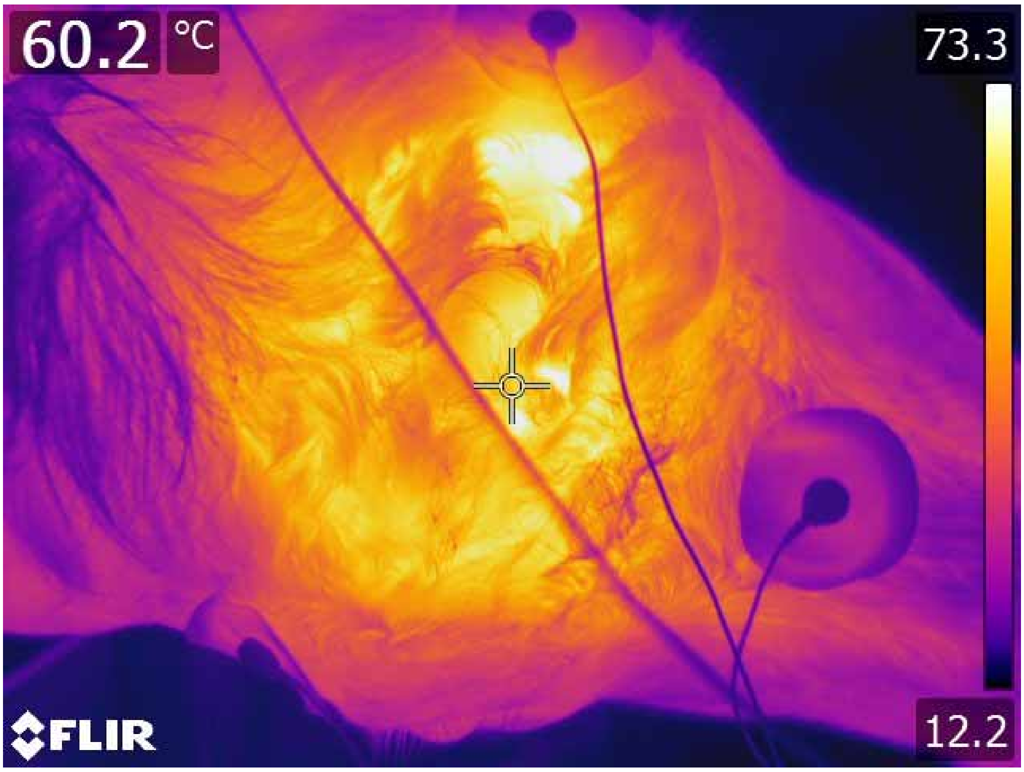
Animal number	Date	time	action
19	6/10/2017	10:09:24	DTS starts
19	6/10/2017	10:09:25	plasma leakage, blinks
19	6/10/2017	10:09:31	eyes closed
19	6/10/2017	10:09:33	rear legs collapse, eyes wide open
19	6/10/2017	10:09:44	not breathing
19	6/10/2017	10:09:51	wand removed
19	6/10/2017	10:10:03	top knot lifting (?)
19	6/10/2017	10:10:03	corneal reflex
19	6/10/2017	10:10:12	chin lift dropped
19	6/10/2017	10:10:33	chinlift raised
19	6/10/2017	10:10:58	chin lift dropped
19	6/10/2017	10:11:14	crook used to free front leg from crush mechanism
19	6/10/2017	10:11:31	head restraint opened
19	6/10/2017	10:11:44	some deep breaths
19	6/10/2017	10:11:44	side opened
19	6/10/2017	10:11:52	corneal reflex
19	6/10/2017	10:11:58	rolled out
19	6/10/2017	10:12:10	leads attached
19	6/10/2017	10:12:19	thermal image
19	6/10/2017	10:12:42	captive bolt
19	6/10/2017	10:13:08	leads removed
19	6/10/2017	10:13:31	skin cut
19	6/10/2017	10:13:41	rodded
19	6/10/2017	10:13:46	clipped
19	6/10/2017	10:13:51	oesophagus cut
19	6/10/2017	10:13:52	exsanguinated

Thermal images

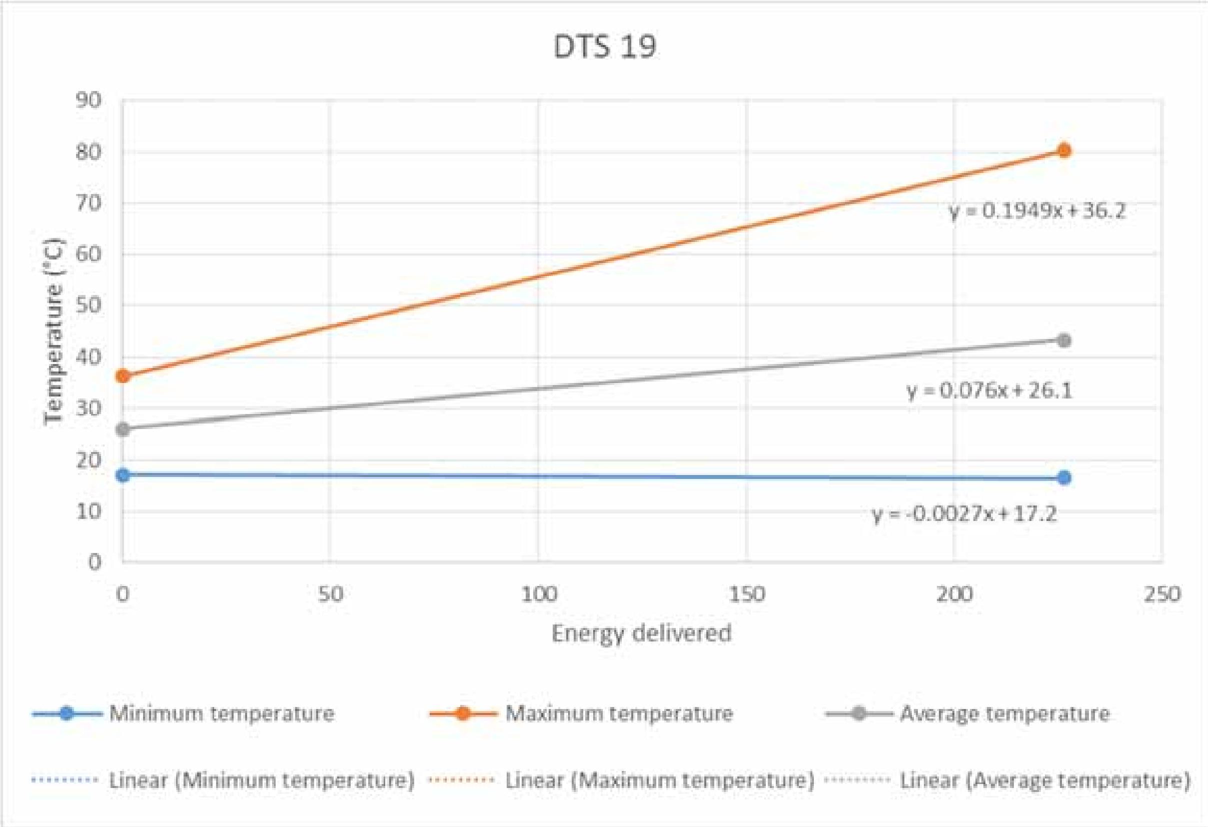
Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 19 lost posture at approximately 8 seconds post onset of DTS application, which is equivalent to approximately 135 kJ of energy delivered. At 135 kJ energy delivered the estimated average forehead temperature is 36.36 °C (17.56 – 62.51).



Animal 19 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 25.1 °C; maximum temperature in image (white): 33.3 °C; minimum temperature in image (black): 13.2 °C.



Animal 19 thermal map of forehead immediately after DTS application. Temperature at cursor: 60.2 °C; maximum temperature in image (white): 73.3 °C; minimum temperature in image (black): 12.2 °C. Heating extending towards the poll is indicative of energy leakage. Disk shapes with cool centres and cables are EEG recording electrode pads and leads.



Animal 19 estimated temperature change on the forehead by energy delivered.

EEG traces

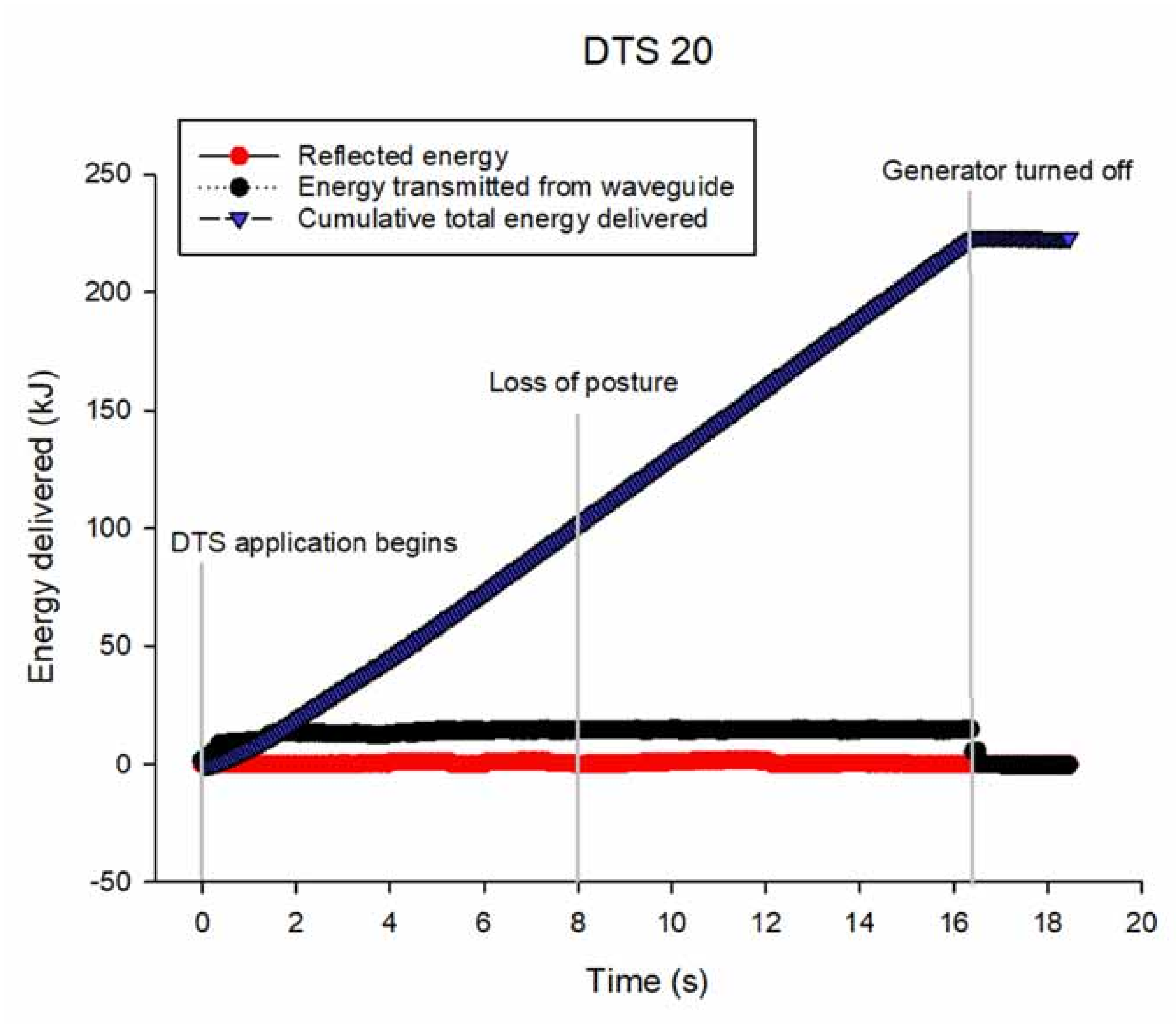
EEG collected on bleed conveyor post roll-out: once movement artefact was removed, there were too little EEG data available prior to the return of the corneal reflex and captive bolt application to generate a meaningful chart.

