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**The development and practical implementation of Single Pulse Ultra-High Current for humane and Halal compliant slaughter of cattle**

# The development and practical implementation of Single Pulse Ultra-High Current (SPUC) stunning for a humane and Halal compliant system of stunning cattle.

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Doctor of Philosophy (PhD) in the Faculty of Health Sciences of the School of Veterinary Sciences.

by

**Awal Fuseini**

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# Abstract

This thesis reports the development of a new system of head-only electrical stunning system, Single Pulse Ultra-High Current (SPUC) for the humane slaughter of cattle. Optimum electrical parameters for the development and construction of the SPUC Stunner were identified through a number of *in vitro* experiments. First, the electric fields in the head and brain were measured by applying voltage from a 250 V, 50 Hz power source with an isolated (from earth) output through two large needle electrodes, via the nose and neck. The electric field in the head was found to be 3.3 $Vcm^{-1}$ and 1.3 $Vcm^{-1}$ in the brain. Secondly, neural membranes were electroporated using a Gene Pulser Xcell electroporation system (Bio-Rad, USA) to determine the effect of energy on the electroporation of brain cells. The results showed that energy was the main factor influencing electroporation of neural membranes.

To date a prototype SPUC stunner has been produced. Using dummy loads, the stunner has been demonstrated to produce the target range of voltage and current. It is undergoing further design and development to better avoid arcing and to provide additional protection for the high voltage switch.

The work for this PhD also included an investigation into the knowledge, views and acceptability of stunning at the time of Halal slaughter across a range of stakeholders as an important precursor for the design and introduction of the SPUC device into full commercial use. A survey of 314 Halal consumers and 66 Islamic scholars in the UK showed that 95% of Islamic scholars and 53% of Halal consumers would accept meat from stunned animals if the method of stunning did not cause the death of animals prior to exsanguination. In a separate study to evaluate veterinary students' perception about Halal slaughter, the majority (95.2%) of the 459 respondents indicated that they would want all animals to be stunned prior to slaughter, including Halal slaughter.

***dedicated to***

*Hamna, Saha, Suglo, Simli and Hajia Fatima*

# Acknowledgements

I will like to extend my heartfelt gratitude to my supervisor, Professor Toby Knowles for his support and guidance from the conception of this project through to its completion. Special mention also goes to Mr. Steve Wotton who was one of my supervisors until his retirement in 2017, I wish Steve a happy retirement. My thanks also go to Dr. Jefferey Lines of Silsoe Livestock Systems, Jeff was instrumental during the development of the electronics for the SPUC stunner and played a significant role in the design of the *in vitro* experiments involving cattle heads and brain cells. I also thank Dr. Jo Murrell and Mr. Andy Grist, who both briefly supervised my work after Steve's retirement. I will also like to thank Mr. Steve Brown and Mr. Stefaan Lescouhier for their assistance during the field experiments. Special thanks also go to Dr. Tristan Cogan for granting me permission to use his laboratory and for his guidance in conducting the *in vitro* experiment on ion migration from neural membranes (described in chapter 4). I am also grateful to the Imaging and Cytometry Laboratory of the University of York for granting me permission to conduct one of the electroporation experiments in their laboratory (described chapter 5).

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# Author's declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is my own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are my own views and not the views of the University of Bristol.

**Awal Fuseini**

[redacted]

DATE: June 2019

# Authored papers

As part of this PhD project, I have published eleven peer-reviewed papers in various scientific journals. However only three of the papers have been fully included as chapters in this thesis to fit into a narrative. In the three papers included as chapters, I can confirm that I was the lead author and wrote the manuscripts as well as analysed the data (where applicable). The following are the published papers included as chapters in the thesis. Appendix A contains copies of all published papers that are not included as full chapters in the thesis.

1. Chapter 1 (from section 1.8): The brain, unconsciousness and death: a critical appraisal with regard to Halal meat production.

Note: I was the sole author of this paper which was published in 2019 in Animal Welfare Journal (*Animal Welfare, 28, 165-171*). I conceived the idea, gathered the relevant scientific information, wrote the paper and submitted it to the journal for review.

2. Chapter 2: The perception and acceptability of pre-slaughter and post-slaughter stunning for Halal production: The views of UK Islamic scholars and Halal consumers.

Note: I was the lead author of this paper which was published in 2017 in Meat Science Journal (*Meat Science, 123, 143-150*). I conceived the idea, collected the data, analysed the data, wrote the paper and submitted it to the journal for review. There were three other authors of the paper, two of whom were my supervisors who provided guidance on data analysis and reviewed the draft publication.

3. Chapter 3: Veterinary students' perception and understanding of issues surrounding the slaughter of animals according to the rules of Halal: a survey of students from four English universities.

Note: I was the lead author of this paper which was published in the Journal of Animals (*Animals, 9, 293*). I conceived the idea, collected the data, analysed the data, wrote the paper and submitted it to the journal for review. There were two co-authors of this paper, both of whom were my supervisors. They both provided guidance on data collection, analysis and reviewed the manuscript before it was submitted to the journal for review.

The remainder of the papers are listed below. I am the primary author on all of these papers, the main body of text within all these papers was primarily written by myself:

1. **Fuseini, A**., Knowles, T.G., Lines, J.A., Hadley, P.J., and Wotton, S.B. (2016). The stunning and slaughter of cattle within the EU: a review of the current situation with regard to the halal market. *Animal Welfare, 25,* 365-376.

Please note that this paper is a summary of the introductory chapter of this thesis (chapter 1). At the start of the PhD project, a 25,000 worded literature review was conducted, this was subsequently summarised and published in Animal Welfare Journal.

2. **Fuseini, A**., Knowles, T.G., Hadley, P.J., Wotton, S.B. (2016). Halal stunning and slaughter: Criteria for the assessment of dead animals. *Meat Science, 119,* 132-137.
3. **Fuseini, A**., Wotton, S.B., Hadley, P.J., and Knowles, T.G. (2017). The compatibility of modern slaughter techniques with halal slaughter: a review of the aspects of 'modern' slaughter methods that divide scholarly opinion within the Muslim community. *Animal Welfare, 26,* 301-310.
4. **Fuseini, A**., Knowles, T.G., Hadley, P.J., Wotton, S.B. (2017). Food and companion animal welfare: the Islamic perspective. *CAB Review, 12,* 1-6.

5. **Fuseini, A.**, Wotton, S.B., Knowles, T.G., and Hadley, P.J. (2017). Halal Meat Fraud and Safety Issues in the UK: a review in the context of the European Union. *Food Ethics, 1,* 127-142.
6. **Fuseini, A**. (2017). Halal food certification in the UK and its impact on food businesses: a review in the context of the European Union. *CAB Reviews, 12,* 1-7.
7. **Fuseini, A**., Sulemana, I. (2018). An Exploratory Study of the Influence of Attitudes toward Animal Welfare on Meat Consumption in Ghana. *Food Ethics, 2,* 57-75.
8. **Fuseini, A**., Teye, M., Wotton, S.B., Lines, J.A., and Knowles, T.G. (2018). Electrical water bath stunning for Halal poultry meat production: animal welfare issues and compatibility with the Halal rules. *CAB Reviews, 13,* 1-7.

# Presentation at conferences

1. Winterbotham Animal Health and Welfare Day (2016). School of Veterinary Sciences, University of Bristol, Langford. March 7, 2016.
2. School of Veterinary Science Post Graduate Research Day (2016). School of Veterinary Sciences, University of Bristol, Langford. June 8, 2016.
3. Halal Food Authority's Halal Industry Conference (2016). Kensington Close Hotel, London. May 26, 2016.
4. Agriculture and Horticulture Development Board (AHDB) Halal Conference (2017). Crowne Plaza, Stratford-upon-Avon. February 22, 2017.
5. QMS/AHDB Livestock PhD Seminar (2017). Stratford Manor Hotel, Stratford. December 7, 2017.
6. School of Veterinary Science Post Graduate Research Day (2018). School of Veterinary Sciences, University of Bristol, Langford. April 30, 2018.
7. Faculty of Health Sciences Research day. Richmond Building, University of Bristol, Bristol. September 19, 2018.
8. Winterbotham Animal Health and Welfare Day (2018). School of Veterinary Sciences, University of Bristol, Langford. March 8, 2018.
9. Winterbotham Animal Health and Welfare Day (2019). The Bristol Hotel, Bristol. March 7, 2019.

# Awards

- *Best PhD presentation winner at the 2017 AHDB/QMS Livestock PhD Seminar*-I was adjudged the best presenter at a seminar where 44 PhD researchers from across several UK universities presented their work.
- *Bristol Plus Award*-I was one of four postgraduate students in the university to receive the Bristol Plus Award in 2015. Recipients were expected to develop and demonstrate a range of skills and experience with a view to improving their employability on completion of their studies.

# Table of Contents


Abstract………………………………………………………………………… ii
Dedication……………………………………………………………………… iii
Acknowledgements…………………………………………………………… iv
Author's declaration………………………………………………………… v
Authored papers……………………………………………………………… vi
Presentation at conferences………………………………………………… ix
Awards…………………………………………………………………………… x
Table of contents……………………………………………………………… xi
List of tables…………………………………………………………………… xv
List of figures………………………………………………………………… xvii
Abbreviations…………………………………………………………………… xx

**Chapter 1. General Introduction ……………………………………... 1**

1.1. Stunning of food animal ………………………….. 1
1.2. Restraint………………………………………………………………8
1.2.1. Restraint for slaughter without stunning……………………… 10
1.2.2. Upright restraint of cattle……………………………………… 12
1.2.3. Use of rotating pens to invert cattle on their backs……………… 14
1.2.4. Restraining cattle in lateral recumbency position………………….15
1.2.5. Restraint for post neck-cut stunning……………………………… 16
1.2.6. Restraint for electrical stunning……………………………………17
1.3. Stunning of cattle…………………………………………………… 18
1.3.1. Electrical stunning…………………………………………………… 22
1.5.1.1. Electrical stunning parameters………………………………………… 24
1.5.1.1.1. Frequency and waveform…………………………………………….. 26
1.5.1.2. Head-only electrical stunning………………………………………...26
1.5.1.3. Head to body electrical stunning……………………………………..29
1.5.1.4. Limitations of electrical stunning……………………………………….30
1.5.1.5. Type of electrical stunners……………………………………… 32
1.5.1.5.1. The Jarvis Beef Stunner ……………………………………………32
1.5.1.5.2. The BANSS Stunning Box MGB………………………………….36
1.5.1.5.3. The use of microwave energy………………………………… 37
1.5.1.5.4. Single Pulse Ultra-High Current (SPUC)……………………… 39
1.5.2. Mechanical Stunning……………………………………………………… 41
1.5.2.1.Penetrative and non-penetrative captive bolt guns…………………… 42
1.5.2.2.Use of free bullet firearms ……………………………………… 44
1.5.2.3.Use of shotgun…………………………………………………………… 45
1.6. Slaughter of food animals…………………………………………………46
1.6.1. Ritual (religious) slaughter………………………………………………48
1.6.1.1. Halal slaughter……………………………………………………………..49
1.6.1.2. Shechita slaughter…………………………………………………………51
1.6.1.3. Animal welfare aspects of ritual slaughter without stunning ……… 53
1.7. Previous studies on stunning and compatibility with ritual (Halal) slaughter…………………………………………………………………...55
1.7.1. High voltage electrical stunning………………………………………56
1.7.2. Electroencephalogram (EEG) and electrocardiogram (ECG)………… 57
1.7.3. Effect of stunning on carcass and meat quality………………………… 58

1.7.4. Effect of slaughter method on carcass and meat quality…………………61
1.7.5. Compatibility of pre-slaughter stunning with ritual (Halal) slaughter… 64
1.7.6. Compatibility of post neck-cut stunning with ritual (Halal) slaughter… 68
1.8. The role of the brain in the control of conscious perception and death……… 71
1.8.1. The brain………………………………………………………………… 71
1.8.2. Unconsciousness………………………………………………………. 72
1.8.3. Death…………………………………………………………………… 75
1.8.4. Halal-compliant methods of stunning based on the definition of death……… 78
1.8.5. Animal welfare implications…………………………………………………. 79
1.8.6. Conclusion ……………………………………………………………….80

**Chapter 2. The perception and acceptability of pre-slaughter and post-slaughter stunning for Halal production: The views of UK Islamic scholars and Halal consumers………………………………………………………………… 82**

2.1. Introduction……………………………………………………………… 82
2.2. Materials and methods……………………………………………………. …… 85
2.2.1. Data collection……………………………………………………85
2.2.2. Questionnaire development………………………………………85
2.2.3. Interviews with Islamic scholars ………………………………86
2.2.4. Survey of Halal consumers………………………………………86
2.2.5. Questionnaire 1- Islamic scholars…………………………….....87
2.2.6. Questionnaire 2- Halal consumers……………………………… 88
2.3. Results……………………………………………………………………92
2.3.1. Requirements of Halal slaughter-Islamic scholars………………….92
2.3.2. Stunning and slaughter-Islamic scholars……………………………97
2.3.3. Halal consumer demographics ……………………………… 98
2.3.4. Requirements of Halal slaughter- consumers………………………102
2.3.5. Stunning and slaughter-Halal consumers………………………… 103
2.4. Discussion………………………………………………… 104
2.5. Conclusion………………………………………………………………108

**Chapter 3. Veterinary students' perception and understanding of issues surrounding the slaughter of animals according to the rules of Halal: a survey of students from four English universities……………………………………… 109**

3.1. Introduction…………………………………………………………………….. 110
3.2. Materials and methods…………………………………………………………..113
3.2.1. Data collection and sampling methods…………………………………… 113
3.2.2. Data analysis…………………………………………………… 113
3.3. Results……………………………………………………………………… 113
3.4. Discussion………………………………………………………………………….119
3.5. Conclusions……………………………………………………………………… 124

**Chapter 4. Measurement of voltage drop across cattle heads and the migration of sodium and potassium ions through neural membranes……………………… 125**

4.1. Introduction…………………………………………………………………… 125
4.2. Materials and methods………………………………………………………… 127
4.2.1. Voltage measurement across cattle heads………………………………………. 127
4.2.1.1. Animals…………………………………………………………………………..127
4.2.1.2. Method of slaughter……………………………………………………………….. 127
4.2.1.3. Voltage application to cattle heads…………………………………………… 127
4.2.2. Measurement of ion migration from brain cell membranes………………………...129
4.2.2.1. Animals……………………………………………………………………………..129

4.2.2.2. Brain sample preparation…… 129
4.2.2.3. Ion migration from neural membranes…… 130
4.2.2.3.1. Use of time constant-pulse protocol……130
4.2.2.3.2. Use of exponential decay-pulse protocol……130
4.3. Results…… 131
4.3.1. Voltage drop in cattle heads…… 131
4.3.2. Ion migration from neural membranes…… 131
4.3.2.1. Use of exponential decay-pulse protocol…… 134
4.4. Discussion…… 135
4.5. Conclusion……136

**Chapter 5. An investigation of the amount of energy, optimum voltage and number of pulses required to electroporate human T-lymphoblastoid cells (Jurkat cells) as a model for the development of Single Pulse Ultra-High Current (SPUC) for the humane slaughter of cattle…… 138**
5.1. Introduction …… 138
5.2. Materials and Methods……140
5.2.1. Jurkat cells…… 140
5.2.2. Bovine brain cells……140
5.2.3. Dyes…… 141
5.2.4. Live/ dead cell markers …… 141
5.2.5. Electroporator…… 141
5.2.6. Electroporation protocol-Jurkat cells……141
5.2.7. Electroporation protocol- Brain cells……142
5.2.8. Measurement of fluorescence-Jurkat cells……143
5.2.9. Measurement of fluorescence-Brain cells……143
5.2.10. Statistical analysis……143
5.3. Results…… 144
5.3.1. Effect of energy……146
5.3.2. Effect of voltage……147
5.3.3. Effect of number of pulses……149
5.4. Discussion and conclusion…… 150

**Chapter 6. Design, construction, assembly and trials of the prototype SPUC stunner…… 154**
6.1. Introduction…… 154
6.2. Pre-design meeting……157
6.3. Rough and 3D sketch of prototype……157
6.4. Construction of restraint and electrodes…… 160
6.5. Test of restraint…… 160
6.6. Design and construction of electrodes……160
6.6.1. Design…… 161
6.7. Incorporation of electrodes into restraint……165
6.8. Trial with dummy load……166
6.9. Installation of CCTV…… 166
6.10. Set-up and initial trial with animals…… 166
6.10.1. Protocol for initial test of SPUC stunner…… 166
6.10.2. Re-design of restraint……169
6.11. Full trial with live animals……170
6.11.1. Ethical approvals …… 171

6.11.2. Experimental protocol…………………………………………………… 171
6.11.2.1. Method ……………………………………………………. 172
6.11.2.2. Preliminary trial -stage 1 (parts 1 and 2) ………………………. 173
6.11.2.3. Overview of the approach to EEG and ECG recording ……… 176
6.11.2.4. Photos of the prototype SPUC stunner……………………………..178

**Chapter 7. Compliance of Single Pulse Ultra-High Current (SPUC) stunning with guidance from the European Food Safety Authority (EFSA) on the criteria for the assessment of the effectiveness of new or modified stunning methods……… 182**

7.1. Introduction…………………………………………………………………… 182
7.2. Application procedure………………………………………………………… 184
7.3. Brief description of the SPUC system …………………………………… 188
7.4. Ethical approval…………………………………………………………………189
7.5. Preliminary laboratory experimentation……………………………………… 189
7.6. Assessment of the effectiveness of SPUC stunning……………………… 190
7.6.1. Step 1: The effectiveness of SPUC stunning under laboratory conditions…………………………………………………………..190
7.6.2. Step 2: The effectiveness of SPUC stunning under abattoir conditions…………………………………………………………..192
7.7. Approach………………………………………………………………….. …… 194
7.7.1. Eligibility criteria………………………………………………… 194
7.7.1.1. Specific EFSA guidance on head-only electrical stunning………… 195
7.7.2. Reporting quality criteria………………………………………………196

**Chapter 8. Lay summary report of major activities and update on planned trial of the SPUC stunner on live animals…………………………………….……………… 201**

8.1. Introduction……………………………………………………………………201
8.2. Summary of literature reviews……………………………………………… 201
8.3. Summary of stakeholders' surveys……………………………………………201
8.3.1. Survey of Islamic scholars and Halal consumers…………………………202
8.3.2. Survey of veterinary students in England…………………………………203
8.4. Other published work within the project…………………………………… 203
8.5. Summary of *in vitro* experiments…………………………………………… 204
8.5.1. Measurement of voltage drop and the resistance of cattle heads………… 204
8.5.2. Measurement of migration of ions from neural membranes………………204
8.5.3. Measurement of electroporation of neural membranes………………… 205
8.6. Assessment of the efficacy of the SPUC stunner on live animals……………………………………………………………………… 205
8.7. Further research………………………………………………………………..207
8.8. General Discussion……………………………………………………………208
8.9. Conclusion…………………………………………………………………… 208

**References………………………………………………………………………..**211
**Appendix 1……………………………………………………………………….**257

# List of tables

Table 1 The acceptability of stunning among UK halal certifiers and the recognition of certificates issued by these certifiers in the major halal-importing countries. (Adapted from Fuseini et al, 2016) …………………………………………………………..68

Table 2 Responses of some UK Halal certifiers as to whether they approve post neck-cut stunning as Halal or not (Email communications, 2015)…………………………70

Table 3 An outline of the questions put forward to the Islamic scholars and Halal consumers……………………………………………………………………………91

Table 4. A summary of the outcome of the scholars' survey………………………..96

Table 5. A summary of the results of the survey of Halal consumers………………102

Table 6. Additional Halal slaughter requirements provided by 105 respondents…..103

Table 7. Cross tabulation of the year of study of respondent and awareness that slaughter without stunning for religious purposes was permissible in the UK……..116

Table 8. Comments by respondents who choose the option 'other' to the question regarding their opinion on the use of pre-slaughter stunning for meat animals…118

Table 9. Cross tabulation of the year of study of respondent and willingness to buy and consume Halal meat derived from animals that have been stunned before slaughter..119

Table 10. Results of voltage measurements across cattle heads……………………..131

Table 11a. The effect of electroporation on ion concentration following time constant-pulsing using 300 V for 10 ms. ……………………………………………………..133

Table 11b. The effect of electroporation on ion concentration following time constant-pulsing using 400 V for 13 ms. ……………………………………………………134

Table 11c. The effect of electroporation on ion concentration following different electrical parameters. ……………………………………………………………135

Table 12. The effect of voltage and number of pulses on cell death, % intact live cells and the percentage of cells electroporated. ……………………………………………148
Table 13. Electrical parameters of the SPUC stunner. ………………………………164
Table 14a. Stage 1, part 1 test regimen (fixed time of 75 ms). ………………………175
Table 14b. Stage 1, part 2 test regimen (fixed voltage of 7.5 kV)……………………175
Table 15a. Revised calculation [stage 1, part 1 test regimen (fixed time of 75 ms)] if resistance vary substantially from estimated 53 Ω. …………………………………175
Table 15b. Revised calculation [Stage 1, part 2 test regimen (fixed voltage of 7.5 kV)] if resistance vary substantially from estimated 53 Ω. ………………………………176
Table 16: Scoring system of post-stun convulsions following a SPUC stun. …… 194
Table 17. A list of variables that will be measured and the methods of measurement of these parameters during the SPUC research. ………………………………………195
Table 18: Specification of the electrical characteristics of the SPUC stunner. ……196
Table 19: Check list of the REFLECT reporting guidance on randomised controlled trials for livestock and food safety. ……………………………………………… 200

# List of figures

Figure 1. An upright restraint with the chin lift component suitable for use during slaughter without stunning. This may be fitted with a belly lift and back pusher. Source: http://www.vcons.be/en/products-detail?parent_id=44&child=53. ………...12

Figure 2. An upright restraining pen with a chin lift, belly lift, forehead bracket and back pusher suitable for the restraint of cattle during slaughter without stunning. [Adapted from Grandin and Regenstein (1994)]……………………………………14

Figure 3. A graphical representation of an AC sine wave showing one complete waveform………………………………………………………………………..26

Figure 4. A Jarvis Beef Stunner showing the brisket, nose plate and neck electrodes..34

Figure 5. The BANSS Stunning Box MGB showing a restrained cow and the various electrode positions. (Source: http://www.banss.de/en/#slaughtering-technology-cattle-stunning/)..........................................................................................................37

Figure 6. The special knife used for Shechita slaughter (Chalaf). …………………..53

Figure 7. Photograph of a cow's head with stimulating and recording electrode array during measurement of voltage across the head…………………………………..128

Figure 8. Illustration of the regions R1, R2, R3 & R4 in flow microscopic plots. R1 is gating on intact cells using forward and side scatter detectors, R2 is gating on intact live cells using DAPI as a live vs dead discriminator (live cells are DAPI negative, y-axis), R3 is gating on intact, live and single cells using pulse width (forward scatter trigger), y-axis and R4 is gating on intact, live single cells that are positive for calcein (y-axis)…………………………………………………………………………… 145

Figure 9. The effect of energy and number of pulses on the uptake of calcein through neural membrane……………………………………………………………………147

Figure 10. A plot of the impact of the amount of energy and number of pulses on (a)

the proportion of cells electroporated, (b) the proportion of cells that have a compromised cell membrane (c) proportion of cells that are dead and (d) proportion of live cells that have taken up calcein………………………………………………… 150

Figure 11. Main stages involved in the design and construction of the restraint and electronics of the SPUC stunner and subsequent trial on live animals………………156

Figure 12a. Initial sketches of the nose and neck electrodes of the SPUC stunner which were later modified for optimal delivery of electricity to the nose and neck of cattle..158

Figure 12b. A second sketch of the nose and neck electrodes showing the level of insulation………………………………………………………………………158

Figure 12c. A 3D sketch of the SPUC stunner showing the major components……159

Figure 13. Outline of the electrical circuit of the SPUC stunner…………………….162

Figure 14. A diagrammatic illustration of the control system of the SPUC stunner showing the main control buttons, see photo of actual unit in figure 16………… 163

Figure 15. Photograph of the control panel of the SPUC stunner showing the functions of the main buttons. This is located in the control room (see figure 20) next to the stunner………………………………………………………………………163

Figure 16. Photograph showing the high voltage unit and control panel of the SPUC stunner in the control room……………………………………………………164

Figure 17. Screenshot from CCTV of electrical arcing during the initial trial of the SPUC stunner on a live cow. The nose plate will be redesigned with improved insulation to prevent arcing in future trials………………………………………….169

Figure 18. A 3D sketch of the proposed changes to the chin lift and nose plate electrode…………………………………………………………………………170

Figure 19. Photograph of the SPUC stunner in the project site in Chimay, Belgium...178

Figure 20. A photograph showing the entry to the SPUC stunner from the raceway………………………………………………………………………...178

Figure 21. Photograph of the SPUC stunner showing the nose plate and neck restraints……………………………………………………………………………179

Figure 22. Photograph of a cow restrained in the SPUC stunner………………… 179

Figure 23. Photograph of the head of a restrained cow showing a side window where EEG and ECG recordings will be taken post stun…………………………………180

Figure 24. An overview of the support mechanisms available to applicants during the approval process for new or modified stunning systems……………………………185

Figure 25. Step by step procedure for EFSA's handling of applications submitted for the approval of new or modified stunning interventions (Adapted from EFSA, 2018)…………………………………………………………………………...186

Figure 26. The criteria adopted by EFSA in assessing the effectiveness of new or modified stunning interventions (Adapted from EFSA, 2013)…………………… 187

# Abbreviations

AC-Alternating Current

AHAW-Animal Health and Welfare

AMPC-The Australian Meat Processor Corporation

AVMA-American Veterinary Medical Association

AWERB-Animal Welfare and Ethical Review Body

BCVA-British Cattle Veterinary Association

BSE-Bovine Spongiform Encephalopathy

BVA- British Veterinary Association

DAPI-4,6-diamidino-2-phenylindole

DC-Direct Current

DCB-Dark Cutting Beef

DEFRA-Department of Environment, Food & Rural Affairs

DFD-Dark Firm Dry

DMEM-Dulbecco's Modified Eagle Medium

DNA-Deoxyribonucleic Acid

EBLEX-English Beef and Lamb Executive

EC-European Commission

ECG-Electrocardiogram

ECOG-Electrocorticogram

EEG-Electroencephalogram

EFSA-European Food Safety Authority

EU-European Union

FAWC-Farm Animal Welfare Committee

FSA-Food Standards Agency

GABA-Gamma Amino 4-Butyric Acid

GCC-Gulf Cooperation Council

HCB-Halal Certification Body

HFA-Halal Food Authority

HMC-Halal Monitoring Committee

HSA-Humane Slaughter Association

MAFF-Ministry of Agriculture, Fisheries and Food

OV-Official Veterinarian

PBS-Phosphate buffered-saline

RSPCA- Royal Society for the Prevention of Cruelty to Animals

SPUC-Single Pulse Ultra-High Current

VEP-Visually Evoked Potential

WASK-Welfare of Animals at the time of Slaughter or Killing

WATOK-Welfare of Animals at the Time of Killing

# Chapter 1. General Introduction

## 1.1. Stunning of food animals

The requirement for humane killing of farm animals for human consumption using scientifically validated methods of stunning within the European Union (EU) is detailed in Council Regulation (EC) 1099/2009 of 24 September 2009 on the Protection of Animals at the Time of Killing, There is a derogation in article 4(4) of this regulation allowing member states to permit the slaughter of animals without stunning when carried out in accordance with religious rites. This is interpreted in the domestic legislation in England, The Welfare of Animals at the Time of Killing (England) Regulation 2015, which requires all animals to be stunned prior to slaughter with the exception of animals slaughtered in accordance with religious rites, these being mainly for followers of Islam (Halal) and Judaism (Shechita). The global demand for meat slaughtered in strict compliance with religious beliefs is very large and it is projected to grow even stronger with its associated economic benefits (Sunkar, 2008 and Mintel, 2009). This has led to a scramble for the Halal market by the mainstream retail multiples and a corresponding increase in the number of unregulated Halal certification bodies (Fuseini, 2017; Farouk, 2012 and Longdell, 1994). In the UK alone, Fuseini (2017) reported that there are over 12 Halal certification bodies all operating according to different standards. Proponents of animal welfare have always questioned the humaneness of traditional methods of ritual slaughter of food animals due to the fact that such methods of slaughter are usually carried out whilst animals are fully conscious. On the other hand, during conventional slaughter in the developed world, cattle are commonly stunned pre-slaughter by the use of a captive bolt gun which delivers a percussive force to induce insensibility through the transfer of kinetic energy to the brain to disrupt normal brain function (Gregory, 2007). It is generally accepted by Muslims that for meat to be regarded as Halal, the animal must not be dead at the time its neck is cut, and that the volume of blood lost must not be obstructed by any form of human intervention (e.g. stunning) during or prior to the neck cut. This is consistent with the teachings of the Holy Quran which forbids the consumption of meat from animals that die naturally, irrespective of whether their necks were cut afterwards or not. An exception to this rule is for animals that die during hunting. However, the name of God must be recited before a hunter shoots the animal. The following Quranic verse reiterates the prohibition of the consumption of carrion and the prohibition of the consumption of meat killed by certain methods including those killed by wild animals:

"Prohibited to you are dead animals, blood, the flesh of swine, and that which has been dedicated to other than Allah, and [those animals] killed by strangling or by a violent blow or by a head-long fall or by the goring of horns, and those from which a wild animal has eaten, except what you (are able to) slaughter (before its death), and those which are sacrificed on stone altars, and (prohibited is) that you seek decision through divining arrows"(Quran 5:3).

Fuseini (2019) explained, that the majority of proponents of pre-slaughter stunning for the production of Halal meat have overcautiously approved only reversible stunning because they deem mechanical stunning as high-risk methods that are likely to result in instantaneous death in some instances. For this reason, the method of stunning generally acceptable for use during Halal slaughter (by the majority of proponents) is electrical head-only stunning (Anil et al. 2006). Captive bolt stunning is therefore not a preferred stunning method for use during Halal slaughter because it may lead to the death of animals if their necks are not cut and causes gross physical damage. Muslims are also prohibited to kill animals by a violent blow (see above Quranic quotation) hence most Muslims reject both penetrative and non-penetrative captive bolt stunning because they believe these methods contravene the teachings of Islam. Despite the doubts surrounding the recoverability of captive bolt stunning, some Muslims in Sweden accept it for Halal slaughter provided stunning is immediately followed by exsanguination (Berg & Jakobsson, 2007). Non-penetrative captive bolt stunning is also accepted by countries in the Gulf Cooperation Council (GCC), made up of Saudi Arabia, Bahrain, Qatar, Kuwait, Oman and the United Arab Emirate. These countries have adopted a 'unified' Halal standard (GSO). Malaysia (JAKIM) also approves non-penetrative captive bolt stunning on condition that it must not fracture the skull (MS1500/2009). A number of animal welfare surveys conducted by the Food Standards Agency (FSA) in Great Britain suggested that some slaughter plants use penetrative captive bolt for the slaughter of Halal beef (FSA, 2012, 2015). The results also indicated that the majority of animals were stunned prior to Halal slaughter despite the continuous resistance against the use of stunning for Halal meat production by some Muslims in the UK.

Electrical stunning methods have been developed to cater for the Halal market (Weaver and Wotton, 2009). Aside the reversibility of head-only stunning which makes it acceptable for some Muslims, electrical stunning also arguably plays a vital role in eliminating the risks associated with the potential spread of brain embolic materials to edible parts of carcasses when cattle are stunned mechanically, due to the gross physical damage caused by mechanical stunning equipment. This was suggested by Anil *et al.* (1999) in a study linking captive bolt stunning to the possible spread of Bovine Spongiform Encephalopathy (BSE). Sadly, conventional electrical stunning (CES) of small and large ruminants is not without its inherent problems; there is a risk of the stunned animal regaining consciousness during the period it is bled-out (Shaw *et al.*, 1990). This is because the duration of insensibility is very short, and the neck-cut may also be significantly delayed due to violent kicking during the clonic phase of epileptiform following electrical stunning. The duration of unconsciousness has been shown to last for on average 50 s in cattle (Wotton *et al.*, 2000) and 18-42 s in sheep (Blackmore and Newhook, 1982), it is therefore an animal welfare and additionally a safety concern for slaughter floor operatives. There is also the problem of blood splash (Weaver and Wotton, 2009; Mpamhanga and Wotton, 2015) brought about by elevated blood pressure with a resulting inferior meat quality (Gregory, 2005). Gregory et al (2008) associated blood splash to the bursting of small capillary beds within muscle groups. In New Zealand, a head-only electrical stunning method, The Jarvis Beef Stunner, was developed to cater for the Halal market (Weaver and Wotton, 2009). In this method, post-stun convulsions are brought under control by the use of low voltage electro-immobilisation (Weaver and Wotton, 2009, Gilbert *et al.*, 1984) which is generally accepted by all Muslim groups who support the idea of pre-slaughter stunning (Anil *et al.*, 2006). There are, however, some welfare concerns regarding the use of low voltage electro-immobilisation. The use of any immobilisation procedure during slaughter, including the one used in the method described above, is contrary to EU welfare legislation (EC 1099/2009) and the English domestic regulation (WATOK, 2015). The concern from an animal welfare perspective is that the use of electro-immobilisation is capable of masking the accurate assessment of effective stunning (Wotton *et al.*, 2000). It is important to note that although use of the Jarvis Beef Stunner (with post-stun electro-immobilisation) is illegal in the EU, meat from animals stunned using this method of stunning are widely imported into the EU from New Zealand. A cardiac arrest cycle was incorporated into the Jarvis Beef Stunner for use in the EU.

Despite the controversial nature of slaughter without stunning from an animal welfare viewpoint, there is an increasing number of people who oppose pre-slaughter stunning of animals during ritual slaughter in Europe and other parts of the world (Grandin, 2010). Some Muslim groups expressly reject the idea of stunning animals before slaughter with the belief that such a procedure was not practiced by the Prophet and it is therefore contrary to the teachings of the Prophet. Opponents of stunning also argue that stunning leads to inferior meat quality and that it can cause instantaneous death and affects the rate and volume of bleed-out. However, Pleiter (2004), Khalid *et al.* (2015), Anil *et al.* (2004, 2006) have demonstrated that stunning does not affect the volume of blood loss, whilst Önenç and Kaya (2004) showed that the slaughter of animals without stunning has no meat quality advantage over meat from animals pre-stunned using percussive captive bolt or electrical head-only stunning before slaughter. Danso et al. (2017) showed that when lambs were killed by three methods of slaughter (i.e. electrical head-only stunning prior to neck-cut, post neck-cut electrical stunning and slaughter without stunning) there was no effect on the quality of lamb meat as a consequence of the method of slaughter.

Proponents of animal welfare have over the years, been calling for an end to the slaughter of animals without stunning. In early 2014, the British Veterinary Association (BVA) with the support of the Royal Society for the Prevention of Cruelty to Animals (RSPCA) launched a government e-petition with the aim of securing 100,000 signatories so that the issue of ending non-stun slaughter in the UK could be debated in the House of Commons. The target was reached and on the 23rd of February 2015, Westminster politicians debated on the subject in a Westminster hall, a full debate is yet to be held in parliament. Some stakeholders are also lobbying for the labelling of meat to indicate whether animals were stunned before slaughter or not, in order to help consumers make informed decisions when buying meat. In late 2017, the UK's Agriculture and Horticulture Development Board (AHDB) launched a public consultation to gauge industry's and public opinion on the need for Halal sheep meat to be labelled according to the method of slaughter and other animal welfare attributes (AHDB, 2017). This initiative was welcomed by the Muslim authorities (e.g. Muslim Council of Britain and the Halal certifiers) and mainstream animal welfare organisations such as the BVA and RSPCA.

It has been suggested that a large quantity of the meat from animals slaughtered in accordance with religious rites (without stunning) are either exported or sold on by the mainstream retail multiples to non-religious consumers. This is consistent with the results of a recent animal welfare survey published by the UK's FSA which showed that 25% of sheep were slaughtered without stunning, 24% of which were exported mainly to EU member states (FSA, 2019). In the case of Kosher meat, the hindquarters from Shechita slaughtered animals are usually deemed unfit for consumption by followers of the Jewish faith unless they go through a process called porging to remove certain tissues and forbidden fats (e.g. tallow), a process which is expensive and time consuming, hence such products are usually passed on to mainstream retailers. In the UK, at least one of the Halal certification bodies accepts and certifies Kosher meat as Halal (see website of Halal Consultations Limited http://halalconsultations.com/kosher/), so there is the possibility that some of the hindquarters from Shechita slaughtered animals could be absorbed into the Halal market. The result of a recent European Commission funded survey (Food Chain Evaluation Consortium, 2015) involving 13,500 meat consumers comprising 500 respondents from each of the 27 member states (study was finalised before Croatia joined the EU and before the UK's referendum to leave the EU) showed that 80% of respondents were generally satisfied with the information currently on meat products. However, when respondents were directly asked whether they would like to have information on the stunning of animals when purchasing meat, 72% of respondents indicated their interest in having information on stunning.

The inability of conventional electrical stunning (CES) to maintain an unconscious and still animal for a longer duration of time calls into question the effectiveness of CES methods as well as its reliability in terms of protecting animal welfare and the health and safety of the slaughter floor operatives. The violent post-stun convulsions also tend to delay the time between the stun and neck-cut, which may lead to the recovery of animals during bleeding-out because of the short duration of unconsciousness induced during CES. There is also the issue of blood splash in the rump region of the carcass (see Weaver and Wotton, 2009) which also highlights the economic loss in carcass and meat value. There is therefore the need for further scientific research to investigate ways of improving electrical stunning methods for beef in order to improve animal welfare, meat quality and operator safety. Recent investigation on the use of a new system of electrical stunning, using single pulse ultra-high current (SPUC) by Robins *et al.,* (2014) showed an improvement over CES by the elimination of post stun tonic/clonic seizures, improvement in carcass and meat quality and the extension of the duration of unconsciousness to around 4 minutes. Animals stunned using this method also showed signs of recovery, which could make the method acceptable as part of religious slaughter.

The present study will use EEG to record the immediacy and duration of unconsciousness, high amplitude and low frequency on the electroencephalography indicates a state of unconsciousness and vice versa. Preliminary laboratory work will use bovine heads and brain cells to estimate the electrical parameters of the stunner.

The effect of energy, voltage, number of pulses and pulse interval on the electroporation of brain cells will be investigated. Electrical energy describes the force required to move an object, that is, the repulsive or attractive force between two charged particles (e.g. electrons). Voltage on the other hand is the electrical potential between two points, in simple terms it describes the 'push' behind the movement of electrons, and it is sometimes referred to as electromotive force. Electrical pulse is a burst of voltage or current and pulse interval describes the duration of the pulse. Frequency and waveform of current are described in 1.5.1.1.1 below.

The objective of this project is to produce a new system of head-only electrical stunning of adult cattle. It is hypothesised that unconsciousness will be induced through the poration of neural membranes. Although the mechanism of induction of unconsciousness through electroporation is still not well understood, it is thought the poration of neural membranes leads to sustained depolarisation of brain cells due to the physical disturbance of neural membranes. Further, the opening of membranous pores may have an effect on ionic concentration within brain cells which may be responsible for the induction of unconsciousness. These mechanisms need to be investigated in future research.

## 1.2. Restraint

The aim of restraining animals is to restrict the movement of the animal in order to carry out procedures such as the confirmation of cattle identity and the pre-slaughter stunning of animals. Research has shown that the slaughter of animals, especially when carried out without stunning is a stressful procedure for animals (Ferguson and Warner, 2008). Efforts must therefore be made to use calm pre-slaughter handling techniques as well as less stressful restraining methods in order to reduce panic, injury and avoidable pain and distress to improve animal welfare, operator safety, carcass and meat quality

(Lambooij *et al.*, 2012). The type of restraining device used must not agitate or make the animal panic as this will affect the correct positioning of the cut (during slaughter without stunning) or the stunning equipment (Holleben, 2007). The use of excessive force and inappropriate restraint can compromise the welfare of animals. Crush restraint can be injurious to the animal and its handler (Grandin and Regenstein, 1994) and it has been shown that cattle find crush restraints aversive (Mitchell et al., 1988). Grandin (2005) reported that when animals struggle during the use of restraints, it demonstrates that the handler is applying excessive force. The natural behaviour of animals is therefore an important consideration in the design and improvement of restraining devices in order to reduce stress and injury to cattle. Improvements in restraint can improve animal welfare and reduce stress and injuries (Grandin, 1995a). Obvious changes made to the design of the crush restraint in a series of surveys were found to reduce injuries to cattle (Grandin, 1975). Mpamhanga and Wotton (2015) recently investigated the responses of cattle post-stun/kill and carcass quality in a Jarvis Beef Stunner under commercial conditions compared with those in which crush restraints (for identification) were not used pre-slaughter. They reported that without the use of the crush restraint (for identification), there was a profound reduction in post-stun/kill limb movement, muscle tone and the prevalence of brain stem activities. In addition, the authors reported a profound reduction in blood splash. Because the identity of cattle can be established post-slaughter without any traceability or food safety risks, Wotton and Mpamhanga (2015) suggested that the use of crush restraints (for identification) should be abolished. Poor pre-slaughter handling and restraint of cattle can be injurious, cause bruising, and result in downgrading and dark cutting beef (DCB) that markedly reduce the market value of beef (Warriss, 1990). The loss in value of meat as a consequence of lapses in pre-slaughter handling of cattle is usually underestimated

(Ferguson and Warner, 2008). In two surveys involving a total of 16,118 cattle, McNally and Warriss (1996) estimated the monetary loss as a consequence of bruising caused by poor pre-slaughter handling. With an average of 0.308kg rejected as a result of carcass bruising, the authors quoted an amount of £616 per 1000 cattle as the estimated financial loss as a result of trimming of bruised tissues at a time when carcasses were marketed at a wholesale value of £2 per kg. Boleman *et al.* (1998) estimated carcass bruising results in a loss of $4.03 per animal which equates to an overall annual loss of $114,452,000 to the beef industry in the USA.

### 1.2.1. Restraint for slaughter without stunning

The two main religious slaughter methods of economic importance in the UK are Halal, for followers of the Islamic faith, and Shechita for Jews. Both the Muslim and Jewish communities around the world demonstrate differences in the interpretation of their own religious doctrines regarding the acceptability of pre-slaughter stunning of animals (see Fuseini, et al., 2016). Although little is usually mentioned in their respective texts about the religious requirements in terms of the type and method of restraint. Derogation exists in the legislation in England that allows for the slaughter of animals without any form of pre-slaughter stunning if it is specifically for religious purposes (WASK, 1995, WATOK 2015). These regulations do, however require bovines to be properly restrained pre-slaughter, during slaughter and post-slaughter until animals are unconscious and in any event not before the expiry of a period of not less than 30 seconds, after it has been bled. Lambooij *et al.* (2012) echoed that animals destined for the slaughter line must be properly restrained in order to avoid struggling, injury and avoidable pain.

There are several ways in which animals can be restrained before religious slaughter. Some of the commonly known restraining methods used over the years have included:

hoisting or suspension of cattle by the hind leg, the use of v-restraint, restraining cattle in a standing or upright position, restraint by the use of a rotary pen (half or full inversion) and casting with a rope (Gregory, 2005). The restraint of cattle by suspension or hoisting is highly aversive and likely to cause pain and unnecessary suffering, given the weight of the animal and anatomy of their digestive system (Grandin, 2014) and as such, it is contrary to European welfare legislative requirements (Gregory, 2005). On the other hand, restraining cattle in an upright position is the preferred method (FAWC, 2012), this improves cattle welfare compared with other systems of restraint such as dorsal recumbency (Hollenben et al., 2010) and the suspension or hoisting of live cattle by the hind leg, which must be avoided (Grandin, 2014). The Farm Animal Welfare Council (FAWC, 1985) recommended that the use of rotary, inversion-pens during religious slaughter must be abolished in the UK by the year 1987. Therefore, the only method of restraint currently accepted for religious slaughter without stunning in the UK, is to slaughter in an upright position (FAWC, 2003, 2012, WATOK, 2015). However, article 15 of Council regulation EC 1099/ 2009 does contradict this provision. It states that bovine animals shall not be restrained by inversion or unnatural position unless such animals are slaughtered in accordance with article 4(4) [religious slaughter]. It further explains that such animals must be fitted with a device that restricts the vertical and lateral movement of the head and must be adjustable to fit different animals depending on their size. It is important to ensure that during religious slaughter without stunning, the head is appropriately restrained with a fully stretched neck to ensure unimpeded access to the neck and this must be kept stretched until the animal loses consciousness to avoid the sides of the wound from touching each other and occluding blood loss. Figure 1 shows an upright restraint with a chin lift suitable for use during slaughter without stunning. The chin lift stretches the neck before the ritual cut is made to ensure that the neck is easily accessible to the slaughter operative. The chin lift also ensures that after the ritual cut is made, the two sides of the wound are kept apart until the animal loses complete sensibility. This aids effective bleeding (Anil, 2012, Dialrel, 2010).

**Figure 1**. An upright restraint with the chin lift component suitable for use during slaughter without stunning. This may be fitted with a belly lift and back pusher.

Source: http://www.vcons.be/en/products-detail?parent_id=44&child=53

## 1.2.2. Upright restraint of cattle

With the enactment of domestic legislation to implement EC Regulation 1099/2009 on the protection of animals at the time of killing (WATOK, 2015), the process of approval of all new upright restraining pens used for non-stun slaughter of cattle in England has passed from DEFRA to the Food Standards Agency (FSA). FAWC in November 2013 reported on the design and operating criteria of upright bovine restraining pens for non-stunned slaughter, for use by the FSA.

Upright restraint of animals allows for the animals to be slaughtered in their natural standing position. This procedure can either be performed in a pen or a box that is usually designed to provide a neck stretch or chin lift to ensure easy access to the neck.

