Animal Welfare 2012, 21(S2): 75-80
doi: 10.7120/096272812X13353700593644
ISSN 0962-7286

Assessment of unconsciousness during slaughter without stunning in lambs

P Rodríguez, A Velarde*, A Dalmau and P Llonch

Animal Welfare Subprogram, IRTA, Finca Camps i Armet s/n, E-17121 Monells, Girona, Spain
* Contact for correspondence and requests for reprints: antonio.velarde@irta.cat

Abstract

The aim of this study was to assess brain activity in lambs during slaughter without stunning and its correlation with heart rate (HR) and the absence of physiological reflexes. The index of consciousness (IoC-view®) assesses consciousness by an algorithm that analyses the EEG and gives an index from 0 (unconscious) to 100 (conscious). Eight lambs (Merino breed) of 20 to 25 kg live weight were individually restrained in a stretcher and three skin electrodes were placed at the level of the frontal bone. The electrodes were connected to the IoC-view®. Two additional electrodes were placed on the chest and the HR was transferred to a watch monitor. Recording time started 2 min prior to sticking to attain the basal IoC and HR value of each animal. During bleeding, presence of corneal reflex and rhythmic breathing were recorded every 10 s until brain death. Rhythmic breathing disappeared at an average time of 44 (± 4.2) s after sticking, ranging between 30 and 60 s. The corneal reflex disappeared at 116 (± 11.01) s, ranging between 80 and 160 s after sticking. Changes in the brain activity occurred between 22 to 82 s after sticking (52 [± 20.2] s). In five out of six animals the HR increased (177 [± 22.7] beats min^{-1}) compared to basal values (139 [± 8.4] beats min^{-1}) at 115 (± 97.5) s after sticking. Both brain activity and physiological reflexes revealed that when bleeding is performed, through a transverse incision across the neck without stunning, the onset of unconsciousness could last 1 min which impaired animal welfare. This prolonged consciousness compared to other authors' findings may be attributable to inefficient bleeding when lambs are slaughtered without head restraint.

Keywords: *animal welfare, consciousness, EEG, lambs, neck sticking, religious slaughter*

Introduction

Stunning prior to slaughter is a legal requirement in the EU to induce unconsciousness and insensibility either immediately or sequentially, so that sticking can be performed without avoidable fear, anxiety, pain, suffering and distress (Council Regulation EC No 1099/2009). For this purpose, the stunning procedure should induce unconsciousness in the animal without pain and has to be prolonged until brain death by exsanguination. However, the OIE recommendations (OIE 2009), and the USA (USDA 2010) and the EU legislations (Regulation EC No 1099/2009) exempt religious slaughter from stunning. During halal and shechita slaughter the animals are restrained and bled through a transverse incision across the neck, severing the skin, muscles, trachea, oesophagus, carotid arteries, jugular veins and major nerves. The bleeding requires some time to provoke brain death. In sheep, after neck cutting, the time to loss of consciousness ranges from 14 to 298 s (Gregory & Wotton 1984). In addition, the cuts involve substantial tissue damage in areas well supplied with nociceptors (Kavaliers 1989). One EEG study developed in calves revealed that ventral-neck incision is associated with noxious stimulation that is expected to be painful prior to loss of consciousness (Mellor *et al* 2009). In sheep, there have been no direct EEG studies, however, based on the physiological similarities between sheep and cattle, it is reasonable to suppose that the neck cut in non-stunned sheep causes pain (Hemsworth *et al* 2009). Furthermore, during bleeding, animals may feel also anxiety, pain, distress and other suffering (EFSA 2004). Thus, there is concern about the extent of time that lambs are conscious or able to perceive pain, anxiety and distress after neck cutting (von Holleben *et al* 2010). One of the most reliable methods of assessing the state of consciousness is monitoring the brain activity by recording the EEG (Raj *et al* 1997; Rodriguez *et al* 2008). The index of consciousness® (IoC, IoC-view®, Morpheus Medical, Spain) is a monitor based on wireless technology that assesses brain activity in non-restrained animals. The IoC analyses the raw EEG giving a unitless scale from 0 (null brain activity) to 99 (awake) (Revuelta *et al* 2008). The IoC-view® monitor is currently used in human patients (Revuelta *et al* 2008), rabbits (Silva *et al* 2011) and pigs (Llonch *et al* 2011). The recommended IoC for surgical anaesthesia is under 60 whereas an IoC under 40 would suggest deep unconsciousness (Revuelta *et al* 2008). In commercial conditions, the state of consciousness tends to be assessed by means of the presence of some physiological reflexes (von Holleben *et al* 2010), such as the corneal reflex and rhythmic breathing. The advantage of these reflexes is that they can be easily monitored in the slaughterhouse. However, these reflexes are indicative only of brain-stem activity and its relationship with cortical function is unclear (Anil & McKinstry 1991). The HR has been used as a physiological index of pain (Mellor *et al* 2000), suffering and stress (von Holleben *et al* 2010).