Animal 20

DTS settings

200 kJ, 15 kW.

The chart below shows energy data from the DTS control software. Blue is the total energy delivered from the waveguide over time; black is the amount of energy being delivered at a single time point; red is the amount of energy reflected at a single time point. Vertical lines indicate time that DTS application began; time the animal lost posture, and time that the DTS generator was turned off, ending the DTS application. Loss of posture, identified from video footage, occurred at approximately 8 seconds post onset of DTS application, which is equivalent to approximately 101 kJ of energy delivered.



Notes from real-time observations

Reduction in power is expected to reduce the rate of energy delivery, i.e. heating will be slower.

Pulling back or aversion type response seen on video footage.

Loss of posture occurred, but slow as opposed to abrupt. This was followed by convulsions then return to standing posture. Eyelids flickering rapidly, eye rolled down, groaning vocalisation on exhalation.

Corneal reflex absent at 45 sec post loss of posture.

Corneal reflex slow/inconclusive at 60 sec.

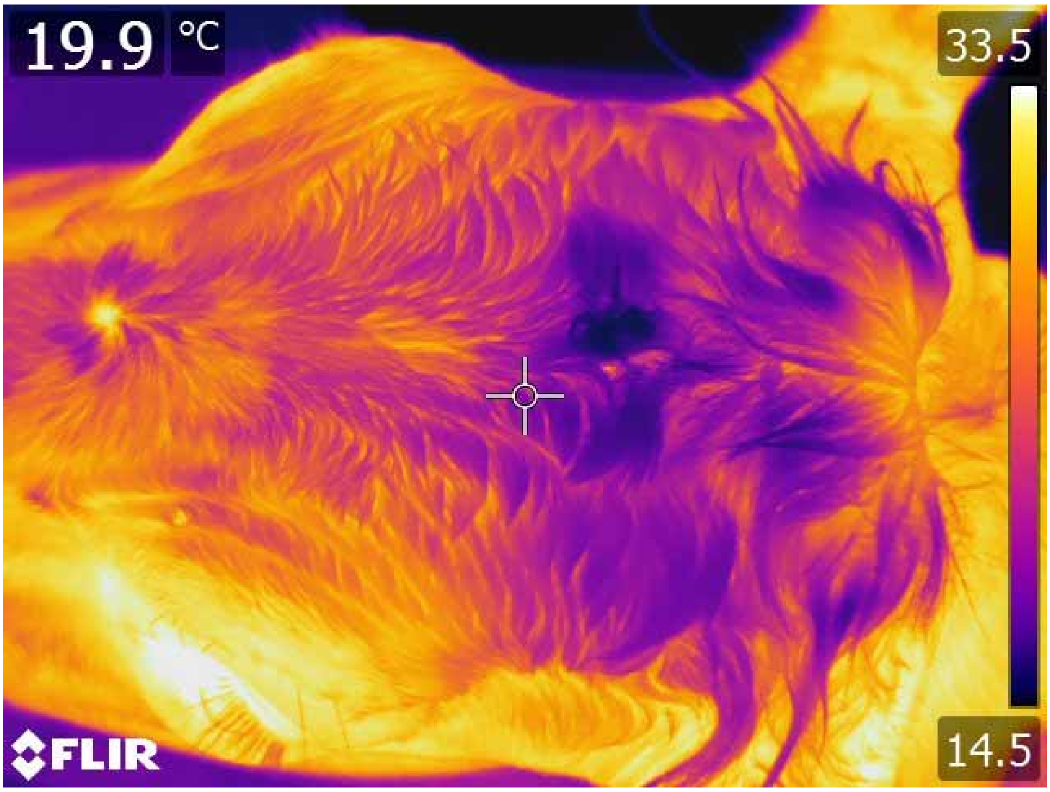
Return of reflex at 72 sec, captive bolt applied.

Detailed event log from video footage

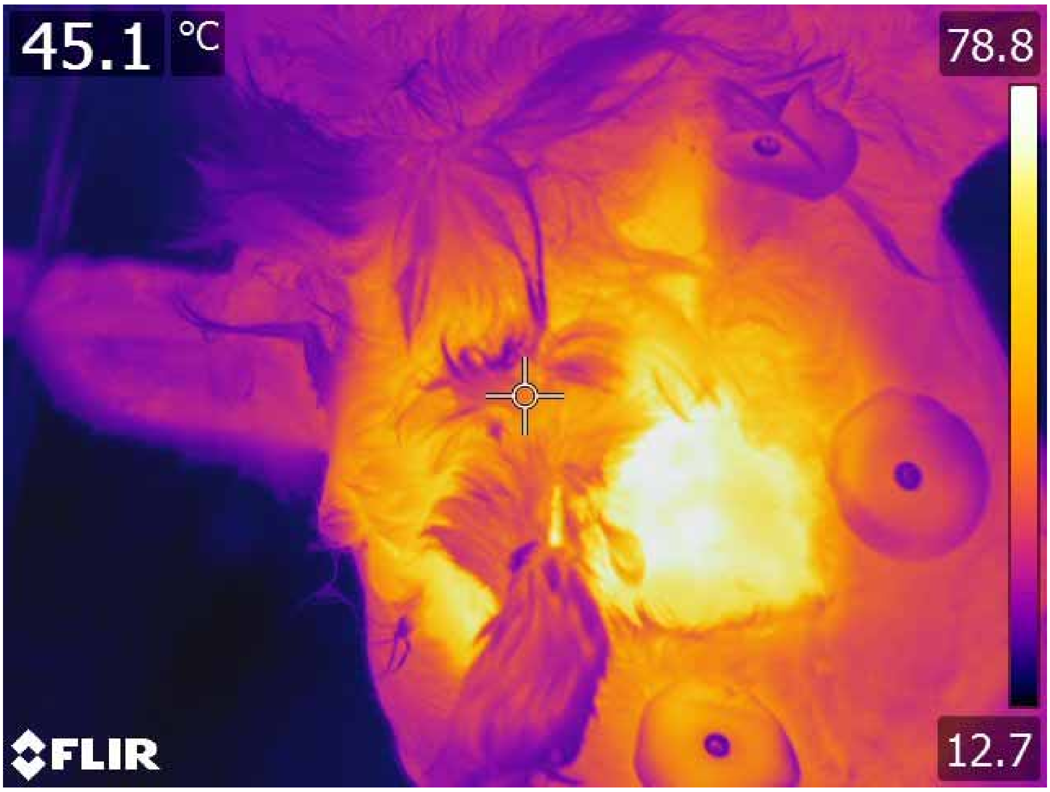
20	6/10/2017	10:28:55	animal in chin lift up wand in place
20	6/10/2017		nb head shot camera not available
20	6/10/2017	10:29:30	DTS starts
20	6/10/2017	10:29:34	pulls back with rear legs
20	6/10/2017	10:29:38	rear legs collapse
20	6/10/2017	10:29:50	legs paddling
20	6/10/2017	10:30:00	wand removed
20	6/10/2017	10:30:18	corneal reflex abd torch
20	6/10/2017	10:30:21	stands-breathing
20	6/10/2017	10:30:35	corneal touch
20	6/10/2017	10:30:39	tail swishing
20	6/10/2017	10:31:00	still standing
20	6/10/2017	10:31:12	captive bolt rear legs collapse
20	6/10/2017	10:31:23	head bale opens, left front leg stuck
20	6/10/2017	10:31:29	eyes wide open
20	6/10/2017	10:31:35	side door opens
20	6/10/2017	10:31:35	on table
20	6/10/2017	10:32:00	thermal image
20	6/10/2017	10:32:16	skin cut
20	6/10/2017	10:32:32	clip and cut oesophagus
20	6/10/2017	10:32:40	stuck.

Thermal images

Thermal images pre-DTS and post-DTS are shown below. The maximum, minimum and average temperatures recorded on the forehead area were used to estimate heating rates as a function of energy delivered (data from the DTS control software), and these are shown in the chart following the images. The equations allow us to estimate the maximum (orange), average (grey) and minimum (blue) temperatures at any given delivered energy level. Animal 20 lost posture at approximately 8 seconds post onset of DTS application, which is equivalent to approximately 101 kJ of energy delivered. At 101 kJ energy delivered the estimated average forehead temperature is 32.92 °C (16.71 – 55.94).



Animal 20 thermal map of forehead immediately prior to DTS application. Temperature at cursor: 19.9 °C; maximum temperature in image (white): 33.5 °C; minimum temperature in image (black): 14.5 °C.