The chin lift also ensures an accurate neck cut by preventing movement of the head and keeps the wound edges apart after slaughter to ensure efficient bleed-out. Double rail conveyor systems have also been developed for the upright restraint of cattle in which the legs of the animal do not touch the ground and the entire body weight is supported on the brisket and belly (Grandin, 1988). At the end of the conveyor a chin lift is positioned to aid neck cutting. Restraining boxes have been adapted by equipment manufacturers for the upright restraint of cattle during religious slaughter. Grandin (1993) identified the Cincinnati, a device with a chin lift, belly plate and back pusher as the most reputable restraining device for the upright restraining of cattle. The belly plate supports the weight of the animal, however, the animal must not be lifted off its feet as this will put excessive pressure on the thoracic cavity (Grandin, 1995, Grandin and Regenstein, 1994). This device is particularly useful when handling wild and flighty animals that are not accustomed to human interaction.

Flaws in the design of some upright restraining devices may, however, affect the effectiveness of the system as well as cause stress and avoidable pain to animals. Some poorly designed upright restraints exert pressure on animals due to defects in the head holder or chin lift (FAWC; 1985, Grandin and Regenstein, 1994). These views were supported by Berg (2007) who found that the poor design and construction of upright restraints compromises the welfare of animals in that it exerts pressure on the animal as a consequence of defects in the head holder, back pusher and the possibility of the sides of the wound touching each other. An important welfare concern with the use of upright restrainers is the possible aspiration of blood and the refluxing of gut content. Gregory *et al*. (2009) examined the respiratory tracts of cattle after slaughter in an upright position in accordance with Halal and Kosher methods without pre-slaughter stunning as well as following the use of captive bolt stunning. They found traces of blood in the inner part of the trachea in 21% of the captive bolt stunned cattle, 58% of the Halal and 19% of the Shechita slaughtered cattle. However, when the larynx were observed, bright red blood-like forms were found in 19% of the Halal, 10% of the Shechita and 0% of the captive bolt stunned cattle. Figure 2 shows an upright restraint system suitable for use during slaughter without stunning. The system is fitted with a belly lift, a chin lift and a rear pusher. The chin lift ensures that the neck is stretched so that the operative can easily make the cut and it also ensures that the wounds are kept apart during bleed-out until consciousness is lost and the animal is released.

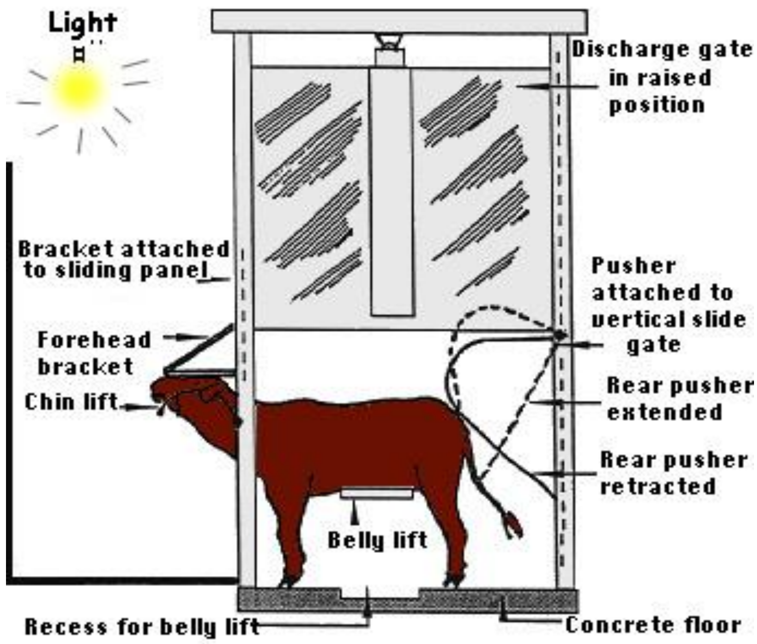



**Figure 2**. An upright restraining pen with a chin lift, belly lift, forehead bracket and back pusher suitable for the restraint of cattle during slaughter without stunning. [Adapted from Grandin and Regenstein (1994)].

## 1.2.3. Use of rotating pens to invert cattle on their backs

During religious slaughter without stunning, cattle may be restrained by inversion, in a rotating pen, on their backs, which remains acceptable in some parts of the world. This method of restraint has significant welfare implications. Scientific investigations comparing this method with the upright restraint have concluded that the restraint of cattle by inversion on their backs is time-consuming, in addition, there is increased vocalisation and laboured breathing as well as increased levels of cortisol, an indication of stress in animals (Koorts, 1991). The Farm Animal Welfare Committee (1985, 2012) recommended for the prohibition of rotary pens due to the stress and discomfort as a result of the rumen exerting pressure on the diaphragm and the thoracic cavity. Gregory (2005) observed more vigorous struggling when cattle were inverted before the use of the head restraint compared to the application of a head restraint before inversion of cattle. Dialrel (2010), a European Commission (EC) funded research project on religious dialogue, to assess the religious standards applied across the EU, looked at the slaughter of animals in regard to economic, cultural and religious differences in selected countries. During plant visits by the Dialrel researchers, it was observed that depending on the extent of the neck cut and the position of the cut, blood and rumen content entered the trachea and larynx when animals were inverted on their backs. This was considered an animal welfare compromise.

### 1.2.4. Restraining cattle in lateral recumbency position

Cattle to be slaughtered without stunning can be restrained in a lateral recumbency position, i.e. restraining the animal in a 90-degree angle to lie on their sides (lying on the left side is preferred during Halal slaughter). Petty *et al.*, 1994, and Pesenhofer *et al.*, 2006 compared the restraint of cattle in lateral recumbency with the restraint of cattle on their backs and concluded that restraint in a lateral recumbency position was less stressful. Pesenhofer et al., 2006 explained that when animals are restrained in a lateral recumbency position, they do not experience breathing problems because there is no pressure of the rumen and other organs on the diaphragm and the thoracic cavity. Additionally, restraining cattle on their sides ensure that their body weight is easily supported during and after the neck cut. It must be noted however, that any system of restraining animals (including lateral recumbency) would have animal welfare implications. Tagawa and others (1994) restrained healthy Holstein breeds of cattle on their sides and then on their backs without slaughtering them, they observed that restraining cattle on their sides exerts pressure on their internal organs. The authors concluded that lateral recumbency and restraining animals on their backs put pressure on the respiratory system and adversely affect the normal functioning of the respiratory system.

### 1.2.5. Restraint for post neck-cut stunning

In countries of the developed world where derogations exist for the slaughter of animals without stunning for religious rites, it is not uncommon for animal welfare charities, academics and veterinarians to lobby with religious communities for the use of post neck-cut stunning, especially in large animals. The main objective of post neck-cut stunning is to abolish consciousness during bleeding-out and so reduces the time interval between the neck cut and the loss of sensibility until death supervenes. In the context of religious slaughter, post neck-cut stunning provides assurances that, at the time of the ritual cut, all animals are alive, an important religious slaughter requirement. Gregory *et al.* (2012) concluded that post neck-cut stunning provides an improvement in animal welfare in comparison with slaughter without stunning. Despite satisfying one of the most important requirements of religious slaughter, post neck-cut stunning is still regarded as incompatible with the religious slaughter rules by some authorities within the Muslim and Jewish communities. The UK's largest certifier of stunned Halal meat, the Halal Food Authority (HFA) gave oral evidence to the All-Party Parliamentary Group (APPG) into religious slaughter of red meat (beef and lamb) (2014). The HFA confirmed that they approved post neck-cut stunning as Halal and that they were convinced that the practice improves animal welfare by reducing the time taken to lose consciousness after the neck cut. However, Shechita UK, in their written evidence, rejected the idea of applying any method of stunning after the Shechita cut. They explained that the Shechita cut renders animals irreversibly unconscious when performed correctly, therefore there was no need for the stunning of animals after the Shechita cut.

The stun must be applied immediately after the cut. However, the application of the stun may be significantly delayed if animals struggle vigorously, this affects the accurate placement of the stunning equipment. Berg (2007) reported that the time between the neck cut and the application of the stun may be influenced by the following factors;

- The level of training, experience and expertise of the slaughter operative
- The breed and behaviour (e.g. temperament) of the animal
- The system of restraint used

Binder (2010) echoed that the system of restraint can have an effect on the time interval between the cut and the stun. Researchers have measured the time interval in cattle to be not less than 40 s (Berg 2007) and around 5 s (Velarde *et al.,* 2010).

During post neck-cut stunning, a neck restraint is required to hold the head in place, this usually has a neck stretch function to ensure unimpeded access during the performance of the cut, and subsequent application of the stun.

## 1.2.6. Restraint for electrical stunning

The successful application of electrical stunning requires the accurate placement of the stunning electrodes, to achieve this objective, animals must be appropriately restrained. A good restraint, in addition to ensuring the accuracy of the stun, reduces agitation, thereby preventing bruising and blood splash in carcasses (Hollenben *et al.*, 2002). The bleeding of electrically stunned animals can be achieved by ejecting and hoisting them by the hind leg or bleeding them in-situ in the restraint. Due to the risk associated with the possible recovery of electrically head-only stunned animals during bleeding-out, it is recommended that animals must be bled-out as promptly as possible after the stun (EFSA, 2004; Hollenben et al., 2002; Ilgert, 1985). Hollenben and others (2010) during a Dialrel project, identified the following as appropriate restraining procedure for the electrical stunning of cattle prior to slaughter;

i. Restraint by the use of a single animal pen with manual application of the stun.
ii. Restraint by the use of a single animal pen with automatic application of the stun as employed in the Jarvis and BANSS Beef Stunners.
iii. Halter handheld.

A well-designed restraint device will improve operator safety, animal welfare and both carcass and meat quality. Ewbank (1992) observed that poorly designed systems which tend to hold the head too tightly increases the level of stress and extends the time it takes to restrain animals. The vision of cattle at the entrance of the restraint must be screened from moving personnel or objects within the slaughter hall.

## 1.3. Stunning of cattle

The Chinese are reported to be the pioneers in using mechanical stunning to stun large animals as early as the 15$^{th}$ century, mainly to improve slaughter operator safety and increase throughput (Mellor and Littin, 2004). The authors pointed out that at that time, animal welfare was not the reason for the use of mechanical stunning, and equipment used to ‘stun’ animals would have been basic with less accuracy. Zivotofsky and Strous (2012) echoed that the stunning of animals to improve animal welfare in the industrialised world only begun some 150 years ago. According to the authors, although the devices used during the early days of stunning were seen as state of the art, 'today's meat consumers would cringe' if those early devices were shown to them. Scientific advancement over the last few decades has led to a better understanding of electrophysiology, anatomy and behaviour of animals leading to improvements in stunning techniques and the development of more accurate and effective stunning devices. There are on-going studies around the globe looking at ways of refining existing stunning techniques or developing new ones to improve animal welfare during slaughter. Stunning is used to induce immediate loss of sensibility prior to slaughter (Gregory, 2007). The three main forms of stunning are electrical, mechanical and the use of controlled atmospheres. Electrical stunning is reported to be the commonly used method of stunning of sheep, rabbits and pigs (Zivotofsky and Strous, 2012) whereas cattle are normally stunned using mechanical devices such as a penetrative captive bolt gun (Gregory and Shaw, 2000). Gregory (1998) reported that rapid bleeding-out is achieved in stunned animals because stunning presents a still animal (in most cases), which aids in the immediate and more accurate severance of the major blood vessels especially in large animals. Hollenben *et al*., (2010) explained that effectively stunned animals (during electrical stunning) are insensible to pain because stunning results in the disruption of normal brain function by affecting the state of neurons through the release of chemicals (neurotransmitters) in the brain. The induction of unconsciousness must be immediate and sustained in order to eliminate any pain associated with the application of the stunning device (particularly during mechanical and electrical stunning) and the neck-cut as well as prevent the recovery of animals during the period they are bled-out. Hollenben and others (2010) reiterated that where a stunning system cannot induce immediate loss of consciousness (e.g. controlled atmosphere stunning), the application of such a method of stunning, and the initiation of the onset of unconsciousness must not be aversive. Only scientifically validated stunning methods or stunning parameters must be used (by trained operatives) to stun animals in order to minimise unnecessary suffering and to prevent situations where animals are immobilised instead of being stunned effectively. This may not always be the case during the stunning and slaughter of certain species of all animals. Data from the UK's FSA showed that the Halal sector used unapproved electrical stunning parameters in the stunning and slaughter of poultry in Great Britain (FSA, 2019).

The accurate use of stunning devices is influenced by many factors. Grandin (2003) observed that the level of training and experience of licensed slaughter operatives influence the proper and successful use of a stunning device. A scientific report on the welfare aspects of animal stunning and killing methods published by the European Food Safety Authority (EFSA, 2004) outlined the following general rules pertaining to the stunning of animals:

i. The method of stunning must be applied once, that is, the method used must be capable of rendering animals unconscious during the first attempt.
ii. There must be a backup stunning method to be used in the event of the failure or unsuccessful application of the main stunning method.
iii. An immediate investigation must be launched in the event of two consecutive failures of a stunning method or device. Stunning and slaughtering can only resume once the fault has been rectified.

The re-stunning of animals is usually necessitated by the failure of slaughter operatives to accurately apply the stunning device, or it may be due to faults with the device itself. Where recoverable or reversible stunning is used for ritual slaughter, stunning must be immediately followed with a lateral cut of the main blood vessels supplying oxygenated blood to the brain. This ensures that the fall in blood pressure is sufficient to prevent the animal from regaining consciousness during the period they are being bled-out. Although not generally acceptable for religious slaughter, research has shown that thoracic sticking ensures a more rapid drop in blood pressure than a transverse cut of the neck (Daly & Warriss, 1986; Shaw *et al.*, 1990) which hastens the death of animals by depriving the brain of oxygenated blood. Anil and his colleagues (1995) showed that the use of thoracic sticking reduced blood pressure to almost 0 within 8 seconds. Gregory *et al.* (2008) explained the reasons behind the possibility of some cattle regaining consciousness during bleed-out after ritual slaughter. They asserted that cattle have an alternative route of blood supply through the vertebral arteries to the brain and that arterial contractions may also be a factor as well as clotting of the severed ends of the carotids (false aneurysms).

Over the years, animal scientists have devised different criteria for the evaluation of the effectiveness of stunning devices. Wotton *et al.* (2000) in an experiment using the Jarvis Beef Stunner, showed that the induction of unconsciousness and ventricular fibrillation in 93% of cattle was associated with rhythmic breathing movements and / or gasping. Analysis of videotapes during the same experiment also showed that rotation of the eye ball was associated with the onset of unconsciousness in 53% of animals. They further established that eye reflexes returned with corneal followed by palpebral reflexes and that the above responses were subsequently lost with no return to consciousness after brain death. Other studies involving the Jarvis Beef Stunner have concluded that 32% of animals exhibited rhythmic breathing after an effective stun (Weaver and Wotton, 2009) and there was a marked reduction in the number of animals showing rhythmic breathing and eye roll when a crush restraint was not used (Mpamhanga and Wotton, 2015). The presence of rhythmic breathing (brain stem reflex) is thought to be due to residual brain stem function in a dying animal.

There are several methods of stunning available for commercial use to render animals insensible to pain; electrical, mechanical and the use of controlled atmospheres. For the purpose of this review, only electrical beef stunning methods will be considered.

### 1.3.1. Electrical stunning

Electrical stunning involves the passage of electric current of sufficient magnitude through the brain in order to induce unconsciousness and insensibility through the depolarisation of neurons (Blackmore and Delaney, 1988), which results in a tonic/clonic epileptic fit (Gregory, 1987). Experiences of epileptic seizures in man, in addition to electrophysiological evidence suggest that human beings experience no pain or other forms of sensations during these seizures (Bager *et al.*, 1992). Gregory and Wotton (1985) and Zivotofsky and Strous (2012) identified electrical stunning as the most common method of stunning animals prior to slaughter, it is however important to note that in recent years, there appear to be a move away from electrical stunning of poultry to the use of controlled atmosphere. Additionally, in many parts of the EU, large ruminants (e.g. cattle) are commonly stunned mechanically with penetrative captive bolt guns. Farouk (2013) explained the possible reason behind the popularity of electrical stunning systems in some parts of the world; the technique requires relatively low capital investment, well suited to high throughput plants and that it can be automated. From an animal welfare perspective, electrical stunning, when carried out successfully, is accepted as a humane pre-slaughter procedure (Bager *et al.*, 1992; Cook *et al.*, 1996; Pleiter, 2005). Electrical stunning produces brain dysfunction through the overstimulation of neurotransmitters in the brain that induces tonic/clonic epilepsy. Dialrel (2010) explained that neurotransmitters are vital for the communication between neurons in the brain and that a physiological equilibrium is reached when Aspartate or Glutamate (excitatory neurotransmitters) interact with gamma amino-4-

butyric acid (inhibitory neurotransmitters). Following the flow of electric current through the brain, there is increased concentration of extracellular glutamate and aspartate which results in the excitation and uncoordinated activity of cellular structures leading to immediate loss of sensibility within 200 ms (Cook *et al.*, 1995). Tonic/clonic epileptic seizures have been shown to be inconsistent with normal neural function, thus produces a state of unconsciousness (Cook *et al.*, 1992; Hoenderken, 1978). Rosen (2004) postulated that the passage of electric current through the brain, a procedure that induces epileptic seizures may be painful. However, in an experiment using humans, Levinger (1976) demonstrated that although the passage of electric current through the brain may be painful, in most cases, by the time the person can perceive the pain, he/she would have been in a state of unconsciousness. It takes animals between 100 and 150 ms to feel pain after the initiation of a noxious stimuli (Liu et al., 2011), whilst the application of an electrical stunning current can induce unconsciousness within 50 ms (Robins et al., 2014). The concern should be around the use of inappropriate electrical parameters, faulty or poorly designed stunning devices, incorrect electrode positioning and the wrong application of stunning devices including 'mis-stuns' that will be aversive and will undoubtedly compromise animal welfare. Gregory (2007) suggested that the stunning equipment must be inspected to ensure that it is in good condition and able to discharge the recommended stunning parameters at the right position and that the reaction of the animal must be carefully observed.

Simmons and Daly (2005) categorised the epileptic state into 3 different phases:

i. ***Phase 1*** is a fully developed epileptic seizure, when there is hypersynchronous activity of all brain cells, that is, brain cells fire in a synchronised fashion. This phase of epileptic seizure prevents even the most basic of reflex activity to function and all activities associated with sensibility are absent during this phase. In this phase, electrical stimulation results in tonic muscle contraction which puts the animal in a rigid state with flexed hind legs and fully extended forelegs. Some features of clonic seizures such as uncontrolled limb movement may develop during this phase. Neck/thoracic sticking is highly recommended during this phase of epilepsy.

ii. ***Phase 2*** of epilepsy is characterised by reduced brain activity. This reduction in brain activity is as a direct result of the release of chemicals called neurotransmitters into the brain responsible for sustaining unconsciousness in the animal with some clonic activities.
iii. ***Phase 3*** is when the brain begins to return to normal function, so the animal begins to recover unless bleed-out occurs to cause death.

The epileptic seizures described above may only last for a short duration which presents a welfare issue since animals may recover before they are bled out if ventricular fibrillation is not induced. The duration of unconsciousness has been estimated by researchers to be between 40s and 60s (Daly and Warriss, 1986; Shaw *et al.*, 1990; Wotton *et al.*, 2000). To avoid the tendency of animals recovering after electrical stunning, thoracic sticking must be used to severe the main arteries and veins close to the heart (Anil *et al.*, 1995; Pleiter, 2005; EFSA, 2004; Cook and Devine, 2002). EFSA (2004) reported that there are currently two forms of electrical stunning.

i. Head-only electrical stunning.
ii. Head-to-body electrical stunning

#### 1.5.1.1. Electrical stunning parameters

During electrical stunning of animals, electric current of sufficient magnitude is applied transcranially in order to induce immediate loss of consciousness by inducing brain dysfunction. Knowledge of the relationship between electrical properties (current, voltage, resistance, energy) and the duration of application is vital to ensure the effective stunning of animals. The relationship between current(I), voltage(V) and resistance(R) is explained by Ohm's Law which states that the amount of current that flows through an electrical circuit is directly proportional to the voltage applied and inversely proportional to the resistance at a constant temperature.

Ohm's Law: $I \propto V/R$

$\therefore$ Ohm's Law: $I=V/R$

Note the following:

- Electric current is measure in Amperes (A).
- Voltage is measured in Volts (V)
- The unit for measuring resistance is Ohms (Ω)

This implies that the current flowing through a circuit (in this case a live animal) increases as the electromotive force (voltage) increases. However, certain parts of the animal such as thick skin or fat layers may act as opposing forces to the current, the opposing forces in an electrical circuit are referred to as resistance or impedance (in the case of AC). An increase in the resistance will decrease the amount of current flow. Some parts of the animal are poor conductors of electricity and will therefore slow down or divert the flow of electricity through the animal during stunning. These structures include the skull, hair, fat layers and thick skin. Water may be carefully sprayed in order to improve electrical conductivity, however, too much water may produce lower resistance pathways that can divert the current away from the brain and result in a painful electric shock without necessarily stunning them (Steve Wotton, Personal Communication, 2014).

#### 1.5.1.1.1. Frequency and waveform

The frequency of current is the number of cycle (waveforms) per second. If 1000 cycles of waveform occur in a second, then the frequency of current will be 1000Hz or 1kHz. Electrical energy or current may be generated as direct current (DC) or alternating current (AC). A waveform is the shape formed as a result of the rate of change in the flow of electrons through a conductor over time. There are different shapes or waveforms that may be generated. For example, a sine wave or sinusoid is formed when the AC voltage produced by an alternator are plotted graphically against the time over which the event occurs (see figure 3). Other waveforms include; triangle waveform, trapezoid waveform, square waveform etc.

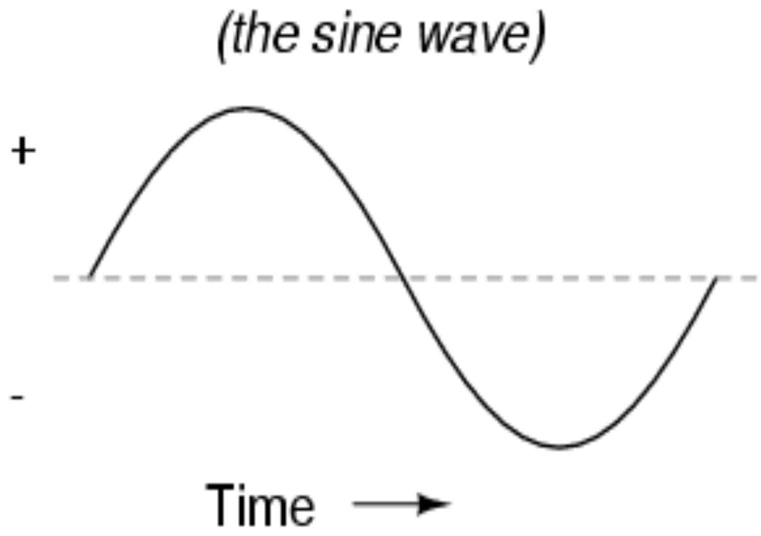



**Figure 3.** A graphical representation of an AC sine wave showing one complete waveform.

### 1.5.1.2. Head-only electrical stunning

In this method of electrical stunning, electric current is applied transcranially in order to produce unconsciousness. Although the animal is made unconscious and insensible to pain as a result of the stun, death does not occur, and the animal is able to make a full recovery if neck cutting or thoracic sticking does not occur. Gregory (1998) reported that head-only electrical stunning results in rapid reduction in heart rate during the course of current application but when the application of electrical current is halted, heart rate rises to above normal values.

The flow of electric current through the brain causes rapid depolarisation of neurons (brain cells) with the development of synchronised electrical responses. In a normal functioning brain, millions of neurons communicate with each other through the perpetuation of electrical signals produced by the controlled release of neurotransmitters into the brain. These neurotransmitters are released into the gap (synapses) between nerve endings resulting in the binding of the neurotransmitters with protein molecules (receptors) within the plasma membranes of brain cells (Farouk, 2013). The 'equilibrium' established in a normal brain by the controlled release of neurotransmitters is usually disrupted as a consequence of stunning. For instance, the levels of the neurotransmitters aspartate and glutamate are markedly increased as a consequence of the electrical stun, this results in the 'over-excitation' of brain cells which lead to epileptic-like seizures (Farouk, 2013). The nature and pattern of electrical signals generated during the state of 'over-excitation' is akin to EEG recordings during tonic/clonic seizures in man. Apart from aspartate and glutamate, another neurotransmitter, gamma amino 4-butyric acid (GABA) is known to increase during head-only electrical stunning. Cook *et al.*, (1992) linked the release of GABA to analgesia since the time profile of the release of GABA corresponds with the period of analgesia. In an experiment using sheep, it was concluded that sheep did not respond to external stimuli such as electrical stimulation of the teeth and pinching of the ears for >9 minutes after they were electrically stunned (Wotton and Gregory, 1998). The period of unresponsiveness to external stimuli represents a period of analgesia. The stimulation of the brain and spinal cord results in rigid muscular contraction (tonic phase). Tonic muscular contractions continue for a short duration after the application of electric current, usually for approximately 10s. This gives way to clonic contractions characterised by convulsions and the absence of rhythmic breathing throughout both tonic and clonic phases. Gregory (1998) explained that the convulsions during the clonic phase are as a result of brain dysfunction and not signs of consciousness. Both tonic and clonic muscular contractions are vital in assessing the effectiveness of the stun (Wenzlawowicz, 2006; EFSA, 2004). Another downside of head-only electrical stunning, apart from the short duration of unconsciousness is that slaughter operatives may sustain injuries due to the violent convulsions during the clonic phase and accurate sticking may be compromised. Wotton and Weaver (2009) reported that the Jarvis Beef Stunner, when used in Halal operations, may use low voltage electro-immobilisation to reduce post-stun convulsions. This is however contrary to EU welfare legislation (EC 1099/2009) since electro-immobilisation may mask the potential recovery of animals. To avoid the recovery of head-only electrically stunned animals, it is recommended that stunning must be followed immediately by chest sticking, this leads to dramatic loss of blood pressure in order to promote the death of the animal as soon as possible. The use of thoracic sticking revealed that blood pressure was reduced to near zero in about 8 s, however, a neck cut in calves produced times 60-180s when false aneurysms occurred (Anil, *et al.*, 1995). It is estimated that the shortest time for the return of breathing is about 37s (Anil et al, 1997) following effective head-only stunning of pigs and the maximum time between an effective thoracic stick in pigs and brain death is 22 s (Wotton and Gregory, 1986) so the recommendation is that sticking must be carried out within 15s (37-22) after stunning (Wotton, et al., 2003).

### 1.5.1.3. Head to body electrical stunning

This method of electrical stunning induces epilepsy in the brain followed by ventricular fibrillation (cardiac arrest) to ensure that the animal does not regain consciousness. This method of stunning has significant animal welfare and health and safety advantages over head-only electrical stunning. Gregory and Wotton (1984) suggested that where there is delayed bleed-out, this method ensures prompt and terminal cessation of the circulation of oxygenated blood to the brain and thus prevents the resumption of consciousness. The irreversible nature of head to body electrical stunning together with spinal discharge also ensures that post-stun convulsions, synonymous with head-only stunning, are prevented and a still animal results, which is safer and easier to work with. The induction of ventricular fibrillation also ensures that the possible bruising of carcasses during stunning and / or slaughter, e.g. during impact whilst rolling out from the stun pen, is reduced (Gregory *et al.*, 1988) and research has shown that overall bleed-out is not affected as result of ventricular fibrillation (Raj and Johnson, 1997, Gregory and Wilkins, 1989).

Wotton and others (2000) explained that fibrillation of the heart was achieved by the Jarvis Beef Stunner when a 550 V sinusoidal alternating current (AC) at 50 Hz using a choke limited current of approximately 3.5 A was applied between nose and brisket electrodes. In an attempt to explain cardiac dysfunction, Hollenben *et al.* (2010) identified the following as factors influencing the induction of ventricular fibrillation during head to body electrical stunning:

i. The frequency and waveform of electrical current used. There is less chance of fibrillating the heart when higher frequencies are used (Gregory *et al.*, 1991).
ii. The rate of current flow through the body.

iii. The pathways of current flow through the body.

iv. The region or part of the heart which receives the current

v. The stage in the cardiac cycle when the current is received.

vi. The species of animal being stunned.

This method of electrical stunning is not consistent with religious slaughter due to the fact that death will occur as a result of the induced ventricular fibrillation and not necessarily through bleed-out.

### 1.5.1.4. Limitations of electrical stunning

The use of electrical devices to stun/ kill animals prior to slaughter has often been criticised due to some drawbacks (Zivotofsky and Strous, 2012). The relatively short duration of insensibility induced by conventional electrical stunning systems, particularly in bovine animals has led to doubts about the effectiveness of this method of stunning. The duration of unconsciousness induced by the insult has been reported to vary between 40 and 60s (Daly and Warriss, 1986, Shaw *et al.*, 1990, Wotton *et al.*, 2000). This period of insensibility may even be shorter in other animals. Velarde *et al.*, (2002) reported that the best way to detect lamb regaining consciousness is to observe for the return of spontaneous breathing which often occurs around 29s after stunning. This presents a welfare problem since animals may recover before or during the neck cut or thoracic stick or even during the course of bleeding-out. Zivotofsky and Strous (2012) suggested that if the duration of the epileptic seizure in bovine animals is shorter (20-90 s) than the time taken for cattle to lose consciousness (up to 2 minutes) after the neck cut, then it implies that many animals would regain consciousness only to lose consciousness again as a result of exsanguination. It is against this backdrop that the authors concluded that the use of electrical stunning, particularly in beef must not be considered an effective method of stunning unless accompanied by ventricular fibrillation at the point of or, immediately after the stun.

Council regulation EC 1099/2009 stresses the need for slaughter operatives to be familiar with the instruction manuals produced by equipment manufacturers for the stunning systems they apply in order to ensure the efficient application of such devices. However, it has been suggested that even when applied by the most skilled and experienced operatives, electrical stunning systems have failed to effectively stun all animals (Stueber, 2000, Aichinger, 2003). This is consistent with the findings of a recent EU funded study on animal welfare concerns in relation to slaughter practices from the viewpoint of veterinary science (DIALREL report, 2010). In a study involving 23 cattle, it was found that head-only electrical stunning failed to effectively stun just over 39% (9) of the animals (Stueber, 2000). Kilgour (1978) demonstrated that when animals are mis-stunned or ineffectively stunned, the animals undoubtedly experience significant stress and anxiety as a result of the production, in large quantities, of the neurotransmitter, epinephrine. Epinephrine production is associated with all stunning methods and it has been shown to be produced in larger quantities during stunning than most environmental stressors (Warrington, 1974). The use of electrical stunning may also have a detrimental effect on carcass and meat quality which eventually affects the marketability and profitability of meat. Leach and Warrington (1976) gave a possible explanation to the incidence of blood splash in carcasses. They asserted that electrical stunning results in elevated blood pressures which force blood out of capillaries onto the carcass. Gregory (2004b) reported that there are four theories that have been put forward to explain the cause of the capillary ruptures that lead to blood splash.

i. Counteracting muscle contractions produced during stunning leads to the tearing of the capillary bed.

ii. Arteriolar dilation with engorgement of capillary bed, which would encourage the rupture of blood vessels when put under pressure.

iii. The blood vessels may be unduly fragile.

iv. During intense generalised muscle body contractions, such as those during electrical stunning, the venous and arterial systems experience severe external pressure.

In addition to the above constraints, there also appear to be variations in the effectiveness of electrical stunning systems. Over the years, scientists have attempted to identify the possible factors influencing these variations. Moreno (2004) found that seasonal variations affected the induction of ventricular fibrillation whilst Gregory (1993) reported that the cleanliness of animals affect the incidence of ventricular fibrillation because heavy contamination impedes the flow of electric current to the heart. Weaver and Wotton (2009) showed that the use of a prototype chest electrode in a Jarvis Beef Stunner improved the chances of fibrillating the heart.

## 1.5.1.5. Types of electrical stunners

There are a number of electrical stunning systems. Below are the various electrical beef stunners and their mode of operation.

### 1.5.1.5.1. The Jarvis Beef Stunner

The Jarvis Beef Stunner was first developed by Jarvis Equipment (NZ) Ltd as a head-only electric stunning system to cater for the Halal market (Weaver and Wotton, 2009). This system meets the Halal slaughter requirement because it is reversible. In the halal mode, the Jarvis Beef Stunner induces epileptic seizures in the brain without ventricular fibrillation. The stun is then followed by the application of low voltage electro-immobilisation to reduce post-stun convulsions to promote operator safety during slaughter and to restrain the animal during sticking and/or bleeding on a horizontal bleeding conveyor. However, the use of any immobilisation procedure during the slaughter of animals is inconsistent with EU welfare legislation (EC 1099/2009) since this may mask any signs of recovery. This makes the use of the Jarvis Beef Stunner with this configuration illegal in the UK and within the EU. Weaver and Wotton (2009) reported that a cardiac arrest cycle was therefore incorporated into the Jarvis Beef Stunner in order to meet EU legislation and welfare requirements (see figure 4). According to the authors, the welfare advantages of using the ‘adapted’ stunner are:

i. It eliminates the risk of animals recovering from the stun during bleed-out.
ii. Death is not dependent on exsanguination.
iii. The time interval between stunning and sticking and the accuracy of the cut is irrelevant.

Electric current is applied to induce a stun, cause ventricular fibrillation and promote spinal discharge in cattle. The 3 consecutive cycles of the Jarvis Beef Stunner as explained by Weaver and Wotton (2009) are as follows:

i. A 3s stun cycle. This involves the application of electric current (sinusoidal AC) between the nose-plate and the neck yoke electrodes in order to induce a tonic/clonic epileptic seizure in the brain.
ii. A 15s-cardiac arrest cycle. During this cycle, electric current is applied between the nose-plate and the brisket electrodes in order to induce ventricular fibrillation.
iii. A 4s spinal discharge cycle. Post-stun convulsions are markedly reduced by the application of electric current between the nose-plate and the rear of the animal where it makes contact with the metal work of the stunning box.

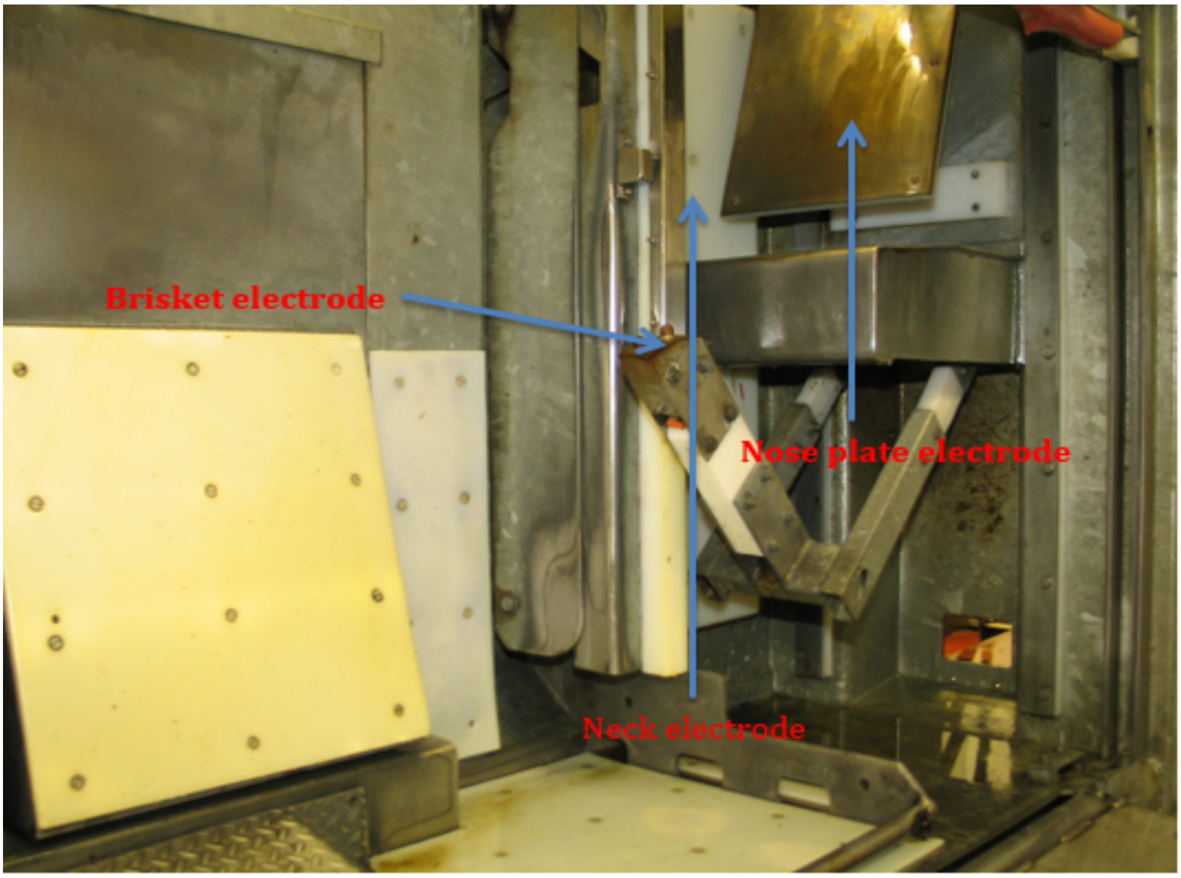



**Figure 4.** A Jarvis Beef Stunner showing the brisket, nose plate and neck electrodes.

Farouk (2013) outlined the modus operandi (Standard Operating Procedure –SOP) of the Jarvis Beef Stunner with Fixed Cradle Immobiliser for head-only electrical stunning used in New Zealand as follows:

- The animal is restrained in the stun box
- The stun button is then switched on which automatically applies the chin lift that helps maintain the head in position
- The nose electrode is then activated. Electric current is applied from the nose to the neck with the following parameters: 2.0-4.0 amp of current, 550V AC over duration of 3.5s. The stun button is continually applied until the pre-set current is achieved.

- The animal is then ejected and the neck-cut is made. It is recommended for the cut to be made within 10s of rollout in order to promote death through the loss of blood.
- Low voltage electro-immobilisation is then carried out whilst the animal is on the cradle. This is achieved by passing current through the body from the fixed electrodes to the head bars.
- The passage of current usually lasts for about 20s (pre-set).

Despite the popularity of the Jarvis Beef Stunner in some parts of the world, some meat quality issues have been reported with its application. There have also been reported variations in the incidence of the induction ventricular fibrillation. Blood splash in hindquarters as well as broken femur (thigh bone) have been highlighted with the use of the Jarvis Beef Stunner (Weaver and Wotton, 2009). Leach and Warrington (1976) explained that electrical head-only stunning results in increased blood pressure capable of forcing blood out of damaged capillaries into muscles which causes blood splash in carcasses. In a study to determine the effect of seasonal variations on the induction of ventricular fibrillation, it was found that there was a reduction in the incidence of ventricular fibrillation from 89% during the summer months to 69% in the winter (Moreno, 2004). This study was conducted in a single plant therefore further studies may be needed. It has also been shown that apart from seasonal variations, the cleanliness of cattle, especially heavy contamination in the brisket area can obstruct the passage of electric current to the heart for a successful ventricular fibrillation (Gregory, 1993). In an effort to avert the occasional poor electrical contact and resultant lack of ventricular fibrillation together with the meat quality problems associated with the Jarvis Beef Stunner, Weaver and Wotton (2009) replaced the brisket electrode with a prototype chest electrode with and without spinal discharge. ECG readings showed the successful induction of ventricular fibrillation in all animals and there was also marked reduction in the incidence of blood splash in the sirloin. However, there was no significant differences between the treatments on the incidence of broken femurs.

Slaughter operatives must be given adequate in-service training on the assessment of the signs of recovery, to ensure that ineffectively stunned animals or those recovering from the stun due to delayed sticking, are re-stunned with a back-up stunning device in line with the requirements of EC1099/2009. As part of the University of Bristol's Animal Welfare Officer (AWO) training programme, the following have been identified as a guide to recognising signs of ineffectively stunned animals. It is recommended that when any of these signs is noticed, a captive bolt gun must be deployed and followed immediately with sticking:

i. Movement (clonic activity) that continuous during and after hoisting, i.e. sign of an effective head only stun
ii. Continued muscle tone in the forelimbs, in the free hind leg and ears erect
iii. Rhythmic breathing after about 50s
iv. Movement seen as paddling of limbs
v. Raising of the head
vi. Recovery of consciousness

#### 1.5.1.5.2. The BANSS Stunning Box MGB

The BANSS Stunning Box MGB is a multifunctional cattle stunning box developed by BANSS Germany Meat Technologies (see figure 5). The box is fitted with breast and abdomen plates which provide support to the animal whilst it is restrained. The breast plate also acts as an electrode. The box is fitted with neck and nose electrodes through which current is applied to traverse the brain in order to induce epilepsy. The breast plate acts as an electrode through which current is applied to induce ventricular fibrillation. The animal is stuck whilst remaining restrained within the box and is subsequently ejected onto a bleeding conveyor prior to further processing. This stunning box may no longer be in commercial production.

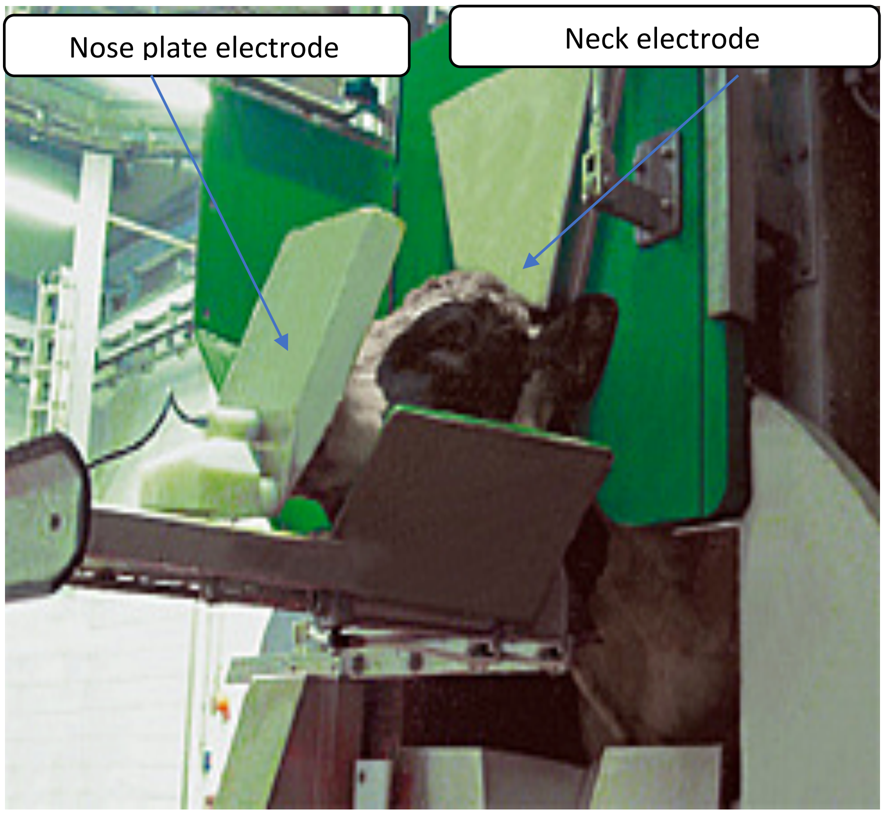



**Figure 5**. The BANSS Stunning Box MGB showing a restrained cow and the various electrode positions. (Source: http://www.banss.de/en/#slaughtering-technology-cattle-stunning/)

#### 1.5.1.5.3. The use of microwave energy

Electrical head-only stunning of animals is generally accepted by some Muslim groups during the Halal slaughter process because it is deemed recoverable, hence providing assurances that the animal is alive at the point of slaughter (Anil *et al.*, 2006). However, the inability of this technique to induce and maintain a longer duration of unconsciousness in cattle (Wotton *et al.,* 2000), has questioned the reliability of this method of stunning for reducing pain and suffering during the entire slaughter process (Zivotofsky and Strous, 2012). This has led to scientific investigations using microwave energy with the aim of inducing a period of unconsciousness long enough to maintain insensibility from the point of stun until death occurs. Animals stunned using this technique must be able to recover from the stun in order for it to be accepted by religious authorities or Halal certification bodies. Although not presently commercially available, patent applications have previously been filed pertaining to the use of microwave energy as a humane method of stunning pigs and poultry (Schwartz, 1974, 1976; Werner, 1976; Takamura and Ishida, 1997). This method of stunning uses microwave energy to generate electromagnetic fields within the brain to raise the temperature of the brain to levels that will induce unconsciousness without protein denaturation and tissue damage. The technique is similar to that experienced during thermal unconsciousness in conditions such as high fever and hyperthermia. Oshima *et al.*, (1992) showed that heat stroke and fever will occur when core body temperatures reach 40 to 45˚C, whilst Guy and Chou (1982) demonstrated that raising the brain temperature of rats by 8˚C caused unconsciousness which was maintained for 4 to 5 minutes. Therefore, the research objectives are to identify the optimum temperatures at which insensibility will occur which may be specie-dependent and ensuring that unconsciousness is immediate and sufficiently long enough to prevent the recovery of animals during bleeding out.