The aim of this study was to assess brain activity in lambs during slaughter without stunning and its correlation with the heart rate and the absence of physiological reflexes.

Materials and methods

Study animals

After the approval of the Institutional Animal Care and Use Committee (IACUC) of the Institut de Recerca i Tecnologia Agroalimentàries (IRTA), eight male lambs of the Merino breed, weighing between 20 and 25 kg, were used. One day prior to the start of the study the lambs underwent a 2-h journey from their farm of origin to the commercial slaughterhouse. Here, they were housed in one pen (8 m^2) and provided with bedding material, food and water.

In order to record brain activity and HR, lambs were restrained individually in a net and remained suspended in sternal recumbency. The restrained animal's limbs were approximately 20 to 30 cm above the ground. Afterwards, three surface electrodes (Blue sensor, AMBU, Spain) were placed on the animal's skull at the level of the frontal bone. Once the skin had been shaved and cleaned, the positive electrode was placed on the breakpoint between the frontal and the parietal bone, the negative electrode was placed on the dorsal part of the occipital bone, and the reference electrode was placed between the positive and the negative electrodes. The IoC-view® monitor was then fitted to the electrodes to record EEG data. IoC data was transferred to a Pocket Personal Computer (iPAQ, HP, USA). Cardenas (2008) suggested that the IoC-view® has an update delay of 7 to 12 s between the state of awareness and IoC recording. In order to obtain the appropriate IoC that corresponds to each second, this delay was averaged to 10 s (Llonch *et al* 2011). At the same time, a belt with two electrodes was placed around the chest to record the HR using a Polar heart frequency watch (Polar, USA). Subsequently, animals were left individually in a pen for 2 min with visual and olfactory contact with the other animals.

The slaughter procedure in all the lambs was performed by a halal-authorised slaughterman. Prior to the sticking, the head of each lamb was pulled dorsally to stretch the neck in order to facilitate sticking. A unique transversal neck cut was performed with a knife with a 20-cm long blade. The neck cut severed skin, muscle, trachea, oesophagus, carotid arteries, jugular veins and major nerves. Two minutes prior to sticking, IoC and HR were recorded until 5 min after the neck cut. In addition, the presence of corneal reflex (through physical stimulation of the cornea) and rhythmic breathing (as indicated by the breathing sound and the movements of the flanks) were monitored for each 10-s interval until brain death. At the end of this period, and after checking the death of the animal by means of a null IoC and the absence of rhythmic breathing and corneal reflex, the carcase was hoisted and all the electrodes removed.

Statistical analysis

Analyses were carried out with the Statistical Analysis System (SAS 9.2, software SAS Institute Inc, Cary, NC, USA 2002–2008). For the data analysis of IoC and HR, the average data prior to the sticking (basal values) were compared to the values recorded each second during and after the neck cutting, using a mixed model analysis (PROC MIXED). When the analysis of variance showed significant differences ($P < 0.05$), the comparison of least square mean values (LSMEANS) was used and adjusted to Tukey multiple comparison test. In all cases, the variables were submitted to symmetrical composition covariance structure (CS). A descriptive statistics with Proc Means of SAS was carried out for the physiological reflexes (corneal reflex and rhythmic breathing). Correlations (PROC CORR) between the brain activity, changes in the HR and the physiological reflexes were also analysed.