Animal 20 thermal map of forehead immediately after DTS application. Temperature at cursor: 45.1 °C; maximum temperature in image (white): 78.8 °C; minimum temperature in image (black): 12.7 °C. Heating extending towards the poll is indicative of energy leakage. Disk shapes with cool centres are EEG recording electrode pads.

Animal 20 estimated temperature change on the forehead by energy delivered.

EEG traces

EEG collected on bleed conveyor post roll-out: once movement artefact was removed, there were too little EEG data available prior to the return of the corneal reflex and captive bolt application to generate a meaningful chart.

CONTACT US

t 1300 363 400
+61 3 9545 2176
e csiroenquiries@csiro.au
w www.csiro.au

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Agriculture & Food
Alison Small
t +61 2 6776 1435
e Alison.small@csiro.au

8.4 Appendix 4 - Assessment of existing data against the EU guidelines for the assessment criteria for studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing

milestone report

Project code: P.PIP.0528

Prepared by: James Ralph, Alison Small, Leisha Hewitt
Wagstaff Food Services

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DTS Diathermic Syncope - controlled trials

Milestone 6. Review validation requirements as per EU guidelines

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Abstract

Dr Leisha Hewitt was subcontracted to carry out an independent review of the work to date and planned trials, mapped against the requiremetns of the European Food Safety Authority (EFSA) guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing. The objective was to identify any gaps in the data provided in terms of meeting regulatory approval of a novel technology for rendering animals insensible prior to slaughter. Regarding the EFSA eligibility criteria, the descriptive information in the study provides sufficient scientific detail on the stunning intervention. However, there is insufficient information to fulfil all the EFSA eligibility criteria regarding the animal welfare outcome. The study does not fulfil the reporting criteria, largely due to the fact that this was a small scale pilot study. As the study did not fulfil the reporting criteria, the methodological criteria was not assessed.

Table of Contents

1 Milestone description

Review validation requirements as per EU guidelines.

2 Project objectives

2.1 Milestone 6 Objective:

Review work to-date in the light of the EFSA guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing (EFSA Journal 2013; 11 (12): 3486), and provide recommendations regarding further research activities, under Objective 01 of the Head Agreement.

3 Methodology

The EFSA guidance defines the assessment process and the criteria that will be applied by the Animal Health and Welfare Panel to studies on known new or modified legal stunning interventions to determine their suitability for further assessment. The criteria that need to be fulfilled are eligibility criteria, reporting quality criteria and methodological quality criteria. The pilot study was conducted in a commercial abattoir, under experimental conditions. The parameters in the study were assessed (Table 3) when applying a stunning intervention based on the application of DTS technology. This technology is not included in Annex I of Council Regulation (EC) No 1099/2009 or the EFSA guidance. The parameters were therefore mapped against the ESFA guidance using previously published scientific findings.

4 Success in meeting the milestone

Milestone Complete.

5 Conclusions/recommendations

The recommendations made under the review are predominantly associated with reporting, particularly with regards to the detailed descriptions of statistical assumptions and parameters. These recommendations will be addressed during preparation of the report to the current project.

Some suggestions as to further work, particularly with reference to recoverability, are made. At present, these activities are outside the scope of the current project, and will require Animal Ethics Committee Approvals. These suggestions will be considered when preparing future project applications.

6 Report prepared by Dr Leisha Hewitt

Sub-contractor activities 01:

Review work to-date in the light of the EFSA guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing (EFSA Journal 2013; 11 (12): 3486), and provide recommendations regarding further research activities, under Objective 01 of the Head Agreement.

6.1 INTRODUCTION

Article 4 (2) of Council Regulation (EC) No 1099/20094 on the protection of animals at the time of killing allows the Commission to amend stunning parameters laid down in Annex I to this Regulation to take into account scientific and technical progress on the basis of an EFSA opinion. Any such amendments shall ensure a level of animal welfare at least equivalent to that ensured by the existing methods. This can be as simple as the revision of a particular stunning parameter, or as broad as the consideration of a completely novel stunning intervention. This report presents a comprehensive review of the use of electro-magnetic energy applied directly to the brain to induce volumetric heating (DTS: Diathermic Syncope® (DTS)), to establish whether scientific studies completed to-date have effectively addressed the criteria outlined in the EFSA guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing (EFSA Journal 2013;11(12):3486) (hereafter referred to as 'EFSA guidance').

The EFSA guidance defines the assessment process and the criteria that will be applied by the Animal Health and Welfare Panel to studies on known new or modified legal stunning interventions to determine their suitability for further assessment. The criteria that need to be fulfilled are eligibility criteria, reporting quality criteria and methodological quality criteria. If the criteria regarding eligibility, reporting quality and methodological quality are fulfilled, i.e. the study provides sufficient detail regarding the intervention and the outcome to allow for a conclusion to be reached about the suitability (or lack thereof) of the intervention, a full assessment of the animal welfare implications of the proposed alternative stunning intervention, including both pre-stunning and stunning phases, and an evaluation of the quality, strength and external validity of the evidence presented would be carried out at the next level of the assessment (Table 1). The EFSA guidance also specifies general aspects applicable to studies on stunning interventions that should be considered. It should be noted that the EFSA guidance only covers stunning interventions that fall into the categories of mechanical, electrical and gas methods (including low atmospheric pressure). The criteria defined in the EFSA guidance apply only to the assessment of stunning procedure itself and do not take into account pre-stunning processes and restraint methods.

Table 1 Summary of the phases and steps involved in the assessment of studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing as outlined in EFSA guidance (2013a)

Phase	Step	Criteria	Content	Detail
1	1	Eligibility criteria	Intervention	The key parameters described in the legislation as well as any others provided by experts on stunning interventions.
			Outcome	Immediate onset of unconsciousness and insensibility or absence of avoidable pain, distress and suffering until the loss of consciousness and sensibility, and duration of the unconsciousness and insensibility (until death).
	2	Reporting quality criteria	REFLECT statement	Designed to increase the transparency and comparability of conducting and reporting scientific studies. The REFLECT and STROBE statements are used as a basis for assessing reporting quality. Only assessed if eligibility criteria are met.
			STROBE statement	
	3	Methodological quality criteria	Assessment of possible biases	Used to describe the level of uncertainty surrounding the evidence presented in the study to inform the next level of assessment. Only assessed if the eligibility and reporting quality criteria are met.
2	1	Full assessment of animal welfare implications	Pre-stunning phase	Quality, strength and external validity of the evidence presented. Only assessed if eligibility criteria and reporting/methodological quality criteria are met.
			Stunning phase	

6.2 Council Regulation (EC) No 1099/2009 definition of stunning

Council Regulation (EC) No 1099/2009 on the protection of animals at the time of killing defines “stunning” in Article 2 (f) as “any intentionally induced process which causes the loss of consciousness and sensibility without pain including any process resulting in instantaneous death”. Article 4 on stunning interventions states that “animals shall only be killed after stunning in accordance with the methods and specific requirements related to the application of those methods set out in Annex I of the Regulation” and “that loss of consciousness and sensibility shall be maintained until the death of the animal”.

Annex I of the Regulation lists the permitted stunning interventions and related specifications. Article 4 (2) of the Regulation allows the Commission to amend Annex I as to take account of scientific and technical progress on the basis of opinion by EFSA. Any such amendment has to ensure a level of animal welfare at least equivalent to that ensured by existing methods.

As the use of DTS is a novel stunning system, no parameters for its use are currently defined by Council Regulation (EC) No 1099/2009.

6.2.1 Eligibility criteria

The eligibility criteria are based upon the legislation and previously published scientific data. They focus on the intervention and the outcome of interest, that being the immediate onset of unconsciousness and insensibility or absence of avoidable pain, distress and suffering until the loss of consciousness and sensibility, and duration of the unconsciousness and insensibility (until death). DTS is not described in the legislation, therefore any suggested intervention and outcome criteria are based on previously published scientific data and expert opinion.

6.2.1.1 Intervention

The EFSA Guidance details parameters for mechanical, electrical and modified atmosphere stunning methods. The parameters and components for mechanical and electrical stunning interventions have been replicated in Appendix 6.1 (column 1-3). The essential parameters for the DTS stunning intervention were identified after a review of the development of the technology to-date (Appendix 6.2) (and associated peer reviewed publications). These were aligned against the criteria established for mechanical and electrical stunning methods (as determined by the EFSA ad-hoc expert working group) and are presented in Appendix 6.1 (column 4-5).

6.2.1.2 Outcome

The EFSA guidance states that for studies researching new or modified stunning interventions, it needs to be demonstrated whether or not the intervention results in immediate unconsciousness and whether or not the stun is reversible. Studies should explain, in detail, how and when the onset of unconsciousness and insensibility is measured. It is required that the methodology used in the determination of the onset of unconsciousness has been accepted in appropriate internationally and stringently peer-reviewed journals. In addition, the chances and potential causes of failure need to be characterized. The neuronal basis of unconsciousness with regard to stunning is detailed in the EFSA report on the welfare aspects of the main systems of stunning and killing in the main commercial species of animals (2004). It states that stunning interventions should disrupt the normal functioning of neurons in the thalamus and cerebral cortex, best demonstrated by the use of EEGs.

The purpose of the EU Slaughter Regulation 1099/2009 is to avoid pain and suffering during stunning and slaughter. Regulation 1099/2009 requires that stunning method should induce immediate loss of consciousness. If the loss of consciousness is not immediate, then the onset of unconsciousness needs to occur without causing avoidable pain and suffering.

As a member of the EFSA AHAW Panel, Raj (2015) outlines two criteria/rules that have to be fulfilled before a stunning intervention is considered not to induce pain, distress and suffering before the onset of unconsciousness and insensibility:

- Animal based indicators from the behaviour response type and animal-based measures from at least one of the two additional response (i.e. physiological or neurological response) relevant to the intervention/species, which must be indicative of the absence of pain, distress and suffering.

- In general, these animal-based indicators should be consistent at the level of the individual animal, depending upon the species and the coping strategies (that is, consistent with respect to their interpretation).

6.2.2 Reporting criteria

During investigations into the use of alternative stunning interventions, several study designs could be applied. The REFLECT statement and the STROBE statement were identified as the most suitable guidelines that could be applied (EFSA, 2013a). STROBE stands for an international, collaborative initiative of epidemiologists, methodologists, statisticians, researchers and journal editors involved in the conduct and dissemination of observational studies, with the common aim of Strengthening the Reporting of Observational studies in Epidemiology. REFLECT stands for Reporting Guidelines for Randomised Controlled Trials for Livestock and Food Safety. The published STROBE guidelines are provided in full in Appendix 6.3. The REFLECT and STROBE statements have been adapted in the EFSA guidance (2013a) as shown in Table 2.