Microwave energy has successfully been used to euthanise chicken (Zeller *et al.,* 1989), laboratory rodents (Anon, 2001) and has also been utilised in the field of histology for the fixation of brain tissues (Moroji *et al.*, 1977). Over the last three decades, there has been extensive scientific research using microwave energy to induce unconsciousness in rodents such as mice and rats (Guy and Chou, 1982; Ikarashi *et al.*, 1984; Lambooy *et al.*, 1989; Delaney and Geiger, 1996; Cosi and Marien, 1998). The technique has also been used in recent years to successfully induce insensibility in larger animals such as sheep (Small *et al.*, 2013) and cattle (Johnson *et al.*, 2014). Small *et al.*, (2013) in an experiment using cadaver heads, showed that brain temperature could be raised to a level (approximately 44˚C) at which insensibility was likely. The authors subsequently used live sheep and were able to show that the application of microwave energy achieved brain temperatures of 43 to 48˚C in 20s. On the contrary, earlier experiments using pigs failed to achieve the desired results (Lambooy *et al.*, 1989). This may be due to the fact that at the time of this investigation, the use of microwave energy to stun animals was still in its infancy and there were no microwave generators capable of producing sufficient power to successfully stun larger animals (Ralph *et, al*., 2011). Ralph *et al.*, (2011) reported that recent developments have led to the discovery of more powerful equipment capable of generating a sufficient increase in temperature in the brain of cattle to induce immediate loss of consciousness. EEG readings have shown seizure-like complexes which are indications of unconsciousness therefore; microwave energy could possibly be used as a humane stunning technique if insensibility could be immediate and sustained until the death of the animal. This has led to the filing of patent applications for the use of microwave energy in the stunning of pigs and poultry (Schwartz, 1974, 1976, Werner, 1976; Takamura and Ishida, 1997). However, a lot of work is still needed to ensure that the technique meets animal welfare requirements. Small and others (2013) identified excessive surface heating as one of the major challenges, a situation that has been shown to be uncomfortable to animals based on previous studies on cats (Rice and Kenshalo, 1962).

#### 1.5.1.5.4. Single Pulse Ultra-High Current (SPUC)

This novel system of stunning has been reported by Robins *et al.* (2014). A reversible, method for head-only electrical stunning using a single-pulse ultra-high current (SPUC) generated by a capacitance current spike of a minimum voltage of 5000 V and a current of 70 A applied for 50 ms. It is suggested that the SPUC system would overcome some of the shortfalls of conventional electrical stunning. The authors proposed that this system will bring about the following improvements over existing electrical stunning systems for beef:

i. Increased duration of unconsciousness to prevent the recovery of animals during exsanguination. Conventional electrical stunning methods have been shown to have relatively shorter duration of unconsciousness (Daly and Warriss, 1986; Wotton *et al.*, 2000).

ii. Reduction or elimination of epileptic seizures to reduce post-stun convulsions with subsequent improvement in operator safety. Conventional electrical stunning systems must trigger epileptic seizure in the brain to be deemed effective (AVMA, 2013) but the SPUC system is hypothesised to employ the principle of electroporation, where insensibility is achieved through the opening of pores in neural membranes to produce brain dysfunction.

iii. Improvement in meat quality by reducing ecchymosis. Conventional electrical stunning has been shown to increase the incidence of blood splash in carcasses (Leach and Warrington, 1976).

Robins *et al.* (2014) outlined the experimental procedure as follows. Cattle were individually restrained in a head yoke, which also acted as one electrode of the electrical circuit. The modified stun box (Jarvis Engineering Technologies, New Zealand) was constructed with a nose plate and a neck electrode. This allowed the flow of current between the nose plate and the neck electrode to ensure that sufficient current was passed through the brain to induce insensibility. The stun was generated from a capacitance discharge producing a single or bipolar pulse/s of current generated by approximately 5000 V (voltage) at 70 Amps applied for approximately 50ms. Cattle were subsequently ejected after the stun onto a bleed table to monitor and evaluate post-stun events such as the duration of unconsciousness produced and any signs of early recovery. A captive bolt gun was used to re-stun cattle followed by slaughter once they exhibited any overt signs of recovery. The welfare and operational aspects of the SPUC were assessed through three progressive experiments.

Stage 1, denoted UHC1, involved the use of video recordings in typical abattoir working conditions to assess the effect and efficacy of the SPUC. The main focus of this stage was to measure the processing rate as well as behavioural assessment of reflexes.

In stage 2, there was modification of the waveform of the SPUC to produce two successive bipolar UHC1 discharges, hereby denoted UHC2. The primary aim of this was to increase the effectiveness of the stun.

Stage 3 involved the recording of EEG readings under laboratory conditions to objectively measure the physiological effects of UHC2. The researchers demonstrated that UHC2 can produce a stun that meets the criteria of an effective stun (EFSA, 2013). However, in some cases there were one or more reflexes immediately after the stun, although these reflexes were not indices of recovery. The implication is that the UHC2 parameters may need to be altered slightly in further trials in order to induce prolonged insensibility. It was also recommended that further studies were required to identify the optimal electrode positions and current path-ways although it appears there is no requirement for the power to be increased.

### 1.5.2. Mechanical stunning

As stated above (see Mellor and Littin, 2004), mechanical stunning of large animals probably started in the 15$^{th}$ century in China. Macnaughten (1932) threw some light on earlier systems of killing and slaughter of animals. The author reported that three main slaughter techniques were used in the nineteenth century; stunning by the use of a pole-axe, killing with the nape-stab and ventral neck incision. Slaughter by ventral neck-cut is currently practiced by both the Muslim and Jewish communities during Halal and Shechita slaughter respectively. Both the pole-axe and nape-stab methods were employed to immobilise animals in order to improve the safety of slaughter operatives and increase throughputs. However, the pole-axe technique was capable of inducing unconsciousness when applied with the thimble end of the pole-axe placed correctly on the forehead with sufficient velocity to cause concussion. Today, mechanical stunning and killing is achieved by the use of penetrative and non-penetrative captive bolt stunning (Anil, 2012, Blackmore and Delaney, 1988). The following are the types of mechanical devices used to stun cattle.

### 1.5.2.1. Penetrative and non-penetrative captive bolt guns

Gregory (2005) reported that captive bolt stunning causes concussion which results in neural dysfunction and the immediate loss of consciousness. The aim of both penetrative and non-penetrative captive bolt stunning is to induce unconsciousness through the transfer of kinetic energy to the brain through the differential acceleration of the head/skull and the brain to cause concussion. However, in the case of penetrative captive bolt stunning, there is physical damage to the brain because the steel bolt penetrates the skull to cause structural damage to the brain. Anil (2012) outlined the factors affecting the effectiveness of captive bolt stunning as follows:

i. The use of a suitable gun in good condition with the correct cartridge strength.
ii. The correct positioning of the gun on the head (specie dependent).
iii. The bolt velocity and impact on the head.

iv. Tissue damage

v. Penetration

vi. The amount of energy

EFSA (2004) recommended the disuse of non-penetrative captive bolt stunning in cattle because of concerns over its effectiveness. Council regulation EC 1099/2009 prohibits the use of non-penetrative captive bolt stunning in ruminants over 10kg. It is worth noting however that during Halal production in many non-EU countries (e.g. Australia, New Zealand etc), non-penetrative captive bolt stunning is still widely used on ruminants with no weight restrictions. The Australian Meat Processor Corporation (AMPC, 2016) commissioned a study to review the effectiveness of non-penetrative captive stunning on ruminants (including those above 10 kg). The report concluded that non-penetrative captive bolt stunning is a humane stunning technique irrespective of the size of the animal, provided the head is properly restrained and animals are stunned with high velocity stunners. The study further recommended the use of pneumatic stunners over cartridge powered ones because of the high failure rate of the latter. Concerns have also been raised about the risk to public health when animals are stunned or killed with captive bolt guns because they have been shown to transfer brain tissues to edible parts of carcasses (Anil *et al.*, 2002). Others have argued that there is no danger to public health (Steve Wotton, Personal Communication, 2015). Gregory (2005) in his review of concerns about stunning and slaughter, suggested that if further scientific evidence shows that captive bolt stunning contributes to the transfer of BSE prions beyond the lungs to edible carcasses, there would be a move away from the use of captive bolt stunning to electrical stunning.

Mechanical stunning is generally not accepted for use during Halal slaughter in the UK, because of doubts over its reversibility and the possible damage or injury to animals.

Farouk *et al.*, (2006) suggested that due to the doubts surrounding the use of mechanical stunning from a Halal perspective, such methods must be avoided. The Malaysian Halal Standard, MS1500 (2004, 2009) accepts mushroom head stunning on condition that the skull must remain intact after stunning. Any physical damage detected during post-mortem inspection of the skull will result in whole carcass condemnation as non-halal. In practice, sufficient velocity to produce an effective stun will always be associated with a depressed fracture of the skull. In conclusion, the use mechanical stunning should be discouraged during Halal slaughter since it does not fully comply with the Halal requirements.

### 1.5.2.2. Use of free bullet firearms

Firearms may be used to humanely kill animals particularly during disease control or emergency slaughter. In line with EU legislative requirements, emergency slaughter must be sanctioned by an Official Veterinarian if the carcass is intended for human consumption. Bovine over 2 years that are subjected to emergency slaughter and intended for human consumption must undergo Brain Stem Sampling to test for Bovine Spongiform Encephalopathy (BSE). In disease control situations, conditions may be different from conditions in an abattoir, for instance, animals may not be restrained in the field, therefore the accuracy of the shot is paramount. Galvin and others (2005) emphasise the need for the operative to be well trained in the operation, maintenance and marksmanship to be able to deliver an accurate shot to render animals instantaneously insensible. The mode of induction of unconsciousness using firearms is similar to that when penetrative captive bolts are used. The free bullet, when fired correctly (usually by trained marksmen), transfers kinetic energy to the head to cause differential movement of the head/skull and the brain to cause concussion and immediate loss of consciousness. The bullet subsequently penetrates the skull to cause gross physical destruction of the brain, this prevents the recovery of animals. When applied correctly, it is a humane killing method (Galvin *et al*., 2005). Due to the fact that free bullets can exit the carcass with sufficient force, the bullet can pose safety concern to operatives and other animals in close proximity. It has been suggested that firearms should not be used when animals are standing on hard surfaces/floors or in enclosed spaces, this is because of the risk of the bullet ricocheting off the skull or, solid objects and endangering operatives or other animals (Close, 1996; Appelt and Sperry, 2007). Galvin *et al*. (2005) noted that shotguns, rifles, and handguns of the correct cartridge strength, calibre and bullet specification can be used to humanely kill different species of animals. Free bullet firearms are best used in the field and it is the preferred humane killing method for horses (Blackmore, 1985; Oliver, 1979) and other species of animals. In a guidance booklet issued to farmers and veterinary surgeons by the British Cattle Veterinary Association (BCVA, 2005), 0.32 and 0.22 calibre rim-fire rifles were recommended for use during emergency slaughter, it is also recommended that for the purpose of health and safety of operators, people in close proximity during the use of fire arms must stand behind the operator.

#### 1.5.2.3. Use of shotguns

When applied correctly, the use of shotgun to kill animals has been shown to be a humane procedure. To ensure the humane death of animals and the protection of operators from injury, the type of shotgun and the distance it is held away from the animal's head are crucial. The Humane Slaughter Association (HSA, 2013) reported that during on-farm destruction of livestock, shotguns are recommended because they are safer to use than free bullet firearms, in that the shot/ammunition disperses within the head of large animals and that, in many cases, it lacks the ability to travel as far as free bullets. Further, according to HSA (2013) guidance notes, immediately after the shot, the animal should collapse and that post-stun convulsions are not indications of consciousness. The BCVA (2005) indicated that the muzzle of the shotgun must be held at least 5 to 10 cm away from the animal and that a 0.410, 12, 16, 20 and 28 bore shotguns with 4 to 6 birdshots are recommended. According to the BCVA (2005) guidance, holding shotguns directly against the head of the animal can cause injury to slaughter operators.

## 1.6. Slaughter of food animals

It is generally accepted that the slaughter of animals is a painful procedure (Ferguson and Warner, 2008). This is supported by scientific investigations into the pain associated with injuries (Gregory, 2004a; Mellor *et al*., 2008; Johnson et al., 2015) and it has also been reported that neck-cutting without any form of anaesthesia is likely to cause pain (Gibson et al., 2009; Mellor et al., 2009; Gregory *et al*., 2010; Nakyinsige *et al*., 2013). Animal welfare legislation such as EU regulation EC1099/2009 is drafted based on scientific research data in order to reduce pain and distress to animals before and during the slaughter process. For instance, the English domestic regulation, WATOK (2015) make it an absolute offence to slaughter any animal without pre-slaughter stunning to make it insensible to pain. However, an exception is made where an animal is slaughtered for religious consumption, mainly for Jews and Muslims. During the slaughter of animals, whether with(out) stunning, a sharp blade in the form of a knife or other mechanical blades must be employed to severe the major blood vessels that supply oxygenated blood to the brain in order to cause the death of the animal through a catastrophic fall in blood pressure and loss of blood. These blood vessels are usually located in the neck or chest regions of the animal. In the case of religious slaughter, a transverse neck-cut is made to ensure that the major blood vessels: carotid arteries, Jugular veins and the trachea and oesophagus, including all soft tissues ventral to the cervical vertebrae (Malaysian Halal Standard, MS 1500, 2009, HFA Halal Standard, 2014) are severed to ensure sufficient blood loss and death (Gregory, 2007). This method of slaughter, when used without pre-slaughter stunning or with short-acting head-only electrical stunning may have some welfare implications especially in the case of bovine animals. This is because cattle have an alternative route of blood supply to the brain through the vertebral arteries (Gregory *et al*., 2008) which are not severed during the ritual cut. Cattle may therefore remain conscious or regain consciousness during bleed-out. It has been demonstrated that an isoelectric EEG was present after 30-127s after both carotid arteries and jugular veins were severed 10s following head-only electrical stunning (Bager *et al.,* 1990). It must be reiterated however that despite the majority of scientific investigations concluding that neck incision can be painful without pre-slaughter stunning, not everyone agrees with this evidence. Levinger (1976) reported that the ritual cut can act as a stun due to the rapid blood loss which results in immediate death of animals however this has not been scientifically proven. Other researchers have suggested that animals showed no overt signs of pain when the ritual cut was made (Bager *et al*., 1992, Grandin and Regenstein, 1994). EFSA (2004) however argues that the fact that an animal shows little or even no reaction to the ritual cut does not necessarily imply that it does not experience pain, which could be due to the prey/predator response.

In most countries around the world, during conventional slaughter, animals are stunned in order to induce immediate loss of consciousness. This is usually followed by chest sticking (thoracic sticking) to ensure a rapid fall in blood pressure through loss of blood. This involves the incision of a sharp knife in the thoracic cavity in order to severe the brachiocephalic trunk and other major blood vessels in that area, which results in dramatic blood loss in order to cause death. Anil *et al*., (1995) showed that the use of thoracic sticking is a more efficient method of bleeding out than the ritual or neck cut.

## 1.6.1. Ritual (religious) slaughter

The slaughter of animals in accordance with religious beliefs remains a contentious issue (Grandin, 2012), this is due to the fact that many religious groups (mainly Muslims and Jews) demand that the slaughter of animals be done whilst they are fully conscious, i.e. slaughter without any form of stunning. The two main ritual slaughter methods of economic and welfare significance are therefore those practiced by Muslims (Halal) and Jews (Shechita) (Farouk, 2013). The exponential increase in the population of Muslims across Europe over the years and the corresponding rise in demand for meat slaughtered in line with religious traditions, highlights the economic significance of ritually slaughtered meat. There is also animal welfare concern surrounding religious slaughter, this is due to the increasing numbers of animals slaughtered without any form of stunning, a procedure which has been shown to be painful and compromises the welfare of animals (Ferguson and Warner, 2008; Gibson et al., 2009; Mellor et al., 2009; Gregory *et al*., 2010). As indicated above, it must be noted that some EU member states including England (WATOK, 2015) exempt religious slaughter from stunning. It has been reported that over 80% of animals slaughtered during Halal meat production in the UK are stunned (FSA, 2012, 2015). The FSA animal welfare surveys further indicate that there has been a recent increase in the number of animals slaughtered without stunning. Some Halal certification bodies and Muslim groups however vehemently disputed some aspects of the FSA's 2013 Animal Welfare Survey. In a statement released by the Halal Food Authority (HFA) (http://halalfoodauthority.com/press-releases/, 2015), they stated that although the survey indicated that 75% of cattle were stunned before slaughter, no Halal certification body in the UK recognises the use of captive bolt or other form of irreversible stunning for Halal beef because these methods of stunning contravene the Halal slaughter guidelines stipulated in the *Quran* and *Hadith*. The survey also highlighted the fact that all animals slaughtered in accordance with Shechita rules were not stunned.

There are similarities between Halal slaughter and that of Shechita. Animals must be healthy, clean and more importantly alive at the point of slaughter. In both methods of slaughter, a surgically sharp knife of appropriate size and length must be used in a single movement across the neck to sever the major blood vessels. The aim of the ritual cut is to stop the supply of oxygenated blood to the brain (Gregory, 2007) and this must be done by a practicing Muslim (HFA Halal Standard, 2014; MS1500/ 2009; Indonesian Standard, MUI HAS 23103, 2012) or an appropriately appointed Jew (Shochet). Blood is considered impure by followers of both faiths and it is therefore forbidden to intentionally consume blood in any form so there is a requirement during religious slaughter for blood to be drained out of the animal as far as practical.

#### 1.6.1.1. Halal slaughter

The dietary requirements for followers of the Islamic faith are laid down in the Holy Quran (the Islamic Holy Book) and the Hadith (The sayings or traditions of the Prophet Mohammed PBUH). However, there are differences in the way these scriptures are interpreted by Muslim Scholars which has often created confusion among Halal consumers and Halal food business operators as to what is permissible (Halal) or not (Haram). The increase in the number of unregulated Halal certification bodies has further compounded the problem since these certification bodies are in direct conflict making it difficult for consumers to identify which practices are more authentic in line with Islamic teachings. It is generally agreed that for meat to be Halal, the animal must be healthy, clean, and alive at the point of slaughter and that the slaughter person must be a practicing Muslim (MS1500 2009; HFA Standard, 2014; Indonesian Standard, MUI HAS 23103, 2012). However, there are disagreements among Muslims regarding other aspects of Halal slaughter. The pre-slaughter stunning of animals and the use of mechanical fixed blades to slaughter chicken have been strongly debated among Muslim Scholars. Also, although the Holy Quran clearly mentions the permissibility of Kosher meat for Muslims (Quran, 5:5), there are scholars who argue that the Quran is only referring to orthodox Jews hence Muslims can only eat meat slaughtered by orthodox Jews, whilst others completely rule out the consumption of Kosher by Muslims. The acceptability of stunning as part of Halal slaughter is now becoming popular and has gained recognition in countries where there is a Muslim majority such as Indonesia, Egypt, Malaysia, Turkey, Kuwait, Saudi Arabia, Yemen, Tanzania and the United Arabic Emirates. In the UK, the Halal Food Authority is the largest certifier of stunned Halal meat and certificates issued by this organisation are widely recognised across the globe. Other UK Halal certification bodies that accept pre-slaughter stunning include: Halal Consultations Ltd, the Halal Authority Board, Universal Halal Agency and the Institute of Islamic Jurisprudence. These organisations accept stunning based on scholarly advice and the only type of stunning accepted is reversible stunning. This is where an animal is able to make a full recovery from the stun if bleed-out does not occur. This type of stunning provides assurance that, although the stun induces a state of unconsciousness, this does not kill the animal. On the other side of the argument is the Halal Monitoring Committee (HMC). The HMC does not accept any form of pre-slaughter stunning as part of their Halal certification procedures. Opponents of stunning have always argued that all forms of stunning result in the death of animals before the ritual cut, however, a number of recovery trials have shown that some forms of stunning are reversible and does not result in the death of animals. They also believe that bleed-out is adversely affected as a consequence of stunning although extensive research has shown that there is no statistical difference between the amount of blood loss in stunned and non-stunned animals (Anil *et al*., 2004; 2006, Pleiter, 2004; Khalid, et al., 2015). There is also a myth that the stunning of animals before slaughter leads to inferior meat quality, however, research has also refuted this claim (Önenç and Kaya, 2004).

#### 1.6.1.2. Shechita slaughter

In terms of attention to detail, the Jewish method of slaughter, Shechita is arguably the most scrutinised method of slaughter. There is a list of requirements regarding the slaughter person (Shochet), the knife (Chalaf) and meat inspection. Regenstein *et al*., (2003) gives a detailed account of the requirements of Shechita slaughter. Religiously accepted species of animals are slaughtered in line with the Halakhah or Rabbinical law by a trained person of the faith, usually a Rabbi (Levinger, 1976). The slaughterman (Shochet) uses an extremely sharp Chalaf (figure 6) to severe the major blood vessels. The Chalaf is required to be straight and surgically sharp and the length must be at least twice the diameter of the neck of the animal (Levinger, 1976). The Shochet must be a practicing Jew with a good character and must undergo training before approval is granted by the Rabbinical Commission, England or the chief Rabbi in Scotland. A Shochet, in addition to the religious approval must also hold a Food Standard Agency certificate of competence (EC 1099/2009).

Animals destined to be slaughtered must be restrained appropriately, the method of restraint is not clearly defined by religious authorities although the legal guidelines can be found in EC Regulation (1099/2009) or WATOK (2015). The Shochet then offers a prayer or blessing to acknowledge the taking of a life and for God's mercy. The Chalaf must severe the major arteries and veins to ensure that there is sufficient blood loss to cause death. Before and after each slaughter, the Shochet checks the sharpness of the

Chalaf and the cut made must be inspected to make sure it meets the Shechita guidelines. If an anomaly is detected with either the knife or the cut, the whole carcass is rejected as spiritually unfit for consumption (Regenstein, 2003). After bleed-out and death of the animal, a Jewish meat inspector makes an incision through the abdominal wall in order to examine the thorax for any anomalies. Carcasses that exhibit any abnormal features are rejected as treife (Regenstein, 2003). The pre-slaughter stunning of animals before Shechita slaughter is unacceptable. Animal welfare survey conducted by the FSA in Great Britain (FSA, 2012, 2015) found that all animals slaughtered as Shechita were slaughtered without any form of stunning. It has however been reported that post neck-cut stunning with captive bolt is used in the UK during Shechita slaughter (Anil, 2012), a claim that is disputed by some quarters of the Jewish community (Shimon Cohen, Personal communication, 2014). The total number of animals slaughtered as Shechita compared to the numbers for Halal is relatively small. FSA (2015) reported that out of 44,216 cattle/calves slaughtered during the course of an animal welfare survey, only 475 (1%) were slaughtered as Shechita whilst 1,437 (3%) were slaughtered for the Halal market. During the same period, the survey also showed that 41% of sheep were Halal slaughtered against 1% by Shechita.

**Figure 6.** The special knife used for Shechita slaughter (Chalaf).

The length of the knife must be at least twice the width of the animal's neck (Grandin and Regenstein, 1994; Levinger, 1976).

#### 1.6.1.3. Animal welfare aspects of ritual slaughter without stunning

The practice of slaughter without stunning to make them insensible to pain remains a controversial issue from an animal welfare viewpoint (Grandin, 2010). It has also been demonstrated that the process is likely to cause pain to animals (Ferguson and Warner, 2008; Mellor *et al*., 2009; Gibson *et al*., 2009; Gregory *et al*., 2010). It is against this backdrop that the Humane Slaughter Act (1958) and EC 1099/ 2009 require the pre-slaughter stunning of all animals with the exception of animals slaughtered for religious reasons. Halal and Shechita slaughter require all animals to be alive and healthy at the time of slaughter and according to the teachings of both religions, a sharp knife must be used to severe the carotid arteries, jugular veins, trachea and oesophagus to ensure rapid blood loss and death. Some concerns regarding the welfare of animals slaughtered by the methods described above, particularly when carried out without stunning have been raised. The stress of the restraining method, the possible pain associated with the ritual cut itself, the likelihood that animals may experience undue distress during bleed-out and the long duration of time animals may take to lose consciousness are some of the concerns from animal welfare perspective (Gibson *et al*., 2009; Gregory, 2005; Grandin and Regenstein, 1994).

In terms of the method of restraint applied prior to slaughter, a poor system will lead to struggling which increases the likelihood of injurious and avoidable pain (Lambooij *et al*., 2012). Also, there are concerns over the use of some obsolete methods of restraint in some parts of the world such as the hoisting of conscious animals by the hind leg before slaughter. When animals are restrained in an upright position, there is the possibility that the animal may aspire blood into the lungs that may cause significant suffering (Gregory *et al.*, 2009; Von Wenzlawowicz and Von Hollenben, 2007). The restraining of animals may also mask the reaction of the animal to the ritual cut (Grandin and Regenstein, 1994). The ventral cut made on the neck of animals during ritual slaughter, particularly when carried out without stunning may be painful and animals may prolong the time to lose brain function (Gregory, 2008; Ferguson and Warner, 2008; Gregory *et al.*, 2010; Nakyinsige *et al.*, 2013). The time taken for animals to lose consciousness has been measured using electroencephalogram (EEG), Somatosensory Evoked Potentials and electrocardiogram (ECG), although this appears to vary (Gregory and Wotton, 1984; Gibson *et al.*, 2009; Gregory *et al.*, 2010; Daly *et al*, 1986.). Gregory and Wotton (1984) suggested that calves lose brain function promptly whilst Bager *et al.*, (1992) suggested that loss of brain function in other animals can take longer. In adult cattle for instance, the ritual cut may not be able to completely stop the supply of oxygenated blood to the brain because cattle have an alternative route through which blood can be supplied to the brain via the vertebral arteries which are left intact during the ritual cut (Gregory *et al.*, 2008). The authors also suggested that cattle may remain conscious during bleed-out as a result of blood clotting at the severed ends of the carotids brought about by false aneurysms thus restricting the loss of blood through the severed carotid arteries.

## 1.7. Previous studies on stunning and compatibility with ritual (Halal)slaughter

Concerns over the inability of CES systems to maintain insensibility until death supersedes (Daly and Warriss, 1986; Shaw *et al.*, 1990) and the possible negative impact of this method of stunning on meat quality (Gilbert and Devine, 1982; Kirton *et al.*, 1978) have led to the trial of different electrical parameters by animal welfare and meat scientists. The most common form of frequency of current used in conventional electrical stunners is 50 Hz sinusoidal AC. This leads to direct muscle stimulation which has been demonstrated to cause blood splash in carcasses, bruising and broken bones (Weaver and Wotton, 2009). Simmons (1995) reported that the use of higher frequency current could reduce the degree of direct muscle stimulation and therefore improve haemorrhaging, reduce the occurrence of broken bones. However, the required current to stun is higher with higher frequencies and the duration of epilepsy induced is relatively shorter and thus poses a welfare concern (Anil, 2012). For electrical stunning to be judged as effective, it must induce a tonic/clonic epileptic seizure in the brain (AVMA, 2013). However, Robins *et al.* (2014) have suggested that it may be possible to effectively stun cattle without inducing tonic/clonic epilepsy. A tonic/clonic epileptic seizure is a pathological condition of neural synchrony, shown to be inconsistent with normal neural function and thus consciousness (Cook *et al.*, 1992, 1995). It has been shown that when electric current of sufficient magnitude was applied for at least 200 milliseconds across the brain, there was an overstimulation of the release of neurotransmitters resulting in generalised epilepsy (Cook *et al.*, 1995). Wotton *et al.* (2000) demonstrated that cattle were immediately and effectively stunned when at least 1.15 A sinusoidal AC at 550 V and 50 Hz was delivered between the neck and nose electrodes for 0.7 s. Many researchers have attempted to explain the principles as well as investigate the efficacy of electrical stunning methods using different electrical parameters and slightly adapting existing stunning systems on different species (Wotton *et al.,* 2000; Weaver and Wotton, 2009; Cook and Devine, 2002; Bager *et al.*, 1992; Devine *et al.*, 1986, 1987; Lambooij, 1982a, b; Lambooij *et al.*, 1983).

## 1.7.1. High Voltage Electrical Stunning

For animals to be effectively stunned, sufficient electrical current must be passed through the brain in order to induced immediate loss of consciousness as a result of the disruption of normal brain function (MAFF, 1995). Council Regulation (EC) No. 1099/2009 specifies the minimum currents that must be applied in order to achieve an effective stun. Bovine animals less than 6 months old must be stunned with a minimum current of 1.25 A, for those aged 6 months and older, the recommended minimum current is 1.28 A. Wotton *et al.* (2000) demonstrated effective stunning of cattle when at least 1.15 A sinusoidal AC at 50 Hz was applied for 0.7s whilst Lambooij *et al.* (1983) used 1.25 A sinusoidal AC at 50 Hz to successfully stun calves.

Animals may be stunned using either low or high voltage electrical stunning devices. It has been shown that the use of a low voltage of 150 V combined with 1.5 A of current at 50 Hz is sufficient to successfully induce immediate loss of consciousness in calves (Gregory *et al.*, 1996; Lambooij *et al.*, 1983). In the case of adult cattle, some researchers have used high voltage electrical stunning devices to effectively stun the animals (Wotton *et al.*, 2000; Weaver and Wotton, 2009; Robins *et al.*, 2014). Robins and his colleagues (2014) investigated the efficacy of pulsed ultra-High current for the pre-slaughter stunning of cattle.

### 1.7.2. Electroencephalogram (EEG) and Electrocardiogram (ECG)

The development of electroencephalography (EEG) in the 1920s is credited to German neurologist, Hans Berger. EEG is a reflection of the electrical activity of a living brain (Kroeger *et al.*, 2013). Murrell and Johnson (2006) described EEG as a phenomenon which represents the functional activity of the brain. An isoelectric or flat EEG is a critical turning point between a living brain and one that is dead, this is usually encountered when human subjects are in a state of coma (Kroeger *et al.*, 2013). During coma, the brain is said to be in the lowest level of metabolism and neural activity, a state known to be inconsistent with consciousness. The electrocardiogram (ECG) on the other hand is a measure of the electrical activity of the heart. In the field of animal welfare science, EEG and ECG techniques are often used to measure indices such as the onset and duration of unconsciousness, the perception of pain, the induction of ventricular fibrillation, etc. during pre-slaughter stunning and exsanguination of animals. Knowledge of changes in amplitude and frequency of waveforms or the absence of electrical activity are vital. The procedure for the placement of electrodes and the recording of both EEG and ECG data are described by Gibson *et al.*, (2007, 2009). Both techniques have been used to measure various parameters during the stunning and slaughter of food animals. Gregory and Wotton (1990) investigated the loss of visual evoked potentials during non-penetrative captive bolt stunning. Other researchers have looked at the time to loss of sensibility and cardiac fibrillation (Blackmore and Newhook, 1982; Wotton *et al.*, 2000; Weaver and Wotton, 2009). The assessment of unconsciousness or death can be done by the reduction or absence of somatosensory, auditory and/or visually evoked potentials and the fibrillation of the heart. Although the absence of evoked potentials is generally seen as an indication of unconsciousness, the presence of evoked potentials may not necessarily denote a state of consciousness, this is because visually evoked potentials have been found in anaesthetised animals (Gregory, 1998; Zeman, 2001; EFSA, 2004) therefore, it is a test of residual consciousness.

Gibson *et al.*, (2009) investigated the electroencephalographic responses in 10 halothane anaesthetised calves using non-penetrative captive bolt stunning. The authors concluded that all animals showed an initial decrease in total power of the electroencephalogram (EEG), which is indicative of the loss of cerebrocortical function needed to maintain sensibility. Similar conclusions were drawn by Blackmore and Delaney (1988).

### 1.7.3. Effect of stunning on carcass and meat quality

The benefits of stunning from an animal welfare viewpoint cannot be underestimated. Stunning is usually carried out to reduce pain and distress during the neck cut and bleed-out (Gregory, 1998). In order to reduce carcass downgrading and its associated financial loss to the industry, the method of stunning used must, in addition to protecting the welfare of animals, have minimal or no negative effect on product quality. It is therefore essential to consider the impact of stunning on carcass and meat quality when assessing the efficacy of the method of stunning (Velarde *et al.*, 2003).

It has been reported that the stunning of animals prior to slaughter may have a detrimental effect on meat quality (Gregory, 1998). Immonen (2000) reported that the level of muscle glycogen at the time of slaughter is an important determinant of meat quality. Devine *et al.*, (1993) explained that stunning is likely to affect meat quality by increasing the stress level in animals which leads to the depletion of muscle glycogen resulting in elevated ultimate pH. The effect of stunning on the pH and the onset of rigor post-mortem has been extensively discussed (Gregory, 1994; Bilgili, 1999; Roth *et al.*, 2003). During stunning, the point of contact of the electrodes and the electrical parameters used may have some effects on carcass and meat quality. During high voltage head-to-back electrical stunning, there is high incidence of broken vertebral bones (Wotton *et al.,* 1992). This may be reduced by changing the waveform and the frequencies of electric current used in order to reduce the strength of muscle stimulation. Increasing the frequency of electric current could reduce the incidence of broken bones in the vertebral column (Steve Wotton, Personal Communication, 2014). However, higher frequencies have been shown to increase the danger of animals recovering from the stun during exsanguination and that such frequencies may fail to fibrillate the heart (Wotton *et al.*, 1992; Wilkins *et al.*, 1998; Mouchoniére *et al.*, 1999). Weaver and Wotton (2009) reported that in addition to inducing ventricular fibrillation, there was a reduction in the incidence of blood splash in the sirloin when the brisket electrode in a Jarvis Beef Stunner was replaced with a prototype chest electrode. Anil (2012) in a review of the effect of slaughter methods on both carcass and meat quality of sheep and cattle, suggested that the incidence of bruising and haemorrhaging may be reduced by observing the following:

i. Reducing the time interval between stunning and sticking in order to avoid the rupturing of blood vessels that cause blood to leak onto the carcass.
ii. Using captive bolt stunning instead of electrical stunning in order to reduce muscle spasm and blood splash.
iii. Applying electrical current continuously without interruptions during electrical stunning.

iv. Adopting stunning methods that induce ventricular fibrillation in lambs, this may reduce blood pressures and consequently the incidence of blood splash.

Blood splash (blood speckle, spotting, petechial haemorrhages, ecchymosis) is not a concern from a hygiene point of view, but because it detracts from the appearance of meat, it is an important problem economically (Steve Wotton, Personal Communication, 2015). The petechial haemorrhages occur in muscle but can be present in other tissues, e.g. fat. Despite considerable research into the subject, its cause has not been fully understood (Warriss, 2000). Gregory (2005) reports that there are four theories that have been put forward to explain the cause of the capillary rupture that leads to blood splash:

i. Counteracting muscle contractions produced during stunning causing tearing of the capillary bed.

ii. Arteriolar dilation with engorgement of the capillary bed, which would encourage the rupture of blood vessels when put under pressure.

iii. The blood vessels may be unduly fragile.

iv. During intense generalised muscle body contractions, such as those during electrical stunning, the venous and arterial systems experience severe external pressure.

Electrical stunning at low frequency (50 Hz) is known to result in direct muscle stimulation (Simmons, 1995). This direct effect on muscle fibres is significantly reduced if higher frequency waveforms (e.g. 1500 Hz) are used for pre-slaughter stunning for example with pigs (Simmons, 1995). Greater than 1500Hz abolished stun induced bone breakages, by reducing stun current induced muscle contractions. Blood splash in the forequarter was reduced.

Önenç and Kaya (2004) compared the meat attributes of cattle slaughtered without stunning to those slaughtered using a percussive non-penetrative captive bolt stunner and electrical stunning using a constant voltage of 400V and 1.5 amps applied for 10s. The authors demonstrated that meat quality parameters were as good or better when cattle were stunned electrically or mechanically compared to those slaughtered without stunning.

### 1.7.4. Effect of slaughter methods on carcass and meat quality

The way animals are handled pre-slaughter, during slaughter and the post-slaughter manipulation of carcasses may have a detrimental effect on product quality. The impact of pre-slaughter handling on animal welfare and product quality has been discussed in the preceding chapters (see chapter 2 above). The definition of meat quality can vary, and it is widely subjective. Meat quality may be described according to the physical, technological, chemical, safety, credence, appearance, table or eating and intrinsic or extrinsic properties of the meat in question (Troy and Kerry, 2010; Joo and Kim, 2011). Farouk *et al.*, (2014) in a comprehensive review of ritual slaughter methods (Halal and Shechita) and meat quality, described meat quality in terms of nutritional, functional, convenience, tactile, chewing or eating quality and the environmental impact attributes of meat. To some consumers, meat quality is defined to reflect religious ideologies, ethnicity, culture, packaging materials, information on a label, political and economic considerations (Ndu *et al.*, 2011; Thompson *et al.*, 2008; Korzen and Lassen, 2010).

Farouk (2013) argued that the ritual slaughter of red meat species should not have a more negative impact on meat quality in comparison with those slaughtered by conventional methods. Daly (2005) suggested that some carcass and meat quality issues are the result of the pre-slaughter stunning of animals whilst Gregory (2007) discussed the causes of blood splash (ecchymosis) in carcasses. As previously stated, blood splash makes carcasses unsightly leading to the downgrading of such carcasses and this affects the value and marketability of the meat (Gregory, 2005). The slaughter of animals without stunning should therefore reduce the incidence of blood splash in carcasses if blood splash in carcasses is attributable to stunning. Kirton *et al.*, (1980) looked at the incidence of blood splash in lambs in relation to the method of pre-slaughter stunning. The authors concluded that the severity of blood splash in lamb carcasses was less prevalent during non-stun slaughter and more pronounced in head-only electrical stunning. They arranged the severity of blood splash in the following (ascending) order: (a) no stun (b) percussion stunning (c) captive bolt stunning (d) head-to-back electrical stunning (e) head-only electrical stunning. Conversely, Velarde *et al.*, (2003) compared the impact of pre-slaughter electrical stunning on the quality of lamb against slaughter without stunning. They found no difference in quality parameters between electrically stunned lambs and those that were slaughtered without stunning. They concluded that the incidence and severity of blood splash in carcasses is not affected by the use of electrical stunning prior to slaughter. Other researchers have suggested ways of reducing the incidence of blood splash in carcasses during the pre-slaughter stunning of animals. Kirton *et al.*, (1978) and Anil (2012) have suggested that the incidence of blood splash in carcasses could be reduced by reducing the time interval between stunning and sticking. The use of minimum stunning currents, reduced stun to stick intervals and ensuring that there is good electrode contact with the animal could reduce blood splash and speckle bruising (Gilbert, 1993). In addition, the application of electric current during stunning must be done in an uninterrupted manner in order to reduce haemorrhaging in carcasses (Kirton and Frazerhurst, 1983). It has also been reported that the use of thoracic sticking within 10s after stunning reduced blood splash (Mulley *et al.,* 2010). In a review of the effect of slaughter methods on carcass and meat quality characteristics, Anil (2012) suggested that haemorrhaging could be reduced by using high frequencies of current with square waveforms instead of the commonly used 50Hz frequency with sinusoidal waveform. Robins *et al.*, (2014) demonstrated that a single pulse ultra-High current generated by a capacitance discharge of around 5000 V using 70 A of current to stun cattle. It is likely that SPUC will reduce or eliminate direct muscle stimulation – the major cause of meat quality defects.

An important aspect of slaughter from a religious perspective is to ensure that blood is drained from the carcass, this is because the consumption of blood is forbidden in Islam (Quran, 5:3) and in Judaism (Leviticus, 7:26-27, Leviticus, 17:10-14). Appropriate slaughtering procedures must therefore be adopted during religious slaughter to make sure the major arteries and veins are severed to ensure rapid and sufficient loss of blood in order to meet religious guidelines. Exsanguination is achieved through a ventral neck cut (ritual slaughter) or chest sticking. Anil *et al.,* (1995) demonstrated that the use of thoracic sticking is a more efficient method of exsanguination which results in a fast loss of brain function in cattle. However, this method of slaughter is not permitted during ritual slaughter. Although it has been reported by Farouk (2013) that thoracic sticking is sometimes employed 30s after the ritual cut in some parts of the world. Proponents of slaughter without stunning often argue that the pre-slaughter stunning of animals may have a negative impact on bleed-out efficiency and the retention of blood in carcasses. This view is supported by a recent study by Nakyinsige *et al.* (2014), in which higher blood loss was reported when rabbits of the New Zealand white breed were slaughtered without stunning compared to their counterparts which were gas killed before exsanguination (presumably due to the absence of a muscle pumping in rabbits that were killed in the gas). This is however contrary to research findings of bleed-out efficiency in sheep and cattle which have shown no difference in total blood loss (Anil *et al.*, 2004, 2006; Chrystall *et al.*, 1981). In a study involving 440 sheep, Khalid *et al.* (2015) showed that there was no significant difference in blood loss and carcass weight when sheep were slaughtered through three treatments: electrical head-only stunning, slaughter without stunning and post-neck cut electrical stunning. In this experiment, animals were restrained in a V-restrainer and slaughtered in either an upright or horizontal position. Some studies have even suggested that stunned animals lose more blood by volume than animals slaughtered without pre-stunning (Velarde *et al.*, 2003; Hopkins *et al.*, 2006). It can be concluded that blood is lost at slaughter due to gravity because venous pressure quickly falls preventing the passive refilling of the heart and although the heart may continue to beat for some time, it cannot pump blood out of the body. It has also been shown that any post-cut movement will aid blood loss through muscle-pumping (squeezing blood from vessels within muscle groups as they contract) and changes in posture.

### 1.7.5. Compatibility of pre-slaughter stunning with ritual (Halal) slaughter

European regulation 1099/2009 stipulates that all animals must be stunned before slaughter in order to reduce pain and distress. However, member states have the right to exercise derogation, which allows religious slaughter without stunning. The practice of slaughtering animals without stunning remains a controversial issue from animal welfare perspective (Grandin, 2010). This may cause animals significant distress particularly during restraint, the pain associated with the ritual cut itself, the latency of the initiation of unconsciousness and events such as the aspiration of blood into lungs during bleed-out may be painful (Gregory, 2005).

The debate surrounding the acceptability of stunning as part of Halal slaughter within the Muslim community is one that is likely to linger on. The fact remains that stunning is not mentioned anywhere in the Quran or Hadith so its acceptance (Halal) or rejection (Haram) is open to the interpretation of Islamic scholars. It is important to note however, that stunning is a relatively new technique that came into practice some centuries after the various Holy books (the Bible, the Tora and the Quran) were revealed. At the time the religious scriptures were revealed, electricity had not been discovered for use in the manufacture of electrical stunners and the idea of stunning animals mechanically had not been thought of. Opponents of pre-slaughter stunning during Halal slaughter vehemently argue that the slaughter of animals without stunning is more humane than slaughter with stunning and that there is no guarantee that animals are alive at the time of slaughter, which is an important theological requirement with respect to Halal slaughter. There is little scientific evidence to support the claim that slaughter without stunning may be humane (Bager *et al.*, 1992; Grandin and Regenstein, 1994). Some groups of Muslims have even suggested that the pre-slaughter stunning of animals during Halal slaughter is contrary to Shariah law because stunning adversely affects the efficiency of bleed-out and meat quality. This is however contrary to research findings which have shown that there is no statistical difference between the rate and volume of blood loss during slaughter with stunning and without stunning (Pleiter, 2004; Anil, 2004, 2006; Khalid *et al.*, 2015), neither is there any improvement in meat quality when animals are slaughtered without stunning (Önenç and Kaya, 2004).

Despite the refusal of some Muslim groups to accept stunning as part of Halal slaughter, the practice is becoming popular among the Muslim community in general, and more recently in Muslim populated countries such as Malaysia, Indonesia, Egypt, Saudi

Arabia, Qatar, Kuwait, the United Arab Emirates, Yemen, Tanzania etc. This is partly due to the fact that Muslim scholars in these countries are now well informed about stunning and there is a realisation through recovery trials (or demonstrations) that some methods of stunning are reversible, thus compliant with the prescribed Islamic slaughter requirements. Stunning is accepted as Halal on condition that it does not kill the animal before the ritual cut is made, therefore all Halal compliant methods of stunning must be reversible. Prominent Islamic scholars around the world are now issuing declarations or rulings (Fatwas) in support of stunning. Notable among the Fatwas issued in support of stunning include the following:

- Fatwa issued in 1977 in Saudi Arabia. This Fatwa was made to accept captive bolt stunning as Halal.
- A counter-Fatwa was issued in 1995 by Al Azhar University in Egypt to dismiss the one made in Saudi Arabia in 1977 because captive bolt may not be reversible and it is similar to delivering a heavy blow to the head which is contra to Islamic teachings (Quran, 5:3).
- Fatwa issued in 1978 by the Egyptian Fatwa Council at Al Azhar University. The Fatwa was made specifically to confirm the suitability of electronarcosis for Halal slaughter.
- Fatwa issued in 1987 by the Fiqh Council in Makkah, Saudi Arabia. This Fatwa was issued regarding reversible electrical stunning during the 10th session of the Islamic Fiqh Council at the Muslim World League held from 24th October to 28th of October 1987.
- Fatwa issued in 2006 by the Council for Legal Verdicts in Yemen. This Fatwa was made in reference to reversible electrical stunning.

It is clear from all the Fatwas issued in support of pre-slaughter stunning of animals during Halal slaughter that there is emphasis on the reversibility of the stunning method. Anil *et al.*, (2006) reported that head-only electrical stunning is generally accepted as Halal by the Muslim community all over the world. Fuseini *et al.,* 2017 carried out a survey of Islamic scholars and Halal consumers in the UK and reported that over 95% of the scholars and 53% of the consumers regarded meat from stunned animals as Halal provided the animals were alive before neck cutting. Despite the clear guidelines issued by Islamic scholars on the need for the stunning method to be reversible, it has been reported by Berg and Jakobsson (2007) that some Muslims in Sweden, in addition to using reversible electrical stunning, also employ stunning methods that result in the death of a high proportion of animals, such as captive bolt stunning. This practice is also prevalent in the UK (FSA, 2012, 2015). Table 1 shows the acceptability of stunning for Halal meat production by some UK Halal certification bodies (Fuseini et al., 2016). It also shows whether these HCBs are recognised by the major Halal meat importing countries (i.e. The UAE, Indonesia, Malaysia and Singapore).

| Halal Certification Body | Acceptance of stunning | Certificate recognition in major halal-importing countries | | | |
|---|---|---|---|---|---|
| | | UAE | Indonesia | Malaysia | Singapore |
| HMC | No | Yes | No | No | No |
| HFA | Yes | Yes | Yes | Yes | Yes |
| HAB | Yes | No | No | No | No |
| HCL | Yes | No | No | No | No |
| Assure-IP | No | No | No | No | No |
| AHDA | No | No | No | No | No |
| IIJ | Yes | No | No | No | No |

**Table 1.** The acceptability of stunning among UK halal certifiers and the recognition of certificates issued by these certifiers in the major halal-importing countries. (Adapted from Fuseini et al, 2016).