Results

The IoC and HR before and after neck sticking and corneal reflex and rhythmic breathing after the sticking are shown in Figure 1.

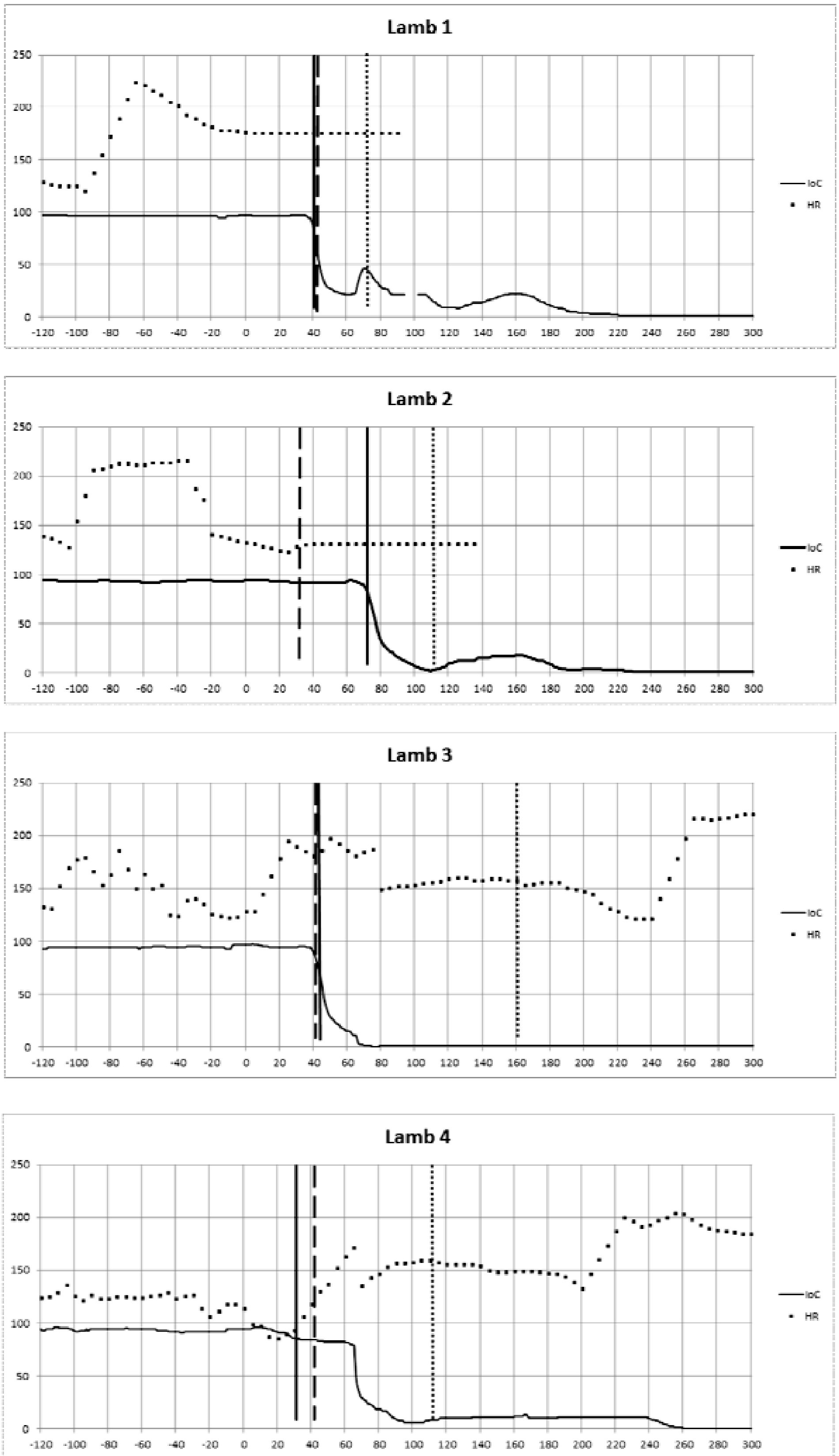
The IoC-view® recordings were successful in all animals assessed. The mean (± SD) basal IoC was 95 (± 1.8). The IoC started to decrease significantly ($P < 0.05$) 52 (± 20.2) s after sticking (IoC = 89 [± 3.7]). It continued decreasing and reached its lowest value on average (IoC = 1) at 215 (± 62.4) s after sticking. The HR was successfully recorded in six animals. In lambs 1 and 2, the signal was lost before the end of the recording time. The average basal HR was 139.1 (± 8.37) beats min^{-1}. The HR increased significantly (177 [± 22.7] beats min^{-1}) in all the animals at 115 (± 97.5) s after sticking except in lamb 8, whose HR never changed compared to basal values.