Table 2 Parameters used to assess the reporting quality of studies on stunning interventions, per section of the study report

Parameter	**Description**
Introduction	
Background and rationale	Explain the scientific background and rationale for the investigation being reported.
Objective	Describe the specific objectives and hypotheses. Clearly state primary and secondary objectives (if applicable).
Materials and methods	
Study population	Give characteristics of the study population (species, breed, animal type (e.g. dairy or beef cattle), and weight) and potential confounders (health status, fasting, water deprivation, husbandry system); indicate the number of animals with missing data for each variable of interest.
Number of animals (sample size)	How was the sample size determined and, when applicable, explanation of any interim analyses and stopping rules. Experimental/intervention units must be described and information on whether true replication was done is needed.
Intervention	Precise details of the interventions intended for each group, how and when interventions were actually administered. In addition, specifications of the requirements for the stunning method are provided in section 3.1.1 (EFSA, 2013a).

Outcome	Clearly define all primary outcomes (onset of unconsciousness/insensibility, absence of pain, distress and suffering and duration of unconsciousness/insensibility) and ancillary outcomes (e.g. heart beat, tail flicking). Report category boundaries when continuous variables were categorised. Specifications of the requirements for the assessment of unconsciousness and insensibility as well as absence of pain, distress and suffering are provided in section 3.1.2.1–3.1.2.3 (EFSA, 2013a).
Bias and confounding	Describe any efforts to address potential sources of bias that are relevant to the study design and could affect the internal and external validity of the study. Concerning external validity, report methods to control for sampling bias. Was any comparison made between the reference population and animals under study? Concerning internal validity, report methods to control for selection bias, information bias and confounding. These may include random allocation, matching, blocking stratification for randomised controlled trials, and multivariable analytical methods.
Blinding (masking)	Specify if blinding was performed or not. If done, describe who was blinded (e.g. the data collector, the data analyst) as well as how and when it was done. If the process was different for outcomes, clarify per outcome (e.g. behaviour data was blinded but electroencephalography data were not).
Statistical methods	Describe all statistical methods used to summarise the data and test the hypotheses, including those used to control for confounding; include information about data transformations. Describe any methods used to examine subgroups and interactions; Explain how missing data were addressed. Guidance can be found in Lang (2013).
Results	
Numbers analysed	Basic information about the distribution of important confounders and effect modifiers in each study group (age, weight, sex). If variables are continuous provide means (SD) if normally distributed, if not provide medians and interpercentile ranges, ranges, or both. Report the upper and lower boundaries of interpercentile ranges and the minimum and maximum values of ranges, numbers of study units (denominator) in each group included in each analysis and whether the analysis was by “intention-to-treat”. State the results in absolute numbers when feasible (e.g. 10/20, not 50 %).
Outcomes and estimations	For each outcome, report a summary of results for each group (although it is recommended that data are made available at individual animal level, at least in studies performed in a controlled environment); give unadjusted estimates and their precision (e.g. 95% confidence interval) and, if applicable, confounder-adjusted estimates and number. If the design includes non- independent observations, ensure variance components are reported. Make clear which confounders were adjusted for.

Adverse events	Describe all important adverse events or side effects in each intervention group and report the number of adverse events in each group and indicate if they appear prior to or after unconsciousness is reached. For example, in the case of electrical stunning, high electrical resistance could cause overheating of the stunning electrodes, leading to poor stunning as well as burn marks on the skin.
Ancillary analyses	Report the outcome of any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory.
Discussion	
Key results and interpretation	Summarise key results with reference to study objectives; provide a well- founded interpretation of results considering objectives and limitations, taking into account sources of potential bias or imprecision, multiplicity of analyses, results from similar studies, and other relevant evidence.
External validity	Discuss the potential for external validation of the study results (e.g. applicability of the stunning method in slaughterhouses in different Member States).
Other	
Funding	Give the source of funding and the role of the funders for the submitted study.

6.2.3 Methodological quality

The methodological quality criteria focus on elements in the report that allow the assessment of the internal and external validity of the submitted study. Internal validity is reached when the study results reflect reality among the animals under study, whereas external validity is reached when the study results are reasonably generalised to the broader reference population. The main biases affecting internal validity are confounding, selection bias and information bias (EFSA, 2013a). The most relevant bias affecting external validity is sampling bias. The EFSA guidance states that the methodological quality of the study will be assessed only if the eligibility and reporting quality criteria are met.

6.2.3.1 Selection bias

Selection bias is defined in the Cochrane handbook (Higgins and Green, 2011) as *"Systematic differences between baseline characteristics of the groups that are compared."* For studies assessing the effectiveness of alternative stunning treatments, selection bias would be present if the allocation of treatment groups did not follow the same rules.

6.2.3.2 Attrition bias

Attrition bias is defined in the Cochrane handbook as *"Systematic differences between groups in withdrawals from the study."* For example, if animals with certain characteristics are withdrawn differentially from the treatment or control groups.

6.2.3.3 Performance bias

Performance bias is defined in the Cochrane handbook as "Systematic differences between groups in the care that is provided, or in exposure to factors other than the intervention of interest." For example, if the observers are aware of details of the intervention and that awareness differentially affects their handling of the treatment or control groups.

6.2.3.4 Confounding

Confounding is bias that arises from the occurrence or mixing of effects of extraneous factors (confounders) with the main effects of interest in the study. For example, effectiveness of stunning intervention may be confounded by other variables that are correlated with some aspect of the intervention.

6.3 DOCUMENTATION ASSESSED

The final report from the pilot study (P.PIP.0395 Final Report Dielectric induction of temporary insensibility in cattle – animal trials) was assessed as part of this review (hereafter referred to as 'the study' or 'pilot study').

6.4 ASSESSMENT APPROACH

The assessment of the submitted studies was carried out in a manner analogous to that described in the guidance document (EFSA, 2013a). For the DTS stunning intervention, the eligibility criteria relating to the intervention were identified from a review of previous scientific findings and mapped against the EFSA guidance for mechanical and electrical stunning interventions. This is shown in Appendix 6.1 (columns 4-5).

6.5 ASSESSMENT OF ELIGIBILITY CRITERIA

6.5.1 Intervention under experimental abattoir conditions (pilot study)

The pilot study was conducted in a commercial abattoir, under experimental conditions to demonstrate the proof of concept. An evaluation of the reported parameters in the submitted study is presented in this section. The equivalent DTS parameters (Appendix 6.1) have been used in the assessment of intervention eligibility criteria (Table 3). Further recommendations based on this assessment are presented in Table 8.

Table 3 **Assessment of eligibility criteria:** Parameters assessed when applying DTS stunning intervention were mapped against those used for mechanical and electrical stunning methods (based on Annex I of Council Regulation (EC) No 1099/2009) (Appendix 6.1) and on further details of requirements as determined by the EFSA guidance (corresponding to Table 3 (EFSA, 2013a))

Parameter	**Component**	**Description presented in study**	**Is equivalent information to that required by the EFSA guidance present? (yes, no or not applicable)**
Position and application of the waveguide	Restraining system	Description of restraint method included in the method section. Animals in both treatment groups moved and restrained using the same equipment and handling methods. Description of head restraint (with images) and reference to the specific design (Grandin.com). Head restraint is of the type used commercially for non-stunned slaughtered, where the neck needs to be kept in extension.	YES
	Position and contact of the waveguide	Following each phase of technology development, the equipment is tested on at least 20 cadaver heads and dummy loads prior to commencement of work at the experimental site. Application of the waveguide is controlled by an operative outside the faraday cage. The applicator is fitted with a limit switch, preventing operation if the animal's head is not pressed firmly against the applicator opening.	YES
	Design of waveguide	The equipment is refined after the completion of each experimental stage. Purpose built applicator head with proximity sensors. Patented design. Prototype 4 designed and tested for use in the next stage.	YES
	Wave penetration site	Waveguide terminated directly over the frontal lobes of the brain (similar position to that used for penetrative captive bolt stunning).	YES
Appropriate energy according to	Microwave generator output and auto-tuner	Microwave rotary joint with stepper motor control, animal load cell. 30-100kW, 922MHz microwave generator. Full description of generator components is provided (confidential information –	YES

animal size and species		patented technology). The purpose of the auto-tuner is to maximize the energy delivered to the brain, and minimize reflected/wasted energy, thereby shortening the required application duration.	
	Safety mechanisms	Safety interlocks throughout, microwave leakage monitors in appropriate areas, screened animal stunning room (faraday cage). Warning lights fitted on the cage and generator. Auto cut-off mechanism which operates if the waveguide is not in contact with the animal's head.	YES
	Type (e.g. beef or dairy cattle), size of animal	Animal type described. Female Aberdeen Angus cattle between 300-450 kg live weight and of calm temperament. All animals were polled.	YES
	Minimum energy (KJ)	Description of how energy is applied to the animal and subsequent monitoring of applied energy. 10 animals received DTS treatment (High energy>290 kj ,n=3; low energy <200kj, n=4 and intermediate, n=3).	YES
	Wavelength of the incident microwave energy	Wavelength controlled. Higher frequencies will achieve lower tissue penetration.	YES
	Animal skin condition	An exhaust vent allows for the extraction of dust and steam from the area of microwave application.	YES
	Depth of penetration	Refers to a wave passing into a dielectric material. Penetration depth calculated using published dielectric properties of tissues. Depth of penetration predicted for animal type.	YES
Duration of intervention		Time of exposure recorded – 10s Exposure time varied between animals if safety mechanism activated	YES
Equipment maintenance, cleaning and storage conditions		Details of equipment maintenance (type of maintenance performed, frequency and time intervals between consecutive maintenance procedures), cleaning and storage conditions not provided	NO

Frequency of calibration of the equipment		Details of calibration (method used, time interval between consecutive calibrations of the equipment) not provided	NO
Maximum stun to stick/kill interval(s)		Stun to stick interval was recorded for each individual animal. Slaughter was performed using a halal throat cut, severing both carotid arteries. The time elapsed between administration of treatment and slaughter was prolonged due to post-treatment EEG recording and behavioural measurements.	YES

6.5.2 Outcome of the pilot study under experimental abattoir conditions

6.5.2.1 Onset of unconsciousness and insensibility

The evaluation of a stunning intervention requires well-controlled studies under laboratory conditions as a first step, to characterise the animals' responses (unconsciousness, absence of pain) using the most sensitive and specific methods available (e.g. electroencephalography (EEG), blood samples) and to establish the correlations between these measurements and non-invasive parameters (e.g. behavioural observations) that can be applied in slaughterhouses (EFSA, 2013a). The EFSA guidance requires that all parameters that are crucial to the assessment of EEG data are specified (for example, position of recording electrodes, configuration of electrodes and background noise filtration methods). Accreditation to internationally recognised methods (e.g. International Standards Organisation) of data recording, acquisition and analysis should be clearly stated in the study. The assessment of the information provided in the study in relation to the onset and duration of unconsciousness and insensibility (Section 3.2.1 of EFSA guidance 2013a) is collated Tables 4 and 5. Further recommendations to be considered before the next stage of experimental work are presented in Table 9.