## 1.7.6. Compatibility of post neck-cut stunning with ritual (Halal) slaughter

Some proponents of animal welfare regard post neck-cut stunning as a better alternative to slaughter without stunning (Gregory et al., 2012; Anil, 2012). This is because the duration of consciousness is shortened if stunning is applied immediately after the neck incision (Casper and Koepernik, 2010; Binder, 2010; Gsandtner, 2005). Velarde and others (2010) recommended the time interval between the cut and stunning to be 5s in cattle and lambs, and that no further manipulation of the carcass should be done during this time. This is because severed nerves in the neck region of animals have been described by Gregory (2004a) to be able to precede signals for around 4s. Berg (2007) however practically measured the time interval between the cut and stunning (in cattle) to be in the region of 40s or more. The delay between the neck cut and stunning may be influenced by factors such as; the requirement of the religious authorities (Berg, 2007), the level of expertise and experience of the slaughter operatives, the temperament of the animal and the type and method of restraint used.

The advantage of this method of stunning from religious perspective is that it provides an assurance that the animal is alive at the point of slaughter, this, being one of the most important requirements for religious slaughter. Despite the assurance of a live animal at slaughter, opponents of stunning still regard this method of post-cut stunning as inconsistent with their religious doctrines. Anil (2012) reported that captive bolt was previously used in the UK after Shechita slaughter although the Jewish authorities no longer accept this. Halal authorities that accept pre-slaughter stunning generally approve post neck-cut stunning as Halal compliant. On the other hand, opponents of stunning during halal slaughter such as the HMC in the UK are opposed to post neck cut stunning and any form of stunning for that matter.

Table 2 is a compilation of the responses of some Halal certifiers in the UK on the acceptability of post neck-cut stunning (email communication, 2015). The authorities were asked whether they would approve post neck-cut stunning as Halal and their reason(s) or comments for its acceptance or rejection.

| Halal Certification Body | Acceptability of post neck-cut stunning as Halal | Reason(s)/comment(s) for acceptance or rejection |
|---|---|---|
| Halal Monitoring Committee (HMC) | No | The HMC does not certify any type of stunning and does not envisage certifying any type of stunned animals in the future |
| Halal Food Authority (HFA) | Yes | Post cut stunning is deemed a welfare measure, which meets the fundamental criteria for halal slaughtering. However, caution must be exercised to ensure that the neck cut is effective and accurate as per Zabiha requirements. Although the HFA recognises this practice as halal, none of their certificated slaughter plants currently employs this technique. All HFA slaughter plants use pre-slaughter stunning. |
| Halal Consultations Limited (HCL) | Yes | For a stunning procedure to be Halal, it must not injure or kill the animal prior to the cut, it must not also affect the efficiency and rate of bleed-out and it should not have adverse effects on the carcass quality and safety. There is no evidence to suggest that post cut stunning where the animal does not die or suffer prior to slaughter contravenes any of the Halal requirements. |
| Halal Authority Board (HAB) | Yes | HAB on the basis of Ijma (concensus) with 20 UK Scholars, have determined that post cut stunning is permissible and such meat is considered halal. Provided a recoverable stun method is used and that the animal is slaughtered by hand with a prayer said on each animal. |
| Universal Halal Agency (UHA) | Yes | The use of post cut stunning is seen as second best and it may be an alternative for slaughter without stunning. We are of the view that the animal must be stunned before slaughter since post neck-cut stunning still exposes the animal to some element of pain. |
| Assure-IP (AIP) | No | Assure-IP does not accept/ approve stunning by any means as part of Halal slaughter and production of Halal and Tayyab meat at any stage. |

Table 2. Responses of some UK Halal certifiers as to whether they approve post neck-cut stunning as Halal or not (Email communications, 2015)

## 1.8. The role of the brain in the control of conscious perception and death

Sections 1.8 to 1.8.6 form part of a peer reviewed publication (Fuseini, 2019) in Animal Welfare Journal (*Animal Welfare, 28, 165-171*) examining the role of the brain in the control of conscious perception and death. The paper further looks at how Halal is defined based on the two main definitions of death; cardiorespiratory and neurocentric deaths.

### 1.8.1. The Brain

The brain is one of the most important organs in both human and non-human animals. For the purpose of assessing and confirming death, the brain stem is one of the most important structures in the brain (Saposnik *et al*., 2009). It is positioned posteriorly to the brain and it consists of the medulla oblongata, the pons and the midbrain. Its main functions include; the control of breathing, circulation and digestion. The brain stem is also involved in the control of sensory and motor nerves. Damage to the brain stem or its permanent loss of function can be catastrophic in that the human or non-human animals could be diagnosed as dead (Conference of Medical Royal College and their faculties in the UK, 1976). Another structure of the brain of significance importance for the slaughter of animals is the cerebral cortex. It consists of about 80% of the brain and it is divided into 4 lobes; frontal, temporal, occipital and parietal lobes. Fischl *et al.* (2004) carried out an automatic parcellation of the cerebral cortex to identify the various lobes and specific points in the cortex and their functions. The authors identified the parietal lobe as an important structure for the control of consciousness. For the purpose of stunning and slaughter of food animals, the parietal lobe therefore plays a significant role. It houses the somatosensory cortex which processes sensory information and the motor cortex which sends out motor information. It has been reported that during penetrative captive bolt stunning, unconsciousness is caused through the transfer of kinetic energy from the bolt to the head which results in a differential movement of the skull and brain (Daly and Whittington, 1989). The subsequent penetration of the bolt into the skull and the gross destruction of parts of the brain prevents the recovery of animals (Gibson *et al*., 2012). Gibson *et al.* (2015) stunned alpacas (*Vicugna pacos*) with penetrative captive bolt guns and observed that contrary to previous findings in other species, unconsciousness in alpacas depended on the level of destruction of certain structures (direct physical trauma) in the brain and not on the differential movement of the brain within the cranium. The authors explained that successful stunning in alpacas was achieved by damaging the parietal and occipital lobes, the brain stem and the thalamus.

It must be noted that while behavioural indicators can be used under commercial conditions to assess consciousness/unconsciousness, it is difficult to diagnose death of animals subjectively under commercial conditions in an abattoir. This therefore makes it almost impossible for halal certification bodies to identify animals that may die on the slaughter line before neck cutting. The following two sections explain unconsciousness and death with regard to halal meat production.

### 1.8.2. Unconsciousness

Unconsciousness can be defined as the loss of sensibility or awareness. When used in relation to the slaughter of food animals, stunning is usually employed to induce unconsciousness through the disruption of neural communication, this can be followed by neck-cutting to ensure prompt and sufficient blood loss and death (Anil, 2012). Terlouw *et al.* (2016a) reported that during slaughter of animals, unconsciousness usually precedes death regardless of whether animals were stunned prior to neck-cutting or not. The authors explained that during slaughter without stunning, the loss of blood for a certain period of time induces unconsciousness and subsequently death, whilst stunning prior to neck-cutting can induce immediate loss of consciousness (e.g. during electrical or mechanical stunning) or progressive loss of consciousness (during controlled atmosphere stunning). Neural communication and the mechanism of induction of unconsciousness during stunning has been widely reported (Anil, 2012; Fuseini *et al*., 2018). Kam & Power (2012) explained that the brain is made up of billions of cells (neurons) and that these neurons communicate between each other via the transfer of chemicals (neurotransmitters) from one cell (pre-synaptic neuron) to the other (post-synaptic neuron) in a synchronised manner. Any intervention which results in the disruption of the equilibrium of neurotransmitters (e.g. the passage of electricity through the brain) can cause brain dysfunction and the induction of unconsciousness. Raj (2003) explained that neurotransmitters are categorised into excitatory (e.g. glutamate) and inhibitory (e.g. GABA-gamma amino butyric acid) amino acid neurotransmitters and that slight changes in the equilibrium of these chemicals can lead to arousal and depression. Cook and colleagues (1995) reported that the application of 1 Amp of current for 4 s (to the brain) was capable of disrupting the equilibrium established by excitatory-inhibitory neurotransmitters to induce unconsciousness in sheep. Mechanical stunning (e.g. penetrative and non-penetrative captive bolts) on the other hand induces unconsciousness by concussion which results in local mechanical damage and subsequent metabolic dysfunction, including neurotransmitters, calcium homeostasis, ATP depletion and other changes (for a review, see Blyth & Bazarian 2010).

There is sufficient evidence to suggest that, when applied correctly, stunning is a reliable means of rendering animals unconscious (Wotton *et al*., 2000; Gibson *et al*., 2009; Robins *et al*., 2014; Wotton *et al*., 2014). It is however important to ensure that animals are continuously monitored after stunning and throughout the bleeding-out period to ensure that they are effectively stunned, this must be maintained until death supervenes through sufficient blood loss. Berg *et al* (2013a) noted that an effective electric stun results in tonic seizure in the brain. In birds, this is characterised by stiffness of the neck, with wings held tightly in close proximity of the body. The authors noted further that after an effective electric stun, there is absence of breathing, fixed eyes and the absence of vocalisation and corneal reflex. Effective captive-bolt stunning of cattle results in fixed eyes and the absence of palpebral, corneal and pupillary reflexes (Berg 2013b). Under laboratory conditions, unconsciousness can be measured using electroencephalogram (EEG) or electrocorticogram (ECOG) (see Lambooij, 1994; Anil *et al*., 2000). An ineffectively stunned animal will vocalise, show amongst other things, spontaneous blinking, presence of righting reflex, failure to lose posture, the presence of rhythmic breathing.

However, despite the overwhelming evidence that stunning is capable of inducing unconsciousness to abolish the pain associated with the neck-cut, many religious authorities are still insistent on animals being slaughtered without any form of stunning. Animal welfare surveys carried out in licensed abattoirs in Great Britain by the UK's Food Standards Agency (FSA, 2012; 2015) indicated that all animals slaughtered during Shechita (slaughter by Jews) were not stunned whilst the majority of halal meat produced in Great Britain was derived from animals stunned before slaughter. Of animal welfare concern is the 20-30% and 100% of animals slaughtered without stunning during halal and shechita slaughter respectively. In their survey of the attitudes of Islamic scholars and halal consumers towards stunning, Fuseini *et al.* (2017b) reported some scholars to be of the view that the animal must be conscious at the time of neck-cutting to be able to hear the tasmiyyah (a short prayer) being recited just before or during neck-cutting. It must be reiterated that the Islamic scriptures are consistent in the requirement for animals to be alive at the time of neck-cutting, however, there do not appear to be any *Quranic* verses or other scriptures requiring animals to be conscious. Proponents of stunning for halal meat production are of the view that since the scriptures only require animals to be alive (not conscious) at the time of neck-cutting, stunning is permissible as long as it does not result in the death of animals before neck-cutting (MS1500, 2009; MUI HAS 23103, 2012; HFA, 2014; Fuseini *et al.*, 2017b).

### 1.8.3. Death

Advancement in the field of neuroscience has led to refinement in the ancient cardiorespiratory (death based on the absence of a heartbeat) based definition of death to a neurocentric (death based on irreversible loss of brain function) one (Laureys, 2005). Laureys (2005) pointed out that the first person to suggest neurocentric diagnosis of death was a medieval Judaism intellectual by name Moses Maimonides (1135-1204). According to Laureys, Maimonides argued that convulsions in decapitated humans did not signify the presence of central control despite the presence of a beating heart. However, Orban *et al.* (2015) implied that care must be taken when defining death based on the 'brain' because it can sometimes be misleading to the family of the dead and they may interpret it to mean there is a difference between brain death and actual death. Further, the presence of spinal reflexes in braindead 'patients' can cause distress to family members who may not agree with the diagnosis of death due to the presence of limb movement. Earlier application of neurocentric diagnosis of death was probably done in Europe in the 1950s (Wertheimer *et al.*, 1959), since then, advancement in neuroscience has led to refinement in the procedure. Despite these advances in neuroscience, there is still no universally agreed criteria for the assessment of death, and there are differences in the way different countries define death. Wijdicks (2002) carried out an extensive literature review of the criteria for the assessment of death in 80 countries and concluded that ten of these had no formal guidelines on the assessment of death. Further, the author reported that whilst the United States of America and Canada define death as the irreversible loss of function of the entire brain (including the brainstem), the United Kingdom and some EU member states define death as the irreversible loss of function of only the brainstem and not the entire brain. In many parts of the world, when an adult 'patient' is suspected of being brain dead, confirmatory tests are mandatory whilst this is optional in some parts of the world including the United States. Wijdicks (2010) reported that these confirmatory tests are categorised into 2; the first involves assessing the electrical activity of the brain whilst the second involves measuring cerebral blood flow. Electroencephalographic (EEG) recordings are useful in assessing the electrical activity of the brain or the absence of cerebral hemisphere function (Plum and Posner, 1972) whilst brain blood flow tests can be done with magnetic resonance angiogram, transcranial Doppler ultrasonographic scan, CT angiogram and others. As stated above, it must be emphasised that from a halal slaughter point of view, it is virtually impossible to assess whether an unconscious (stunned) animal has died or otherwise prior to the neck-cut under commercial conditions. It is therefore vital that stunning systems approved for halal slaughter must not affect normal cardiac rhythm, further, such stunning systems should not cause physical damage to the brain (see Halal Food Authority Standard, HFA 2014).

Similar to the medical profession, there appear to be no universally agreed definition of death within the Islamic scholarly fraternity. Pernick (1988) reported that in ancient Egypt and Greece, the presence of a beating heart was associated with vital spirits, and death was diagnosed based on the absence of a beating heart. Despite the advancement in neuroscience and the redefinition of death in the medical field in recent years, it appears some communities still define death based on the absence of a beating heart. Grandin (2015) indicated that some religious authorities define death of animals as the absence of a heartbeat, this view is shared by Fuseini and others (2016b). This is unsurprising when you consider that at the time the religious scriptures were revealed over 1400 years ago, neurology was either not a branch of science or it was still in its infancy, and death would not have been defined in terms of the irreversible loss of function of the brain stem at that time. The failure of Islamic scholars to agree a unified definition or assessment of death has meant that some halal certification bodies accept irreversible stunning methods (e.g. penetrative and non-penetrative captive bolt stunning, controlled atmosphere stunning etc.) as long as there is a beating heart in the animal. Grandin (2015) however pointed out that if death is defined based on the absence of a beating heart, then both penetrative and non-penetrative captive bolt stunning may be used for halal because the heart can continue to beat for 8 to 10 minutes after stunning (although a high proportion of animals would be brain dead). Fuseini *et al.* (2016b) looked at the different criteria used by halal certification bodies to identify and remove animals that may die before the ritual cut and the authors concluded that the criteria used by the halal authorities were not reliable in assessing death. Islamic scholars need to agree a more reliable definition of death. However, even if they decide to adopt the medical definition of death based on irreversible loss of brain function, assessing the death of animals as a result of stunning, prior to neck-cutting will be impossible under commercial condition. A more practical definition will be the one suggested by Jerlstrom (2014). Jerlstrom suggested that death of an animal from animal welfare perspective should be defined as irreversible loss of brain and cardiac functions.

### 1.8.4. Halal-compliant methods of stunning based on the definition of death

Historically, and up until the 19th century, Muslims and non-Muslims usually slaughtered sheep and goats without any form of stunning, however in some countries, the use of the poleaxe to stun cattle and pigs was common. Proponents of religious slaughter without stunning insist that this method is of high spiritual significance because it is the method that was practiced by the Prophet of Islam (Farouk *et al*., 2014). Others argue that at the time of the Prophet, stunning had not been discovered so he could not have used a technology that was not in existence (Fuseini *et al*., 2016a). Farouk, Pufpaff and Amir (2016) reported that for stunning to comply with the halal rules, 3 main criteria must be met; the animal must remain alive before neck-cutting, the stunning itself must not be painful or cause any distress and the stunning method must not adversely affect the volume of blood loss. Yaqoob (2010) explained that the religious scriptures emphasise *Ihsaan* (humaneness or proficiency) during halal slaughter, therefore if stunning is objectively shown to reduce or abolish the pain associated with neck-cutting, then it should be promoted for halal meat production. Proponents of stunning for halal meat production generally accept head-only electrical stunning (Anil *et al*., 2006) because these methods of stunning do not usually affect normal cardiac rhythm and are therefore unlikely to cause the death of animals before neck incision due to the continued supply of oxygenated blood from the heart to the brain. However, depending on the definition of death adopted by Islamic jurists; whether based on neurocentric or cardiorespiratory death, irreversible stunning may be acceptable to some Muslims. Where death is defined based on cardiorespiration, both penetrative and non-penetrative captive bolt stunning may be used for halal meat production because they will not stop the heart. As highlighted above, this is because although these methods of stunning may result in death, the heart can keep pumping for up to 10 minutes. In fact penetrative captive bolt stunning is widely used in some parts of Europe during halal meat production (Berg and Jakobsson, 2007; FSA, 2012; 2015) and the use of non-penetrative captive bolt stunning is also approved for the slaughter of cattle by some Muslim-majority countries such as Malaysia (see Malaysian halal standard MS1500, 2009) and the Gulf Cooperation Council, which includes Saudi Arabia, UAE, Qatar, Bahrain, Oman and Kuwait (see GSO 993 halal standard). The author of this paper previously worked in halal certification in the UK and is aware of the use of controlled atmosphere stunning for the slaughter of halal poultry in Germany and the Netherlands. Some Halal certification bodies which approve irreversible stunning do so on condition that there is a beating heart whilst those who approve reversible stunning are of the view that the animal must be alive at the time of neck cutting. In some countries, demonstration of the reversibility of the stunning method is required by the halal certification bodies (e.g. Halal Food Authority, UK) before approval.

### 1.8.5. Animal welfare implications

The lack of clarity on halal slaughter rules has meant that thousands of animals are slaughtered without stunning, with the belief that this method of slaughter is of the highest spiritual quality because it is the only method that was practiced by the Prophet of Islam. There are increasing numbers of Islamic jurists who approve stunning on condition that the method of stunning must not cause the death of animals prior to exsanguination. However, among the proponents of stunning, there is a disagreement as to the meaning of death. Those who define death based on the absence of a beating heart continue to approve all forms of stunning, including reversible and irreversible methods of stunning. On the other hand, those who base their definition on irreversible loss of brain function are of the view that irreversible stunning methods are not acceptable because they will eventually cause the death of animals, albeit not always instantaneously.

To safeguard the welfare of animals during halal slaughter, Islamic jurists need to agree on the rules of halal slaughter. If, indeed, the halal rules do not require animals to be conscious during exsanguination, then some forms of stunning may meet the requirements of halal slaughter (e.g. electrical head-only stunning) in order to protect animal welfare. Islamic jurists also need to agree a definition of death so that there can be clarity on acceptable methods of stunning for halal meat production.

### 1.8.6. Conclusion

The requirements of halal slaughter continue to confuse meat processors, animal science researchers and halal meat consumers due to differences of opinion regarding some aspects of the rules. The rules require animals to be alive at the time of neck-cutting but there appears to be no consensus on the correct definition and assessment of death within the Muslim community. At the time the religious slaughter rules were written some 1,400 years ago, no one knew about the function of the brainstem so death was probably defined based on the absence of a beating heart. However, advancement in the field of neuroscience has meant that death in humans is now defined based on the irreversible loss of function of the brain. Whilst some Muslims have overcautiously approved head-only stunning because it neither causes brain death nor cardiac arrest, others have opted for penetrative and non-penetrative captive-bolt stunning as long as there is still a beating heart prior to neck-cutting. On the other side of the debate are those who insist on approving the slaughter of conscious animals although the scriptures do not appear to command Muslims to slaughter animals while they are fully conscious. Slaughter without stunning was the method used by the Prophet of Islam, but one may argue that at that time electricity had not been discovered so there was no way the Prophet would have been able to use electrical stunning and, at that time, mechanical stunning was yet to be discovered. Proponents of halal stunning need to agree on a unified definition of death, in order to provide clarity as to which methods of stunning should be approved for halal meat production. The current situation has resulted in several halal standards which confuses halal consumers and abattoir operators as to the true definition of halal slaughter.

# 2. The perception and acceptability of pre-slaughter and post-slaughter stunning for Halal production: The views of UK Islamic scholars and Halal consumers.

**Linking Narrative**: This chapter is a published peer-reviewed paper (Fuseini *et al.*, 2017) in Meat Science Journal (*Meat Science, 123, 143-150*). The aim of the paper was to examine the level of understanding and acceptance of pre-slaughter and post-slaughter stunning among Halal consumers and Islamic scholars in the UK. Islamic scholars are the main decision makers with regard to the approval or rejection of stunning for Halal meat production, therefore their opinion is important. The scholars were recruited mainly from mosques (places of worship for Muslims) and madrasas (Islamic schools). The scholars' approval of electrical head-only stunning of cattle for Halal slaughter is vital to the development of the SPUC stunner, thus why this study was carried out and forms part of this thesis. Interestingly, the results showed that over 95% of Islamic scholars and 53% of Halal consumers would accept stunning if the main cause of death was exsanguination.

## 2.1. Introduction

Animals slaughtered for Muslim consumption must meet specific requirements laid down in the Islamic Holy Book, the Quran, and the teachings of the Prophet contained in the various Ahadith (Grandin & Regenstein, 1994; Regenstein *et al*., 2003; Nakyinsige *et al*., 2013; Fuseini *et al*., 2016). These Islamic dietary laws prohibit the consumption of meat (and by-products) from carnivorous animals, pigs, animals that die naturally and all forms of intoxicants (MS1500, 2009; HFA, 2014). The prohibited products are collectively referred to in Arabic as Haram products. For meat to be considered Halal (permissible), the animal must be a permissible species, handled sympathetically prior to and during slaughter, must be alive at the point of slaughter and the person bleeding the animal must have attained the age of discretion (MS1500, 2009; MUI HAS 23103, 2012; HFA, 2014; HMC, 2016). It is preferred that the slaughterer be Muslim, however, the Quran permits Muslims to consume meat slaughtered by Christians and Jews (Quran 5:5). Of paramount importance within the Halal slaughter requirements is that the animal must be alive during the ritual cut (Quran 2:173, 5:3; Regenstein *et al*., 2003; Masri, 2007; Fuseini *et al*., 2016). In many industrialised countries, there is a requirement for the pre-slaughter stunning of animals in order to render them insensible to the pain associated with slaughter (EC 1099/2009; WATOK, 2015). However, some Muslims have questioned the compatibility of pre-slaughter stunning with Halal production (EHDA, 2016; HAIP, 2016). Whilst some Muslims agree that animals may be stunned before slaughter (MS1500, 2009; MUIHAS 23103, 2012), others are of the view that stunning is contrary to the Islamic food laws (HMC, 2016). Proponents of pre-slaughter stunning for Halal production usually accept 'reversible' stunning (HFA, 2014; MS1500, 2009; MUI HAS 23103, 2012), this is where a stunned animal can make a full recovery if bleeding-out does not occur, thereby demonstrating that the death of reversibly stunned animals is caused by blood loss following neck cutting (EBLEX, 2009; Wotton *et al*., 2014). Due to the differences of opinion that exist within the Muslim community regarding some aspects of Halal slaughter, and the lack of understanding of the requirements of other religious slaughter methods such as Shechita, a European Commission funded project, DIALREL (2010), looked at ways of promoting good religious slaughter practices and how to promote dialogue among key players in the industry for the protection of animal welfare. The Halal Monitoring Committee (HMC), the UK's largest certifier of un-stunned Halal meat, conducted an online survey through Ajax Consultants to understand the acceptability of pre-slaughter stunning among Islamic scholars in the UK (HMC, 2009). They concluded that 90% of the 282 scholars rejected electrical stunning of poultry whilst 9% asked for further research to be done on the subject. On whether electrical stunning of large animals was compliant with Halal slaughter, 85% of the scholars said no. In Belgium, a study conducted by animal welfare group GAIA (2010) reported that 21% of the 261 Muslims surveyed said they prefer stunned Halal meat, 30% were identified as neutral, 36% rejected stunning whilst 49% disagreed with stunning for Halal slaughter. This study provided a platform for researchers to interact with Islamic scholars and Halal meat consumers to get a better understanding of the Halal slaughter requirements and the differences that exist among Islamic jurists in the interpretation of the Islamic dietary laws in the UK. There were also discussions on pre-slaughter stunning of food animals for Halal production, and whether simple stunning, defined in European Council Regulation, EC 1099/2009 as a method of stunning that does not result in instantaneous death, may be accepted for Halal slaughter. The acceptability of post-cut stunning was also covered in the study. Post-cut stunning is where the slaughter cut is made on a conscious animal, but the animal is then immediately stunned to prevent further suffering due to the cut and exsanguination.

## 2.2. Materials and methods

### 2.2.1. Data collection

Data were collected from October 2015 to March 2016 using the SurveyMonkey online software and questionnaire web service. Respondents were recruited through telephone calls, emails, and through the use of the Snowball sampling technique, this is where the respondents, after taking part in the study, further distributed the research questionnaires among their family and friends who also take part in the study. We contacted mosques, Islamic centres, Islamic schools (Madrasas) and Muslim Associations through email and telephone communication. Two types of questionnaire were administered; a questionnaire for Islamic scholars and a Halal consumers' questionnaire. Questionnaires were completed remotely (through a web-link or by completing hard copies) or through face-to-face interviews. The study was approved by the University of Bristol Ethical Review Board (ID 26164).

### 2.2.2. Questionnaire development

Draft questionnaires were pilot-tested on 20 respondents at a mosque in West London to ensure that the questions were easy to understand and interpret. The respondents were 6 female and 14 male with varying levels of formal education. The draft questionnaires were slightly modified following the pilot survey, as it was found that many respondents did not actually understand some of the technical terms such as 'stunning', used in the draft questionnaire, open-ended questions. The survey used only closed questions with the exception of two final questions in which respondents were provided a free-text space for comment. In order to get an understanding of the requirements of Halal slaughter and relate it to stunning, the nature of the questions in the two questionnaires (scholar and consumer) were grouped into two parts, the first section was related to the conditions that must be met for meat to be classed as Halal.

The second part was concerned with evaluating the understanding of Islamic scholars and Halal consumers regarding pre-slaughter and post-slaughter stunning and whether reversible stunning (for pre-slaughter) and post-cut stunning may be accepted for Halal slaughter.

### 2.2.3. Interviews with Islamic scholars

Efforts were made to interview all Islamic scholars in-person. Out of 129 mosques and other Islamic organisations contacted to arrange meetings with their scholars, 29 mosques, 15 Islamic centres, 6 Islamic schools (Madrasa), 3 Halal Certification Bodies, 1 Muslim chaplaincy of Her Majesty's Prison and 1 umbrella group for mosques agreed to participate in the study. One organisation emailed to say they did not want their scholars to be interviewed and 73 organisations did not respond to our requests after at least 2 email or telephone reminders. A total of 66 scholars from 17 UK cities or towns were surveyed, 49 (74%) of them were interviewed through face-to-face meetings whilst the remaining 17 (26%) completed the questionnaire through a weblink sent to them through email. The scholars identified themselves as Muftis, Imams, *Ulemah* (male) and *Ulumah* (female), in all, 3 (5%) *Ulumah* and 63 (95%) Muftis, Imams or *Ulemah* participated in the study. A Mufti is a person well versed with Islamic law, such a person can interpret Islamic law regarding what is Halal and what is not. An Imam is a Muslim leader who normally leads the mosque in prayers whilst an *Ulemah* (*Ulumah*- female) in Arabic means a learned person.

### 2.2.4. Survey of Halal consumers

A total of 314 Halal consumers took part in the study. Two hundred and fifteen (68%) of the respondents were recruited through emails, telephone and word-of-mouth. Out of 666 hard copies of the Halal consumer questionnaires given to mosques, Islamic centres and volunteers, 19% (61 respondents) questionnaires were completed. Also, 54 email invitations were sent with a link to the online survey, 12% (38) consumers participated, the rest were recruited using the Snowball sampling technique. Respondents were from 54 towns/cities across the UK.

## 2.2.5. Questionnaire 1- Islamic scholars

The scholars were asked to provide personal information; name, age, gender, name of organisation they are associated with and the nearest city/town of residence. They were given a brief introduction to the aims of the survey and they were assured that data collected would be anonymous. They were asked to select Halal slaughter requirements from a list of 6 possible options, this was followed with a question in which the scholars were provided a free text box (unlimited amount of space) to list any other requirements which were not covered in the preceding question. The scholars' perception and interpretation of pre-slaughter and post-slaughter stunning of food animals with regard to Halal slaughter were evaluated with the following closed questions:

• Stunning of meat animals prior to slaughter has been shown to reduce the pain associated with slaughter. Do you agree with this statement?

• Some methods of stunning have been shown to be reversible, that is, such methods do not lead to the death of animals prior to slaughter (bleeding-out). Are you aware of these methods of stunning?

• If an animal is stunned and then slaughtered by a Muslim and the method of stunning does not injure or result in the death of that animal before slaughter, and blood loss is not adversely affected, would you regard this meat as Halal? Scholars who provided a “No” answer to this question were then provided with a free-text box to give a reason as to why stunned meat is not Halal.

• If an animal is slaughtered whilst it is alive, followed immediately with stunning, would you regard this practice as Halal? A question on the pain associated with slaughter without pre-slaughter stunning was included: If an animal is slaughtered without any form of stunning, which of the following do you consider to apply?

• The animal will feel reduced pain because the knife acts as a stun.

• The animal will feel pain.

## 2.2.6. Questionnaire 2- Halal consumers

The Halal consumer's questionnaire differed slightly from that of the scholars'. The consumers did not have to give their names or any association with an Islamic institution. However, from a series of closed questions, they were asked to indicate their highest academic qualifications from a list of options, whether they considered themselves practicing Muslims, the frequency of their prayers (Salat) with the following options; I pray 5 times a day, I pray occasionally and I do not pray. They were also asked to select one of the following two options that

apply to them;

• I eat only Halal meat.

• I occasionally eat non-Halal meat, but not pork.

The rest of the questions were the same as those in the scholars' questionnaire on the subject of the pain associated with slaughter without stunning, pre-slaughter and post-slaughter stunning. However, on the question regarding the acceptability of reversibly stunned animals for Halal production, consumers were asked to select one of three options (Yes, No or Not Sure) whilst the scholars only had two options (Yes or No). Table 3 is an outline of the questions put forward to the scholars and consumers. Where appropriate, the results are presented as a percentage followed by a 95% confidence interval (CI) for the estimate calculated using Wilson's Method (Altman, Machin, Bryant, & Gardner, 2000).

| Scholars' Questions | Consumers' questions |
| --- | --- |
| 1. Full name of scholar/Imam | 1. Nearest city/town |
| 2. What is your age<br>• Less than 20<br>• 21-30<br>• 31-40<br>• 41-50<br>• 51-60<br>• Over 60 | 2. What is your age<br>• Less than 20<br>• 21-30<br>• 31-40<br>• 41-50<br>• 51-60<br>• Over 60 |
| 3. Gender<br>• Male<br>• Female | 3. Gender<br>• Male<br>• Female |
| 4. Name of organisation/mosque/Islamic centre | 4. Highest academic qualification<br>• GCSE/O-Level<br>• A-Level<br>• Further education qualification<br>• Graduate<br>• Postgraduate qualification<br>• N/A |
| 5.Nearest city/Town | 5. Do you consider yourself a practicing Muslim?<br>• Yes<br>• No |
| 6. What do you consider as the requirements of Halal slaughter (you may tick more than one option)?<br>• Animal must be alive at the point of slaughter<br>• Animal must be healthy and not injured<br>• Age of the animal is important<br>• The slaughterman bleeding the animal must be a Muslim<br>• In the absence of a Muslim, a Jew or Christian may perform Halal slaughter<br>• All flowing blood must be drained from the carcass | 6. Which of the following applies to you?<br>• I eat only Halal meat<br>• I occasionally eat non-Halal meat, but not pork |
| 7. Are there any other requirements for Halal slaughter not covered in question 6 above? If you consider there are, please detail them or write 'None' | 7. Which of the following applies to you?<br>• I pray 5 times a day<br>• I pray occasionally<br>• I do not pray |
| 8. Stunning of meat animals prior to slaughter has been shown to reduce the pain associated with slaughter. Do you agree with this statement?<br>• Yes | 8. What do you consider as the requirement(s) of Halal slaughter (you may tick more than one answer)?<br>• Animal must be alive at the point of slaughter |

- No

9. Some methods of stunning have been shown to be reversible, that is, such methods do not lead to the death of animals prior to slaughter (bleeding-out). Are you aware of these methods?
    - Yes
    - No

10. If an animal is stunned and then slaughtered by a Muslim and the method of stunning does not injure or lead to the death of that animal before slaughter (bleed-out) and does not affect blood loss, would you regard this meat as Halal?
    - Yes
    - No

11. If you answered No to question 10, please state why?

12. If an animal is slaughtered without any form of stunning, which of the following do you consider to apply?
    - The animal will feel reduced pain because the knife acts as a stun
    - The animal will feel pain

13. If an animal is slaughtered whilst it is alive, followed immediately with stunning, would you regard this practice as Halal?
    - Yes
    - No

- Animal must be healthy and not injured
- Animal must be of a certain age
- The slaughterman bleeding the animal must be a Muslim
- In the absence of a Muslim, a Jew or Christian may perform Halal slaughter
- All flowing blood must be drained from the carcass

9. Are there any other requirements for Halal slaughter not covered in question 8? If you consider there are, please detail them or write 'None'

10. Stunning of meat animals prior to slaughter has been shown to reduce the pain associated with slaughter. Do you agree with this statement?
    - Yes
    - No

11. Some methods of stunning have been shown to be reversible, that is, such methods do not lead to the death of animals prior to bleeding-out. Are you aware of these methods?
    - Yes
    - No

12. If an animal is stunned and then slaughtered by a Muslim and the method of stunning does not injure or lead to the death of that animal before slaughter (bleed-out) and does not affect blood loss, would you regard this meat as Halal?
    - Yes
    - No
    - Not sure

13. If you answered No to question 12, please state why?

| | |
|---|---|
| | 14. If an animal is slaughtered without any form of stunning, do you consider any of the following to apply? (you may choose more than one option).<br>• The animal will feel reduced pain because the knife acts as a stun<br>• The animal will feel pain<br>• There is improved blood loss in comparison with stunned animals<br>• Other (Please specify)<br><br>15. If an animal is slaughtered whilst it is alive, followed immediately with stunning, would you regard this practice as Halal?<br>• Yes<br>• No |

**Table 3.** An outline of the questions put forward to the Islamic scholars and Halal consumers

## 2.3. Results

Of 129 Islamic organisations contacted, 55 agreed to participate. A total of 66 scholars from 55 organisations completed the questionnaire. One scholar was excluded from the analysis because of an almost empty answer set.

### 2.3.1. Requirements of Halal slaughter- Islamic scholars

Table 4 is a summary of the outcome of the scholars' survey. The scholars were provided with a list of Halal slaughter requirements to select the main requirements that must be met during Halal slaughter (Table 3). The respondents were allowed to select one or more options. Out of 65 respondents, one respondent skipped the question, 98% of the respondents indicated that the animal must be alive at the time of slaughter, 80% said the person bleeding the animal must be a Muslim, 59% indicated that there is a need for all the flowing blood to be drained out of the carcass, 38% said the animal must be healthy and not injured, 36% indicated that in the absence of a Muslim, a Jew or Christian may slaughter and 17% of respondents indicated that the age of the animal is important. Although 36% of respondents agreed that animals slaughtered by Jews and Christians may be suitable for consumption by Muslims, 61% of these respondents explained that the slaughterer must be a practicing Jew or Christian and that the tasmiyyah (a short prayer) must be recited before slaughter by the Christian or Jew, 17% were of the view that only Shechita (Jewish) slaughter is suitable for consumption by Muslims but not Christian slaughter, 13% said the slaughterer must believe in the monotheism of God and that it is a must for them to recite the tasmiyyah before slaughter. A further 9% explained that any Christian or Jew could slaughter for Muslims to consume as long as they believe in the “original text”. The respondents were then provided a free-text box to list any additional Halal slaughter requirements they felt were not included in the question. Forty-three of the 65 respondents provided at least one additional Halal slaughter requirement, 51% respondents indicated that the sharpness of the knife is important, another 51% said the appropriate blood vessels must be severed, 21% said the name of God must be recited before the slaughter of each animal, 5% indicated that the animal must be a permissible species, 2% said animals must be fed and watered before slaughter and 1 said the slaughterer must be given sufficient training.

| Question | Outcome | | |
|---|---|---|---|
| Age | • Less than 20 | 3 (5%) | |
| | • 21-30 | 11(17%) | |
| | • 31-40 | 16 (25%) | |
| | • 41-50 | 26(40%) | |
| | • 51-60 | 5(8%) | |
| | • Over 60 | 4(6%) | |
| Gender | • Male | 62(95%) | |
| | • Female | 3(5%) | |
| What do you consider as the requirements of Halal slaughter? (you may tick more than one option) (**Question skipped by 1 respondent**) | • Animal must be alive at the point of slaughter | 63 (98%) | CI 91.7 to 99.7% |
| | • Animal must be healthy and not injured | 24 (38%) | CI 26.7 to 49.7% |
| | • Age of the animal is important | 11 (17%) | CI 9.9 to 28.2% |
| | • The slaughterman bleeding the animal must be a Muslim | 51 (80%) | CI 68.3 to 87.7% |
| | • In the absence of a Muslim, a Jew or Christian may perform Halal slaughter | 23 (36%) | CI 25.3 to 28.2% |
| | • All flowing blood must be drained from the carcass | 38 (59%) | CI 47.1 to 70.5% |

| | | | |
|---|---|---|---|
| Are there any other requirements for Halal slaughter not covered in question 6 above? If you consider there are, please detail them or write 'None' **(43 out of 65 respondents provided at least 1 requirement)** | • Knife sharpness is important | 22 (51%) | CI 36.8 to 65.4% |
| | • Appropriate blood vessels must be severed | 22 (51%) | CI 36.8 to 65.4% |
| | • The name of God must be recited before slaughter | 9 (21%) | CI 11.4 to 35.2% |
| | • The animal must be a permissible species | 2 (5%) | CI 1.3 to 15.5% |
| | • Animal must be fed and watered before slaughter | 1 (2%) | CI 0.4 to 12.1% |
| | • The slaughterer must be given sufficient training | 1 (2%) | CI 0.4 to 12.1% |
| Stunning of meat animals prior to slaughter has been shown to reduce the pain associated with slaughter. Do you agree with this statement? (**Question skipped by 3 respondents**) | • Yes | 19 (31%) | CI 20.6 to 43.0% |
| | • No | 43 (69%) | CI 57.0 to 79.4% |
| Some methods of stunning have been shown to be reversible, that is, such methods do not lead to the death of animals prior to slaughter (bleeding-out). Are you aware of these methods? (**Question skipped by 1 respondent**) | • Yes | 27 (42%) | CI 30.9 to 54.4% |
| | • No | 37 (58%) | CI 45.6 to 69.1% |

| | | | |
|---|---|---|---|
| If an animal is stunned and then slaughtered by a Muslim and the method of stunning does not injure or lead to the death of that animal before slaughter (bleed-out) and does not affect blood loss, would you regard this meat as Halal? (**Question skipped by 2 respondents**) | • Yes<br>• No | 60 (95%)<br>3 (5%) | CI 86.9 to 98.4%<br>CI 1.6 to 13.1% |
| If you answered No to the preceding question, please state why? (**There were 3 respondents**) | • Stunning is against the guidance of the Prophet on slaughter<br>• Questioned the validity of any research confirming the reversibility of some methods of stunning | 2<br>1 | CI 20.8 to 93.9%<br>CI 6.1 to 79.2% |
| If an animal is slaughtered without any form of stunning, which of the following do you consider to apply? (**Question skipped by 14 respondents**) | • The animal will feel reduced pain because the knife acts as a stun<br>• The animal will feel pain | 40 (78%)<br>11 (22%) | CI 65.4 to 87.5%<br>CI 12.5 to 34.6% |
| If an animal is slaughtered whilst it is alive, followed immediately with stunning, would you regard this practice as Halal? (**Question skipped by 14 respondents**) | • Yes<br>• No | 45 (88%)<br>6 (12%) | CI 76.6 to 94.5%<br>CI 5.5 to 23.4% |

Table 4. A summary of the outcome of the scholars' survey.

## 2.3.2. Stunning and slaughter- Islamic scholars

Respondents were asked whether they agree with the following statement: 'Stunning of meat animals prior to slaughter has been shown to reduce the pain associated with slaughter'. Out of 65 participants, three did not respond to the question, 31% said yes whilst 69% answered no. The respondents were then asked whether they were aware that some methods of stunning have been shown to be reversible, one respondent skipped the question, 58% said no and 42% said yes. When the respondents were asked whether meat would be considered Halal when animals are stunned with a method of stunning that does not result in death, cause physical injury to animals or adversely affect blood loss, 2 scholars did not respond to the question, 95% said the meat would be considered Halal whilst 5% said the meat would not be Halal. The 2 respondents who did not respond to the question explained that they did not have any practical experience with stunning and that they would prefer to see it in practice before making a decision. Of the 60 respondents who indicated that the meat would be suitable for consumption by Muslims, 25% explained that despite the fact that the meat would be Halal, the act of stunning animals is prohibited in Islam because it is, in their opinion, inhumane and cruel and that stunned animals would only be Halal if it is proven beyond any reasonable doubt that the animal was alive at the time of slaughter, 12% indicated that although reversibly stunned meat is Halal, stunning is not a preferred method of slaughter for Halal whilst 7% of respondents indicated that stunning should only be used for large animals and not birds due to the possibility of some birds dying as a result of the stun. The 3 respondents who indicated that all forms of stunning are not Halal gave the following reasons for their stance; 2 of them said stunning is against the guidance of the Prophet of Islam whilst 1 respondent questioned the validity of any research which suggests that some methods of stunning do not result in instantaneous death. On the issue of the pain associated with the slaughter of animals without any form of stunning, respondents were asked the following question; If an animal is slaughtered without any form of stunning, which of the following do you consider to apply? Fourteen of the 65 respondents did not respond to the question, 78% said the animal will feel reduced pain because the knife acts as a stun whilst 22% said the animal will feel pain. The 14 respondents who did not answer the question insisted that the slaughter of animals without stunning, in their opinion, causes no pain, however, there were only two answer options none of which included the answer they provided. The scholars were also asked for their interpretation of post-cut stunning for Halal production. A total of 14 respondents did not answer the question, 88% of those who responded said the practice is Halal compliant whilst 12% said it is against the Halal slaughter rules. Of those who agreed that post-cut stunned meat is suitable for consumption by Muslims, 31% of them suggested that the act of any form of stunning is unacceptable although the meat would be Halal, 2% said the meat is acceptable on condition that the volume of blood loss is not impeded as a result of the stun and 7% suggested that the practice is disliked although the meat would be Halal. Two of the respondents who suggested that the practice is not consistent with Halal slaughter called for more research into the effect of pre-slaughter and post-slaughter stunning on the volume of blood loss.