At the end of the sticking procedure, all the animals showed a corneal reflex and rhythmic breathing (Figure 1). The first animal that lost rhythmic breathing was at 30 s after sticking and the last at 60 s (44 [± 11.9]). The corneal reflex disappeared between 80 and 160 s after sticking with an average time of 116 (± 11.0) s. No significant correlations were found between the time to decrease brain activity, changes in the HR and the absence of the physiological reflexes.

Discussion

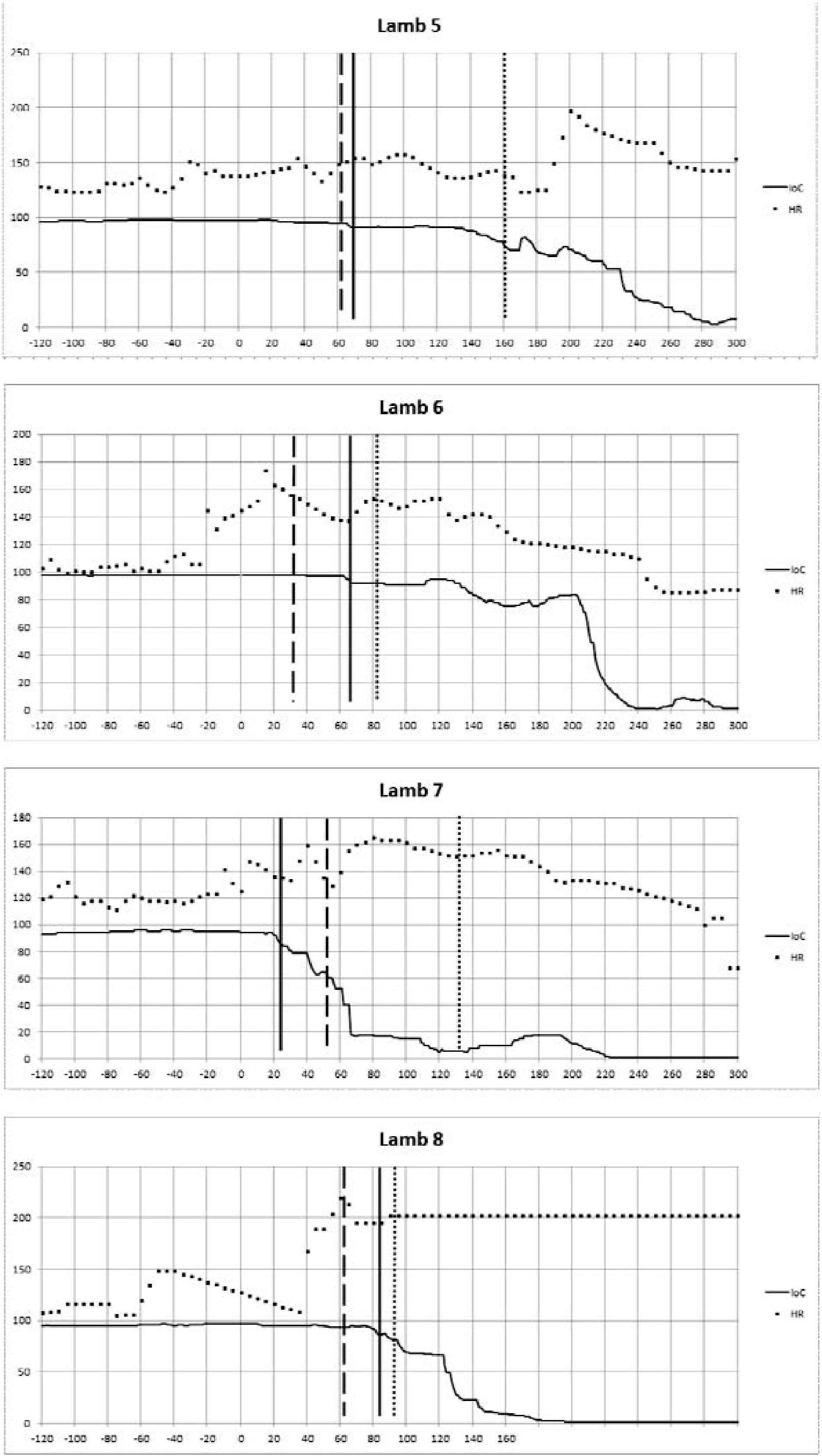
The IoC-view® monitor has been used to assess the depth of anaesthesia in human patients (Revuelta *et al* 2008), as well as in veterinary medicine for assessing consciousness in rabbits (Silva *et al* 2011) and pigs (Llonch *et al* 2011). The basal IoC, recorded before sticking, was around 95 which was similar to that obtained in conscious human patients (Revuelta *et al* 2008) and pigs (Llonch *et al* 2011). In previous studies, Rodríguez *et al* (2008) and Llonch *et al* (2011) concluded that a significant decrease in brain activity is considered a sign of the onset of unconsciousness. In our study, an IoC significantly lower than basal values occurred 52 (± 20.2) s after the sticking. These results are in contrast