6.5.2.1.1 Start and end of EEG measurement

The information provided is summarised in Table 4. The EEG was recorded pre and post-stunning intervention and immediately prior to exsanguination. It was not recorded during bleeding out.

6.5.2.1.2 EEG measurement

The information provided is summarised in Table 4. The EEG criteria were selected based on peer reviewed scientific studies. References for the techniques used were provided in the study. EEG analysis also involved the identification and rejection of artifacts, with reference to video footage to identify event-related artefact.

6.5.2.1.3 EEG recording and analysis

The information provided is summarised in Table 4.

6.5.2.1.4 EEG results

The information provided is summarised in Table 4. The DTS treatment was applied for a duration of 10 seconds, during which time EEG response was not recorded, due to the applied electromagnetic energy over-riding EEG measurements. This means that the actual time to observed changes (deemed incompatible with consciousness) in the EEG was not measured. The authors relied on post-treatment EEG analysis to determine whether the animals were stunned effectively, however, time to onset of unconsciousness was determined by the observation of animal based measures (ABMs).

Table 4 Information provided by the submitted study in relation to the onset and duration of unconsciousness and insensibility (Section 3.2.1 of EFSA, 2013a)

Parameter	**Information provided in the study**	**Is the information required by the EFSA guidance present? (yes, no or not applicable)**
Start and end of EEG measurement	Pre-treatment EEG activity was recorded for a period of up to 2 minutes until a stable clear signal lasting 30 seconds was recorded (T1). Immediate post-treatment EEG was recorded while the animal remained in head restraint (for a maximum of 1 minute). A terminal EEG measurement was made after the animal was removed from restraint and immediately prior to exsanguination. The total period of EEG recording varied between animals.	YES
EEG measurement	Detailed description of EEG measurement presented in the study, with justification for the techniques used. Position and application of EEG recording electrodes described. References to peer reviewed literature included .	YES
EEG recording analysis	The EEG files were stored and both a qualitative and quantitative assessment was carried out. A state of unconsciousness was said to have been induced when the post-stun EEG showed grand mal epileptiform activity or there was a prolonged period during which the total power of the EEG was less than 10% of the pre-stun power (EFSA, 2004). Quantitative assessment included measures of total power (Ptot); Amplitude; RMS (as an alternative measure of power); Median Power Frequency (F50), which measures the frequency below which 50% of the total power is located and Spectral Edge Frequency (F95), which is the frequency below which 95% of the power is located, can be derived. These give numerical values that can be compared more objectively than qualitative assessment of traces.	YES
EEG results	Qualitative assessment of the post-treatment EEG showed a reduction in Amplitude with intermittent activity. Terminal EEG measurement tended towards the isoelectric state. No significant difference in entire EEG spectrum (baseline, post-treatment and terminal values of Mean Power; RMS; amplitude; Median Power Frequency (F50); or 95% Spectral Edge Frequency (F95) between DTS animals and captive bolt animals.	YES
ABM[1] to detect onset of unconsciousness	Under live observation of the animals, the indicators used to assess unconsciousness were: Corneal reflex response; Response to pain (pinprick on the nose); Assessment of visual function. Other indicators observed included: Loss of posture；Rhythmic breathing； Vocalisation； Spontaneous blinking. The entire process was video recorded and annotated against the ethogram.	YES

[1] ABM: Animal-based measure

6.5.2.1.5 Animal-based measures to detect signs of unconsciousness

Animals were monitored throughout the process by live observations and via real-time video. Live behavioural observations during treatment application were recorded against an ethogram (copy provided in the study). Post-treatment observations were performed by two observers; one assessing the animal for signs of unconsciousness (absence of corneal reflex, absence of controlled eye movement and absence of somatic responses), while the second assessed post-treatment behavioural responses against the ethogram. Rhythmic breathing persisted in all of the animals following treatment. The actual time in seconds to onset of unconsciousness based on ABMs was not recorded during the pilot study. There were no significant differences between DTS and captive bolt in terms of physiological measurements.

6.5.2.1.6 Absence of pain, distress and suffering until the loss of consciousness and sensibility

The EFSA guidance requires that for any intervention that does not lead to an immediate onset of unconsciousness and insensibility, the time to loss of consciousness from the beginning of the application of the stunning intervention, and the signs of pain, distress and suffering until the onset of unconsciousness should be recorded in all animals and reported as individual animal-level data.

Loss of consciousness is not immediate with the DTS stunning intervention and there is insufficient evidence from the pilot study to show that skin temperature does not exceed the critical level (where pain is experienced) before unconsciousness is achieved. Work on cadaver heads concluded that the heating effect on the skin was likely to be insufficient to cause a painful response (<9ºC), yet in the pilot study there were marked changes, in the form of scorching to the skin, indicating temperatures in excess to those seen in cadavers. This will be investigated in the next stage of the research using prototype 4 (the development of which has focused on the reduction of surface heating).

Rhythmic breathing and vocalisation was also recorded in some animals. These animal based measures are regarded as indicators of consciousness when other stunning interventions are used (e.g. mechanical and electrical methods). In this particular study, they were not thought to be signs of consciousness. If they were considered to be signs of consciousness, then the interpretation should be that these animals were not stunned. This requires further investigation in the next stage of the planned study.

6.5.2.1.7 Duration of unconsciousness and sensibility

Council Regulation (EC) No 1099/2009 states that unconsciousness and insensibility induced by stunning should last until the animal is dead. Studies under controlled laboratory conditions should determine the duration of unconsciousness using EE.G. The maximum stun to stick interval should be defined (based on the shortest time to recovery of consciousness minus 2 standard deviations). Proof of concept studies require the duration of unconsciousness to be determined without sticking.

The incidence of recovery of consciousness from the stunning intervention itself was not measured as all animals were slaughtered whilst unconscious.

Table 5 Information provided by the submitted study in relation to animal-based measures (ABMs) associated with pain, distress and suffering during the induction of unconsciousness (Section 3.2.1 of EFSA, 2013a)

Parameter	Group of ABMs	Information provided in the study	Is the information required by the EFSA guidance present? (yes, no or not applicable)	Do the ABMs observed suggest pain, distress and suffering? (yes, no or not possible to assess)
Behaviour	Vocalisation	Recorded and subdivided into vocalisation associated with inhalation (snoring) and exhalation (calling). The vocalisation was present when other ABMs absent (no response from eye and absent pain response) Number of animals that vocalised recorded on the individual animal ethogram Bellowing vocalisations also recorded as an indicator of distress (animals euthanased immediately with penetrative captive bolt pistol if present)	YES	Not possible to assess from pilot study data. Not associated with the presence of other ABMs.
	Postures and movements	Body posture (tense, struggling, jump, kick, pull back),	YES	Not possible to assess from pilot study data. Not associated with the presence of other ABMs.
		Movement of nostrils (flared, dilated, pinched shut)	YES	NO
		Movement of ears (pricked up, pinned back, drooping, floppy)	YES	NO
		Grimace	YES	NO
		Tail flicking	YES	NO
		Animals struggling against restraint also recorded as an indicator of distress (animals euthanased	YES	NO

		immediately with penetrative captive bolt pistol if present)		
	General behaviour	Rhythmic breathing	YES	Not possible to assess from pilot study data. Not associated with the presence of other ABMs
		Response to pain (withdrawal effect)	YES	NO
		Blinking and nystagmus during application	YES	NO
Physiological response	Hormone concentration	Plasma concentrations of Cortisol, ACTH, Beta-endorphin, Catecholamines (Adrenaline and noradrenaline) Blood samples taken prior to restraint (baseline) and from exsanguinate at slaughter	YES	Not possible to determine whether the noted increase was due to the application of restraint or the stunning intervention.
	Blood metabolites		NO	NO
	Autonomic responses		NO	NO
Neurological response	Brain activity	EEG – Qualitative and quantitative assessment of EEG (pre and post application)	YES	Not possible to assess during application of treatment. NO – Observed Post-treatment

6.6 ASSESSMENT OF REPORTING QUALITY

The assessment of reporting quality using the EFSA guidance (Table 2) is presented in Table 7. Further recommendations based on this assessment are detailed in Table 10.

6.7 ASSESSMENT OF METHODOLOGICAL QUALITY

The assessment of methodological usually takes the form of a peer review of a manuscript submitted for publication. The assessment focuses on the level pf uncertainty surrounding the evidence presented (Table 6) and the potential limitations of the conclusions. EFSA (2013a) recommends that the assessment of methodological quality is undertaken after confirmed fulfillment of eligibility and reporting criteria. Consequently, in theory, this is beyond the scope of this report. However, the extent to which the study's design, implementation, data acquisition, analysis and interpretation of results aims to minimise systematic errors (biases) that compromise the study's internal validity and therefore fulfil methodolocial criteria is summarised in Table 10.

Table 6 Evaluation of methodological criteria

Parameter	Description
Selection bias	Assess whether systematic differences between characteristics of animals allocated to treatment and control groups exist.
Attrition bias	Assess whether the characteristics of the animals withdrawn from the study/analysis differ systematically between control and treatment groups.
Performance bias	Assess whether the observers were blinded to the details of the intervention or whether differential handling (that could affect comparisons between treatments and control) might have occurred.
Confounding	Assess whether confounding has been addressed properly.