### 2.3.3. Halal consumer demographics

Out of 314 respondents, 73% were male, 27% female and one person did not indicate the gender. The highest level of education of the respondents are 23% graduates, 20% postgraduates, 20% further education, 16% A-level, 13% GCSE/O-Level and 8% selected N/A (not applicable). Most of the respondents, 98% considered themselves as practicing Muslims (14 participants did not respond to this). When asked whether they eat only Halal meat or they occasionally eat non-Halal meat but not pork, 24 of the 314 respondents skipped the question, 98% indicated that they eat only Halal meat, 2% said they occasionally eat non-Halal meat but not pork. On whether they practice their religion, 29 respondents skipped the question, 88% said they pray 5 times a day, 11% pray occasionally and 0.7% said they do not pray at all. Table 5 is a summary of the outcome of the Halal consumers' survey.

| Question | Outcome | | |
|---|---|---|---|
| What is your age? | • Less than 20<br>• 21-30<br>• 31-40<br>• 41-50<br>• 51-60<br>• Over 60 | 31 (10%)<br>65 (21%)<br>111 (35%)<br>71 (23%)<br>27 (9%)<br>9 (23%) | |
| Gender (**Question was skipped by 1 respondent**) | • Male<br>• Female | 228 (73%)<br>85 (27%) | |
| Highest academic qualification (**Question was skipped by 3 respondents**) | • GCSE/O-Level<br>• A-Level<br>• Further education qualification<br>• Graduate<br>• Postgraduate qualification<br>• N/A | 41 (13%)<br>50 (16%)<br>61 (20%<br>73 (23%)<br>62 (20%)<br>24 (8%) | |
| Do you consider yourself a practicing Muslim? (**Question was skipped by 14 respondents**) | • Yes<br>• No | 293 (98 %)<br>7 (2%) | CI 95.3 to 98.9%<br>CI 1.1 to 4.7% |
| Which of the following applies to you? (**Question was skipped by 24 respondents**) | • I eat only Halal meat<br>• I occasionally eat non-Halal meat, but not pork | 283 (98%)<br>7 (2%) | CI 95.1 to 98.8%<br>CI 1.2 to 4.9% |

| | | | |
|---|---|---|---|
| Which of the following applies to you? (**Question was skipped by 29 respondents**) | • I pray 5 times a day<br>• I pray occasionally<br>• I do not pray | 251 (88%)<br>32 (11%)<br>2 (0.70%) | CI 83.8 to 91.3%<br>CI 8.1 to 15.4%<br>CI 0.2 to 2.5% |
| What do you consider as the requirement(s) of Halal slaughter (you may tick more than one option)? | • Animal must be alive at the point of slaughter<br>• Animal must be healthy and not injured<br>• Animal must be of a certain age<br>• The slaughterman bleeding the animal must be a Muslim<br>• In the absence of a Muslim, a Jew or Christian may perform Halal slaughter<br>• All flowing blood must be drained from the carcass | 298 (95%)<br>226 (72%)<br>97 (31%)<br>225 (72%)<br>93 (30%)<br>165 (53%) | CI 91.9 to 96.8%<br>CI 66.8 to 76.7%<br>CI 26 to 36.2%<br>CI 66.4 to 76.4%<br>CI 24.8 to 34.9%<br>CI 47 to 58% |
| Stunning of meat animals prior to slaughter has been shown to reduce the pain associated with slaughter. Do you agree with this statement? (**This question was skipped by 2 respondents**) | • Yes<br>• No | 131 (42%)<br>181 (58%) | CI 36.6 to 47.5%<br>CI 52.5 to 63.4% |
| Some methods of stunning have been shown to be reversible, that is, such methods do not lead to the death of animals prior to bleeding-out. Are you aware of these methods? | • Yes<br>• No | 122 (39%)<br>192 (61%) | CI 33.6 to 44.3%<br>CI 55.7 to 66.4% |

| | | | |
|---|---|---|---|
| If an animal is stunned and then slaughtered by a Muslim and the method of stunning does not injure or lead to the death of that animal before slaughter (bleed-out) and does not affect blood loss, would you regard this meat as Halal? | • Yes | 165 (53%) | CI 47 to 58% |
| | • No | 101 (32%) | CI 27.2 to 37.5% |
| | • Not sure | 48 (15%) | CI 11.7 to 19.7% |
| If you answered No to the preceding question, please state why? (**This question was skipped by 279 respondents**) | • Pre-slaughter stunning is against Islamic law | 20 (57%) | CI 40.9 to 72% |
| | • Stunning is painful/can injure animals | 5 (14%) | CI 6.3 to 29.4% |
| | • Stunning may result in the death of animals before slaughter | 5 (14%) | CI 6.3 to 29.4% |
| | • Would avoid stunned meat because they have little or no knowledge about it | 4 (11%) | CI 4.5 to 26% |
| | • Stunning obstructs bleed-out | 1 (3%) | CI 0.5 to 14.5% |
| If an animal is slaughtered without any form of stunning, do you consider any of the following to apply? (you may choose more than one option) (**This question was skipped by 32 respondents**) | • The animal will feel reduced pain because the knife acts as a stun | 169 (60%) | CI 48.3 to 59.3% |
| | • The animal will feel pain | 94 (33%) | |

| | | | |
|---|---|---|---|
| | • There is improved blood loss in comparison with stunned animals | 56 (20%) | CI 25.1 to 35.2%<br><br>CI 14.0 to 22.4% |
| If an animal is slaughtered whilst it is alive, followed immediately with stunning, would you regard this practice as Halal? (**This question was skipped by 82 respondents**) | • Yes | 159 (69%) | CI 62.3 to 74.2% |
| | • No | 73 (31%) | CI 25.8 to 37.7% |

Table 5. A summary of the results of the survey of Halal consumers.

### 2.3.4. Requirements of Halal slaughter- consumers

Halal consumers were asked to select at least one condition they regarded as the requirement(s) of Halal slaughter from six options. A total of 95% of respondents said animals must be alive at the time of slaughter, 72% said the animal must be healthy and not injured, 72% indicated that the slaughterman bleeding the animal must be a Muslim, 53% said all flowing blood must be drained out of the carcass, 31% indicated that the animal must be of a certain age, 30% indicated that in the absence of a Muslim, a Jew or Christian may slaughter. The respondents were then provided a free-text box to list any additional Halal slaughter requirements not listed in the 6 options. Of the 314 respondents, 105 provided at least one more additional Halal slaughter requirement(s), these additional requirements are summarised in Table 6 below.

| Additional Halal slaughter requirement | No. of respondents | 95% CI |
|---|---|---|
| The name of Allah (God) must be recited | 63 | 50.4 to 68.9% |
| The knife/ blade must be sharp | 9 | 4.6 to 15.5% |
| Animal welfare is important | 26 | 17.5 to 33.8% |
| Animals must not see other animals being slaughtered or the sharpening of the knife | 5 | 2.1 to 10.7% |
| Animal must be orientated to face Qibla (Grand mosque in Mecca) | 2 | 0.5 to 6.7% |
| Animal must be a Halal animal | 2 | 0.5 to 6.7% |
| The slaughterer must be of a sound mind | 1 | 0.2 to 5.2% |
| Animal must be slaughtered with a single movement of the knife | 2 | 3.5 to 6.7% |
| The animal must be in a state of fear, this aids rapid bleed-out | 1 | 0.2 to 5.2% |

Table 6. Additional Halal slaughter requirements provided by 105 respondents

## 2.3.5. Stunning and slaughter- Halal consumers

Halal consumers were asked whether they agreed with the following statement: Stunning of meat animals prior to slaughter has been shown to reduce the pain associated with slaughter. The majority of respondents 58% selected 'no' to imply that they disagreed with the statement whilst 42% said 'yes' they agreed with the statement and 2 did not respond to the question. The consumers were subsequently asked whether they were aware that some stunning methods have been shown to be reversible; 39%

replied yes they were aware of such methods whilst 61% indicated that they were not aware of reversible stunning methods. When asked whether they would regard meat as Halal if animals were pre-stunned with a method of stunning that does not result in death, cause physical injury or obstruct bleed-out, 53% respondents indicated that such meat would be Halal, 32% said the meat would not be Halal whilst 15% were unsure. Respondents who indicated that the meat would not be Halal were provided a free-text box to explain why such meat would not be Halal, 35 of them responded; 57% respondents said pre-slaughter stunning is against Islamic law, 3% respondents said stunning obstructs bleed-out, 14% respondents said stunning is painful/can injure animals, 14% respondents said stunning may result in the death of animals before slaughter and 11% respondents said they had little or no knowledge about stunning so they would rather avoid stunned meat. On post-cut stunning, 82 participants did not respond to the question, 69% of the respondents said they regard post-cut stunning as Halal whilst 31% indicated that they did not recognise it as Halal. Consumers were asked about the consequences of slaughter without pre-stunning (with an option of selecting one or more answers), 60% said the animal will feel reduced pain because the knife acts as a stun, 33% said the animal will feel pain and 20% said there would be improved blood loss in comparison with stunned animals. Thirty-two respondents skipped the question.

## 2.4. Discussion

This study confirms that the interpretation of the Shariah law by Islamic jurists within the UK (and other parts of the world) regarding the acceptability of slaughter practices for Halal production differs between scholars. With guidance from the Quran and Hadith, one would expect these jurists to make unanimous decisions regarding what is Halal and Haram (prohibited), however, a number of differences of opinions exist among the scholars. Fuseini et al., (2016) suggested that the differences in scholarly opinion may sometimes be the result of differences between the two main sects, Sunni and Shia or the subdivision of the Sunni sect into four different schools of law; the Maliki, Hanbali, Shafii and Hanafi schools of law. Although there are several differences within the Muslim community regarding Halal slaughter, the most important of these differences is whether pre-slaughter stunning is Halal compliant, this is due to concerns that some methods of stunning may violate the Halal rules by causing the death of animals before slaughter. During our face-to-face meetings with Islamic scholars in the UK, some of them admitted that they had never witnessed the stunning of animals in practice. One scholar indicated that he would not believe any research demonstrating the reversibility of stunning until he sees it in practice. Islamic scholars are expected to advise Halal consumers on the suitability of various slaughter methods for Halal production, however, the lack of understanding of stunning among some scholars has resulted in the issuance of confusing Fatwas (religious rulings) on the suitability of stunned meat for consumption by Muslims. There is an urgent need for these scholars to be given theoretical and practical education on stunning and other modern slaughter techniques such as mechanical slaughter, this will help them make informed decisions about the suitability of these techniques for Halal production. Our findings also revealed that the majority of UK scholars (95%) would regard meat from stunned animals as Halal on condition that it is proven to be reversible, however, 25% of those who would regard stunned meat as Halal did not think stunning should be encouraged during Halal slaughter because they believe the practice impedes bleeding out, leads to poor meat quality and that it is an inhumane and cruel practice. Research has however demonstrated that the stunning of animals before slaughter has no adverse effects on blood loss (Anil *et al*., 2004, 2006; Khalid *et al*., 2015), meat quality (Önenç

& Kaya, 2004) and the humaneness of stunning has been objectively demonstrated with EEG recordings (Newhook & Blackmore, 1982). A further 7% did not think stunning is suitable for Halal poultry, according to the scholars, this is because of a high risk of birds dying due to their size. Our findings differ from the results of a previous study conducted in the UK by the Halal Monitoring Committee (HMC, 2009) in which it was reported that 85% and 90% of the scholars surveyed indicated that they would reject electrical stunning of large animals and birds respectively. Whilst the present study specifically related the question to electrical head-only stunning, there is lack of information on the type of electrical stunning the HMC study related to, especially in large animals where the type of electrical stunning may be adapted to induce cardiac fibrillation, a situation that would not support recovery and would therefore be rejected by the majority of Muslims. Also, this study included both Islamic scholars and Halal consumers whilst the previous study only involved Islamic scholars. As far as we are aware, this is the first time a survey of this nature has included both scholars and consumers. In line with the Quranic position on Halal slaughter, the majority of UK Islamic scholars (98%) and consumers (95%) agreed that, for meat to be Halal, the animal must be alive at the time of slaughter. Conversely, although the Quran permits the consumption of the food of Jews and Christians (Quran 5:5), with the exception of specifically prohibited foods such as pork and intoxicants, only 36% of scholars and 30% of consumers think such meat is acceptable for Muslims to eat. Some of the scholars who rejected Christian and Jewish slaughter were of the opinion that Jewish (Shechita) and Christian slaughter were incompatible with Islamic law because there is no requirement for the name of God to be recited on every single animal. Others argued that only practicing Christian and Orthodox Jewish slaughter may be suitable for Muslims to consume on condition that the life of the animal is dedicated to God before slaughter. Our study also revealed that 80% of UK scholars and 72% of UK Halal consumers were of the view that the slaughterman must be a Muslim and 59% of scholars and 53% of consumers said there is a requirement for all the flowing blood to be drained out of the carcass. Just over half (52%) of the Halal consumers surveyed indicated that they would regard meat as Halal if animals are stunned with methods that do not result in the death of animals before the ritual cut is made, in addition, there is a requirement for the method of stunning to have no adverse effect on blood loss or cause physical injury to the animal. Our results corroborate a study conducted in Belgium by a national animal welfare group, GAIA (2010). They reported that up to 51% of Belgian Muslims do not oppose the use of stunning during Halal slaughter. There were more Halal consumers (42%) than Islamic scholars (31%) who were convinced that pre-slaughter stunning of animals could be used to reduce or abolish the pain associated with slaughter, but the majority in each of the two groups believe that stunning does not play any role in reducing pain. Despite the fact that research has demonstrated the reversibility of some methods of stunning (Velarde *et al*., 2002; EBLEX, 2009, Wotton et al., 2014), the majority of scholars (58%) and consumers (61%) indicated that they were unaware of the availability of such methods. It is interesting to note that even though Islamic scholars are expected to advise Halal consumers on the suitability of stunned meat for consumption by Muslims, a higher proportion of the consumers than scholars are aware of the existence of reversible stunning. One would expect the scholars to be better informed about these methods of stunning, regarded by the majority of proponents of Halal stunning as Halal (MS1500, 2009; MUI HAS 23103, 2012; HFA, 2014). The consumers, apart from getting their advice from the scholars on the suitability of stunning for Halal production, may be investigating the available stunning methods more than the scholars. Many Islamic scholars were willing to participate in demonstrations of the reversibility of stunning, this may help in assuring the scholars that some methods of stunning may be Halal compliant. Video evidence is available to support the reversibility of head only electrical stunning with sheep (EBLEX, 2009). This study will have been subject to a degree of bias, as is the case with any study. In this case all questions were in English, but with a translation facility for those who could not read and understand English. Muslims with no formal education and those who could not speak English did not have equal opportunity to participate in the study. Additionally, snowball recruitment of consumers would be likely to recruit among people from similar backgrounds and with common connections perhaps to the exclusion of some sub-communities.

## 2.5. Conclusion

There is no common agreement among Muslims on the suitability of stunning for Halal production. Many Islamic scholars and Halal consumers would regard pre-slaughter stunned meat as Halal if there were some level of assurance that the type of stunning used does not cause the death of animals before the Halal cut is made. However, many Islamic scholars, despite agreeing that reversible stunning is compatible with Halal slaughter, would still not recommend its use during Halal slaughter because of the belief that pre-slaughter stunning is a cruel and inhumane practice and violates the guidelines of Halal slaughter. Many of these scholars hold an opinion that pre-slaughter stunning of animals adversely affects the volume of blood loss during exsanguination and that stunning produces inferior meat quality. There is an urgent need for stakeholders in the meat industry to involve Islamic scholars in research on pre-slaughter stunning to enable them to make informed decisions about the aspects of stunning that continue to divide opinions of the scholars.

# 3. Veterinary students' perception and understanding of issues surrounding the slaughter of animals according to the rules of Halal: a survey of students from four English universities.

**Linking Narrative**: This chapter is a published peer-reviewed paper (Fuseini *et al.*, 2019) in the journal of Animals (*Animals-in press*). The paper is a survey of veterinary students from four English universities to examine their perception and level of understanding on issues surrounding Halal slaughter. Of the 459 students surveyed, 437 (95.2%) indicated that they would want all animals to be stunned before slaughter, including during religious slaughter, 17 (3.6%) either did not have an opinion or indicated 'other' as their preferred option and 5 (1.1%) indicated that religious slaughter should be exempt from stunning in order to comply with traditional religious values. This paper gives an insight into the debate surrounding calls for an end to the slaughter of animals without stunning by the veterinary profession (e.g. the British Veterinary Association-BVA). Contrary to the views of the BVA, this study showed that some veterinary students would like an exemption for religious slaughter to be carried out without slaughter, albeit this was a minority of respondents. Following the publication of the paper, the BVA communicated with the lead author with a view to sharing the findings with the BVA's Welfare at Slaughter Working Group. As potential future enforcers of religious slaughter regulations, veterinary students were identified as key stakeholders in Halal meat production, thus the need for this study. The paper was therefore included as a chapter in the thesis to highlight the important role veterinarians play in Halal meat production.

## 3.1. Introduction

The slaughter of food animals, whether stunned or not, is an emotive issue that has long divided opinion. Those against the production of farm animals, their slaughter and the subsequent consumption of meat, have often cited the effect of livestock agriculture on the environment (Tew *et al*., 1992; Johnson *et al*., 1991; Feber *et al*., 1997; Dale *et al*., 2007), a decline in the population of wild animals as a consequence of cultivating animal feed (Edge, 2000) or simply put a case for animal rights (Regan, 1983) or religiosity (Fuseini and Sulemana, 2018). However, the perceived importance of meat in the diet of man cannot be underestimated. Meat is seen by many as an important source of proteins, amino acids, vitamins and other essential nutrients required for the sustenance of life (Kauffmann, 2001; Font-i-Furnols and Guerrero, 2014). The slaughter of animals in many industrialised economies is a highly regulated procedure, these regulatory measures are put in place to protect the welfare of animals (and the health and safety of operatives) and to ensure that meat is fit for human consumption. Within the European Union, the protection of animals at the time of slaughter is regulated under Council Regulation, EC1099/2009 [10] which specifies acceptable pre-slaughter procedures, and approved slaughter methods for food animals. To protect animal welfare, EC1099/2009 requires the stunning of all animals prior to death (itself caused by bleeding out), with the exception of animals slaughtered in accordance with religious rites, this being mainly for Shechita and Halal slaughter. Halal slaughter is practiced by followers of the Islamic faith, animals are required to be alive prior to bleeding and a prayer is said by the slaughterer at the time of neck-cutting on every animal. Shechita slaughter on the other hand is practiced by followers of the Jewish faith, again animals are required to be alive and a prayer is said, however, during Shechita slaughter, there is no requirement for the prayer to be recited on every animal. Whilst some Muslims accept stunning during Halal slaughter, the Jewish community unanimously reject all forms of stunning. Member states can apply a derogation to permit slaughter without stunning, and this derogation is in place in the English domestic regulation, the Welfare of Animals at the Time of Killing (WATOK 2015) Regulation. Research using, for example, EEG recordings of the electrical activity of brain has demonstrated that this method of slaughter does compromise animal welfare (Gibson *et al*., 2009; Mellor and Littin, 2004; Gregory *et al*., 2012). Contrary to the findings of Gibson and colleagues (2009), other researchers (Grandin and Regenstein, 1994) subjectively observed the behaviour of some three thousand cattle slaughtered without stunning (in line with the Shechita rules), and concluded that, 'in their opinion', the animals did not exhibit overt behaviours that were consistent with pain. The UK and other member states have used the derogation to permit slaughter without stunning under strict conditions. In Great Britain, the domestic regulation, (WATOK, 2015) requires animals to be individually and mechanically restrained during slaughter without stunning, and that ruminants must not be moved after the neck-cut until they lose sensibility. As a regulation, sheep must standstill for at least 20 s whilst cattle must not be moved for at least 30 s following the neck-cut. The essence of the standstill time is to ensure that animals lose sensibility due to blood loss before they are moved in order to avoid any additional pain or distress associated with the process. Further, abattoirs slaughtering animals without stunning must also have a backup stunner (a requirement also of abattoirs using pre-slaughter stunning) to be used in the event of delayed loss of consciousness after the neck-cut.

Animal rights and welfare groups continue to publicise the negative aspects of slaughter (both stun and non-stun) with a view to highlighting welfare compromises during slaughter. Over the last decade or so, UK-based animal welfare charity, Animal Aid, has released several covert recordings taken in abattoirs that have highlighted animal suffering and have used this as an argument for veganism (Animal Aid, 2017; Animal Aid, 2019). Harper and Makatouni (2002) suggested that consumers are becoming well informed about the welfare aspects of livestock agriculture and are opting for welfare-friendly products. Some consumers are well informed about the role official veterinarians (OVs) play in safeguarding animal health and welfare. Wall (2014) reported that the role of OVs is paramount in the implementation of the 'one health' initiative which is a collaborative, multi-disciplinary approach to ensuring the optimal attainment of good health and welfare of human and non-human animals globally. OVs receive specialist veterinary training to be licenced to work in abattoirs to safeguard animal welfare and human health. However, Spinka (2012) noted that there are gaps in the level of knowledge of OVs across the EU due to differences in the modules taught and the depth of subjects covered by different EU universities.

As potential future enforcers of religious slaughter laws in the UK (and other parts of the world), veterinary students at four English universities were recruited to participate in this study. The aim was to evaluate their perception and understanding of the regulations governing religious slaughter as it stands in the UK. The paper further examines the difference in the level of understanding of these issues amongst different year groups. To the best of our knowledge, there is no prior published data on veterinary students' perception and understanding of religious slaughter.

## 3.2. Materials and methods

### 3.2.1. Data collection and sampling methods

A total of 459 veterinary students from four universities in England participated in the study; University of Bristol (n=344), University of Nottingham (n=57) University of Liverpool (n=45) and Royal Veterinary College (n=13). Prior to the survey all students were provided information on the aims and objectives of the study and all respondents gave informed consent to participate in the study. Respondents' data were anonymised. Data were collected using 'SurveyMonkey' online software by sending a weblink to students to allow them to participate at a time convenient to themselves. One of the possible limitations of this study is that respondents were not asked about their religion. The University of Bristol's Ethical Review Board granted ethical approval for the study (ID75001).

### 3.2.2. Data analysis

Responses to questions are reported as percentages with actual number of respondents contributing following in brackets. Exact Chi Square tests were used to test for associations between categorical variables, where there were ordered categorical variables, a test for trend was carried out using an exact Gamma statistic (IBM SPSS Statistics v25, IBM Inc, NY).

## 3.3. Results

One respondent was dropped from the analysis because he/she did not answer a majority of the questions. Elsewhere, where there were occasional missed questions, those respondents are not included in the count or calculation of percentage. Omissions are treated as missing at random [there were 12 questions requiring a response and there were 8 individual (respondents) omissions in total across 7 of these questions]. The mean age of respondents was 22 with a range of 18-39. Respondents were recruited from four universities in England offering veterinary degrees, the majority of respondents indicated their programmes of study as veterinary science 98.9% (454), with the remaining 1.1% (5) selecting 'other' (Veterinary Medicine and Surgery, Veterinary Medicine, Bioveterinary science and Veterinary Medicine with Intercalation) as their programmes of study. The levels or years of study of respondents were 22.9% (105) in the fourth year, 22.7% (104) in the third year, 21.2% (97) in the second year, 18.3% (84) in the fifth year and 14.9% (68) in the first year. Respondents' homes of origin were from cities and towns across the UK with the larger proportions of respondents indicating they came from London 6.1% (28), Bristol 6.1% (28), Nottingham 2.8% (13) and Manchester 2.8% (13) areas.

Respondents were asked whether they were meat eaters and if so, their level of meat consumption. One respondent did not answer this question. The majority of respondents indicated that they were meat eaters 81.3% (372) whilst 19.2% (88) indicated that they did not eat meat and 0.9% (4) chose only dietary exclusions by choice. Of the 372 meat eaters, 62.5% (286) indicated that they ate meat regularly whilst 18.8% (86) said they ate meat occasionally. The 88 respondents who indicated that they did not eat meat were 15.3% (70) vegetarians and 3.9% (18) vegans. Respondents were presented with the statement: 'At slaughter, the death of an animal takes place because the major blood vessels are severed, and critical blood loss occurs. This process is thought to be painful' and were then asked if they agreed with a series of statements. Two respondents did not answer these questions. The majority of respondents, 90.4% (413) indicated that 'pre-slaughter stunning abolishes the pain associated with the neck-cut during slaughter', whilst 9.6% (44) selected the option 'pre-slaughter stunning cannot abolish the pain associated with the neck-cut during slaughter'. There was a significant association between respondents' year of study and their response to whether stunning is capable of abolishing the pain associated with the neck-cut (Chi Sq. =33.0, df=4, $p<0.001$). The proportion of those agreeing that pre-slaughter stunning abolishes the pain associated with the neck-cut during slaughter were 79.4% (54), 80% (77), 95.2% (99), 98.1% (103), 95.2% (80) of years 1, 2, 3, 4, and 5 respectively (proportions per year). The perception and understanding of respondents with regards to halal slaughter in the UK was evaluated (one respondent did not answer this question). The majority of respondents, 36.9% (169) selected the following option: 'the majority of animals are not stunned, but some Muslims will accept meat from stunned animals as being Halal', 29% (133) selected 'all animals are required to be slaughtered without stunning', 23.1% (106) selected 'the majority of animals are stunned as most Muslims accept meat from stunned animals as Halal', whilst 10.9% (50) respondents indicated that they were not sure about the situation of Halal slaughter in the UK. The results indicated a significant association between the year of study and respondents' understanding regarding the situation with Halal slaughter in the UK; in later years of study, students understanding tended to improve (Chi Sq.=84.2, df=12, $p<0.001$). Respondents' awareness about the permissibility of slaughter without stunning for religious slaughter was evaluated (see table 7). One respondent did not answer the question, 90.4% (414) indicated 'Yes', whilst 9.6% (44) indicated 'No'. There was a significant association between respondents' year of study and their response to the above question, with a trend across years (a trend of increased awareness) (Gamma=0.659, df = 4, $p<0.001$). Table 7 shows a cross tabulation between respondents' year of study and their understanding of the permission of the slaughter of animals without stunning under UK animal welfare regulations.

| Year of study | UK welfare regulations do permit the slaughter of animals without stunning, but only for religious slaughter. Were you aware of this? | | |
|---|---|---|---|
| | No | Yes | Total |
| 1 | 29% (20) | 71% (48) | 100% (68) |
| 2 | 14% (14) | 86% (83) | 100% (97) |
| 3 | 5% (5) | 95% (99) | 100% (104) |
| 4 | 4% (4) | 96% (101) | 100% (105) |
| 5 | 1% (1) | 99% (83) | 100% (84) |
| Totals | 44 | 414 | (458) |

**Table 7.** Cross tabulation of the year of study of respondent and awareness that slaughter without stunning for religious purposes was permissible in the UK.

In a separate question, respondents were asked to share their opinion on the use of pre-slaughter stunning during meat production; 95.2% (437) of respondents indicated that all animals must be stunned before slaughter, including during religious slaughter, 2.2% (10) selected 'other' with the option to leave comments. (Table 8 shows the comments left by these 10 respondents), 1.5% (7) indicated that they did not have an opinion on stunning and 1.1% (5) indicated that religious slaughter should be exempt from stunning to comply with traditional religious values. Respondents were also asked for their views on whether there is a need for meat to be labelled according to the method of slaughter (i.e. whether meat is from an animal that has been stunned or not). A total of 97.2% (446) indicated that meat should be labelled according to whether it was derived from stunned or non-stun animals, 1.3% (6) indicated that there is no need to label meat, whilst 1.5% (7) indicated they did not have an opinion. To gauge respondents' acceptability of meat derived from animals that had been effectively stunned during Halal slaughter, respondents were asked: ‘If animals are effectively stunned before Halal slaughter, as an ordinary consumer, would you wittingly purchase and consume this type of Halal meat?’. Two separate analysis were made, first, with all respondents (including vegans and vegetarians) and a second, excluding vegans and vegetarians. In the first analysis, one respondent did not answer the question, 79.0% (362) answered ‘Yes’ and 21% (96) indicated ‘No’. The results showed a significant trend with respondents’ year of study and their willingness to purchase and consume Halal meat from effectively stunned animals (Gamma=0.210, df = 4, p=0.009), an increasing proportion answering ‘yes’, with increasing year of study (Table 9). In the second analysis which excluded vegans and vegetarians, the majority of respondents, 88.4% (327) indicated that they would wittingly purchase Halal meat from stunned animals whilst 11.6% (43) indicated that they would not purchase Halal meat from stunned animals. This still indicated a trend of increased acceptance of Halal meat from stunned animals as respondents progressed in their years of study, but this time the trend was lesser and not statistically significant (Gamma=0.147, df=4, p=0.203). Data were then further analysed to examine the attitudes of vegetarians and vegans alone towards Halal meat. The results showed a significant increase in the percentage of vegetarians and vegans who would wittingly purchase Halal meat from stunned animals as year of study increased (Gamma=0.311, df=4, p=0.042).

| |
|---|
| There is no such thing as humane slaughter, no animal wants to die, therefore it is not humane. |
| I do not agree with the exemption of stunning pre-slaughter and believe that stunning should be performed. This being said, I respect the choices and beliefs of religions other than my own. |
| All animals should be stunned before slaughter if is the most humane way - it is insanity that religion could come before the welfare of sentient beings. |
| I think a method should be employed to ensure the animal can't feel the pain of the true cause of death. I was told by a vet that stunning renders the animal essentially 'brain-dead and prevents the feeling of pain during slaughter-any other method that achieves the same effect without causing further pain or discomfort to the animal would also be appropriate. |
| Conflicting between the two I have an opinion, but it changes whether I draw from my cultural upbringing or my veterinary knowledge. |
| Animals should not be slaughtered for meat at all, however whilst this continues to happen, they should all be stunned including for religious slaughter. |
| If animals aren't stunned, meat in stores should be labelled accordingly so that people can be informed/choose not to eat non-stunned meat. |
| I think it is difficult as people want to follow what their religion says, and I think they have that right, but I also think the welfare of the animal is important so I'm not sure if religious slaughter should be exempt from stunning or not. |
| Stunning makes us feel better at the animal doesn't display a classic pain response. It's impossible for us to actually know the level of pain the animal feels after stunning. If stunning does eliminate the pain, then it should be done in all cases of slaughter regardless of religious beliefs. |
| I agree most closely with the first option however I would rather non recoverable stun / killing methods were used to eliminate pain from recovery. |

Table 8. Comments by respondents who choose the option 'other' to the question regarding their opinion on the use of pre-slaughter stunning for meat animals.

| Year of study | If animals are effectively stunned before Halal slaughter, as an ordinary consumer, would you wittingly purchase and consume this type of Halal meat? | | |
|---|---|---|---|
| | Yes | No | Total |
| 1 | 69% (47) | 31% (21) | 100% (68) |
| 2 | 75% (73) | 25% (24) | 100% (97) |
| 3 | 82% (85) | 18% (19) | 100% (104) |
| 4 | 81% (85) | 19% (20) | 100% (105) |
| 5 | 86% (72) | 14% (12) | 100% (84) |
| Total | 362 | 96 | 458 |

Table 9**.** Cross tabulation of the year of study of respondent and willingness to buy and consume Halal meat derived from animals that have been stunned before slaughter.

## 3.4. Discussion

The results of this study give an insight into the perception and understanding of religious slaughter issues by veterinary students at various levels of their studies from four universities in England. The results suggest some lack of a clear understanding of Halal slaughter with regard to the regulations and animal welfare issues surrounding the two main methods of slaughter, stun and non-stun. An understanding of these issues does appear to improve as they progress in their studies. The importance of the role of an independent, official veterinary surgeon in protecting animal welfare and public health cannot be underestimated. Due to the significance of their role, they require a better understanding of the burning issues around slaughter, particularly religious slaughter. The slaughter of animals under religious rites continues to attract public interest because of the insistence by a section of the religious communities that animals be slaughtered by severance of major blood vessels whilst they are fully conscious. There is scientific evidence to suggest that the slaughter of animals without stunning is painful (Gibson *et al*., 2009), and loss of consciousness may be protracted (Gregory *et al.*, 2010), especially in the case of cattle were the vertebral artery is able to maintain blood supply to the brain (Gregory *et al*., 2006). However, a minority of the respondents in this study did not agree that stunning was necessary, by indicating that they believed 'stunning of animals prior to slaughter cannot abolish the pain associated with the neck-cut during slaughter'. The majority of the respondents 90.4% (413), however, indicated that they understood the slaughter of animals without stunning to be painful and that stunning is capable of abolishing the pain associated with neck cutting. There was a trend of increased awareness of respondents' responses to whether stunning is capable of abolishing the pain associated with the neck-cut. This suggests that the first-year students may not have yet undertaken any lectures on the science of stunning and slaughter and the rest of the years may have varying degrees of teaching, understanding and retention of the concept of stunning. Main (2010) suggested that variation in veterinary curriculum and the way veterinary students are taught may account to variation in the level of understanding of students in the ever-evolving animal welfare module.

On the situation with regard to stunning of animals before Halal slaughter, the responses generally showed some lack of understanding of the facts surrounding Halal slaughter in the UK and suggests that this material needs to be better presented to students. This corroborates the conclusion made by Main (2010) who noted that there is a need for veterinary institutions to include some core components of animal welfare in their curriculum in order to offer students a better appreciation of welfare science, ethics and standards. There is sufficient evidence from animal welfare surveys to show that the majority of animals are stunned during Halal slaughter in the England and Wales (FSA, 2018) and also within the EU (Dialrel, 2010). There were, however, 23.1% (106) of respondents who indicated that they thought the majority of animals are stunned before Halal slaughter, this is consistent with the current situation in the England and Wales and parts of Europe (FSA, 2018; Dialrel, 2010). Respondents' understanding on this issue (situation with regard to stunning of animals before Halal slaughter) tend to improve in later years of their study, describing the situation in line with current practices. On the acceptability to veterinary students of Halal meat from animals that have been stunned, the majority of respondents indicated that they would buy and consume Halal meat from stunned animals. Respondents who indicated that they would avoid stunned Halal meat may have done so with the believe that Halal stunning is not as humane as conventional stunning, or they may have done so for reasons not related to animal welfare. Levine and colleagues (2015) observed differences in the level of understanding of humane procedures between US veterinary students with aspirations to work with food animals from those aspiring to work with companion animals, this may account for why some students consciously avoided meat derived from animals stunned with stunning methods they may perceive to be inhumane. Similarly, Mariti and others (2018) observed that veterinary students in Italy gave more consideration to the welfare of companion animals than that of food animals, this may affect their perception and understanding of animal welfare issues around food animals. It must be noted that there is no real procedural difference between stunned Halal and conventional slaughter (with the exception of a short prayer during Halal slaughter). Therefore, the humaneness of stunned Halal slaughter is not inferior to the humaneness of conventional slaughter, one cannot therefore use humaneness (or the lack of it) as a reason to avoid meat from effectively stunned Halal slaughter. Interestingly, the results also showed a significant increase in the percentage of vegans and vegetarians with year of study who would wittingly buy Halal meat from stunned animals. Although, presumably, vegans and vegetarians will usually avoid purchasing meat, their responses may have been for a number of reasons; i) some respondents may have been vegetarians who would consume meat if the humaneness of slaughter was guaranteed, ii) the level understanding of vegetarians and vegans on animal welfare issues (particularly humane slaughter) may have improved as they progressed in the level of study or iii) they may just have given hypothetical answers to the question as they were only given the 'yes' or 'no' alternatives and did not feel they could miss a section of the questionnaire. In retrospect, more thought should have gone into the questionnaire to avoid ambiguity in interpretation of the responses made by the vegetarians and vegans to this question. Beardsworth and Keil (1991) in a study on vegan and vegetarian trends, reported that ethics and welfare were the main reasons why some consumers avoided meat. One may argue that if some vegans and vegetarians can be assured of the highest welfare of animals, it may change their consumption pattern, which may explain this finding.

In line with scientific opinion on the welfare aspects of slaughter without stunning (Gibson *et al*., 2009; Mellor and Littin, 2004; Gregory *et al*., 2012), the majority of respondents indicated that all animals must be stunned before slaughter. This is consistent with the observation made by Broom (1999), who reported that there is increased awareness around animal welfare at slaughter which has led to an increasing number of consumers demanding humanely slaughtered products or avoiding those associated with poor welfare. A small proportion of respondents indicated that they did not have an opinion on stunning. With the recent rise in campaigning for restrictions or a ban on non-stun slaughter by the veterinary profession and other animal welfare charities (e.g. the British Veterinary Association, Royal Society for the Prevention of Cruelty to Animals), one would expect veterinary students to be well informed and hold an opinion on pre-slaughter stunning. The issue is further highlighted by the ten respondents who selected 'other' and left comments to support their choice (see table 8). The comments showed variation in the opinion of veterinary students with regard to stunning and slaughter; some respondents questioned the humaneness of stunning and others called for the current exemption of religious slaughter from stunning to be withdrawn. One respondent cited their cultural upbringing as a factor that conflicts with their profession and opinion on stunning; the influence of the culture of students on their perception of animal welfare issues has been discussed by Philip and McCulloch (2010) who reported that students' attitudes to animal welfare were influenced by their cultural upbringing, with students from Europe and the USA less likely to 'condone cruelty to animals'. A minority of respondents indicated that in their opinion, religious slaughter should be exempt from stunning to comply with traditional religious values. This is the case in some but not all EU member states (e.g. the UK, France, Germany and others), where a derogation is applied to permit the slaughter of animals without stunning for religious rites.

The results showed a higher proportion of vegans 3.9% (18) compared with the general UK population which is estimated at around 1.16% (The Vegan Society, 2018). This may be in part due to an increased empathy for animals by veterinary students due to their close association and everyday contact with animals. A minority of the respondents (less than 1%) indicated that they did not eat meat due to 'dietary exclusion by choice. These respondents neither identified themselves as vegans, vegetarians, nor meat eaters. The assumption is that they probably had an intolerance, allergic or medical reason for not consuming meat.

## 3.5. Conclusions

As future enforcers of the law at the time of slaughter (including Halal), it is important for veterinary institutions in the UK to introduce students to the science and politics surrounding religious slaughter at all stages of their veterinary education so that students will be well informed about these issues on qualifying. It appears that, for the majority of veterinary students the debate surrounding Halal meat production is not concerned with religious ideas, but animal welfare, with the majority of respondents indicating that they would consciously consume Halal meat if it was obtained from animals that have been effectively stunned. Vegetarianism and veganism are slightly increased among veterinary students in comparison with the general UK population. It is recommended that future studies on this topic should consider evaluating the curriculum of different universities to examine whether there are disparities in teaching on religious slaughter, and how it might be improved in general.

# 4. Measurement of voltage drop across cattle heads and the migration of sodium and potassium ions through neural membranes.

**Linking Narrative:** This chapter describes two *in vitro* experiments conducted with bovine heads and brain cells to estimate the electrical parameters required to develop the prototype SPUC stunner. The first part of the experiment involving bovine heads enabled an estimation of the impedance of the heads as well as confirm the feasibility of applying voltage through nose and neck electrodes. The second experiment involving brain samples enabled the estimation of the factors influencing the electroporation of neural membranes.

## 4.1. Introduction

Mechanical stunning followed by chest sticking to sever the brachiocephalic trunk is the most common method used for humane slaughter of cattle (Wotton *et al.,* 2000). This method is however not without its shortfalls. The Humane Slaughter Association (HSA, 1995) estimated that the average time interval between stunning and sticking is 73.6 s, this presents animal welfare issues in situations where there are inadequate tissue damage and animals recover before they are bled-out, or during the period they are bled-out. The longer stun to stick duration may be due to the violent post-stun convulsions associated with mechanical stunning, this can be injurious to slaughter operatives when they are kicked. The use of mechanical stunners (particularly those operated pneumatically) have also been associated with the spread of brain embolic materials to edible offal of cattle carcasses, thus linking it to the possible spread of Bovine Spongiform Encephalopathy (Garland *et al.,* 1996). Effective electrical stunning provides an alternative means for the humane slaughter of cattle, whilst eliminating some of the animal welfare and human health and safety issues associated with mechanical stunning. The Jarvis Beef Stunner is a commercial system which is used in Europe and other parts of the world to effectively stun cattle. Wotton et al. (2000) reported that the Jarvis Beef Stunner operates in three sequential cycles; first, the animal is stunned (head-only) via a three-second current application to the head, the second cycle involves the fibrillation of the heart with a 15 s current application to the brisket and the final cycle involves a 4 s application of current to discharge the spinal cord with a view to abolishing post-stun convulsions.

The development of any new or modified electrical stunning system depends on a knowledge of the resistance of the location where the electrodes are placed to deliver the voltage, this ensures an estimation of the amount of the applied voltage which reaches the brain to cause neural dysfunction. To this end, this experiment was conducted (at the abattoir on the campus of Bristol University's Veterinary School) as part of the development of the SPUC stunner to identify the electrical parameters for the development of the SPUC stunner. A further experiment was conducted to ascertain the effect of voltage on the influx or efflux of ions from neural membranes. The two main experiments in this chapter are: i) Use of cattle heads to measure resistance and how voltage develops within the head using a 250 V, 50 Hz power supply and ii) Use of brain cells to evaluate the electrical parameters needed to induce ion influx/efflux from neural membranes using a Gene Pulser Electroporation System (Bio-Rad Laboratories Inc., USA).

## 4.2. Materials and methods

### 4.2.1. Voltage measurement across cattle heads

The aim of this experiment was to measure the resistance of cattle heads and how voltage develops within the head.

#### 4.2.1.1. Animals

The five animals used for the experiment were: one male Aberdeen Angus and 4 females; 3 Hereford X and 1 Aberdeen Angus. The age of animals ranged between 20 and 24 months.

#### 4.2.1.2. Method of slaughter

Cattle were slaughtered according to the normal slaughter routine at the University of Bristol Veterinary School's abattoir, Langford, UK. Carcasses from all five animals were passed fit for human consumption by the Official Veterinarian (OV). The slaughter method involved pre-slaughter stunning of cattle with a penetrating captive bolt gun followed by a thoracic (chest) stick to ensure rapid loss of blood. The OV subsequently inspected the heads before the experiment was conducted.

#### 4.2.1.3. Voltage application to cattle heads

A 250 V, 50 Hz power supply with an isolated (from earth) output was used to apply voltage through 2 large needle electrodes, one inserted in the nose and the other in the neck. Two probes were inserted close to the nose and neck electrodes (Kelvin connections) to measure (a) the voltage developed across the head and (b) to be able to calculate the resistance of the application to the head. A Kelvin connection is a set of precision electrodes used to deliver current in a manner that reduces contact impedance between the electrodes and the contact surface (in this case cattle heads). A set of probes were manufactured from stainless steel insulated apart from the recording surface at the tips and inserted into the brain through carefully drilled holes through the skull. These probes measured the voltage that was developed within the cranium across neural tissue. The objective of this experiment was to estimate the proportion of the applied voltage across the head and brain, and to calculate the resistance of the head. This information was vital in the design of the electrical component of the SPUC stunner. The experimental set-up and data obtained from the experiment are shown below in figure 7 and table 10 respectively.

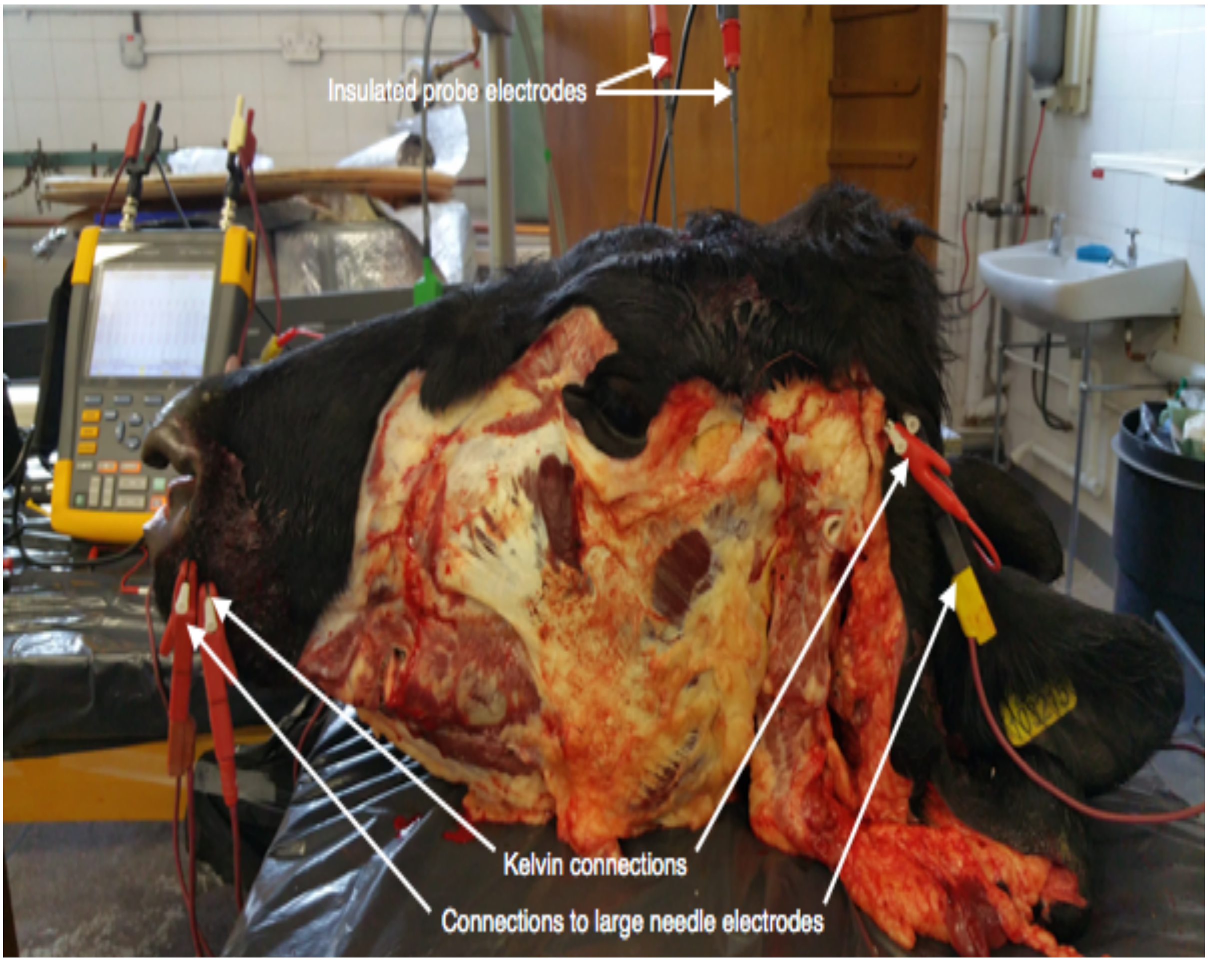



Figure 7. Photograph of a cow's head with stimulating and recording electrode array during measurement of voltage across the head.

## 4.2.2. Measurement of ion migration from brain cell membranes

### 4.2.2.1. Animals

Brain samples were obtained from two animals slaughtered at the Langford abattoir according to the method of slaughter described in chapter 4.2.1.2. The animals were; one each of male and female Aberdeen Angus, aged 22 months and 24 months respectively.

### 4.2.2.2. Brain sample preparation

Brain samples were collected using a core borer immediately after slaughter and post-mortem inspection according to the normal slaughter protocols at the Langford abattoir (see slaughter method in 4.2.1.2 above). Samples were immediately placed into test tubes containing ice in order to keep the cells alive and also to increase the efficiency of the electroporation of cells. Potter *et al* (1984) reported that the electroporation of rat brain cells at low temperatures (around 0° C) increased the efficiency of transfection by three-fold. The brain samples were then washed three times in de-ionised water to remove all remnant ions and other contaminants. Cells were dissociated into smaller fragments using a scalpel and samples were placed in test tubes and 5 ml of de-ionised water added. The cell solution was pipetted to remove all lumps and then centrifuged at 800G for 5 minutes. After centrifugation, the top solution was discarded leaving the cells at the bottom of the tubes. De-ionised water was then added and pipetted continuously to get a clear solution of cell suspensions. Using 0.2 cm cuvettes, the cells were subsequently electroporated using a Gene Pulser Xcell electroporation system (Bio-Rad, USA). Both single and multiple pulse protocols were used. The concentrations of sodium and potassium ions were measured using Horiba sodium and potassium meters (Horiba Instruments, Northampton-UK). This PhD project is based on the principles of electroporation, the aim of this part of the experiment was therefore to investigate the feasibility of electroporating bovine neural membranes and to identify optimal electrical parameters needed to do so. A knowledge of the electrical parameters needed to electroporate brain cells, combined with the measurement of the voltage and resistance across the heads of cattle were used to design the electronics of the SPUC beef stunner.