Figure 1



Index of consciousness (IoC) and heart rate (HR) in lambs 1 to 4 slaughtered in relation to time (s) before and after the neck cut (at time 0). The continuous bar shows the time when the IoC started to decrease significantly ($P < 0.05$), the broken line shows the time to lose the rhythmic breathing and the dotted bar shows the time when the corneal reflex was lost.

Figure 1 (cont)



Index of consciousness (IoC) and heart rate (HR) in lambs 5 to 8 slaughtered in relation to time (s) before and after the neck cut (at time 0). The continuous bar shows the time when the IoC started to decrease significantly (P < 0.05), the broken line shows the time to lose the rhythmic breathing and the dotted bar shows the time when the corneal reflex was lost.

to Gregory and Wotton (1984) who found that time to reach unconsciousness was significantly less (14 s) when a complete section of both carotid arteries and jugular veins (including checking vessels for any obstruction during bleeding) were applied in sheep. As was suggested by Blackmore (1984), a slow rate of blood loss can extend the period of consciousness during slaughter without stunning. In fact, in previous experiments performed by the authors in pigs and rabbits, once the IoC starts to decrease, it falls rapidly (10 s approximately) to deep unconsciousness values (< 40; Revuelta *et al* 2008). According to the results of this study, this pattern was only performed by four lambs (1, 2, 3 and 4) whereas in lambs 5, 6, 7, and 8 the fall to deep unconsciousness lasted longer. This prolonged consciousness could be due to inefficient bleeding, as the neck was not stretched after sticking to facilitate the outflow, which could delay the rate of bleeding, the decline in blood pressure and hence lead to sustained brain function (Anil *et al* 2004). In reference to that, this study was carried out in a commercial slaughterhouse but under experimental conditions. Although the slaughter was performed by a halal slaughterman, the procedure was different to that during halal slaughter. For instance, Velarde *et al* (2010) stated that after visiting six slaughterhouses around Europe which perform slaughter without stunning, in all of them the lambs were restrained by either hoisting before neck cutting, manually on the side or mechanically on the side. In our experiment, lambs remained restrained in sternal recumbency in order to easily monitor brain activity and HR. The impact that this posture could have on the bleeding efficiency and thus on the time to reach unconsciousness is unclear.