6.8 CONCLUSIONS AND RECOMMENDATIONS

The EFSA guidance defines the assessment process and the criteria that will be applied by the Animal Health and Welfare Panel to studies on known new or modified legal stunning interventions to determine their suitability for further assessment. The criteria that need to be fulfilled are eligibility criteria, reporting quality criteria and methodological quality criteria. The pilot study was conducted in a commercial abattoir, under experimental conditions. The parameters in the study were assessed (Table 3) when applying a stunning intervention based on the application of DTS technology. This technology is not included in Annex I of Council Regulation (EC) No 1099/2009 or the EFSA guidance. The parameters were therefore mapped against the ESFA guidance using previously published scientific findings.

Regarding the EFSA eligibility criteria, the descriptive information in the study provides sufficient scientific detail on the stunning intervention (Table 3). However, there is insufficient information to fulfil all the EFSA eligibility criteria regarding the animal welfare outcome

(Table 5). The study does not fulfil the reporting criteria, largely due to the fact that this was a small scale pilot study. As the study did not fulfil the reporting criteria, it was not possible to assess the methodological criteria, however, the important aspects for further consideration before the next experimental stage are summarised in Table 10.

In the study, the methods used in the experiments were supported by citations to studies and protocols used in previously published articles. The use of live animals in the experimental procedures was minimized as far as possible; specifically, the 3R principles (replacement, reduction and refinement) were considered in accordance with Directive 2010/63/EU.

Recommendations are presented in Tables 8-11. The recommendations should be considered prior to and during the commercial level validation in the next stage of the experimental work.

Table 7: Parameters used to assess the reporting and methodological quality of the pilot study

Parameter	EFSA description	Description in study	Fulfilment criteria (Yes, No, in-part)
Introduction			
Background and rationale	Explain the scientific background and rationale for the investigation being reported	The study explains that DTS is a potential alternative to other stunning methods that are not accepted by some communities e.g. Muslim and Jewish. The scientific rationale for the study refers to previous studies which show that electromagnetic energy can lead to unconsciousness. Previously, scientific studies have been performed on cadavers and anaesthetized animals.	YES
Objective	Describe the specific objectives and hypotheses. Clearly state primary and secondary objectives (if applicable)	The objective of the study was to complete a pilot investigation into the induction of insensibility in conscious cattle (rather than anaesthetised animals) by the application of microwave energy. Additional objectives included adding to the EEG and behavioural data to determine what behavioural signs might be examined experimentally for future assessment of the method in a practical environment. The next generation prototype was also used in this study.	YES
Materials and methods			
Study population	Give characteristics of the study population (species, breed, animal type (e.g. dairy or beef cattle), and weight) and potential confounders (health status, fasting, water deprivation, husbandry system); indicate the number of animals with missing data for each variable of interest	The characteristics of the study population were provided. The number of animals with incomplete data sets was indicated. Potential confounders were not identified. Potential confounders which were not identified at this stage include:	NO

		• the use of female cattle in the study rather than mixed sex; • a narrow age range; • a narrow weight range between 350-400kg; and • the use of animals assessed as being quiet in temperament. The animals were rested in lairage for 4 days prior to the trial and provided with hay and water *ad lib.* Animals were moved individually to the knocking box.	
Number of animals (sample size)	How was the sample size determined and, when applicable, explanation of any interim analyses and stopping rules. Experimental/intervention units must be described and information on whether true replication was done is needed	Eighteen animals used in the study (Data from 17 animals analysed). It was determined that approximately 10 replicates were sufficient to give sufficient preliminary information to allow a power analysis for design of a statistically sound study. It was not clear in the study report how this was calculated, though this information is available. 10 animals received DTS treatment (High energy>290 kj ,n=3; low energy <200kj, n=4 and intermediate, n=3). The method and criteria for determining the energy groups was not sufficiently detailed. There were no true replicates in the experiments as all of the animals were from the same source population and were not allocated to the controls and treatments in a truly random manner as treatment order was pre-selected, however, animals arrived at the treatment site in random order. Animal 17 excluded from the analysis as it received low dose of energy (35.55kj) which did not render it unconscious. Criteria for exclusions was not described.	NO
Intervention	Precise details of the interventions intended for each group, how and when interventions were actually	See table 3 – It should noted that specifications for the DTS stunning method are not included in the EFSA report,	YES

	administered. In addition, specifications of the requirements for the stunning method are provided in section 3.1.1 (EFSA AHAW Panel, 2013)	therefore they were developed by mapping the parameters against existing stunning methods (mechanical and electrical)	
Outcome	Clearly define all primary outcomes (onset of unconsciousness/insensibility, absence of pain, distress and suffering and duration of unconsciousness/insensibility) and ancillary outcomes (e.g. heart beat, tail flicking). Report category boundaries when continuous variables were categorised. Specifications of the requirements for the assessment of unconsciousness and insensibility as well as absence of pain, distress and suffering are provided in section 3.1.2.1–3.1.2.3 (EFSA, 2013a)	See tables 4 and 5.	IN-PART
Bias and confounding	Describe any efforts to address potential sources of bias that are relevant to the study design and could affect the internal and external validity of the study. Concerning external validity, report methods to control for sampling bias. Was any comparison made between the reference population and animals under study? Concerning internal validity, report methods to control for selection bias, information bias and confounding. These may include random allocation, matching, blocking stratification for randomised controlled trials, and multivariable analytical methods.	Potential sources of bias are discussed in the methodology section, though not in sufficient detail. Although treatments were randomised within the study and animals were brought to the box in a random order, performance bias was introduced through the selection of animals for the treatment group to fit the head restraint. Animals that could not fit the head restraint, where good contact with the waveguide could not be established were shot with a captive bolt pistol (ie allocated to the control).	NO
Blinding (masking)	Specify if blinding was performed or not. If done, describe who was blinded (e.g. the data collector, the data analyst) as well as how and when it was done. If the process was different for outcomes, clarify per outcome (e.g. behaviour data was blinded but electroencephalography data were not)	Blinding was performed and is described in the methodology section. Observers making assessments of animal-based measures were not blinded to the experimental groups (captive bolt and DTS treatment).	IN-PART

Statistical methods	Describe all statistical methods used to summarise the data and test the hypotheses, including those used to control for confounding; include information about data transformations. Describe any methods used to examine subgroups and interactions; Explain how missing data were addressed. Guidance can be found in Lang (2013)	Statistical methods described - Data were analysed using *nlme* package within R studio (R Core Team 2014). Data were checked for normality using the Shapiro-Wilkes test, and transformed where required; differences between treatments were assessed using a mixed model and ANOVA, and considered significant at the $P<0.05$ level. No explanation as to how missing data would be addressed. This is a pilot study with sufficient replicates to identify problems with the technology and allow a power analysis for the design of a statistically sound study in the future.	IN-PART
Results			
Numbers analysed	Basic information about the distribution of important confounders and effect modifiers in each study group (age, weight, sex). If variables are continuous provide means (SD) if normally distributed, if not provide medians and interpercentile ranges, ranges, or both. Report the upper and lower boundaries of interpercentile ranges and the minimum and maximum values of ranges, numbers of study units (denominator) in each group included in each analysis and whether the analysis was by "intention-to-treat". State the results in absolute numbers when feasible (e.g. 10/20, not 50 %).	Data presented in absolute numbers. This is a pilot study with sufficient replicates to identify problems with the technology and allow a power analysis for the design of a statistically sound study in the future. The data generated was not statistically representative.	NO
Outcomes and estimations	For each outcome, report a summary of results for each group (although it is recommended that data are made available at individual animal level, at least in studies performed in a controlled environment); give unadjusted estimates and their precision (e.g. 95% confidence interval) and, if applicable, confounder-adjusted estimates and number. If the design includes non-independent observations, ensure variance components	Summary report for each group is presented, with data made available at individual animal level. Unadjusted estimates and their precision (95% confidence interval) presented.	IN-PART

	are reported. Make clear which confounders were adjusted for		
Adverse events	Describe all important adverse events or side effects in each intervention group and report the number of adverse events in each group and indicate if they appear prior to or after unconsciousness is reached. For example, in the case of electrical stunning, high electrical resistance could cause overheating of the stunning electrodes, leading to poor stunning as well as burn marks on the skin	Full timeline of events for each individual animal provided. The use of live animals in experimental procedures was minimised as far as possible: specifically, the 3R principles (replacement, reduction and refinement) was considered in accordance with Directive 2010/63/EU. When a particular parameter aimed at achieving effective stunning and slaughter failed to fulfil the criteria in consecutive animals, the procedure was terminated and the animal was humanely killed.	YES
Ancillary analyses	Report the outcome of any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory	Outlined in methodology, e.g. Meat quality components.	YES
Discussion			
Key results and interpretation	Summarise key results with reference to study objectives; provide a well- founded interpretation of results considering objectives and limitations, taking into account sources of potential bias or imprecision, multiplicity of analyses, results from similar studies, and other relevant evidence	Key results discussed with reference to study objectives. Sources of potential bias not discussed in detail.	IN-PART
External validity	Discuss the potential for external validation of the study results (e.g. applicability of the stunning method in slaughterhouses in different Member States)	External validation discussed and planned for the next stage of the experimental work (Table 11).	IN-PART
Other			
Funding	Give the source of funding and the role of the funders for the submitted study.	The source of funding was presented – Wagstaff Food Service Pty Ltd; MLA and AMPC.	YES

Table 8: Summary of recommendations for **eligibility criteria** (intervention) where further consideration is required before the next stage of the study

Parameter	Component	Refinements to equipment and protocols prior to the next stage of experimental work	Further recommendations
Position and application of the waveguide	Restraining system	The head capture unit has been re-engineered with hydraulic rams, which are more rigid than the pneumatic rams used during the pilot study. This reduces the chance of compression during application. The head capture unit has been completely re-designed (report 160606 Head Restraint Installation.pdf supplied). The restraining system does not deviate substantially from that associated with conventional stunning and slaughter processes.	• In the pilot study, no detail of the following was included: Use of rump pusher (included in ethogram but not in description), use of electric goad, time in restraint prior to stun application (Included in ethogram but not in commentary). Animal handling techniques and restraint methods used should be fully described in the next stage study. Engineering diagrams and a photograph of the installed head capture unit should be included.
	Position and contact of the waveguide	Restraint, head capture and waveguide set-up re-engineered to ensure low stress for the animal, efficient processing, and limit leakage and automatic cut-out of the generator. The flexible waveguide arrangement being developed as mentioned above will allow the waveguide to be applied at an angle away from vertical, such that the waveguide can be brought to the animal's head, rather than brining the animal's head to the waveguide, as was done in the pilot studies. Contouring and flexibility within the waveguide tip will also allow for maintenance of good contact.	• In the pilot study report there was insufficient detail on the selection of the appropriate site for the application of the waveguide. • Consider refining figure 2 (in the study) to be more representative off the position on the animal's head in the chin lift and the application of the waveguide. The reason for selection of the application site should be described. A description of the position on the frontal bone, including the angle of application should be included.
	Design of waveguide	Re-engineered to be more flexible, and the tip shaped so that is fits more snugly to the head (Final Commissioning Report – Applicator 08-09-2016.pdf).	• Evaluation of performance in next stage of experimental work. • Full description of modifications should be provided in the study report.