### 4.2.2.3. Ion migration from neural membranes

Two electroporation protocols; time constant and exponential protocols were used to measure the effect of electroporation on the migration of ions between neural membranes.

#### 4.2.2.3.1. Use of time constant-pulse protocol

The time constant-pulse protocol involved the use of two set of electrical parameters (input parameters). First, 300 V was applied for 10 ms (input electrical parameters), repeated four times. Then 400 V was applied for 13 ms and repeated four times. The concentration of sodium and potassium ions were measured before and after electroporation.

#### 4.2.2.3.2. Use of exponential decay pulse protocol

Voltage of 3000 V, capacitance of 50 F and resistance of 100 were inputted into the Gene Pulser Xcell electroporation system using the exponential decay-pulse protocol. The experiment was repeated four times with the following mean output parameters; 4.8 s time constant and a voltage of 2990.8 V.

## 4.3. Results

## 4.3.1. Voltage drop in cattle heads

Table 10 shows the applied electrical parameters and the average kelvin and probe recordings. The results showed that the amount of voltage that developed in cattle heads (after the application of the 250 V) were 163.3, 157.8, 138, 148, 144.3 V in animals 1,2,3,4,5, respectively. The average electric field in cattle heads was found to be 3.3. $Vcm^{-1}$ whilst that in the brain was calculated to be 1.3 $Vcm^{-1}$. The average current was found to be 0.9 A and the mean resistance of the heads was calculated to be 279.8 Ω.

| | | | | Kelvin connections | | | Brain Probes | | |
|---|---|---|---|---|---|---|---|---|---|
| Cow | Mean applied voltage, V1 (V) | Mean current(A) | Resistance (Ω) | Voltage, V2 (V) | Separation (cm) | Eelectric field, E (V/cm) | Voltage, V3 (V) | Separation (cm) | Electric field, E (V/cm) |
| 1 | 250 | 0.99 | 253 | 163.3 | 43 | 3.8 | 1.48 | 4 | 0.37 |
| 2 | 250 | 0.90 | 278 | 157.8 | 45 | 3.5 | 6.50 | 5 | 1.30 |
| 3 | 250 | 0.74 | 337 | 138.0 | 50 | 2.7 | 7.61 | 5 | 1.52 |
| 4 | 250 | 0.99 | 253 | 148.0 | 46 | 3.2 | 6.76 | 5 | 1.35 |
| 5 | 250 | 0.90 | 278 | 144.3 | 46 | 3.1 | 9.47 | 5 | 1.90 |

Table 10. Results of voltage measurements across cattle heads.

## 4.3.2. Ion migration from neural membranes

The average output electrical parameters produced for the experiments using 300 V for 10 ms were; time constant of 9.9 ms, voltage of 296.8 V, capacitance of 100 F and resistance of 100 Ω. The average output parameters for the experiment using 400 V applied for 13 ms were; 12.3 s time constant, 397 V, capacitance of 93.8 F and resistance of 112.5 Ω. The concentration of sodium ions ($Na^+$) before the experiments were 17, 15, 12, 13 for the first, second, third and fourth repetitions respectively, whilst the final concentrations of the same ion were 20, 12, 12 and 14. Changes in potassium ion concentration in the four experiments were; 41 to 37, 32 to 26, 35 to 29 and 29 in the first, second, third and fourth experiments respectively. Tables 11a and 11b show the input and output electrical parameters and the changes in ion concentrations.

| Ion concentration before electroporation | Inputted electrical parameters | Output electrical parameters | Ion concentration after electroporation |
|---|---|---|---|
| $Na^+$ 17<br>$K^+$ 41 | 300 V<br>10ms | TC=7.8ms<br>Voltage= 298 V<br>Capacitance=100<br>Resistance= 100 | $Na^+$ 20<br>$K^+$ 37 |
| $Na^+$ 15<br>$K^+$ 32 | 300 V<br>10ms | TC=10.6<br>Voltage= 297<br>Capacitance= 100<br>Resistance= 100 | $Na^+$ 12<br>$K^+$ 26 |
| $Na^+$ 12<br>$K^+$ 35 | 300 V<br>10ms | TC=10.6<br>Voltage= 296<br>Capacitance= 100<br>Resistance= 100 | $Na^+$ 12<br>$K^+$ 29 |
| $Na^+$ 13<br>$K^+$ 29 | 300 V<br>10ms | TC=10.6<br>Voltage= 296<br>Capacitance= 100<br>Resistance= 100 | $Na^+$ 14<br>$K^+$ 27 |

Table 11a. The effect of electroporation on ion concentration following time constant pulsing-using 300 V for 10 ms.

| Ion concentration before electroporation | Inputted electrical parameters | Output electrical parameters | Ion concentration after electroporation |
|---|---|---|---|
| $Na^+$ 9<br>$K^+$ 20 | 400V<br>13ms | TC= 15.5<br>Vol= 398 V<br>Cap= 100<br>Res= 150 | $Na^+$ 9<br>$K^+$ 19 |
| $Na^+$ 9<br>$K^+$ 20 | 400V<br>13ms | TC= 15.3<br>Vol= 398<br>Cap= 100<br>Res= 150 | $Na^+$ 8<br>$K^+$ 17 |
| $Na^+$ 68<br>$K^+$ 160 | 400V<br>13ms | TC= 14.8<br>Vol= 394<br>Cap= 75<br>Res= 0 | $Na^+$ 69<br>$K^+$ 150 |
| $Na^+$ 77<br>$K^+$ 170 | 400V<br>13ms | TC= 16.6<br>Vol= 398<br>Cap= 100<br>Res= 150 | $Na^+$ 72<br>$K^+$ 160 |

Table 11b. The effect of electroporation on ion concentration following time constant-pulsing using 400 V for 13 ms.

### 4.3.2.1. Use of exponential decay-pulse protocol

Table 11c shows the concentration of $K^+$ and $Na^+$ ions before and after electroporation.

| Ion concentration before electroporation | Inputted electrical parameters | Output electrical parameters | Ion concentration after electroporation |
|---|---|---|---|
| $Na^+$ 7<br>$K^+$ 21 | Voltage= 3000 V<br>Capacitance= 50<br>Resistance= 100 | TC= 4.7ms<br>Voltage= 2994 V | $Na^+$ 9<br>$K^+$ 18 |
| $Na^+$ 7<br>$K^+$ 21 | Voltage= 3000 V<br>Capacitance= 50<br>Resistance= 100 | TC= 4.8<br>Voltage=2987 | $Na^+$ 8<br>$K^+$ 18 |
| $Na^+$ 9<br>$K^+$ 19 | Voltage= 3000 V<br>Capacitance= 50<br>Resistance= 100 | TC= 4.8<br>Voltage= 2990 | $Na^+$ 9<br>$K^+$ 18 |
| $Na^+$ 9<br>$K^+$ 20 | Voltage= 3000 V<br>Capacitance= 50<br>Resistance= 100 | TC=4.9<br>Voltage= 2992 | $Na^+$ 9<br>$K^+$ 18 |

Table 11c. The effect of electroporation on ion concentration following different electrical parameters.

## 4.4. Discussion

The results obtained from the voltage measurements in cattle heads (Table 10) showed that the electric field in the head was found to be 3.3 Vcm $^{-1}$ and 1.3 Vcm $^{-1}$ in the brain It was also observed that the application of electric current to the heads of cattle was not as effective as when stunning animals commercially (Jarvis Beef Stunner) due to a higher than normal impedance between the electrodes and the heads in the experimental set-up. This may have contributed to the high voltage drop from the point of application (250 V) to the Kelvin connections (mean voltage of 150V) and the high resistance of the cow's head (250 Ω). This differs from the resistance of 98 Ω in the cow's head recorded by Wotton and his colleagues (2000) during the electrical stunning of cattle. Another reason for the higher drop in voltage may be due to the electrodes used (need electrodes) in the experiment, the electrodes may not have made good contact with tissues to prevent the large drop in voltage. The second experiment involved the electroporation of neural membranes *in vitro*, followed by the measurement of $Na^+$ and $K^+$ concentrations. The aim was to estimate the percentage of electroporation using the number of ions in solution to predict the amount of ion channels opened as a result of the application of electric current. It was observed that the experimental results were not reproducible, hence did not give an accurate measure of the percentage electroporation of these cells. It was therefore decided that further tests were needed in order to accurately measure the percentage of electroporation. To this end, further investigations were conducted at York University with brain samples collected from a beef abattoir in York. Instead of measuring ionic concentrations, percentage electroporation was measured using a dye, calcein (fluorexon), this dye does not normally penetrate the bi-layer cell membrane unless pores are created through the membrane. Cell density, pore size, imaging and the number of viable cells were determined using DAPI and a confocal microscope (see chapter 5 for experimental results).

## 4.5. Conclusion

The measurement of voltage drops in cattle heads suggested that the best route of voltage application to the brain is through nose and neck electrodes. This ensures that sufficient current reaches the brain to induce neural dysfunction and unconsciousness. Using this information, the SPUC stunner was designed with a nose plate electrode and a pair of neck restraints that acted as electrodes so that current is simultaneously applied between the two electrodes to traverse the brain. The second part of the experiment involved the electroporation of brain cells and the subsequent measurement of ion migration from neural membranes.  This was vital in understanding the effect of electroporation on potassium and sodium ion migration from neural membranes and using this to estimate the proportion of cells electroporated. The results indicated that electroporation does result in ion migration, however using ion migration in estimating the percentage of brain cells electroporated was found to be an unreliable technique. It was therefore seen that further tests were required to be done to measure the factors influencing electroporation of neural cells using a different technique and this led to the work reported in the following chapter, Chapter 5.

# 5. An investigation of the amount of energy, optimum voltage and number of pulses required to electroporate bovine brain cells to determine the electrical parameters to estimate electrical parameters for the development of Single Pulse Ultra-High Current (SPUC) for the humane slaughter of cattle.

## 5.1. Introduction

Electroporation is a technique in molecular biology that involves the application of strong transverse electric fields to cell membrane suspensions (Dimitrov and Sowers, 1990) or other biological structures, so as to induce pores (Knight & Scrutton, 1986, Neumann et al, 1989, Weaver, 2003, Chen et al, 2006). Granot and Rubinsky (2007) explained that there are two types of pores that may be induced through electroporation; reversible and irreversible pores. A reversible pore is a temporary pore capable of resealing shortly after the application of voltage, whilst an irreversible pore is a permanent pore that cannot reseal. Chen et al. (2010) noted that electroporation has a wide range of applications, including the transportation of different therapeutic molecules to the cytoplasm of target cells through the induced porous membrane. The advantage of delivering drugs through electroporation is that drugs can be applied directly to the target cells or organs, this comparatively reduces the dosage of the drug that would otherwise be required using conventional drug delivery systems (through hypodermic needles or oral ingestion) and the associated side effects (Rice et al., 2008). Other applications of the technique include the delivery of proteins *in uteru* (Tabata &Nakajima, 2001), the delivery of DNA *in vivo* and *in ovo* (Muramatsu et al., 1997) and the *in/exo* uteru expression of plasmids in DNA (Saito & Nakatsuji, 2001). In the field of humane slaughter of animals for human consumption, it has been suggested that the electroporation of brain cells may be the mechanism by which insensibility is induced during certain types of pre-slaughter stunning procedures that are applied to animals prior to slaughter in order to abolish the pain associated with the slaughter process itself (Robins et al., 2014). Stunning at slaughter is defined by the European Council Regulation (EC 1099/2009) as "any intentionally induced process that causes loss of consciousness and sensibility without pain, including any process resulting in instantaneous death". The clinical application of electroporation is becoming more popular and widespread in recent years. Rubinsky (2007), however, argues that although electroporation is often viewed as a new technique, its discovery may date back to as early as 1754. Research investigating the effect of electrical discharge on the skin from a static generator (Nollet, 1754) and the conclusion by Fuller (1898) that "multiple high voltage discharges have bactericidal effect on a water sample" may have been the earliest applications of irreversible electroporation. Despite the suggestion that the technique has been known for over two centuries, Tieleman (2004) proposes that the molecular basis of electroporation is still not well understood. Nevertheless, attempts have been made to explain the factors influencing electroporation and the mechanism of pore formation through theory and computer simulation (Tieleman et al., 1997, Smith et al., 2004, Chen et al., 2006). Factors affecting electroporation may be intrinsic (biological) or extrinsic (physical). Potter et al (1984) suggested that the efficacy of electroporation depends on the temperature at which cells are electroporated. Indeed, it has been demonstrated that the electroporation of rat brain tissues at 0°C was 3-fold more effective than electroporation at a higher temperature of 20°C (Li et al., 1997). Other factors, which have been associated with the efficacy with which tissues are electroporated, include voltage, waveform, pulse duration, number of pulses and electroporation medium/buffer (Rols & Teissié, 1990, Vanbever & Préat, 1995).

The aim of this study was to investigate the number of pulses and range of voltage required to successfully electroporate bovine brain cells, and human T-lymphoblastoid cells as a model for the effectiveness with which a pulsed ultra-high current approach to pre-slaughter electrical stunning might be operating in terms of electroporation.

## 5.2. Materials and methods

### 5.2.1. Jurkat cells

293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) media supplemented with 10% Fetal Bovine Serum, L-glutamine, minimum essential amino acids and subcultured at 1:10 when 90% confluent.

### 5.2.2. Bovine brain cells

Brain samples were taken after slaughter and post-mortem inspection at a beef abattoir in York, UK. The samples were obtained from a 29-month-old British Blue Cross cow which was stunned with a penetrative captive bolt gun prior to slaughter by thoracic sticking. Brain samples were removed from the head using a water hose that was applied through the hole created by the captive bolt. The samples were placed in a container and immediately put in a box containing ice. A section of the brain tissue was subsequently carefully dissected, all connective tissues and other attachments were removed from the brain tissues.

### 5.2.3. Dyes

3 µl of calcein (Sigma, Cat. No. C0875) was mixed with the cell suspension in a 0.2 cm gap cuvette before treatment. Calcein was used because it does not readily penetrate intact cell membranes, therefore the efficacy of electroporation was measured by the migration of calcein through the cell membrane.

### 5.2.4. Live/dead cell marker

DAPI (4',6-diamidino-2-phenylindole dihydrochloride) (New England Laboratories, Cat. No. 4083) was used as a live/dead cell marker. DAPI is a fluorescent stain that excludes dead cells due to their compromised lipid bi-layer cell membrane.

### 5.2.5. Electroporator

Gene Pulser Xcell Electroporation System (Bio-Rad Laboratories Inc., USA) was used to deliver single, double and triple pulses via 0.2 cm gap cuvette electrodes (Bio-Rad Laboratories, Cat. No. 1652082). Both time constant and exponential decay protocols were used. Square waveforms were used with pulse duration of 5 ms for single pulses and increment of 0.05 ms for double and triple pulses. Double and triple pulses were achieved by pre-setting the protocols. The gap between the electrodes in the cuvette were 0.2 cm.

### 5.2.6. Electroporation protocol-Jurkat cells

The Jurkat cell lines were cultured in Phosphate buffered saline (PBS) and electroporated using single and multiple pulses. A volume of 300 µl of the cell suspension was pipetted into a 0.2 cm cuvette followed by the addition of 3 µl of calcein. Calcein is a cell permeant dye that is used to determine the effectiveness of electroporation. The dye is impermeable to intact cell membranes and permeability of the cell membrane is only possible when pores are created through the membrane. The cell suspension was then electroporated using Gene Pulser Xcell electroporation system (Bio-Rad Laboratories Inc., USA). Exponential decay and time constant protocols were used (see table 12 for the applied voltages and energy). The successful uptake of calcein through the cell membrane into the cytoplasm was an indication of membrane poration and electroporation. It is worth noting that a small fraction of cells from the control group, that is, cells that were stained with the dye (calcein) but with no application of electric pulses, still showed some fluorescence. The experiment was repeated a number of times (see table 12 below) for each set of electrical parameters (except the treatment involving the application of 160 V). All samples were pulsed using a capacitance of 1000 μF, this is the recommended capacitance for use on Jurkat cells by the equipment manufacturer (Bio-Rad Laboratories Inc, USA). Immediately after pulsing, each sample was transferred from the cuvette into a test tube and washed twice by centrifugation; first, 1ml of PBS was added to the sample in the test tube and centrifuged (300 x g 4 minutes). The supernatant was carefully discarded, and a further 1 ml of PBS added for the second wash. After washing, 400 μl of PBS was added for bio-analysis. DAPI (concentration 1.4 μg $(ml)^{-1}$) was added to the cell suspension for the assessment of cell viability.

### 5.2.7. Electroporation protocol-Brain tissues

Brain tissues were washed using the same procedure described above for washing the Jurkat cell lines. After washing, a volume of 300 μl of PBS was pipetted into a 0.2 cm cuvette, a small tissue of brain (Approximately 1 mm x 1 mm) was then added to the PBS (in the cuvette) followed by the addition of 3 μl of calcein. Samples were then shocked using a Gene Pulser electroporation system (Bio-Rad, USA). After electroporation, the samples were washed twice in PBS, calcein was then used for imaging using a confocal microscope.

### 5.2.8. Measurement of fluorescence-Jurkat cells

A CyAn ADP flow cytometer (Beckman Coulter, USA) was used to analyse the uptake of calcein into the cytoplasm. Dead cells were excluded from live cells using DAPI. Intact cells were identified using forward and scatter profiles, they fell within the region R1 in Figure 8 below. The detectors used were for DAPI excited with the 405nm laser and the detector was a photomultiplier tube with a 450/50-band pass filter. Calcein was excited with the 488nm laser and the detector was a photomultiplier tube with a 530/40-band pass filter. The threshold trigger was FS 488nm. DAPI and calcein were used to determine the number of; intact cells (R1), intact live cells (R2), intact, live and single cells (R3) and intact, live and single cells that were calcein positive (R4). The proportion of cells successfully electroporated (R4/R3), characterized by the proportion of intact, live and single cells with high uptake of calcein into the cytoplasm, was used to determine the extent of pore creation and molecular uptake.

### 5.2.9. Measurement of fluorescence-Brain cells

The post-electroporation analysis was carried out to determine the amount of calcein that penetrated the cell membrane. Cell viability was determined by using DAPI (2-(4-amidinophenyl)-1H -indole-6-carboxamidine). A confocal microscope (Zeiss, Germany) was used to analyse brain tissues for the uptake of calcein post-electroporation.

### 5.2.10. Statistical analysis

To assess the form of the relationship between the total energy supplied during the pulse and the total live, single, porated cells as a percentage of the total intact cells, the data were modelled using polynomial regression in SPSS v23. Polynomials of increasing order were fitted until an additional order failed to reach statistical significance at $\alpha = 0.05$.

## 5.3. Results

The effects of energy and different electrical parameters (voltage, pulse duration and number of pulses) on the efficacy of cell membrane electroporation were investigated. The samples were examined post-electroporation using a flow cytometer to determine the percentage of cells electroporated. Figure 8 is shown as an example of the plot, for one sample, of the different regions, R1, R2, R3 and R4 of a flow cytometer image using DAPI as a live vs. dead discriminator and the uptake of calcein as evidence of membrane poration.

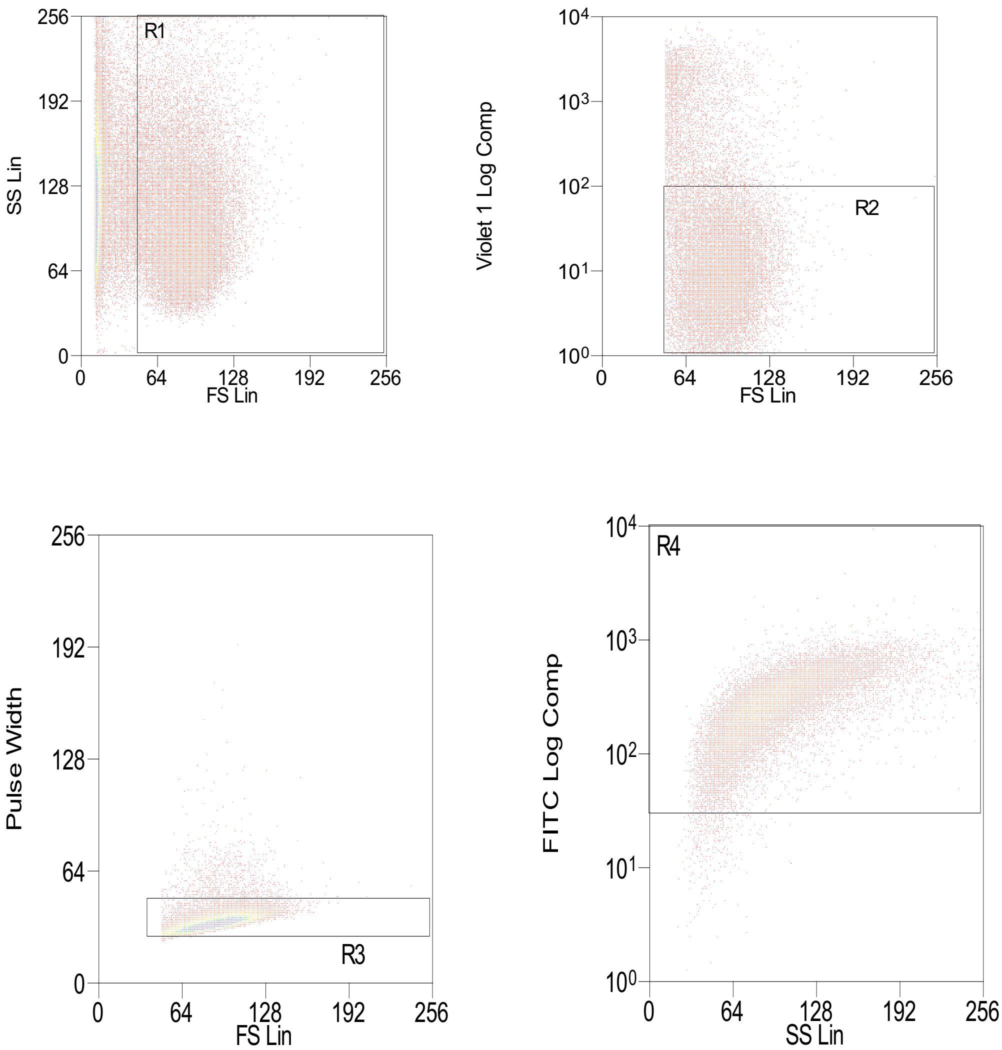



Figure 8. Illustration of the regions R1, R2, R3 & R4 in flow microscopic plots. R1 is gating on intact cells using forward and side scatter detectors, R2 is gating on intact live cells using DAPI as a live vs dead discriminator (live cells are DAPI negative, y-axis), R3 is gating on intact, live and single cells using pulse width (forward scatter trigger), y-axis and R4 is gating on intact, live single cells that are positive for calcein (y-axis).

### 5.3.1. Effect of energy

As stated above, the total cell count may contain cell debris and dying and/or dead cells. This was accounted for by observing the behaviour or viability trend of cells in the control group (the sample that was not electroporated). It was established that the proportion of debris/dead cells in the total cell count was 0.367, therefore, the percentage of cells electroporated was calculated using the formula (R4/(Total cell count*0.9633)*100. Figure 10 illustrates the relationship between the energy supplied and the proportion of cells that were successfully electroporated (R4/Total cell count). A cubic polynomial best described the overall form of the relationship ($p = 0.003$ for the cubic term). However, once the statistical significance of the overall form of the relationship is established it is better shown by a more data driven curve fitting procedure. In Figure 9, a Loess fit, using a biweight kernel fitting 45% of the points is used. The results showed that increasing the amount of applied energy resulted in a corresponding rise in the uptake of calcein through the cell membrane. As the energy continued to increase, so did the percentage of electroporation, until it reached a threshold where electroporation begun to sharply decrease with increasing amount of energy. From Figure 9, it can be seen that there was no difference in effect between single and double pulses, suggesting that the total energy delivered was the main driver of the poration of the cell membrane.

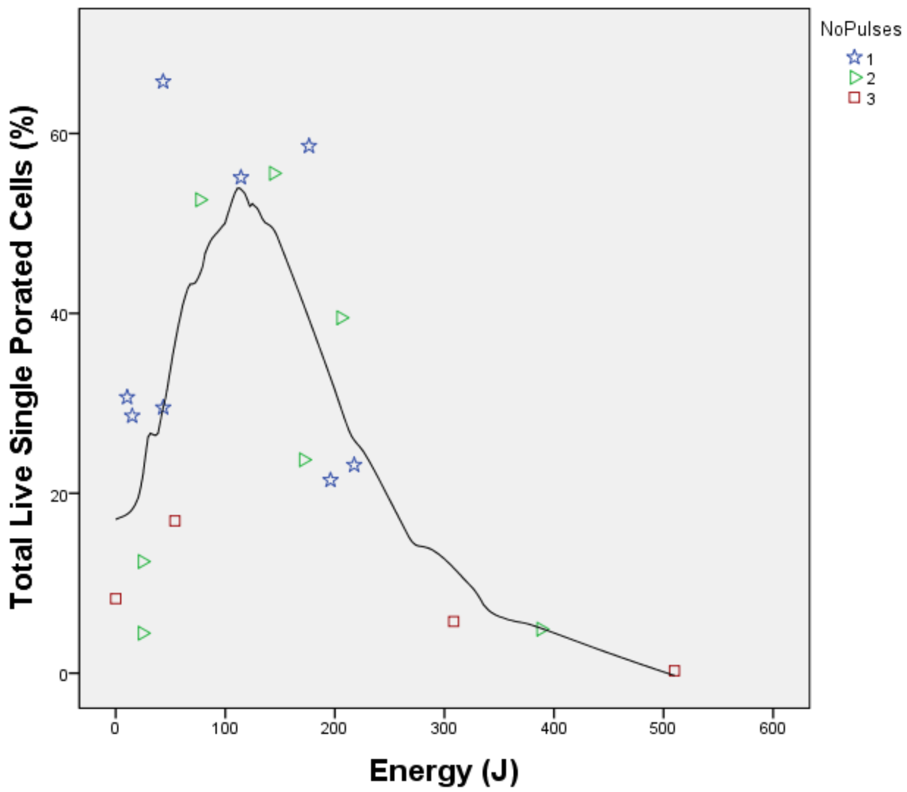



Figure 9. The effect of energy and number of pulses on the uptake of calcein through neural membrane.

## 5.3.2. Effect of voltage

The applied voltage was varied between 50 and 200 V with varying numbers of pulses. The voltage applied had an effect on the percentage of intact live cells, dead cells and the uptake of calcein (Table 12). The percentage of intact live cells was stable between 50 and 140 V. However, an increase in voltage to 160 and 200 V resulted in the decline in the percentage of intact live cells. As seen in Table 12 below, the optimum voltage for the electroporation of Jurkat cells under the conditions performed in this experiment was 140 V. Above 140 V, there was an increased number of cell deaths, albeit there was high uptake of calcein in the cells that remain alive. Below 140 V, there is reduced uptake of calcein into single intact live cells, this drops sharply at 50 V. Figure 10 below shows the effect of energy and number of pulses on electroporation efficiency.

| Applied voltage (V) | Pulse | Energy | Total cells | % Intact live cells | % of cells electroporated | Dead cells |
|---|---|---|---|---|---|---|
| 200 | 1 | 176.42 | 32666 | 79.18 | 100 | 7734 |
| 200 | 1 | 217.58 | 56903 | 49.70 | 93.53 | 28818 |
| 200 | 1 | 196.02 | 63418 | 67.07 | 89.50 | 40873 |
| 200 | 3 | 510.00 | 48131 | 45.38 | 96.06 | 47763 |
| 200 | 2 | 388.09 | 22162 | 36.65 | 97.93 | 18702 |
| 160 | 3 | 0 | 75256 | 76.59 | 46.71 | 57329 |
| 140 | 1 | 114.26 | 29735 | 82.18 | 89.07 | 5789 |
| 140 | 3 | 308.51 | 62619 | 78.63 | 96.95 | 57832 |
| 140 | 2 | 171.40 | 27111 | 81.24 | 35.12 | 3710 |
| 140 | 2 | 205.68 | 49992 | 84.25 | 98.59 | 26137 |
| 100 | 1 | 43.22 | 31528 | 75.14 | 49.31 | 5716 |
| 100 | 1 | 43.22 | 27429 | 82.62 | 96.76 | 4148 |
| 100 | 2 | 76.83 | 35073 | 87.48 | 89.17 | 11006 |
| 100 | 2 | 144.06 | 37915 | 86.59 | 88.35 | 9339 |
| 50 | 1 | 10.37 | 37547 | 76.51 | 49.02 | 3768 |
| 50 | 3 | 54.02 | 21375 | 83.93 | 48.44 | 12613 |
| 50 | 1 | 14.98 | 26311 | 83.55 | 40.41 | 3378 |
| 50 | 2 | 24.30 | 27594 | 84.65 | 6.48 | 4377 |
| 50 | 2 | 24.30 | 27789 | 85.69 | 17.61 | 4744 |
| 0 | 0 | 0 | 29406 | 96.33 | 0.06 | 725 |

Table 12. The effect of voltage and number of pulses on cell death, % intact live cells and the percentage of cells electroporated.

### 5.3.3. Effect of number of pulses

Single and multiple pulses were applied to cell suspensions. It was observed that the number of pulses applied had an effect on the percentage of cells electroporated and the number of dead cells (Table 12). The application of a single pulse and 140 V for instance resulted in less proportion of cell deaths than double and triple pulses (See table 12 above). We also observed that the percentage of cells electroporated were higher when single (89.1%) and triple pulses (97.0%) were applied, however, the application of a double pulse led to reduced percentage of cells electroporated (35.1%). Additionally, when the voltage was increased to 200 V, single pulsing resulted in a mean cell death of 25,808 (over 3 treatments) whilst double and triple pulses led to 18,702 and 47,763 cell deaths respectively. There was similar percentage of cells electroporated during the application of single (100%), double (97.9%) and triple (96.1%) pulses (see table 12). This suggests that although triple pulsing of the cells resulted in more cell deaths, all three forms of pulsing were effective in electroporating cell membranes. This is consistent with the results obtained when voltage was varied at 100 V and 140 V. However, at 50 V, there was a reduction in the proportion of cells that were successfully electroporated in comparison with the proportion of cells electroporated at 100 V, 140 V, 160 V and 200 V. In addition, at 50 V, double pulsing led to reduced percentage of electroporation than single and triple pulses. To ensure efficient electroporation with reduced cell death, voltage should be applied at 140 V single or multiple pulses. It is important to note that the number of dead cells recorded by the cytometer must always be read with caution, this is because this number may include cell debris that may be recorded as dead cells.

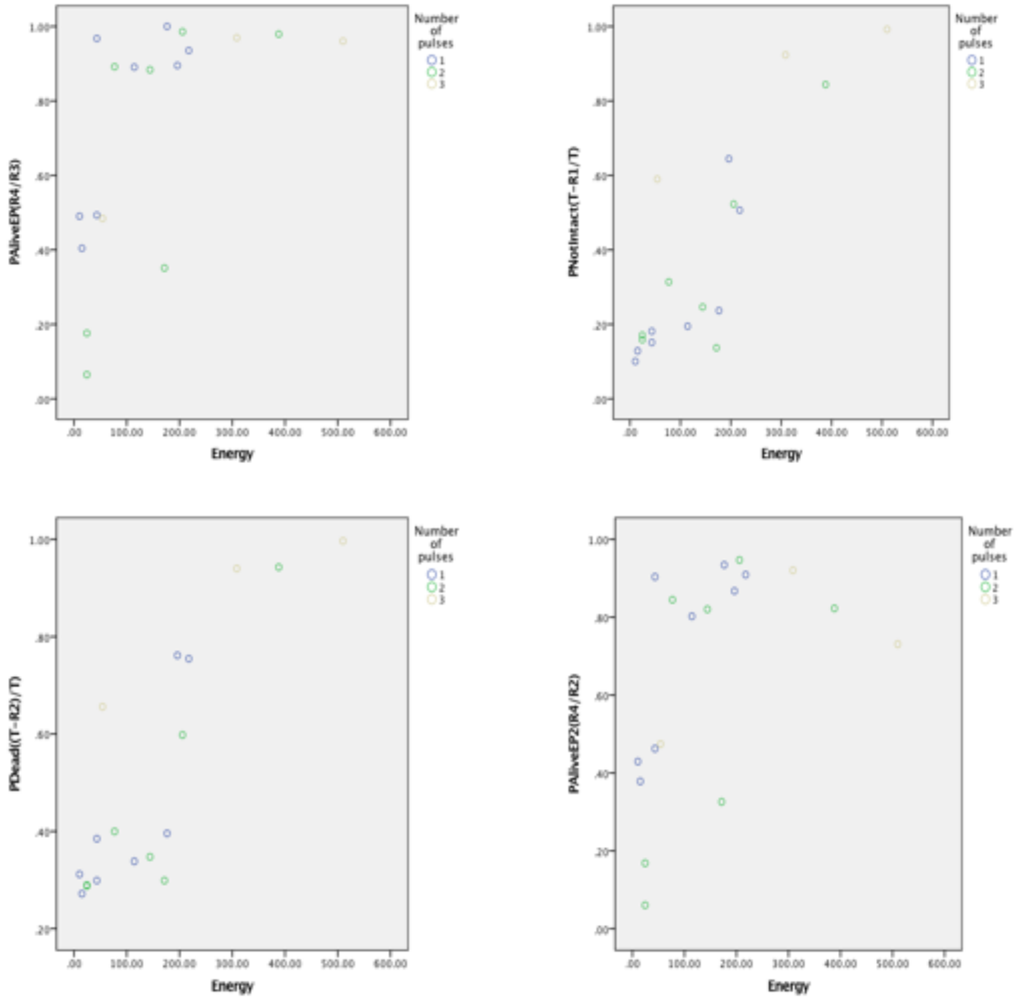



Figure 10. A plot of the impact of the amount of energy in Joules and number of pulses on (a) the proportion of cells electroporated, (b) the proportion of cells that have a compromised cell membrane (c) proportion of cells that are dead and (d) proportion of live cells that have taken up calcein.

## 5.4. Discussion and conclusion

This study investigated the amount of applied energy, optimal voltage and number of pulses required to effectively electroporate Jurkat cells using a Gene Pulser Xcell Electroporation System (Bio-Rad Laboratories Inc., USA). Different levels of energy and voltages were applied; 50 V, 100 V, 140 V, 160 V and 200 V with varying number of pulses. Exponential decay and time constant protocols were used. The successful uptake of calcein through the cell membrane into the cytoplasm was an indication of cell membrane poration and electroporation. The findings revealed that an increase in energy resulted in an initial sharp increase in the uptake of calcein. However, as the energy was continually increased, the percentage of cells electroporated begun to decrease. The possible reason for the sharp fall in the uptake of calcein as the energy continued to increase may be due to some cell lysis brought about by the sustained increase in energy. Energy was therefore an important factor in the poration of neural membranes. This is consistent with the findings of Joshi et al (2002) who reported that energy is the most important factor influencing the formation of pores during cell membrane electroporation. Also, high voltage pulsing resulted in increased uptake of calcein through neural membranes. However, at the upper limit of 200 V, there was a high percentage of cell death. This is consistent with the findings reported by Prausnitz et al (1996). The authors investigated the viability of using low and high voltage, with constant and pulsed electric fields respectively on the transport of a model drug, calcein, across human epidermis. They observed that high voltage pulsing increased the number of molecules transported across the skin, however, this was associated with noxious sensation. As stated above, Robins et al (2014) suggested that electroporation may be responsible for the induction of unconsciousness during SPUC electrical stunning of food animals prior to humane slaughter. Further investigation of the technique would be needed to ensure that its application is not aversive as suggested by Prausnitz et al (1996). Sung et al (2003) applied high voltages at 100 V, 300 V and 500 V and concluded that the uptake of the drug, valbuphine (and its prodrugs) through the skins of rats and mice increased with increasing voltage. The authors did not however give any indication of the amount of cell death. The high level of cell deaths associated with high voltage pulsing in the present study was due to the inability of the pores created to reseal, this process is termed irreversible electroporation. As stated above, there was also the issue of the flow cytometer counting cell debris as dead cells. The number of dead cells recorded by the cytometer must therefore be interpreted with caution. If electroporation were proven to be responsible for the induction of unconsciousness prior to slaughter, then irreversible electroporation of brain cells during stunning would result in improved animal welfare, in that, animals would not be able to recover from the stun when they are being bled-out, therefore slaughter operations could be carried out on an unconscious animal without the animal regaining consciousness. However, this method of stunning will not appeal to the Halal industry since many Halal authorities require that animals are able to recover from the stun to provide a form of assurance that pre-slaughter stunning does not result in the death of animals before bleeding-out. Therefore, the mechanism and principle of reversible electroporation would be best suited for the development of a stunning system for Halal slaughter. We also observed that the percentage of intact live cells were stable with the application of voltages between 50 and 140 V, however, the uptake of calcein was low at 50 V and high at 140 V. Increasing the voltage to 160 V and 200 V resulted in a reduction in the number of intact live cells, suggesting that high voltages may have deleterious impact on cell viability. Therefore, we estimate that, in order to increase electroporation efficiency with reduced cell death, the optimum pulsing voltage should be around 140 V. It is worth noting that a small fraction of cells from the control group, that is, cells that were stained with the dye (calcein) but with no application of electric pulses, still showed some fluorescence. Chen et al (2010) suggested that these "false positive cells" may have resulted from ineffective washing of the calcein off the surface of cell membranes before cytometric analysis, and the uptake of calcein through the compromised membrane of cells that may have died during sample preparation.

The number of pulses applied also had an effect on the efficiency of electroporation and cell death. The application of single and multiple pulses at high voltage had similar effect on the percentage of cells electroporated, however, high level of cell death was associated with triple pulses in comparison with single and double pulses. The effect of the number of pulses in this study was however not consistent on the proportion of intact live cells; single pulsing at 200 V resulted in a high percentage of intact live cells than double and triple pulsing, albeit the percentage of cells successfully electroporated were high in all 3 pulsing protocols. However, at 50 V and 140, the effect of single and multiple pulses on the percentage of intact live cells were similar. From the results of this experiment, it is suggested that high percentage of electroporation of Jurkat cells with reduced cell deaths can be achieved with single or double pulses applied with a voltage of 140 V.

# 6. Design, construction, assembly and trial of the Prototype SPUC Stunner

## 6.1. Introduction

As indicated in the preceding chapters of this thesis, the SPUC stunner is a high voltage head-only electrical stunning system which comprises a system of mechanical restraint designed to hold one animal at a time in compliance with European Council Regulation EC1099/2009. The restraint aims to reduce movement of animals and ensures the accurate application of the neck and nose electrodes of the SPUC Stunner. When the animal is in place, a pair of neck restraints are applied to reduce head movement, followed by a chin lift and a nose place. Both the neck restraint and the nose plate act as electrodes. The mode of operation of the SPUC stunner is similar to the Jarvis beef stunner which is widely used in New Zealand (head-only application) and some parts of Europe (head-to-body application). However, whilst the Jarvis Beef Stunner may have cardiac arrest (for non-halal operation) and spinal discharge (for halal operation in New Zealand) cycles, the SPUC stunner is a head-only electrical stunning system with no secondary application of current. Figure 11 below is a summary of the stages involved in the design and construction of the hardware of the SPUC stunner as well as the trials conducted to evaluate the efficacy of SPUC as a welfare-friendly method of stunning cattle. The restraint component of the SPUC stunner was designed and constructed by VCONS Abattoir Engineers Group, Belgium, with input from Bristol University, Silsoe Livestock Systems, Bedfordshire, UK and the author. The electronic component was designed and assembled by Silsoe Livestock Systems, under the supervision of Dr Jeff Lines in conjunction with Solutions for Research Ltd, Silsoe, Bedfordshire.

The mechanical restraint was manufactured by Vcons and installed in the project abattoir at Les Abattoir du Hainaut, Chimay, Belgium. The electronics were then incorporated into the mechanical restraint in the abattoir, a process with which the author assisted. There were a number of preliminary tests conducted, first, with a dummy load resistor to mimic the resistance of a cow and then once the functioning of the stunner was assured, with a cow to observe any behavioural (subjective) signs of loss of consciousness.

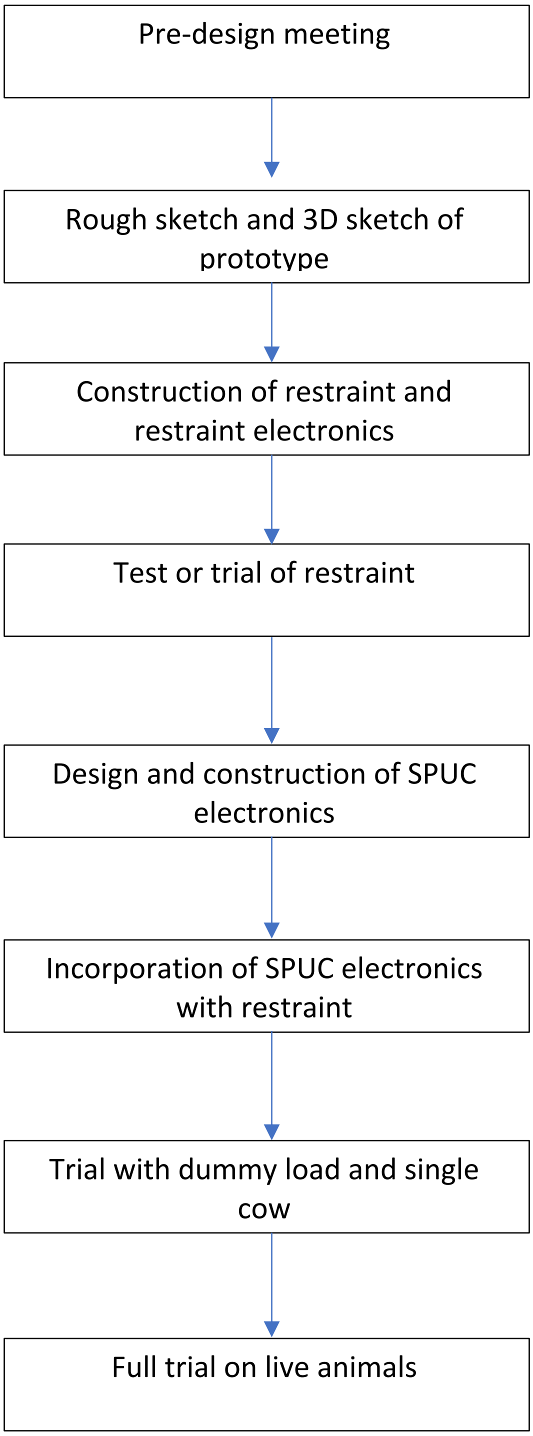



Figure 11. Main stages involved in the design and construction of the restraint and electronics of the SPUC stunner and subsequent trial on live animals.

## 6.2. Pre-design meeting

The project team met at the School of Veterinary Sciences, University of Bristol, Langford to discuss the design of the restraint and the electrodes. It was agreed that the design of the prototype stunner should include a neck restraint (which acts as a set of electrodes), a chin lift and a nose plate electrode. It was agreed that the best route of application of current would be through the nose and neck to ensure successful application of current to the brain. *In vitro* experiments conducted using cattle heads obtained from the Langford abattoir suggested that application of current through the nose and neck was probably the best route, additionally, current application in a commercial beef stunner, the Jarvis Beef Stunner is through a nose and neck electrodes.

## 6.3. Rough and 3D sketch of prototype

The cattle restraining pen was designed to allow its integration into an existing restrainer in the research abattoir. The construction and assembly of the hardware was carried out by Vcons Abattoir Engineers Group, Belgium with input from Silsoe Livestock Systems, the University of Bristol, UK, the author and the Euro Meat Group, Belgium. The entire restrainer, with the exception of the neck restraint and the nose plate were insulated using polyamide blocks to limit the passage of electrical current to the nose and neck electrodes.

An initial sketch of the SPUC prototype stunner was made with emphasis on the neck and nose electrodes (see figure 12a) by the project team at Bristol University. In the sketch, the neck restraints were made up of two metal electrodes applied on either side of the neck. A second sketch was made by Vcons (see figure 12b), in this design, there was one metal bar above the neck and one below. The aim was to restrain the neck of animals in a horizontal manner. It was later agreed that the best way to retrain the neck of cattle would be with the vertical neck restraints (figure 12a). The sketch in figure

12a was further developed with slight modifications into a 3D design by the production team at Vcons Abattoir Engineers Group (see figure 12c) with input from the project team in Bristol University (author included) and Silsoe Livestock Systems.

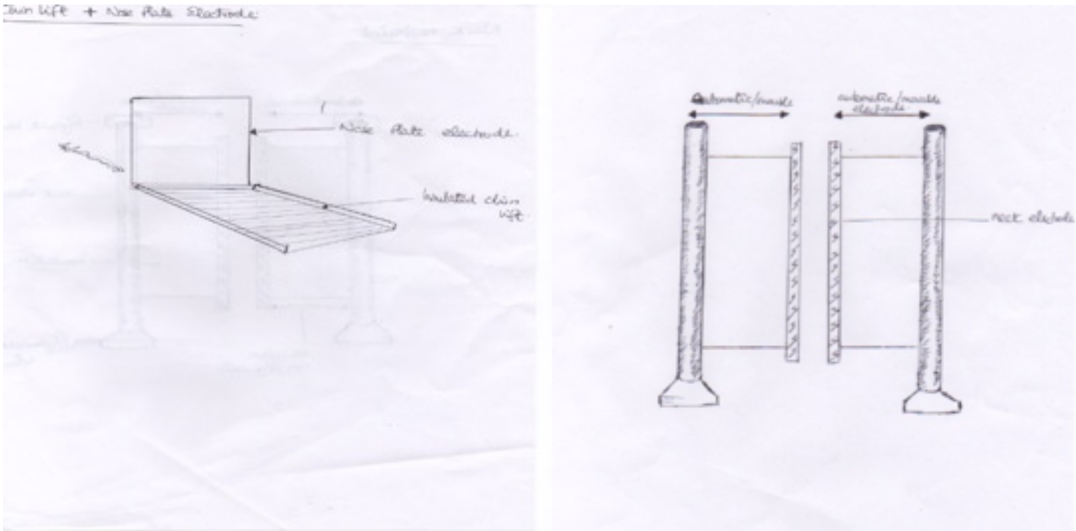


Figure 12a. Initial sketches of the nose and neck electrodes of the SPUC stunner which were later modified for optimal delivery of electricity to the nose and neck of cattle.