	Wave penetration site	Addition of the Teflon window to reduce the risk of arcing, which will in turn reduce the risk of triggering the automatic cut-out.	• Evaluation of performance in next stage of experimental work. • Full description of modifications should be provided in the study report.
Appropriate energy according to animal size and species	Microwave generator output and auto-tuner		• Establish differences between low, medium and high energy applications, including the differences in physiological responses between animals.
	Safety mechanisms	See previous comments on re-design of waveguide to limit leakage and prevent auto cut-out.	• Investigate the impact of the integral safety mechanisms on the stunning outcome, for example, automatic cut-out and containment within faraday cage.
	Type (e.g. Beef or dairy cattle), size of animal	A range of animal types will be used to investigate the influence of sex (entire males, steers, heifers and cows), maturity and live weight (increased range of 200 - 600kg).	• Full description of animal type and the likely impact on the applied treatment and subsequent outcome should be included.
	Minimum energy (KJ)	Energy selection based on previous work by Rault (2014). Development of the applicator in the latest prototype further focuses heating into the brain rather than the peripheral tissues. The settings on the microwave generator do not correlate with the energy to the brain.	• In the pilot study, the settings on the microwave generator does not actually predict the overall energy applied to the brain. The next stage of the study should demonstrate that changes to the design of the applicator have resulted in more accurate focusing of microwave energy into the brain.
	Wavelength of the incident microwave energy		• Further examination of the relationship between wavelength of the incident microwave energy and the depth of penetration should be undertaken. • Further examination of temperature profile in live animals (e.g. skin temperature), particularly in light of the refinements to the technology since the work on cadavers was completed.
	Animal skin condition	Work on cadaver heads concluded that the heating effect on the skin was likely to be insufficient to cause a painful response (<9ºC), yet in the pilot study there were marked changes, in the form of scorching to the skin, indicating temperatures in excess to those seen in cadavers. This will be investigated in the next stage of the research using	• Reported investigation into the use of prototype 4 to reduce the occurrence of surface heating.

		prototype 4 (the development of which has focused on the reduction of surface heating).	
	Depth of penetration	Investigation into the effect of animal type on depth of penetration.	• Reference to a scientific study to support the assumption that the microwave energy travels perpendicular to the plane of each biological material.
Duration of intervention			• Investigation into the use of shorter application times (with refinement of equipment to give increased power application and depth of penetration).
Equipment maintenance, cleaning and storage conditions		Details of equipment maintenance (type of maintenance performed, frequency and time intervals between consecutive maintenance procedures), cleaning and storage conditions not provided.	• Details of required equipment maintenance, cleaning and storage conditions should be developed.
Frequency of calibration of the equipment		Details of calibration (method used, time interval between consecutive calibrations of the equipment) not provided.	• Details of required calibration of the equipment should be developed.
Maximum stun to stick/kill interval(s)		The knocking box has been re-designed, with a neck yoke that lifts away, and a slight side-step arrangement. The side-step is commonly used in commercial knocking boxes to help roll the body out, by tipping it off-balance. The box used in the pilot trials did not have the side-step, so the body was balanced, and difficult to tip out, which may have extended stun to stick intervals. The research group are also investigating the use of side clamp and tipping technologies to improve animal removal and reduce the overall stun to stick interval.	• The next stage of the study should evaluate the maximum stun to stick interval which will still allow animals to bleed out fully (when Halal slaughter method used) prior to the recovery of consciousness/sensibility.

Table 9: Summary of recommendations for **eligibility criteria** (outcome) where further consideration is required before the next stage of the study

Parameter	Recommendations
Start and end of EEG measurement	• Continued EEG measurement through the bleeding period until the point of death to fully understand the maximum acceptable stun-stick interval to allow animals to bleed out fully prior to the return of consciousness/sensibility.
EEG measurement	• There is an expectation within the EFSA guidance that EEG measurement methodology is supported by cited publications. • In the study, EEG measurement techniques are supported by cited publications. Further citation of relevant references and validation data would be beneficial. • Accreditation to internationally recognised methods (e.g. International Standards Organisation) of data recording, acquisition and analysis should be clearly stated in the study.
EEG recording analysis	• EEG data was analysed off-line and artefacts were identified and removed manually, with reference to video footage to identify event-related artefacts. Reference material for manual removal or event-related artefacts to demonstrate that this is common practice and is not introducing bias into the results. Include description of how it was established that the activity was in-fact artifact related rather than meaningful EEG activity.
EEG results	• There is an expectation within the EFSA guidance that EEG measurements are correlated with ABM observations. • Confirmation of the correlation between EEG evidence and the observed behavioural patterns ad physical reflexes (ABMs).
ABM to detect onset of unconsciousness	• The EFSA guidance requires that the indicators used to assess recognition of unconsciousness are relevant to the particular stunning intervention, based on scientific knowledge of each indicator's sensitivity and specificity. There is little information in this area for the use of the DTS stunning intervention. The sensitivity and specificity of the ABMs used to assess recognition of unconsciousness should be evaluated and investigated further in the next stage of experimental work. Particularly, the use of the 'fixed staring eye' as an indicator of unconsciousness. • Estimation of time to onset of unconsciousness using the proxy 'fixed, staring eye' as an indicator. • The use of a sham operation allows the comparison of the two observations to differentiate between pain and suffering due to the stunning intervention and that caused by the handling procedure. Animal-based measures for pain, distress and suffering should be examined for animals exposed to a sham operation (ie. same handling, restraint and application of the apparatus without actual stunning). • Further investigation into the occurrence and cause of vocalisation, rhythmic breathing and body movements during application.

Table 10: Summary of recommendations for **reporting and methodological criteria** where further consideration is required before the next stage of the study

Parameter	**Recommendations**
Background and rationale	• No further recommendations
Objective	• No further recommendations
Study population	• Single animal type was used in the pilot study, which was not representative of the range of cattle slaughtered in commercial abattoirs (female cattle, 350-400kg, calm temperament, polled). It is proposed that the next stage of the study examines the factors that may affect the critical limits of the equipment and energy application (e.g. Sex, maturity, age, temperament, anatomical differences). Confounders to be fully described and considered within the report.
Number of animals (sample size)	• The pilot study was a small-scale pilot study to prove that DTS could induce unconsciousness, whilst maintaining acceptability from an ethical perspective. • Outcomes from the small scale pilot study require validation with a larger number of replicates. This is planned for the next stage of experimental work. • 300 animals will be used in the next validation stage – calculated as the minimum to predict with confidence a 1% failure rate.
Intervention	• No further recommendations.
Outcome	• Estimated time to onset of unconsciousness to be investigated further using ABMs. • See Table 9.
Bias and confounding	• A range of animal types will be used to investigate the influence of sex (entire males, steers, heifers and cows), maturity and live weight (increased range of 200 - 600kg). • Likely sources of bias and confounding to be described in more detail.

	• As animals were subjected to human contact, physical handling and restraint, which are confounding factors, physiological measurements failed to yield useful results. • To fulfill methodological criteria, all sources of bias require consideration.
Blinding (masking)	• Detailed description of any blinding to be included.
Statistical methods	• Reference to Lang and Altman (2013) regarding the identification of appropriate statistical methodology - Recommended in EFSA (2013a) – STROBE criteria.
Numbers analysed	• The pilot study utilised a small number of replicates and the data was not statistically representative. • Increase the number of replicates to 30 in each group to generate statistically valid data. • Provide basic information about the distribution of important confounders and effect modifiers in each group.
Outcomes and estimations	• No further recommendations.
Adverse events	• Adverse events were not described in sufficient detail. Describe all important adverse events or side effects in each intervention group and report the number of adverse events in each group and indicate if they appear prior to or after unconsciousness is reached. For example, in the case of high power DTS, application could lead to scorching of the skin.
Ancillary analyses	• No further recommendations.
Key results and interpretation	• No further recommendations.
External validity	• See Table 11.
Funding	• No further recommendations.

Table 11: Summary of commercial slaughterhouse parameters to be considered during the next stage of the study to allow for the **full assessment of animal welfare implications** (Quality, strength and external validity) of the evidence presented as required by EFSA

Process	Commercial slaughterhouse parameter to be considered
Pre-stunning phase	Investigation into the use of the stunning intervention at commercial processing speeds.
	Investigation into the effect of handling animals in groups and the impact of the process on animals waiting in the lead-up race immediately prior to the box.
Stunning phase	Further identification of potential causes of failure (for example, animal position, equipment failure, restraint method), particularly with range of animal types.
	Development of guidelines for assessing efficacy and animal welfare status. Checks for outcomes of unconsciousness at each stage of the the process need to be outlined in the Toolbox approach (EFSA, 2013b) (Example in Appendix 6.4).
	Optimisation of restraint method to allow for prompt ejection and immediate slaughter.
	Investigation into the identification of ineffective stunning and application of a back-up stunning method in a commercial setting.
	Understand the physiological responses and variability between animals. Report on outcomes for different animal classes.
	Investigation into the reversibility of the technique to ensure that it satisfies Kosher and Halal markets.
	Development and monitoring of critical limits for microwave power and application time for range of animal types.
	Investigation into whether the stunning intervention provides a sufficient period of unconsciousness to allow for death through bleeding (with halal stick only).
	Development of procedures for the maintenance and calibration of equipment.

6.9 REFERENCES

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Higgins JPT and Green S (editors), (2011) Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. The Cochrane Collaboration. Available from www.cochrane-handbook.org.

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Raj M. (2015) Pain and suffering during induction of unconsciousness. Meeting on Amending Annex I of Regulation 1099/2009 on the protection of animals at the time of killing. Brussels 9th June 2015: European Food Safety Authority.

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6.10 GLOSSARY

Parameter	Recommendations
Acceptable alternative	An alternative stunning method that is at least as good as those listed in Council Regulation (EC) No 1099/2009. Specifically, the alternative procedure must induce immediate onset of unconsciousness/insensibility or absence of pain, distress and suffering until the onset of unconsciousness/insensibility and the animal must remain unconscious/insensible until death
Adverse event	Any observation in animals that is unfavourable and unintended and occurs after the intervention
AMPC	Australian Meat Processor Corporation
Immediate unconsciousness	Induce immediate (e.g. in less than one second) and unequivocal loss of consciousness and sensibility. This is a state of unawareness (loss of consciousness) in which there is temporary or permanent impairment of brain function and the individual is unable to respond to normal stimuli, including pain
Insensibility	An animal can be presumed to be insensible when it does not show any reflexes or reactions to stimulus such as sound, odour, light or physical contact
Maximum stun-to- stick/-kill interval(s)	This is the legal parameter describing the time interval between the end of the stunning and the moment of killing by any method (e.g. sticking, neck cutting)
MLA	Meat and Livestock Australia
True replicate	This means that more than one (statistically independent) experimental or observational unit was subjected to the same treatment. Each unit with the same treatment is called a replicate. True replication permits the estimation of variability within a treatment. Without estimating variability within treatments, it is impossible to do statistical inference, hence most models for statistical inference require true replication

Appendix 6.1: Determination of intervention parameters for DTS – Establishing equivalency with the parameters to be provided when applying a mechanical or electrical stunning intervention, based on Annex I of Council Regulation (EC) No 1099/2009 (and on further details of requirements as determined by the EFSA ad-hoc expert working group).

Intervention	Parameters	Component	Equivalent DTS parameters	Equivalent DTS Component
Mechanical stunning – Penetrative captive bolt	Position and direction of the shot	Restraining system	Position and application of the waveguide	Restraining system
		Position of the captive bolt gun		Position and contact of the waveguide
		Bolt penetration site		Wave penetration site
	Appropriate velocity, bolt length and diameter of the bolt according to animal size and species	Captive bolt gun characteristics	Appropriate energy according to animal size and species	Design of waveguide
		Cartridge or compressed air specifications		Microwave generator output
		Type (e.g. beef or dairy cattle), size of animal		Type (e.g. beef or dairy cattle), size of animal
		Equipment maintenance, cleaning and storage conditions		Equipment maintenance, cleaning and storage conditions
	Maximum stun to stick/kill interval(s)[2]		Maximum stun to stick/kill interval(s)	
Mechanical stunning – Non-penetrative captive bolt	Position and direction of the shot	Restraining system	Position and application of the waveguide	Restraining system
		Position of the captive bolt gun		Position and contact of the waveguide
		Bolt impact site		Wave penetration site

[2] Provide information on mean or median and range and standard deviation or interquartile range of the detailed parameter

	Appropriate velocity and shape of the bolt according to animal size and species	Captive bolt gun characteristics	Appropriate energy according to animal size and species	Design of waveguide
		Cartridge or compressed air specifications		Microwave generator output
		Bolt dimensions, mass and velocity		Description of waveguide
		Type (e.g. beef or dairy cattle), size of animal		Type (e.g. beef or dairy cattle), size of animal
		Equipment maintenance, cleaning and storage conditions		Equipment maintenance, cleaning and storage conditions
	Maximum stun to stick/kill interval(s)		Maximum stun to stick/kill interval(s)	
Head-only and head-to-body electrical stunning	Minimum current (A or mA)	Current type	Minimum energy (KJ)	
		Waveform		
		Minimum current		
		Latency		
	Minimum voltage (V)	Exposed minimum voltage (V)		
		Delivered minimum voltage (V)		
	Maximum frequency (Hz)	Maximum frequency (Hz)		
		Minimum frequency (Hz)		
	Minimum time exposure		Duration of intervention	
	Minimum stun to stick interval		Minimum stun to stick interval	

	Frequency of calibration		Frequency of calibration	
	Optimisation of the current flow	Electrode characteristics	Optimisation of the energy applied	Waveguide characteristics
		Electrode appearance		
		Animal restraining		Animal restraining
	Prevention of electrical shocks before stunning			
	Position and contact surface area of electrodes	Position of the electrodes	Position and application of the waveguide	Position of the waveguide
		Type of electrode		Design of waveguide
		Animal skin condition		Animal skin condition

Appendix 6.2: History of DTS equipment development

Activity	**Status**
Cadaver experiments on sheep brains in order to estimate the likely power requirements that would raise the temperature by 8-10°C	**Completed 1986** Conceptually feasible, but generators of predicted output not available Rankin et al 1986
Literature review on potential for use of Microwave Technology for stunning beef cattle	**Completed 2005** Conceptually feasible, and generators of predicted output are now available Owen and Shaw 2005
Microwave stunning technology development stage 1	**Completed 2009** Generators of suitable output are now utilised in industrial applications. Work was carried out on cadaver heads to demonstrate that the required rise in temperature in the brain could be achieved. McLean 2009
Microwave induced insensibility for animals stage 2	**Completed 2010** Anaesthetised sheep were used to demonstrate that the expected temperature rise as predicted in stage 1 would occur when there was an active cerebral circulation. Small 2010
Stage 3: Non-recovery anaesthetised animal trials	**Completed 2013** Anaesthetised cattle were used to demonstrate induction of insensibility as measured by EEG Rault et al 2013
Stage 4: Proof of concept demonstration that insensibility can be induced	**Completed 2015** Ten non-anaesthetized cattle received DTS treatments and were rendered insensible. No detrimental effects on meat quality or endocrine responses were demonstrated AEC 29.13
Stage 5: Commercial level validation	The subject of the current application The aim will be to confirm the outcomes of stage 4 in a larger field trial
Stage 6: Optimisation of the power/time parameters to allow the stun to be reversible	To be planned The concept of reversibility is vital to the Kosher and Halal markets This is likely to involve another phase of anaesthetised animal trials Preliminary data from the proposed current study will inform this phase

Appendix 6.3: STROBE Statement

STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

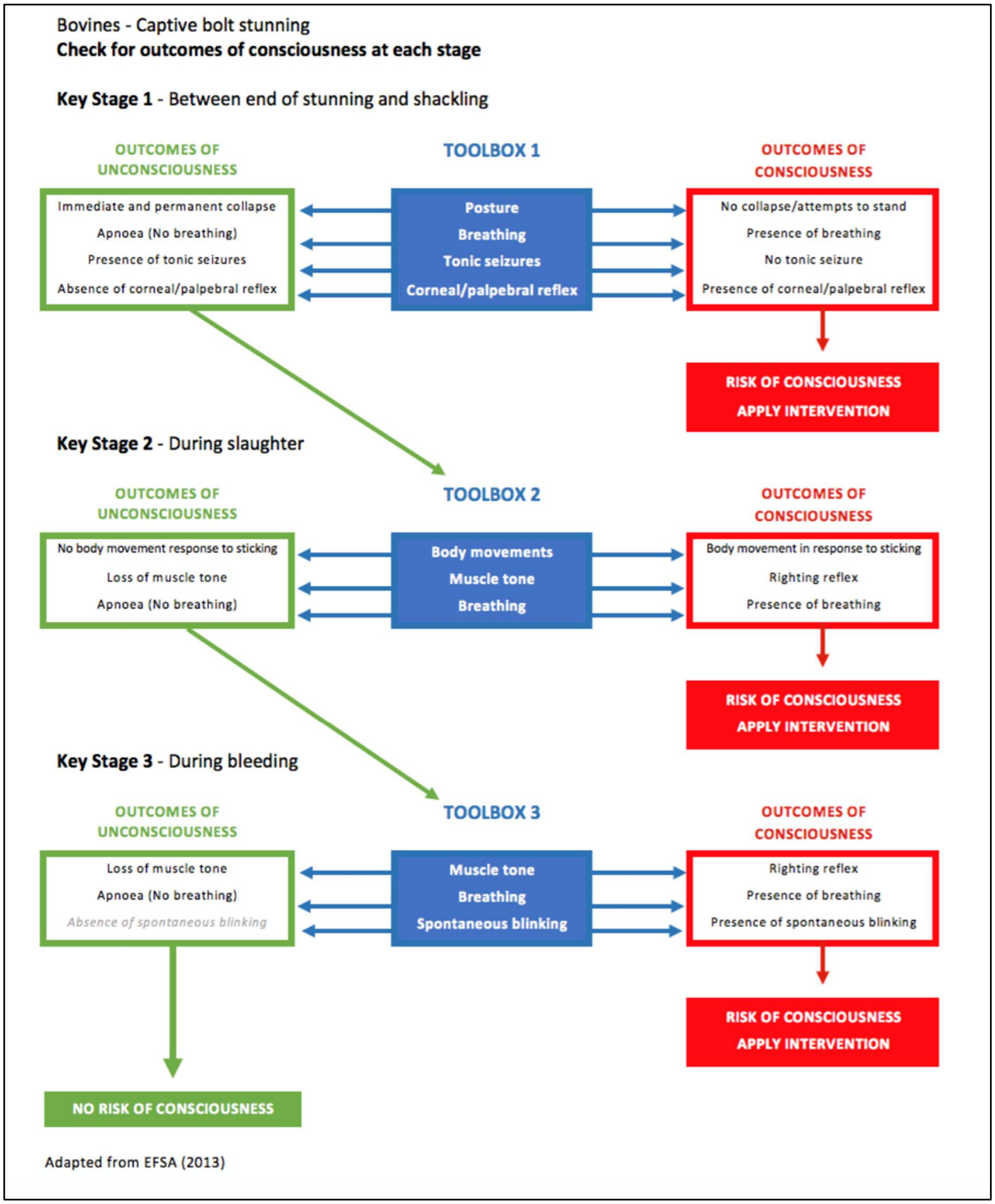
	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram

Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Appendix 6.4: Example of the toolbox approach for captive bolt stunning (adapted from EFSA, 2013b)



Adapted from EFSA (2013b)