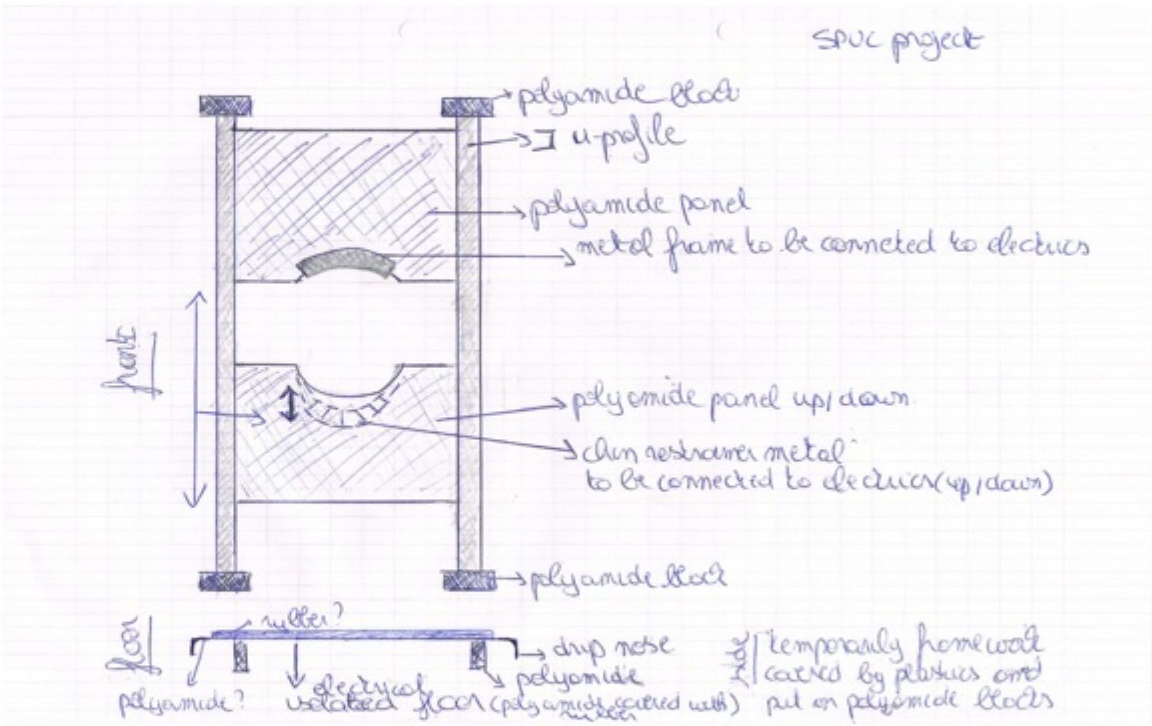



Figure 12b. A second sketch of the nose and neck electrodes showing the level of insulation.

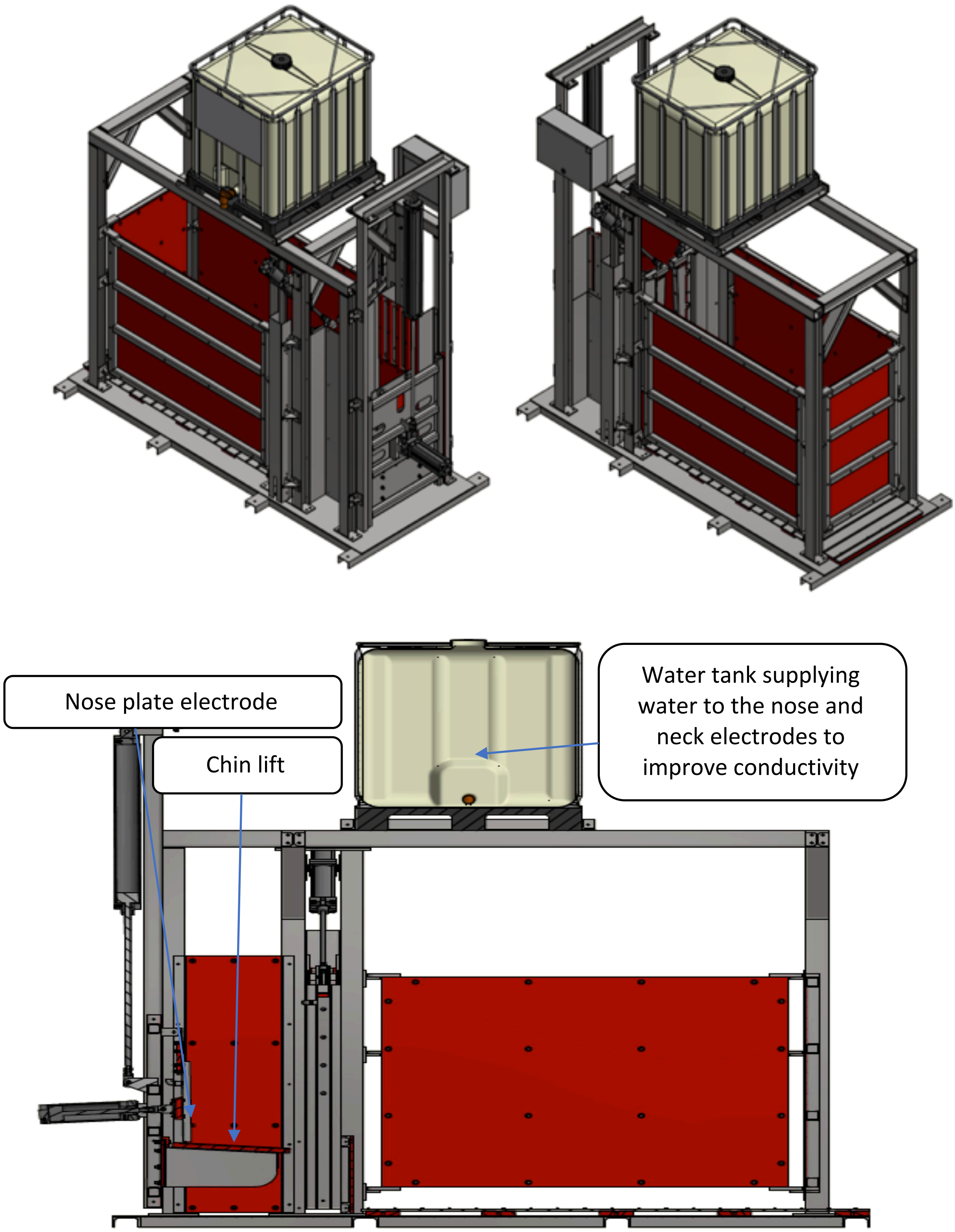



Figure 12c. A 3D sketch of the SPUC stunner showing the major components

## 6.4. Construction of restraint and electrodes

The animal restraint component of the SPUC prototype stunner, including the neck restraint and nose plate was manufactured by Vcons Abattoir Engineers using the rough and 3D sketches in figures 12a, b, c (above). The restraint was incorporated into an existing cattle restraining pen in a low throughput commercial abattoir.

## 6.5. Test of restraint

The restraint was tested on cattle to assess its effectiveness in restraining different size of cattle. A total of 12 Belgium Blue cattle were used in the presence of staff from Euro Meat Group, Vcons, the author, researchers from Bristol University and Silsoe Livestock Systems. All 12 animals were restrained by first applying the neck restraints, followed by the chin lift and nose plate. The aim was to assess the effectiveness of the neck restraint and the practicality of the nose plate, this was applied without the application of any electrical current. A captive bolt gun was then deployed to stun animals in order to induce immediate loss of consciousness. Further modifications were made to the neck restraints and nose plate after the test. The neck restraints were adjusted to make full contact with the opposite sides of the neck by increasing the height of the metal bars. The nose plate on the other hand was slightly curved at both ends to form two horizontal curved ends (instead of the original straight plate design), this was done to improve both the location of the animal's nose and the contact with the electrode plate. In addition, the nose plate and neck restraints were perforated, and water was applied from an overhead water tank. The purpose of keeping the neck and nose electrodes wet was to increase conductivity by reducing the contact resistance.

## 6.6. Design and construction of electrodes

The design and construction of the low and high voltage electronics were carried out by Silsoe Livestock Systems, Bedfordshire, UK, under the supervision of Dr Jeff Lines with input from the project team at Bristol University (including the author). Optimal electrical parameters (voltage, energy, pulse duration) were estimated from the results of the two *in vitro* experiments conducted using bovine heads and brain tissues (Chapters 4 and 5).

### 6.6.1. Design

The SPUC stunner was designed with a 3500 μF capacitor bank consisting of multiple capacitors interconnected to one another with a precision electronic switch (with rating of 800 A for 200 μS), these are housed in a large unit to provide protection to all the electrical components (see housing unit in figure 16). The electronics are designed to allow the discharge of 8 kV over a short time (mS duration). Figure 13 is an illustration of the electrical circuit of the SPUC stunner, figure 14 is a diagrammatic illustration of the control panel and figure 15 is a photograph of the high voltage unit. The electrical parameters are outlined in table 13. A number of assumptions were made;

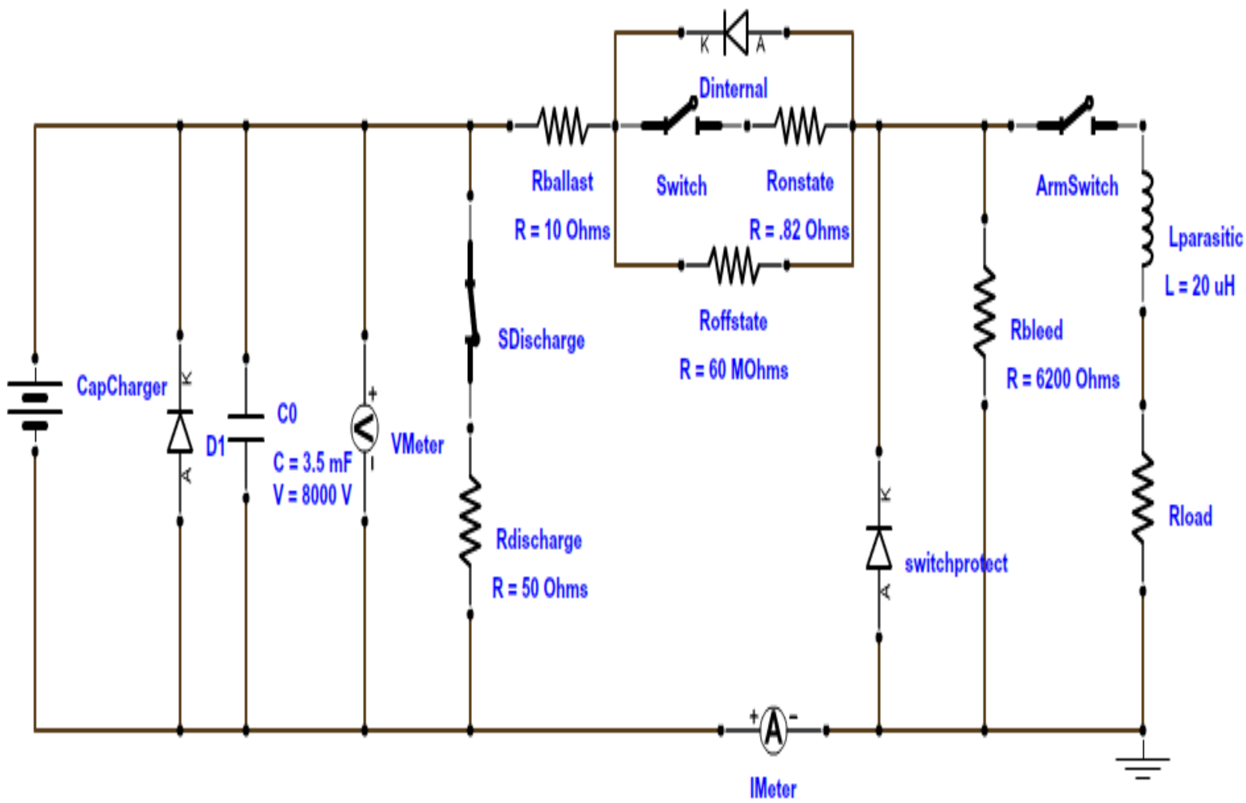
- Impedance of the animal's head (Rload) was assumed to range between 70 and 200 Ohms with little inductance or capacitance
- Parasitic inductance: 10 m leads = 20 μH
- Usage of not more than 1 stun per 200 ms and not more than 1 capacitor discharge in 300 s.

The possible modes of operation of the stunner are detailed below:

**Mode 1**: **Normal operation**- In this mode of operation, the stunner is set to deliver up to 100 mS pulse to Rload with initial voltage of approximately 7 kV across Rload dropping to at least 4 kV at the end of the pulse.

**Mode 2: Capacitance discharge**- Set to reduce the capacitor voltage to 40 V in 1 s on closing switch SDischarge (see figure 13). This is a rapid discharge for the purposes of safety.

**Mode 3: Accidental short circuit-** In this mode of operation, a potential current overload is detected by the self-protection circuit and the Behlke switch is opened to ensure that the current does not exceed the switch rating of 800 A for 200 µS.




Place a diode in line with each charger and across the chargers: UX F15B
15 kV 200 mA

Figure 13. Outline of the electrical circuit of the SPUC stunner

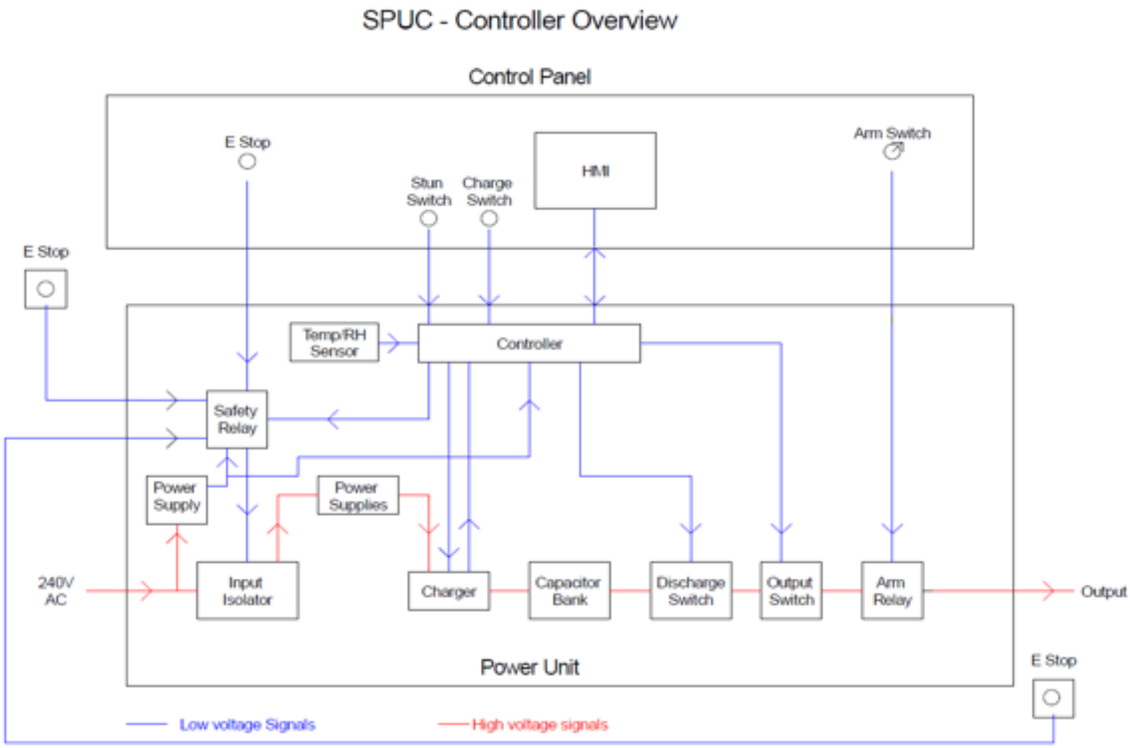



Figure 14. A diagrammatic illustration of the control system of the SPUC stunner showing the main control buttons, see photo of actual unit in figure 15.

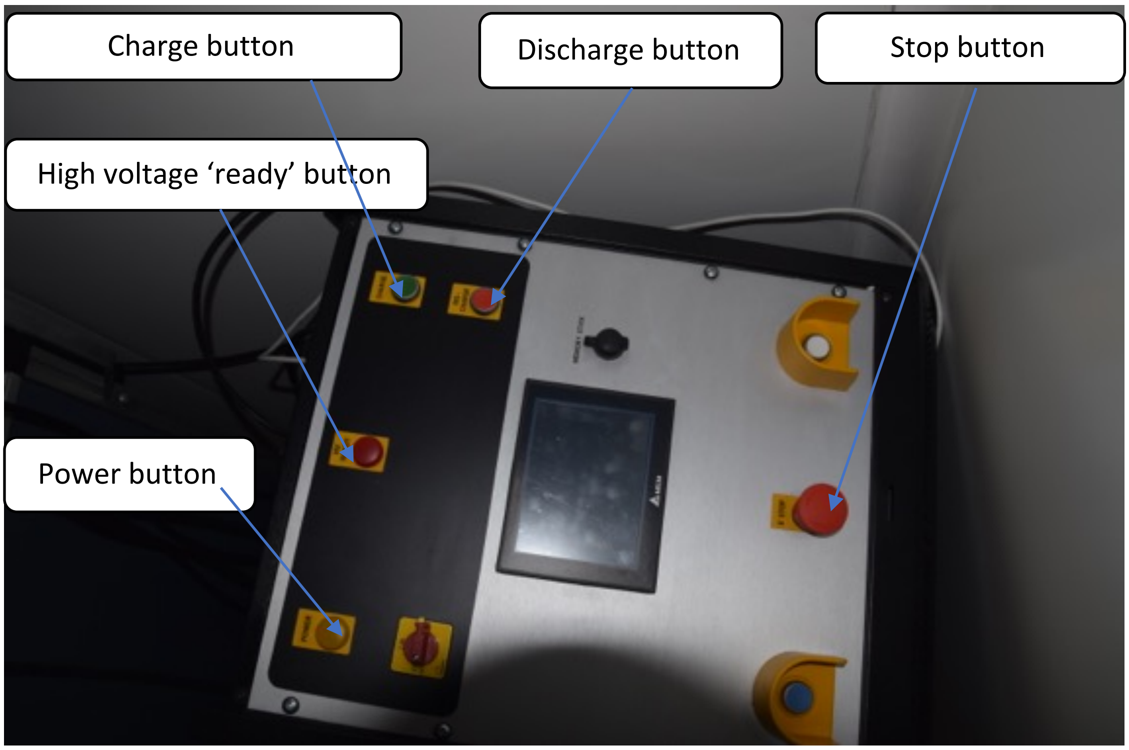



Figure 15. Photograph of the control panel of the SPUC stunner showing the functions of the main buttons. This is located in the control room (see figure 20) next to the stunner.

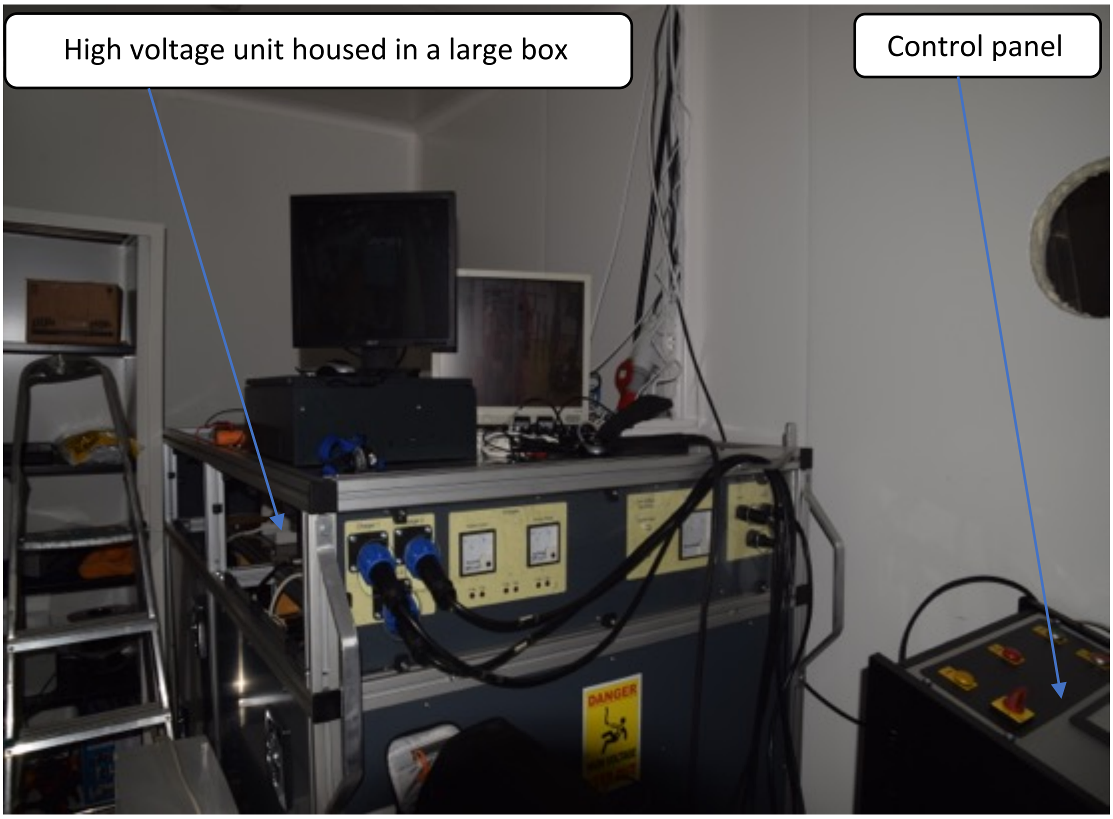



Figure 16. Photograph showing the high voltage unit and control panel of the SPUC stunner in the control room.

| **Parameters** | R ballast | R discharge | R bleed |
|---|---|---|---|
| Value | 10 Ω | 50 Ω | 6200 Ω |
| Energy in normal operation (100mS) | 11.4 kJ | 0 | 790 J |
| Max current in normal operation | 131 A | 0 | 1 |
| Energy in discharge (1 s) | 0 | 112 kJ | 0 |
| Max current in discharge | 0 | 160 A | 0 |
| Energy in Short (200uS) | 1093J | 0 | 0 |
| Max current in Short | 739 A | 0 | 0 |
| Max average power | 57 W | 373 W | 4 W |
| Resistor recommendations following thermal analysis: | 1032AS100KDS resistor. | Parallel assembly of three 1038AS151KDS This OK but marginal at a 300 second repetition rate such a high very unlikely. | 256AS622KDS (6200 Ohm) |

Table 13. Electrical parameters of the SPUC stunner

To explain the electrical parameters employed, it is worth noting that for cells to be electroporated, a high voltage is required to force the opening of pores in neural membranes. Robins *et. al.* (2014) applied a voltage of 5000 V to achieve a SPUC stun. In the present study, the maximum voltage was set at 8000 V to ensure that a range of voltages could be applied during the trial to examine the best set of electrical parameters. It must be noted that although the capacitors are charged up to the 8000 V, not all this voltage is delivered to the animal because of the 10 Ohms ballast resistor (see figure 13 above). For illustration of the interaction between the cow, ballast resistor and the applied voltage, assuming a cow's resistance is 70 Ohms, then the maximum voltage delivered to the cow will be as follows:

$$\text{Voltage delivered to cow} = 8000 * 70 / 10 + 70 = 7000 \text{ V}$$

Power, which is proportional to voltage, is the rate of transfer of energy in a circuit. The results of the experiment reported in chapter 5 identified energy as one of the main factors responsible for electrical. Therefore, the SPUC stunner was designed to deliver sufficient energy during its application using the following power equation:

$$P = V^2/R$$

## 6.7. Incorporation of electrodes into restraint

After the design and construction of the electronic system (control and high voltage units-see figures 16 and 17) at Silsoe Livestock Systems, the units were transported to the project site in Belgium and connected to the mechanical restraint. A process with which the author was involved. Further modification of the neck and nose plate electrodes were required after the control unit was integrated into the restraining pen. These modifications, which included changes in the design of the nose plate to improve contact, the chin lift and the neck restraints were carried out by Vcons Abattoir Engineers in Belgium.

## 6.8. Trial with dummy load

A dummy load was developed by Silsoe Livestock Systems to mimic the resistance of a cow (assumed to range between 70 and 200 Ohms in this experiment). This was tested on the prototype SPUC Stunner to check for any modifications needed in the electrode design. This led to changes in the safety apparatus because of issues encountered during tests with the dummy load. The following changes were made after tests with the dummy load:

- Improvement in the isolation of the nose electrode from the rest of the restraint
- Improvement in the isolation of the neck electrodes
- Improvement in the water flow into the nose and neck electrodes to increase conductivity.

## 6.9. Installation of CCTV

In order to capture and collect ethological data of animal behavioural responses prior to, during and after stunning, 4 CCTV cameras (Swann DVR8-4578, Swann, UK) were installed at vantage positions in and around the SPUC Stunner. This aspect of the trial was the sole responsibility of the author.

## 6.10. Set-up and initial trial with animals

After the trial of the stunner using a dummy load, an initial trial was carried out on two cattle to test the effectiveness of the SPUC stunner. The protocol for this trial is outlined below:

### 6.10.1. Protocol for initial test of SPUC stunner

- All procedures were video recorded to allow for post hoc assessment of behaviour.
- The stunning parameters were recorded digitally, time course of the current supplied over the pulse were logged.

- A CASH Special 0.22 calibre captive bolt was used to shoot the animal immediately before the pulse was applied. This was done to ensure there was sufficient electrode contact and confirm that the equipment was functional (on an already stunned animal) before its use on live animals
- Signs of unconsciousness and recovery were tested using the EUWelNet's indicators; immediate collapse, absence of righting reflex, absence of vocalisation, absence of rhythmic breathing, absence of corneal reflex and absence of nose pinching response.

After the first animal was successfully shot with a captive bolt, followed by the application of a pulse, a live animal was then stunned with the SPUC stunner, without the use of the captive bolt first. The CASH Special 0.22 calibre captive bolt was loaded and kept near the SPUC stunner, to be deployed in the event of a failed or ineffective stun. The SPUC stunner did not result in an effective stun, for instance, there was no immediate collapse of the animal and eye reflexes were still present. In line with the protocol, the animal was immediately shot with the backup stunner to render it immediately unconscious, followed by bleeding to ensure the death of the animal, and prevent recovery from consciousness. A decision was then made to check the system again and reapply it on a dummy load before any potential use of live animals. The issues encountered during the test with the live cow included the following:

- An ineffective stun was recorded
- Arcing of electric current at the nose and neck electrodes and some parts of the restraint (see figure 17)
- No EEG recording on electroencephalogram
- Issues with neck and nose plate electrodes

When the dummy load was redeployed, there was arcing which resulted in failure of the safety systems, this led to the destruction of the main switch which is one of the most important components of the stunner. A decision was then made to abort the trials and carry out the following:

- Reinforce the safety systems to ensure that future trials do not lead to destruction of any electrical components
- Redesign the electrodes to improve contact with the animal with a view to eliminating arcing and current escape from electrodes to the frame of the restraint.
- Improve the insulation between the electrodes (neck and nose plate) and the rest of the restraint.

The following figures describe the setup and some of the issues identified during the initial trial.

Figure 17. Screenshot from CCTV of electrical arcing during the initial trial of the SPUC stunner on a live cow. The nose plate will be redesigned with improved insulation to prevent arcing in future trials.

## 6.10.2. Re-design of restraint

As indicated above, a few structural issues were identified with the restraint. Whilst the neck restraints are capable of making contact with the animal, the nose plate electrode needed to be redesigned. It was observed, that animals were able to move their heads to one side, leading to poor contact with the nose plate (see figure 17). This resulted in arcing and poor delivery of current to the head. The sides of the nose plate and chin lift will be extended to restrict head movement and ensure good contact between the nose plate and the nose of animals (see figure 18 below). Further, the chin lift will be completely isolated from the nose plate and the rest of the restraint to prevent any current escape or diversion. Additionally, the sides of the chin lift will be raised with polyamide blocks to restrict the lateral movement of the head (see changes in new design below in figure 18). Although the neck restraints/electrodes were found to establish good contact with the neck, the allowance between the height of the animals used and the height of the restraint was small. It was suggested that the height of the restraint be raised slightly to cater for larger or taller animals. The isolation of the nose plate, chin lift and neck electrodes will be improved to avoid shorting of electric current.

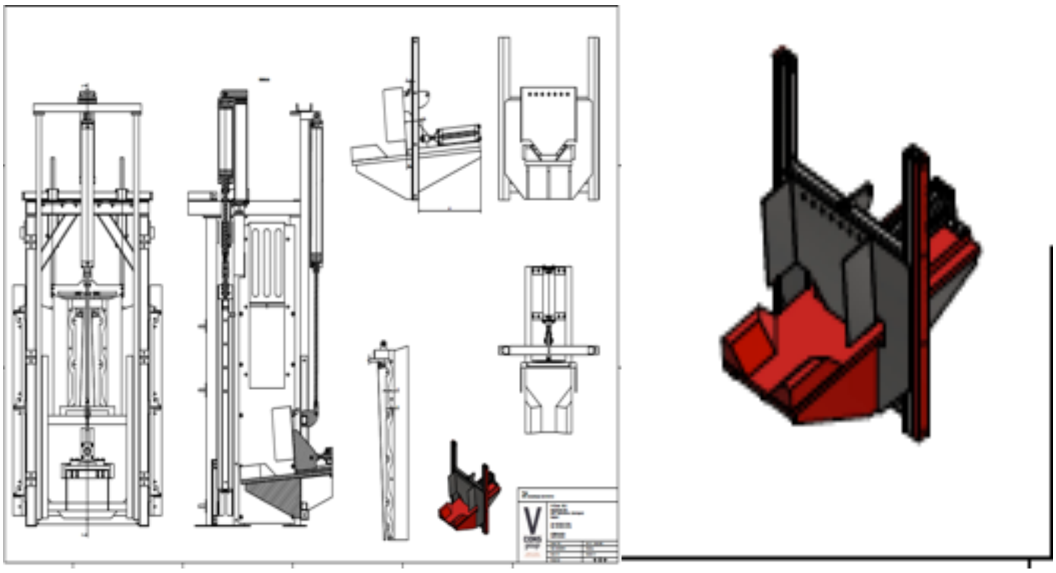


Figure 18. A 3D sketch of the proposed changes to the chin lift and nose plate electrode.

## 6.11. Full trial with live animals

Due to delays in obtaining ethical approval for the use of live animals in this research, coupled with changes that needed to be made to the restraint, a request was made to the university of Bristol to grant an extension to the project. A 5-month extension was granted to allow for changes to be made to the stunner and also for procurement of a Behlke switch to replace a blown one. The Behlke switches are custom-made to order, this took 6 months to arrive (December 2018 to June 2019), it is currently being incorporated into the high voltage unit. Re-designing of the nose plate and improvement in the insulation of the electrodes are currently underway. However, the changes will not be made in good time for the live animals' trial inclusion in this thesis, this due to the impending deadline for the submission of the thesis. Nonetheless, the use of live animals to demonstrate the efficacy of the SPUC stunner should be carried out by August 2019, this section outlines the approach to be adopted during the trial. The author participated in the design of the study given below and will continue to work with the project beyond submission of the thesis, to see it through to completion.

### 6.11.1. Ethical approvals

Due to the planned use of live animals in the trial, and to comply with European Council Directive 2010/63/EU, a number of ethical approvals were obtained from the UK and Belgium. Section 7.4 outlines the details of all three ethical applications made as part of this research. One approval was obtained from Bristol University whilst the other two were granted by the Universities of Liege and Ghent in Belgium.

### 6.11.2. Experimental protocol

The aim of using alive animals in this study is to identify the potential operating range of a commercial SPUC system. There are criteria that have to be met and possible trade-offs to be made in terms of the balance between voltage and duration of the pulse:

- The period for which the animal is unconscious must be sufficient to ensure that the animal does not recover before death due to exsanguination has taken place. Retailers generally require the duration of unconsciousness to be not less than 60 seconds when a captive bolt is used, therefore, for the SPUC to be acceptable to retailers, it must meet this minimum requirement.
- The duration for which the animal is immobile from the start of unconsciousness must be sufficient to ensure that all manual operations such as rolling out,

sticking and shackling can be safely carried out by operators, with an acceptable margin of safety.

- Whilst meeting the above criteria, it is also desirable to use as low a voltage as possible as this increases the safety of the system for the operators, reduces the risk of the stun killing brain cells, which would affect recovery and also reduces the cost and complexity of the actual stunning system.

In addition to quantifying head resistivity in dead cattle heads, the *in-vitro* work with bovine brain cells describes in chapter 5 indicated that it is the energy imparted by the stun that is the main variable dictating stun effectiveness. This means that, potentially, any loss of effectiveness caused by a reduction in stun voltage may be offset by increasing the duration of the pulse width at a lower voltage, so for example, the effect of a 5kV pulse lasting 50mS is likely to be similar to a 3.5kV pulse lasting 100mS or an 8kV pulse lasting 20mS.

The principle aim of the study is to identify a voltage and pulse duration combination, that whilst reducing the voltage required, will still achieve an immediate, effective and long-lasting, but recoverable stun, and a safe operator environment.

### 6.11.2.1. Method

Given the novelty of this approach and the use of an as yet untested piece of equipment, the trial will take place in two stages. An initial scoping trial (Stage 1, Parts 1 & 2 (see below) will be carried out involving up to 20 animals, and in which only gross, behavioural measures of unconsciousness and early signs of recovery will be measured, whilst the acceptable operating range of the device is identified.

When an acceptable operating range of the SPUC stunner has been identified, up to 140 animals will be used in a second trial (Stage 2) which will explore in more detail the relationship between voltage and pulse duration and the response of the animals. In addition to the behavioural measures of unconsciousness and movement, ECG, EEG and full meat quality measurements will be taken. Section 6.11.2.3, below, contains a detailed description of ECG and EEG recording methods. In a final Stage 3, four animals will be allowed to fully recover post stun to demonstrate possible compliance with the requirements of Halal slaughter.

### 6.11.2.2. Preliminary Trial -Stage 1 (Parts 1&2)

All procedures will be video recorded to allow for *post hoc* assessment of behaviour. The stunning parameters will be recorded digitally. The time course of the current supplied over the pulse will be logged.

The very first four animals to be tested will be shot with a captive bolt pistol immediately before the pulse is applied (these four animals are in addition to the 20 animals to be used within Stage 1). This is simply to ensure that there is sufficient electrode contact and that the equipment is functional and capable of delivering a pulse of sufficient amplitude and duration before it is tested on a conscious animal.

After a pulse has been applied, each animal will be tested for signs of unconsciousness and timed to recovery, using the following general indicators (EUWelNet):

- Immediate collapse
- Absence of righting reflex
- Absence of vocalizations
- Absence of rhythmic breathing
- Absence of eye movements
- Absence of positive corneal reflex
- Absence of nose pinching response

At a point of recovery identified by all of the following: the return of rhythmic breathing, a positive corneal reflex and a positive response to a nose prick with a

hypodermic needle, the animal will be shot with a captive bolt pistol. Should an animal show any signs of an ineffective stun immediately following the application of the pulse the animal will be immediately shot with the captive bolt pistol and recorded as a failure to stun. It is important that a true failure to stun due to level of treatment applied is identified, and that the cause is not an equipment failure, or poor electrode contact, etc. Previous work (Robins et al., 2014) identified a 5 kV 50 mS pulse (at 70 A) as effective for 75% of the animals. They gave insufficient detail regarding duration, however, by calculation, their maximum possible energy must have been 35kJ at a resistance of 70 Ω. Thus, as a precaution we should start our treatments at greater than 35kJ and greater than 5kV. However, within acceptable boundaries we are searching for a minimum voltage (This will also be dictated by capacitance, but this is a fixed factor in the study). The main variables of interest in this preliminary trial are duration of stun, and duration of immobilisation from the start of stun. Our search strategy will fall into two Parts within Stage 1. Firstly, the pulse is fix duration at 75 mS and progressively test a stepped fall in energy, starting from 40 kJ by reducing the voltage (see table 14a below). Secondly, for Stage 1, Part 2, at a fixed voltage of 7.5 kV a stepped fall in energy will be progressively tested from 40 kJ by reducing pulse duration (see table 14b below).
Within each stage an endpoint is identified thus: If at any treatment level an animal is not immediately, effectively stunned, the preceding treatment will be retested. If the second test of the preceding treatment level is again successful, the failed treatment level will be retested just one more time to establish repeatability at this limit. With this strategy we should require a maximum of ten animals for each of Parts 1 and 2.

| Energy (kJ) | 40 | 35 | 30 | 25 | 20 | 15 | 10 | 5 |
|---|---|---|---|---|---|---|---|---|
| Volts (kV) | 7.94 | 7.42 | 6.87 | 6.27 | 6.61 | 4.86 | 3.97 | 2.81 |

Table 14a. Stage 1, part 1 test regimen (fixed time of 75 ms)

| Energy (kJ) | 40 | 35 | 30 | 25 | 20 | 15 | 10 | 5 |
|---|---|---|---|---|---|---|---|---|
| Time (ms) | 87 | 73 | 60 | 48 | 37 | 27 | 17 | 8 |

Table 14b. Stage 1, part 2 test regimen (fixed voltage of 7.5 kV)

At the end of Stage 1, two endpoints may be determined with similar energy levels, if not, then a minimum voltage would have been identified for a 75 mS pulse, and a minimum time for a 7.5 kV pulse.

The calculations for the treatments depend upon the estimates for head resistance. Should the actual resistance of the head vary substantially from the estimate, it will be necessary to recalculate the treatments. Preliminary trial in June 2018 using one cow, 7500V, 50 mS pointed to an impedance of 53 Ω. On this basis the above tables are revised as given below (Tables 15a and 15b).

| Energy (kJ) | 40 | 35 | 30 | 25 | 20 | 15 | 10 | 5 |
|---|---|---|---|---|---|---|---|---|
| Volts (kV) | 7.42 | 6.94 | 6.43 | 5.87 | 5.25 | 4.54 | 3.71 | 2.62 |

Table 15a. Revised calculation [stage 1, part 1 test regimen (fixed time of 75 ms)] if resistance vary substantially from estimated 53 Ω.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Energy (kJ) | 40 | 35 | 30 | 25 | 20 | 15 | 10 | 5 |
| Time (ms) | 73 | 61 | 50 | 40 | 30 | 22 | 14 | 7 |

Table 15b. Revised calculation [Stage 1, part 2 test regimen (fixed voltage of 7.5 kV)] if resistance vary substantially from estimated 53 Ω.

### 6.11.2.3. Overview of the approach to EEG and ECG recording

The EEG will be recorded from the left and right hemispheres of the brain using needle electrodes that will be placed subcutaneously immediately after application of the electrical stun. The active electrodes will be placed midline between the medial canthi of the eyes, the reference electrodes will be placed over the mastoid processes and two ground electrodes will be placed caudal to the poll on the dorsal neck. Electrodes will be connected to a signal amplifier (DAM 50 bioamplifier) via and electrode adapter. The EEG will be recorded with an amplifier gain ratio of 1000:1 in alternating current mode, a high pass filter setting of 0.1 Hz and a lowpass filter setting of 100 Hz. The data will be digitized at a rate of 1 KHz (Powerlab) and continuously recorded on a personal computer. The EEG signal will be inspected visually for return of normal spontaneous EEG signals after the stun.

The ECG will be recorded by placement of three subcutaneous needle electrodes in a base apex configuration across the heart. Ideally, we would place the electrodes before the animal is stunned but if this proves logistically challenging, we will place them immediately after application of the stun. Electrodes will be connected to a signal amplifier (DAM 50 bioamplifier) via and electrode adapter. The ECG will be recorded with an amplifier gain ratio of 1000:1 in alternating current mode, a high pass filter setting of 0.1 Hz and a lowpass filter setting of 100 Hz. The data will be digitized at a rate of 1 KHz (Powerlab) and continuously recorded on a personal computer. The ECG signal will be inspected visually for the presence of normal PQRST complexes.

The essence of the EEG recording is to evaluate if the stun is immediate, the duration of unconsciousness will also be recorded. It is worth noting that due to the mode of induction of unconsciousness in SPUC, there may be some deviation from the normal reading of EEG as observed during conventional stunning. For instance, during conventional electrical stunning, there is grand mal epileptic seizure in the brain which results in the release of neurotransmitters which trigger hyper-synchronised activity giving rise to increased amplitude in the EEG. During SPUC stunning, there may not be the release of sufficient neurotransmitters to trigger hypersynchronised activity, secondly, the cell membrane is compromised (poration of membranes) during a successful SPUC which can alter the 'normal' state of cells observed during grand mal epileptic seizures. Nonetheless, an attempt will be made to use EEG to record the electrical activity of the brain in order to examine the presence of high amplitude and low frequency activity in the brain which are the indicators of unconsciousness during conventional electrical stunning, in addition to subjective assessment of unconsciousness (e.g. absence of vocalisation, absence of corneal reflex, absence of rhythmic breathing etc.). Robins *et. al*. (2014) reported that during SPUC stunning of cattle, there was no obvious recording of high amplitude and low frequency in the EEG.

### 6.11.2.4. Photos of prototype SPUC stunner

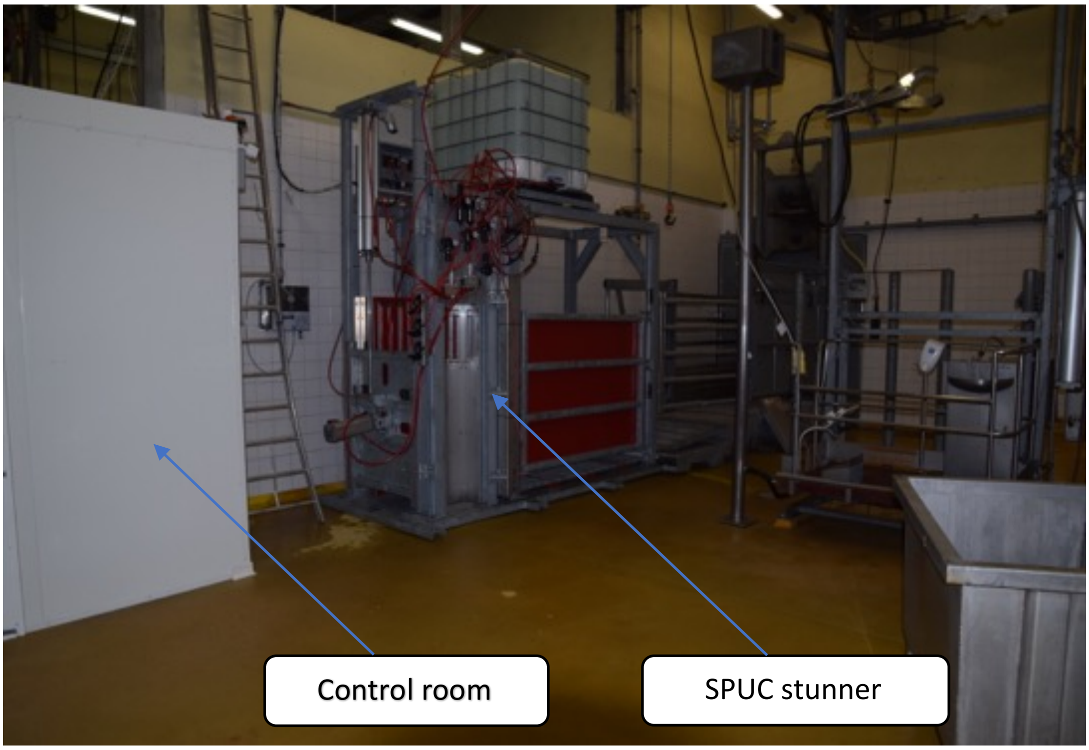



Figure 19. Photograph of the SPUC stunner in the project site in Chimay, Belgium.

Figure 20. A photograph showing the entry to the SPUC stunner from the raceway.

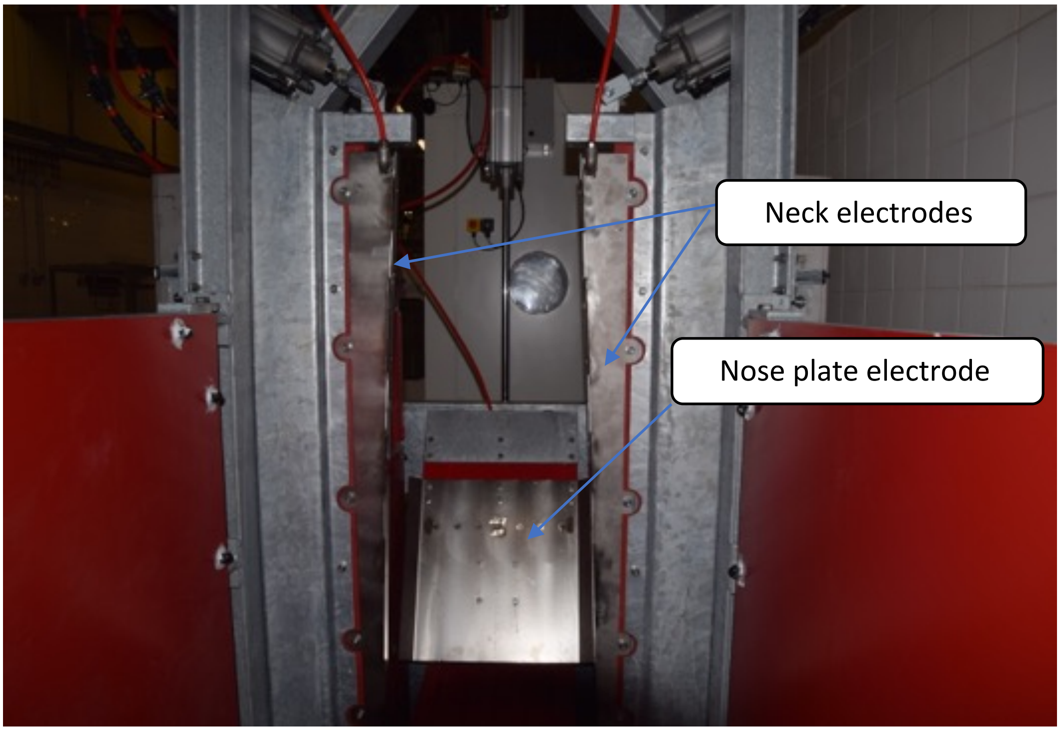


Figure 21. Photograph of the SPUC stunner showing the nose plate and neck restraints.


Figure 22. Photograph of a cow restrained in the SPUC stunner

Figure 23. Photograph of the head of a restrained cow showing a side window where EEG and ECG  recordings will be taken post stun.

# 7. Compliance of Single Pulse Ultra-High Current (SPUC) stunning with guidance from the European Food Safety Authority (EFSA) on the criteria for the assessment of the effectiveness of new or modified stunning methods

**Linking Narrative**: This chapter describes the European Food Safety Authority's procedure for the approval new or modified stunning methods and explains how the SPUC stunner fits into this framework. The essence of the guidance is to ensure that new or modified stunning systems provide sufficient protection to animal welfare at slaughter. The SPUC project has complied with important aspects of the guidance, for instance, three ethical approvals were obtained to demonstrate the compatibility of proposed system with the EFSA guidance and more importantly, with European Council Directive 2010/63/EU. The researchers have also demonstrated how unconsciousness will be assessed during the use of live animals in the research to comply with the EFSA guidance, this will be achieved through the use of the EUWelNet (2013) guidance for signs of unconsciousness. In the event of a failed stun, there are procedures in place to use a backup mechanical stunner to stun animals in order to induce immediate loss of consciousness.

## 7.1. Introduction

As indicated in the preceding chapters, European Council Regulation, EC1099/2009 requires the pre-slaughter stunning of all animals, with the exception of those slaughtered in accordance with religious rites. Stunning is defined according to EC1099/2009 as "any intentionally induced process that causes loss of consciousness and sensibility without pain, including any process resulting in instantaneous death". Article 4 (2) of the Regulation permits the European Commission to amend Annex I (approved stunning methods) to accommodate new or modified methods of stunning. A new or modified stunning method is defined by EFSA (2018) as any method that does not meet the definition of 'approved stunning methods' (see Annex I of EC1099/2009). It must be reiterated that this guidance does not cover stunning methods used mainly for emergency slaughter (e.g. on-farm slaughter) or depopulation, it primarily covers new or modified legal stunning methods used for commercial slaughter at licenced abattoirs in EU member states. In terms of the species of animals, this guidance is only applicable to stunning methods used to stun vertebrate animals (excluding reptiles and amphibians) in line with Article 2 of EC1099/2009. The stunning of animals may be achieved through mechanical and electrical means, or through the use of gaseous mixtures. Researchers around the world continue to modify existing methods or develop new systems of stunning for the humane slaughter of animals, notable among these are the development of Single Pulse Ultra-High Current (SPUC) and the use of microwave energy. The SPUC system is aimed at improving animal welfare at slaughter through the extension of the duration of unconsciousness induced by the stun, and the elimination or reduction of post-stun convulsions (in comparison with conventional head-only electrical stunning systems). Due to its mode of application (head-only), a SPUC stun is unlikely to affect the normal functioning of the heart, there is therefore the possibility that this system of stunning would be reversible and may be acceptable to some Muslims for use during Halal meat production. However, for the SPUC stunner to be accepted as an animal welfare-friendly system and approved for use within the European Union (EU), it must comply with guidance issued by the European Food Safety Authority (EFSA) (EFSA, 2013, 2018). These guidance detail the requirements for the assessment of the effectiveness of all new or modified stunning interventions. The aim is to ensure that new or modified stunning interventions offer protection for the welfare of animals to the same level (or higher) as existing stunning systems (see Annex 1 of EC1099/2009). The format for reporting new or modified stunning methods is as follows:

a. Introduction
    - Background and rationale
    - Objective
b. Materials and methods
    - Method
    - Measurement of the outcomes
c. Reporting the results
    - Reporting outcomes and estimations
    - Reporting uncertainty
d. Discussion and conclusions
    - Reporting interpretation of results
e. Conflict of interest
f. Overall integrity of findings from all studies
g. Demonstration of equivalents with existing methods

- Qualitative approaches
- Quantitative approaches

h. Overall discussion and conclusions

- Results regarding welfare impact
- External validity
- Discussion on equivalence with existing methods

## 7.2. Application procedure

Application for approval or authorisation for a new or modified stunning system is made through the European Commission, this is then forwarded to EFSA. Figure 24 shows the support mechanism available to applicants during the application process. Once an application is received, EFSA acknowledges receipt of the application by issuing a letter to both the European Commission and the applicant. The application is given a unique identification number and registered in EFSA's Register of Questions, the progress of the application can be monitored by the applicant. Below is a step-by-step procedure for the handling of applications by EFSA (see a summary in Figure 26 below) which the SPUC technique will have to go through before it can be granted approval as a new stunning technique by the European Commission:

- An application is submitted by the applicant to the European Commission (EC), the EC reviews and forwards the application to EFSA for scientific assessment.
- EFSA's Applications Desk Unit (APDESK) conducts a completeness check on the application. If the application is complete and deemed to meet the initial checks, it continues to the next stage, otherwise it is returned to the applicant for review.
- Assessment Phase 1- EFSA's Animal Health and Welfare (AHAW) panel then verifies the information and data submitted with a view to scientifically

evaluating the methods used e.g. animal welfare measures, statistical methods etc. EFSA may return the application at this stage if it the methods and information are insufficient.

- Assessment Phase 2- This phase involves risk assessment conducted by EFSA's AHAW panel. The assessments conducted in phase 2 include the following:
  - Assessment of animal welfare risks (e.g. outcome risks based on animal welfare indices: pain, distress, suffering etc).
  - Assessment of the efficacy of the new stunning method against an existing system (see Annex I of EC 1099/2009) to ascertain whether the new system is capable of offering the same or better level of protection for animal welfare.
- The AHAW panel then provides the European Commission with a scientific opinion on the outcome of the welfare assessment, this is usually published in the EFSA Journal in line with the requirements of Article 29 of EC178/2002.
- The European Commission then makes a decision on whether to approve or reject the new or modified method of stunning.

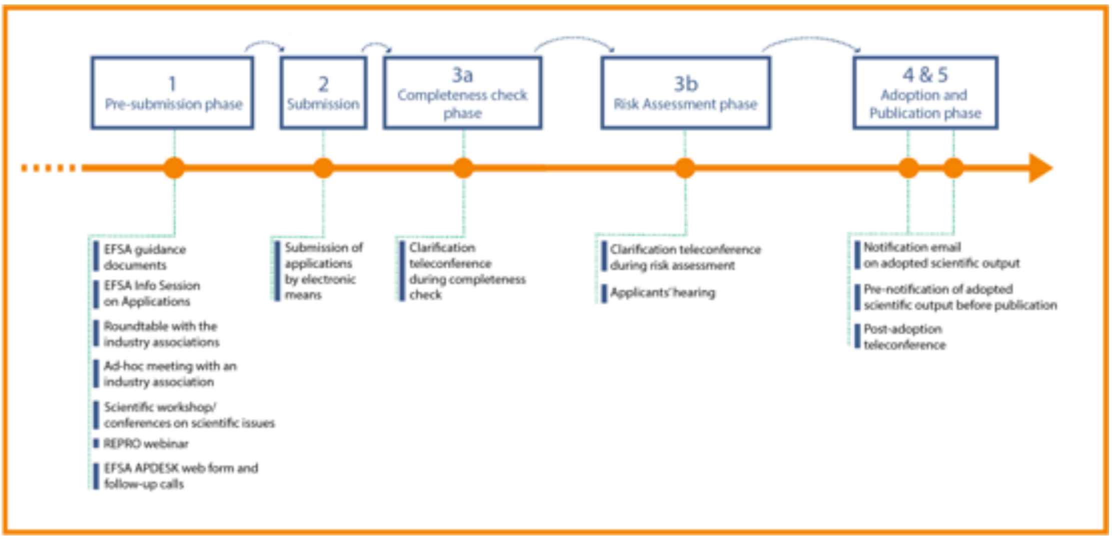


Figure 24. An overview of the support mechanisms available to applicants during the approval process for new or modified stunning systems.

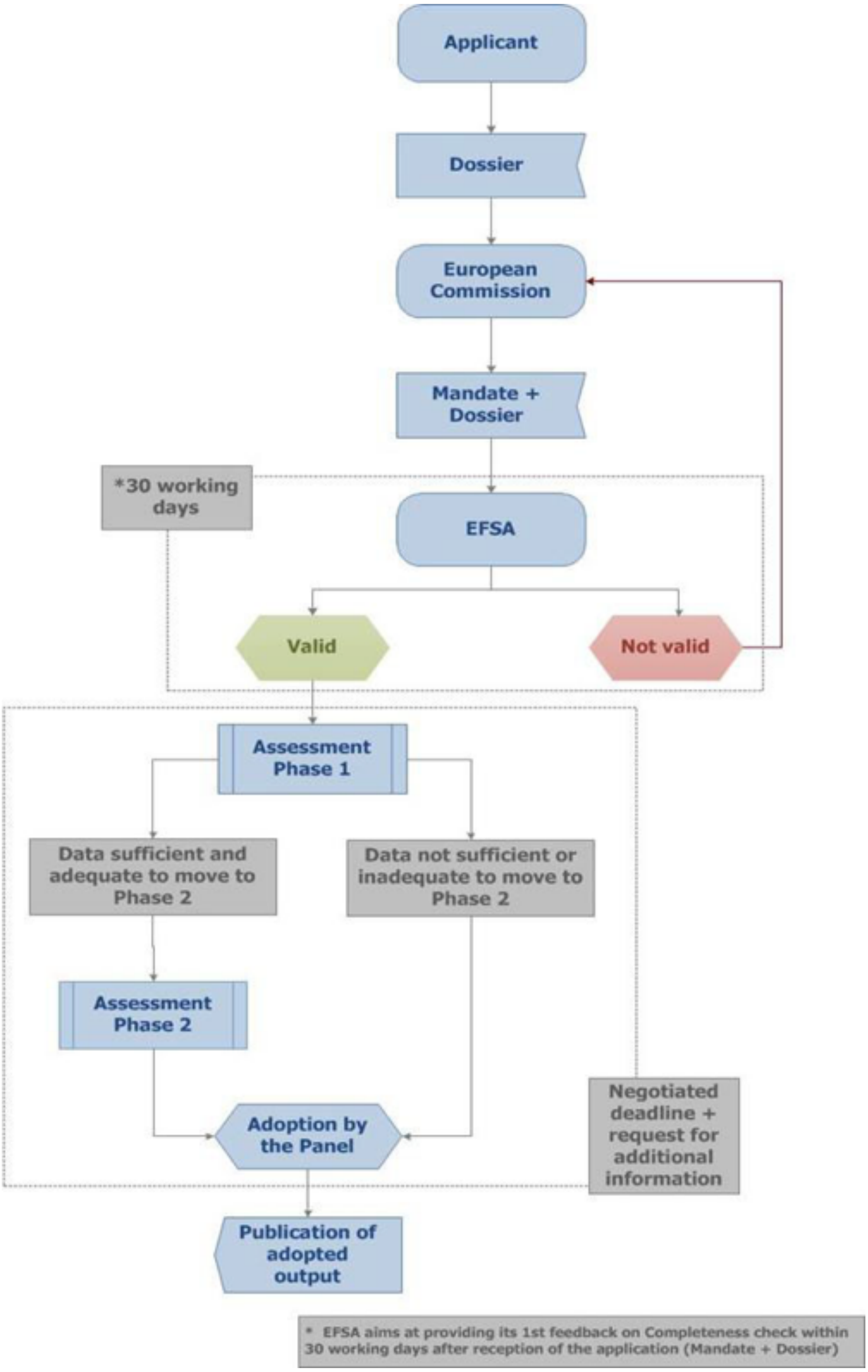



Figure 25. Step by step procedure for EFSA's handling of applications submitted for the approval of new or modified stunning interventions (Adapted from EFSA, 2018).

It should be noted that the first level of assessment of new or modified stunning systems by EFSA does not take into consideration pre-slaughter procedures such as lairaging, movement of animals and restraint prior to stunning. The pre-slaughter aspect of the stunning system is evaluated during the second level of assessment (see figure 26, below adapted from EFSA 2013).

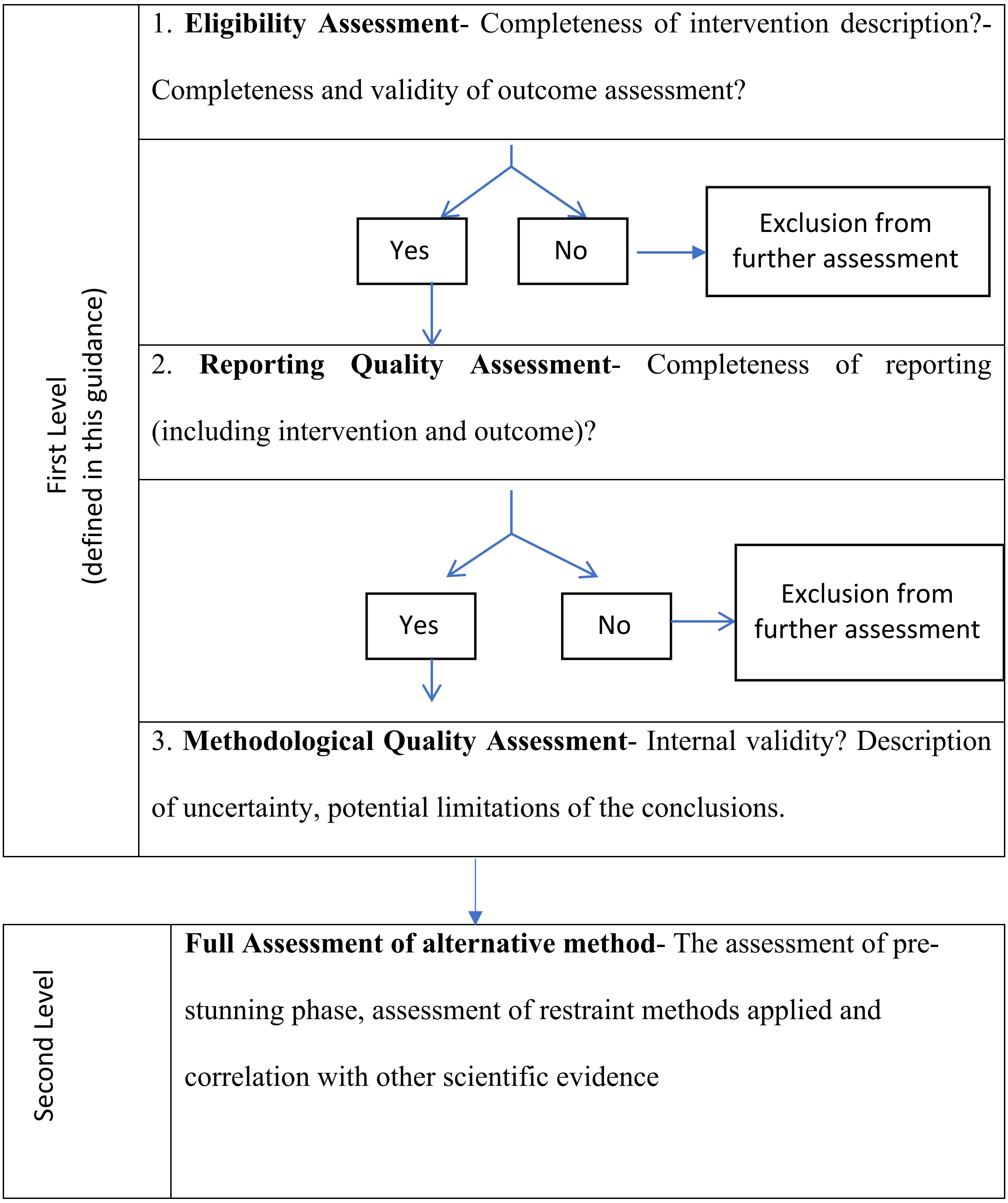



Figure 26. The criteria adopted by EFSA in assessing the effectiveness of new or modified stunning interventions (Adapted from EFSA, 2013).

## 7.3. Brief description of the SPUC system

The main objective of the Single Pulse Ultra-High Current (SPUC) project is to develop and commercialise a new system of head-only stunning of cattle that uses a very high single pulse of current to achieve a stun of long duration combined with an absence of the convulsions usually associated with electrical stunning. A very short duration (2-100 ms) capacitive discharge at a voltage of up to 7.5 kV driving up to 150 A of current will be used to stun cattle. Robins and others (2014) carried out an initial trial using SPUC to stun cattle. The researchers came to the following conclusions:

- Their SPUC system of stunning induced an immediate and sustained duration of unconsciousness, and that the induction of unconsciousness and sustenance of insensibility were independent of grand mal epilepsy in comparison with conventional head-only electrical stunning systems.
- SPUC stunning was also characterised by the reduction or absence of post-stun convulsions in comparison with conventional head-only electrical stunning. This may be attributable to the fact that the induction of unconsciousness during SPUC stunning was not thought to be through the generation of epileptic seizures in the brain.
- The induction of unconsciousness was considered to be perhaps due to the electroporation of neural membranes. Electroporation is the creation of pores through brain cell (neurons) membranes, this technique is routinely applied in human and veterinary medicine to introduce drugs, DNA and other foreign materials into cells (Zimmermann, 1982, Chen et al, 2006).

Using information from the Robins et al (2014) studies, this PhD work conducted at the University of Bristol attempts to develop and implement a commercial SPUC stunning system. The following describes how the SPUC system could comply with the EFSA guidelines.

## 7.4. Ethical approval

The SPUC stunning system under investigation will be tested using live animals when modifications to the electrical insulation have been completed. To this end, three ethical applications were submitted, and approvals have since been granted for all applications in line with European Council Directive 2010/63/EU on the protection of animals used for scientific research. The first was obtained from the University of Bristol's Animal Welfare and Ethical Review Body (AWERB) (reference UB/15/049). A second approval was obtained from the University of Ghent Ethical Commission for Animal Experimentation (reference EC 2016/23) to demonstrate compliance of the experimental protocol with Belgian domestic regulations. The application was made to Ghent University because the experiment was planned to take place in an abattoir (Rue de l'Abattoir 46, 7700 Mouscron) in the Flemish region of Belgium, which is under the jurisdiction of Ghent University.  A decision was later made to move the location of the project to a site (Les Abattoirs du Hainaut, Route Charlemagne 10b, 6464 Chimay, Belgium) in the Wallonia region under the jurisdiction of the University of Liege. This triggered the submission of a third application, this time, to the Department of the Development of Quality Management Service for Animal Welfare University of Liege (Approval reference LA1610414).

## 7.5. Preliminary laboratory experimentation

Three *in vitro* experiments were conducted; first, electroporation of cell membranes was carried out the at the Department of Biology, University of York to ascertain the relationship between the applied voltage gradient and the level of electroporation together with the levels of any associated cell death.  A second study aimed to measure voltage drop in cattle heads between different electrode application sites, and was undertaken at the School of Veterinary Sciences, University of Bristol. A third experiment was conducted to measure the influx or efflux of ions from neural membranes.

## 7.6. Assessment of the effectiveness of SPUC stunning

The EFSA guidance (EFSA, 2013) requires the assessment of the effectiveness of all new or modified stunning systems to be conducted under two conditions:

- Step 1-Assessment of the effectiveness of stunning under laboratory conditions
- Step 2-Assessment of the effectiveness of stunning under abattoir conditions

Below is an outline of the procedure for the assessment of the effectiveness of the SPUC Stunner.

### 7.6.1. Step 1: The effectiveness of SPUC stunning under laboratory conditions

The initial assessment of the efficacy of the SPUC Stunner will be carried out under laboratory conditions at an abattoir in Belgium, Les Abattoir du Hainaut, Chimay. The stunning equipment will be stationed in a spacious and dry area in the slaughter hall. The area is away from the main abattoir operations and the trial will be conducted under controlled conditions.

The trial of the SPUC stunner under laboratory conditions will involve the testing of a combination of stunning parameters on animals in pairs, unless there is a 'failure' to stun the first animal, at which point that parameter combination will be abandoned. Once acceptable stunning parameters have been identified, a representative sample of animals will be SPUC stunned followed by the objective assessment of the immediacy and sustenance of the stun. Brain activity will be objectively recorded using an electroencephalogram (EEG) needle electrode array that will be inserted sub-dermally, immediately after the application of the single pulse ultra-high current stunning treatment. The experimental protocol is fully described in chapter 6.11.2. This project will make use of the methodologies currently used by researchers to evaluate effective conventional electrical stunning in cattle, for example, the presence of high amplitude low frequency (HALF) activity in the EEG, followed by EEG suppression, which is the agreed criteria for effective electrical stunning (Wotton, et al., 2000). In addition, if necessary, new techniques will be developed to further our understanding of stunning effectiveness in cattle based on detailed analysis of EEG and the recording of visual evoked potentials (VEPs). The neurophysiological assessment in the laboratory will be used to identify physical symptoms that can subjectively identify whether cattle are stunned or not by this new method.

In addition, ECG recording electrodes will be applied following restraint and prior to the stun to record heart function immediately pre and post stun.

After stunning, all animals will be observed for the return of brain stem reflexes as describes in 1 above. Following assessment all animals will be immediately re-stunned with a captive bolt gun and then subsequently bled-out.

To safeguard the welfare of animals used in the recovery experiment they will not be allowed to recover fully. The return of signs of recovery outlined above together with signs of a righting reflex will be sufficient to demonstrate that the method can be used for halal, i.e. to ensure that the animals would make a full recovery from the stun. Once this is achieved, a captive bolt gun will be used to induce immediate loss of consciousness and the animals will subsequently be slaughtered by bleeding-out. All procedures will be video-recorded for subsequent analysis and to produce evidence for the use of this method for halal slaughter of cattle.

### 7.6.2. Step 2: The effectiveness of SPUC stunning under abattoir conditions

The applicability of the SPUC Stunner will be tested under commercial conditions in a medium throughput abattoir in Belgium, Euro Meat Group, Rue, de l'Abattoir 46, 7700 Mouscron, Belgium. The research team will aim for 100% efficacy and a confidence interval will be calculated for the performance of the methodology.

The animals to be used for the abattoir trials will be part of the abattoir's normal daily throughput. The SPUC researchers will assess post-stun events such as the onset of unconsciousness, duration of unconsciousness, convulsion, signs of return of sensibility and signs of recovery through objective and subjective means and correlate these with EEG and ECG findings recorded in the laboratory. In line with the EFSA guidance, the SPUC researchers propose to conduct the following assessments in the abattoir:

- Detailed record of the stunning parameters
- The use of animal-based measures to assess the initiation and duration of unconsciousness
- The use of behavioural, physiological or neurological animal-based measures the absence of pain, suffering and distress
- An ethogram of the outcome assessment
- The restraint to be used for the SPUC Stunner will incorporate the best features of an existing system, The Jarvis Beef Stunner. It is not anticipated that any pain, distress or suffering will be greater than that inflicted by existing commercial stunning systems. However, a control or 'sham operation' will be conducted as part of the trial by moving animals into the restraint without the

application of the stun to assess any pain, distress or suffering associated with the use of the SPUC restraint.

The effectiveness of the SPUC stunner will be assessed subjectively using criteria that are generally accepted for the assessment of unconsciousness (EUWelNet, 2013). These criteria include:

- Immediate collapse
- Absence of righting reflex
- Absence of vocalizations
- Absence of rhythmic breathing
- Absence of positive palpebral reflex
- Absence of positive corneal reflex
- Absence of a nose prick response

As stated above, conventional electrical head-only stunning systems induce unconsciousness through tonic/clonic epilepsy. However, this route to the induction of unconsciousness is characterised by profound post-stun/kill convulsions that pose a significant health and safety concern from the viewpoint of the slaughter operative. Robins et al (2014) reported that the application of a SPUC stun eliminated these post-stun movements. Therefore, the assessment of the levels of post-stun movements will form part of the study. Table 16 shows the proposed scoring system to be used for this assessment.

| Score | Descriptor | Description |
|---|---|---|
| 0 | No activity | Very little movement. |
| 1 | Mild activity | Some physical movement of limbs, but not enough to present a danger to operators. |
| 2 | Moderate activity | Considerable physical movement of the limbs, posing a potential danger to operators. |
| 3 | Severe | Gross physical movement presenting a clear danger to an operator. |

Table 16: Scoring system of post-stun convulsions following a SPUC stun.

## 7.7. Approach

The following is an explanation of how the SPUC Stunner will meet EFSA's eligibility criteria, reporting quality criteria and methodological quality criteria.

### 7.7.1. Eligibility criteria

All new or modified stunning technologies must meet the eligibility criteria. This is defined as the ability of a stunning system to induce immediate (with the exception of modified atmosphere systems) and sustained unconsciousness (EFSA, 2013). The induction of unconsciousness must not cause pain, suffering and distress. In the event of simple stunning (such as the proposed SPUC stunner), the procedure must be followed quickly with a procedure that ensures the death of animals (e.g. bleeding-out). As stated above, Robins et al (2014) objectively demonstrated (with EEG recordings) that SPUC stunning is capable of inducing immediate and prolonged unconsciousness. The present research seeks to refine the electrical parameters and restraint in order to produce a commercial SPUC unit that fully complies with the EFSA guidance. Table 17 below outlines the key indicators that would be measured and how these measurements would be conducted.

| Key Indicators | Measurement |
|---|---|
| Immediate onset of unconsciousness and insensibility | EEG and animal-based behavioural measures |
| Duration of unconsciousness | EEG and animal-based behavioural measures |
| Effect on cardiac function | ECG recordings |
| Aversiveness/pain/suffering/distress | SPUC is capable of inducing immediate unconsciousness hence it is unlikely to be painful or aversive. However, behaviour measures of aversiveness and pain will be assessed during the trial |

Table 17. A list of variables that will be measured and the methods of measurement of these parameters during the SPUC research.

#### 7.7.1.1. Specific EFSA guidance on head-only electrical stunning

The EFSA guidance (EFSA, 2013) requires all new or modified electrical head-only stunning systems submitted for approval to be capable of generating generalised epileptiform activity in the EEG. Due to the hypothesised mode of action of the SPUC stunner, the induction of unconsciousness may not be through epileptic seizures in the brain but through a process called electroporation (see above section on electroporation of neural membranes). To comply with the EFSA guidance, Table 18 (EFSA, 2013) is an outline of the electrical parameters of the SPUC stunner.

| Variable | | Parameters/comment |
|---|---|---|
| Voltage | | Up to 7,500 V |
| Nature of voltage | | Unipolar with almost uniform voltage over the whole pulse duration |
| Pulse duration | | 2-100 ms |
| Current | | Up to 150 A. Current delivered is determined by impedance of cow's head. |
| Impedance of cow's head | | 50-100 Ω |
| Maximum stun-to-stick-/kill interval(s) | | 15 s |
| Frequency of calibration of equipment | | Annually |
| Position and contact surface area electrodes | Position of the electrodes | Nose and neck |
| | Type of electrodes | Nose plate and neck restraint |
| | Animal skin condition | Wet |
| Optimisation of current flow | Electrode characteristics | Nose plate and neck metal bars |
| | Electrode appearance | |
| | Animal restraining | Individually and mechanically restrained in an upright position |

Table 18: Specification of the electrical characteristics of the SPUC stunner.

## 7.7.2. Reporting quality criteria

The findings of the SPUC research should be presented in a clear and consistent manner. To achieve this objective, the findings will be reported in line with guidelines of the REFLECT (www.reflect-statement.org/statement/) and STROBE (www.strobe-statement.org/) statements. The REFLECT statement is 'the reporting guidelines for randomised controlled trials for livestock and food safety', whilst the STROBE statement is a collaborative initiative by experts aimed at 'strengthening the reporting of observational studies in epidemiology. EFSA (2013) recognises both the REFLECT and STROBE reporting guidelines for new and modified stunning interventions. The reporting methodology of the SPUC research should be designed to comply with the REFLECT checklist below in Table 19. Prior to submission of the findings to EFSA for evaluation, it is suggested that the SPUC researchers aim to submit the findings for thorough peer review through a scientific journal in line with the EFSA guidance.

| Paper section and topic | Item | Descriptor of REFLECT statement item |
|---|---|---|
| Title & Abstract | 1 | How **study units** were allocated to interventions (e.g., "random allocation," "randomized," or "randomly assigned"). **Clearly state whether the outcome was the result of natural exposure or was the result of a deliberate agent challenge.** |
| Introduction Background | 2 | Scientific background and explanation of rationale. |
| Methods Participants | 3 | Eligibility criteria **for owner/managers and study units at each level of the organizational structure,** and the settings and locations where the data were collected. |
| Interventions | 4 | Precise details of the interventions intended for each group, **the level at which the intervention was allocated,** and how and when interventions were actually administered. |
| | 4b | **Precise details of the agent and the challenge model, if a challenge study design was used.** |
| Objectives | 5 | Specific objectives and hypotheses. **Clearly state primary and secondary objectives (if applicable).** |
| Outcomes | 6 | Clearly defined primary and secondary outcome measures and the levels at which they were measured, and, when applicable, any methods used to enhance the quality of measurements (eg, multiple observations, training of assessors). |
| Sample size | 7 | How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules. **Sample-size considerations should include sample-size determinations at each level of the organizational structure and the assumptions used to account for any non-independence among groups or individuals within a group.** |
| Randomization -- Sequence generation | 8 | Method used to generate the random allocation sequence **at the relevant level of the organizational structure**, including details of any restrictions (eg, blocking, stratification) |
| Randomization -- Allocation concealment | 9 | Method used to implement the random allocation sequence **at the relevant level of the organizational structure**, (eg, numbered containers ~~**or central telephone**~~**),** clarifying whether the sequence was concealed until interventions were assigned. |

| | | |
|---|---|---|
| Randomization -- Implementation | 10 | Who generated the allocation sequence, who enrolled **study units,** and who assigned **study units** to their groups **at the relevant level of the organizational structure**. |
| Blinding (masking) | 11 | Whether or not those administering the interventions, **caregivers** and those assessing the outcomes were blinded to group assignment. If done, how the success of blinding was evaluated**. Provide justification for not using blinding if it was not used.** |
| Statistical methods | 12 | Statistical methods used to compare groups for all outcome(s); Clearly state the level of statistical analysis **and methods used to account for the organizational structure, where applicable;** methods for additional analyses, such as subgroup analyses and adjusted analyses. |
| Results **Study** flow | 13 | Flow **of study units** through each stage **for each level of the organization structure of the study** (a diagram is strongly recommended). Specifically, for each group, report the numbers of **study units** randomly assigned, receiving intended treatment, completing the study protocol, and analysed for the primary outcome. Describe protocol deviations from study as planned, together with reasons. |
| Recruitment | 14 | Dates defining the periods of recruitment and follow-up. |
| Baseline data | 15 | Baseline demographic and clinical characteristics of each group, **explicitly providing information for each relevant level of the organizational structure. Data should be reported in such a way that secondary analysis, such as risk assessment, is possible.** |
| Numbers analysed | 16 | Number **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 (eg, 10/20, not 50%). |
| Outcomes and estimation | 17 | For each primary and secondary outcome, a summary of results for each group, **accounting for each relevant level of the organizational structure**, and the estimated effect size and its precision (e.g., 95% confidence interval) |
| Ancillary analyses | 18 | Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory. |
| Adverse events | 19 | All important adverse events or side effects in each intervention group. |
| Discussion Interpretation | 20 | Interpretation of the results, taking into account study hypotheses, sources of potential bias or imprecision, and the dangers associated with multiplicity of analyses and outcomes. **Where relevant, a discussion of herd immunity should be included. If applicable, a discussion of the relevance of the disease challenge should be included.** |

| | | |
|---|---|---|
| Generalizability | 21 | Generalizability (external validity) of the trial findings. |
| Overall evidence | 22 | General interpretation of the results in the context of current evidence. |

Table 19: Check list of the REFLECT reporting guidance on randomised controlled trials for livestock and food safety.

# 8. Lay summary report of major activities and update on planned trial of the SPUC stunner on live animals.

## 8.1. Introduction

This chapter provides a general summary of the main work and background work leading to the development of the prototype SPUC stunner as well as a description of where the project currently is and future plans. Specifically, it details the laboratory experiments, literature reviews, survey of key stakeholders and published work on the social and politics of Halal meat production, as well as gives an update on the current state of the practical aspects of the development of the SPUC project.

## 8.2. Summary of literature review

After registration as a PhD student on the 1st of July 2015, a 25,000-worded literature review was conducted within the first three months. The review covered topics such as design of raceways, pre-slaughter handling, stunning and the welfare aspects of slaughter with and without stunning. The review was further summarised and published in a peer reviewed journal (see Fuseini et al., 2016: *Animal Welfare, 25,* 365-376).

## 8.3. Summary of stakeholders' surveys

Two stakeholder surveys were carried out as part of this PhD project to evaluate stakeholders' perception and understanding of key aspects of religious slaughter, particularly, the acceptability of pre-slaughter stunning of food animals. These surveys were vital in informing our understanding of the acceptability of pre-slaughter stunning for Halal meat production and how it affects the outcome of the SPUC stunner.

Throughout the research, I presented at several seminars and developed a healthy rapport with Halal certifiers and Islamic scholars, these stakeholders will be invited to observe the final trial of the SPUC stunner on live animals. This will ensure that they are confident in the stunner and it will enable them to make informed decisions about the acceptability (or otherwise) of the stunner for Halal meat production.

### 8.3.1. Survey of Islamic scholars and Halal consumers

Due to the debate surrounding the acceptability of stunning for Halal slaughter, a survey of Islamic scholars and Halal consumers was carried out to get a better understanding of issues affecting the acceptability of stunning for Halal meat production, and how the current project could cater for these. The results showed that Islamic scholars and Halal consumers were generally not well informed about the different methods of stunning, however, 95% of scholars and 53% of consumers indicated that they would only consume meat from stunned animals if it could be proven that the stun did not cause the death of animals. This information was vital in developing the SPUC stunner because if it can be demonstrated that the stunner does not result in instantaneous death of animals (and only induces unconsciousness), it is likely to be acceptable to proponent of Halal stunning and may be approved for use during Halal slaughter. If the SPUC is approved for Halal slaughter, this could potentially reduce the number of animals currently slaughtered without stunning for the Halal market. There are plans to involve key scholars in the trial, this will afford us the opportunity to demonstrate to them that the SPUC stunner is reversible. They will then be confident that the SPUC stunner does not cause the death of animals prior to exsanguination. The results of the scholars and consumers surveys was published in Meat Science Journal in 2017 (see chapter 2).

### 8.3.2. Survey of veterinary students in England

As veterinarians play an important role in the protection of animal welfare and safeguarding meat safety in Halal abattoirs, a survey of veterinary students from four universities in England was carried out to examine their perception and level of understanding on issues around Halal slaughter. Respondents' understanding of the regulation of Halal slaughter, the stunning of animals during Halal meat production and whether they would consume Halal meat from effectively stunned animals were evaluated. Whilst the majority of respondents were of the view that all animals must be stunned before slaughter (including Halal), there was a minority who thought religious slaughter should be exempt from stunning in order to comply with traditional religious values. Further, a minority of respondents questioned the humaneness of stunning, vegetarianism and veganism among veterinary students surveyed were found to be above the national average. This paper was published in the *journal of Animals* in 2019 (see chapter 3).

## 8.4. Other published work within the project

As declared at the beginning of the thesis, the author has published a number of peer reviewed papers on animal welfare, Halal meat certification, Halal meat fraud and the science of stunning and slaughter as part of the work for this PhD project. However, to keep the narrative of the thesis concise these have not been specifically included within the text of the thesis. There is some overlap between these papers, which have highlighted the significance of animal welfare in Islam and argued that Muslims should consider using 'modern' slaughter technologies (e.g. stunning) to improve animal welfare. The importance of Halal certification was highlighted in the papers, which led to further work on the prevalence of fraudulent activities in the Halal meat industry. In collaboration with a Ghanaian academic, the author also published a paper on the attitudes of Ghanaian meat consumers towards animal welfare and how concern for animal welfare influence meat consumption. The full list of papers is given before the contents pages. Appendix A contains copies of all published papers that are not included as full chapters in the thesis.

## 8.5. Summary of *in vitro* experiments

Three *in vitro* experiments were conducted to determine optimum electrical parameters for the design and construction of the SPUC stunner. These experiments showed the effect of voltage on cattle tissues, neural membranes and cattle cadaver. The following is a summary of all three *in vitro* experiments.

### 8.5.1. Measurement of voltage drop and resistance of cattle heads

The objective of this experiment was to evaluate the resistance of cattle heads and the development of voltage within the head. A 250 V power source was applied to the head through large needle electrodes connected to the nose and neck. In addition to evaluating the resistance and how voltage develops within the head, this experiment also confirmed that the best route of application of current to the head (and ultimately the brain) is through the placement of electrodes on the nose and neck of cattle. This informed the development of the SPUC electrodes. The results of this experiment are reported in chapter 4 of this thesis.

### 8.5.2. Measurement of migration of ions from neural membranes

A gene pulser Xcell electroporation system (Bio-Rad Laboratories Inc., USA) was used to pulse brain cells to establish the effect of voltage on the migration of ions between neural membranes. Calcium, potassium and sodium ions were the main ions found to have moved across cell membranes. The aim of this experiment was to establish the proportion of neural membranes that were electroporated (see chapter 4). However, on further review of literature about electroporation of neural membranes, it was established that measurement of ion migration from cell membranes did not give an accurate estimation of the successful poration of cell membranes. This led to the use of a new technique which is reported in chapter 5 and summarised below in 8.5.3 below.

### 8.5.3. Measurement of electroporation of neural membranes

The objective of this experiment was to identify the factors responsible for the electroporation of neural membranes. Brain samples were dissociated and placed in a cuvette in a PBS medium together with calcein. The samples were then electroporated using a gene pulser xcell electroporation system (Bio-Rad Laboratories Inc., USA) with single and multiple pulsing protocols. The proportion of cells successfully electroporated was determined with a cytometer A CyAn ADP flow (Beckman Coulter, USA). Energy was the main factor responsible for the electroporation of neural membranes, the findings are reported in chapter 5.

## 8.6. Assessment of the efficacy of the SPUC stunner on live animals

This research has led to the development of a new system of head-only electrical stunning for the humane slaughter of adult cattle. The system is designed to induce unconsciousness through the application of sufficient voltage to the brain through two set of electrodes; a nose plate and neck electrodes, to cause neural dysfunction. It is hypothesised that the system is unlikely to affect normal cardiac rhythm, which could otherwise result in the fibrillation of the heart (cardiac arrest). Due to significant delays in granting ethical approvals by the authorities in Belgium, for the use of the stunner on live animals, the final part of the project is yet to be carried out. All approvals are now in place, however, with the constraint of the University of Bristol's time allowance for the duration of a PhD project, the final part of the project is to be conducted at a later date, following submission of this thesis. The author continues to remain fully involved with the project with support continuing from the Humane Slaughter Association (HSA), AHDB – Beef and Lamb and EuroMeat Group. The final part of the research will involve the use of the stunner on live animals, assessment of unconsciousness will be carried out by employing both objective (EEG recordings) and subjective (behavioural indices) methods (see section 6.11.2 for the proposed protocol for the planned trial). Work is ongoing to modify the restraint with regard to the electrodes and electrical isolation and also the safety, self-protection systems of the high voltage electrical switch. Work is ongoing and when completed, a full trial will be conducted.

Once the working conditions and efficacy of the stunner are established, a demonstration of its operation and potential reversibility will be carried out to key stakeholders in the Muslim community, mainly Islamic scholars and Halal certification bodies (HCBs). This is crucial to ensuring that the SPUC stunner gains approval from the Muslim authorities and has the potential of improving the welfare of animals during religious slaughter by reducing the number of animals slaughtered without stunning. The two ethical approvals granted in Belgium for the SPUC project allow for reversibility of stunning to be demonstrated using live animals in that country. A number of Islamic scholars and HCBs within the EU have already been contacted, the following HCBs have agreed to attend the licensed demonstration:

- Halal Consultations Limited, UK
- Halal Certification Organisation, UK
- Halal Food Authority, UK

- Halal Food Council of Europe, Belgium
- Halal Quality Control, The Netherlands

A maximum of 4 animals will be stunned and allowed to recover in order to demonstrate to the Halal authorities that the SPUC stunner does not result in the death of animals. All animals used in the demonstration will be immediately stunned with a penetrative captive bolt gun once they show satisfactory signs of recovery. To safeguard animal welfare, animals will not be allowed to fully recover from the stun, animals will be deemed to have recovered with the return of rhythmic breathing.

## 8.7. Further research

Further research is needed before commercial units of the SPUC stunner can be produced. First, there should be further research using brain cells to understand the mechanism of induction of unconsciousness through the poration of neural membranes. This should include how the creation of pores in brain cells influence ion stability and the concentration of both excitatory and inhibitory neurotransmitters. This will ensure proof of conception, a requirement that must be met during EFSA's approval new stunning techniques. Alternative route of application of voltage to the head of cattle may also need to be explored further. Whilst application through nose and neck electrodes have been used widely in existing stunners, alternative routes of application should be investigated.

One of the challenges encountered during the development of the SPUC prototype stunner was the issue of insulating the metal frame (restraint) from the electronics to prevent current escape and arcing. Further research may need to be conducted to identify suitable insulating materials before the commercialisation of the SPUC stunner.

## 8.8. General Discussion

The development of the SPUC stunner required an understanding of the amount of current, voltage, and time required to effectively stun cattle. To achieve this, a number of *in vitro* experiments were conducted. First, the impedance of cattle heads was estimated by connecting the heads to a voltage source and measuring the amount of the applied voltage that reached the brain. The results showed a higher impedance than previously reported (Wotton et. al., 2000), this may have been due to poor contact between the electrodes and cattle heads. A second experiment involved the use of brain cells in a Gene Pulser Xcell Electroporating system to determine the main factors responsible for the electroporation of bovine cells. Energy was identified as the main factor responsible for cell membrane poration. The number of pulses on the other hand affected the proportion of cell survival or death. The least amount of cell deaths was observed during single pulsing, whilst triple pulsing resulted in more cell deaths than double pulsing. It was decided that double pulsing should be used in the SPUC to ensure optimal neural poration and less cell deaths. Whilst these experiments provided guidance to the choice of electrical parameters for the development of the SPUC stunner, final decision on the use of specific pulse duration, current and voltage were made based on the input of an electrical engineer (Dr Jeff Lines), animal physiologist (Steve Wotton) contributions from the author and Professor Toby Knowles.

A prototype SPUC beef stunner has been produced, however, it is yet to undergo full efficacy testing to evaluate its capability of inducing immediate and sustained loss of sensibility and unconsciousness. There is ongoing work to improve the insulation of the stunner to prevent current escape and arcing. It is anticipated that work on improving the insulation will be completed by the end of November 2019 and a trial using live animals will then be carried out in the first week of December 2019.

If shown to be reversible, the SPUC stunner will appeal to Muslim authorities who exclusively approve reversible stunning. The majority of Muslims do not currently approve any method of stunning for beef because they are not convinced about the reversibility of existing beef stunning methods such as captive bolt stunning.

## 8.9. Conclusion

Whilst this PhD project has led to the development of a prototype stunner, work is still needed to improve some aspects of the stunner before it can be submitted to EFSA for assessment. The insulation around the nose plate needs to be improved to ensure that any applied voltage enters the head to span the brain, this should also prevent arcing of current. It is hypothesised that the induction of unconsciousness is through electroporation, however, the mechanism of induction of unconsciousness is still not well understood. Further research is therefore needed to better understand this mechanism.

If successful, the SPUC stunner is likely to improve animal welfare, initial trials with the SPUC has shown that the duration of unconsciousness are relatively longer than that duration conventional electrical head-only stunning. The advantage of longer duration of unconsciousness from animal welfare perspective is that it prevents animals from recovering (regaining consciousness) from the stun during the period they are bled. Other potential advantages of the SPUC stunner over existing beef stunners include 1) Reduction of post-stun convulsions, this is likely to improve slaughter operator safety, 2) Reversibility of stunning to make it compatible with the Halal slaughter requirements 3) Improvement in carcass and meat quality through the reduction of blood splash in carcasses

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Quran (Q5:3). Prohibited to you are dead animals, blood, the flesh of swine, and that which has been dedicated to other than Allah, and [those animals] killed by strangling or by a violent blow or by a head-long fall or by the goring of horns, and those from which a wild animal has eaten, except what you [are able to] slaughter [before its death], and those which are sacrificed on stone altars, and [prohibited is] that you seek decision through divining arrows.

Quran (Q5: 5). This day [all] good foods have been made lawful, and the food of those who were given the Scripture is lawful for you and your food is lawful for them.

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# Appendix 1