Cranial nerve reflexes assist in achieving an overall picture of brain dysfunction. If all are negative, this is a good indication of impaired midbrain or brainstem activity and unconsciousness can be inferred (Gregory 1998). According to our results, the first animal to lose rhythmic breathing did so 30 s after sticking and the last animal that lost corneal reflex — which is generally the last reflex to disappear during loss of consciousness or onset of death (EFSA 2004) — did so at 160 s after sticking. The presence of these reflexes does not distinguish accurately between consciousness and unconsciousness, but when they are absent, it is likely that the animal is unconscious (Anil & McKinstry 1991). Taking into account both physiological reflexes it can be stated that under the conditions of this study, lambs lost consciousness between 30 to 160 s after sticking. These results are consistent with the results of the IoC since the decrease of the brain activity started in a range from 22 to 82 s. However, the fact that no correlation was found between the decrease in brain activity and the loss of physiological reflexes and the statement that both physiological reflexes may be present for several minutes after the cut in unconscious animals (Blackmore 1984) suggest that the loss of rhythmic breathing and corneal reflex does not accurately reflect the onset of unconsciousness.

In the majority of lambs assessed (five out of six), the HR increased significantly after sticking. According to von Holleben *et al* (2010), an acute stress during bleeding may increase the HR. However, in our study, it occurred on average 115 s after sticking. Hence, it is likely that the HR increase was not due to sticking but to the activation of sympathetic tone when blood pressure decreased (EFSA 2004). Moreover, the acute stress suffered by the animals during restraint and the manipulation during the placement of the electrodes increased the basal HR, which may mask the rise after sticking.

Then, the increase of HR is more likely to be related to the compensatory response to a haemorrhagic shock caused by bleeding (von Holleben *et al* 2010) which aims to enhance cardiac output and maintain perfusion pressure, especially in heart, brain and adrenal glands (Gutierrez *et al* 2008).

Animal welfare implications and conclusion

The IoC-view® is likely to be a valuable tool for brain monitoring during slaughter. The index of consciousness revealed that when bleeding is performed through a transverse incision across the neck without stunning, the loss of consciousness can extend from 22 until 82 s (52 [± 20.2] s). In non-stunned animals, the rate of bleeding may have a large impact on the reduction of brain function. Since this study prioritised the EEG recording rather than the quality of the bleeding, further investigations are needed in order to find ways to guarantee a rapid loss of consciousness after sticking in non-stunned lambs. According to the IoC results, the physiological reflexes are not a reliable method to assess the loss of consciousness during slaughter in non-stunned lambs. After sticking, the heart rate may increase in order to cope with haemorrhagic shock and it is not a useful indicator of unconsciousness in lambs slaughtered without stunning.

References

Anil MH and McKinstry JL 1991 Reflexes and loss of sensibility following head-to-back electrical stunning in sheep. *The Veterinary Record 128*: 106-107. http://dx.doi.org/10.1136/vr.128.5.106

Anil MH, Yesildere T, Aksu H, Matur E, McKinstry JL, Erdogan O, Hughes S and Mason C 2004 Comparison of religious slaughter of sheep with methods that include pre-slaughter stunning, and the lack of differences in exsanguination, packed cell volume and meat quality parameters. *Animal Welfare 13*: 387-392

Blackmore DK 1984 Differences in behaviour between sheep and cattle during slaughter. *Research in Veterinary Science 37*: 223-226

Cardenas K 2008 *Analysis of the udate delay over time for changes in the index of consciousness of the IoC-view® Monitor.* Morpheus Medical. http://www.morpheus-medical.com/fileadmin/morpheus_files/ioc_view/update_delay_over_time.pdf. (Accessed 15 September 2011)

Council Regulation (EC) No 1099/2009 2009 On the Protection of Animals at the Time of Killing. *European Community Official Journal L303*: 1-30

EFSA 2004 Welfare aspects of animal stunning and killing methods. *Scientific report of the Scientific Panel for Animal Health and Welfare on a request from the Commission. Question. Adopted on the 15th of June 2004. Brussels.* http://www.efsa.eu.int/science/ahaw/ahaw_opinions/495/opinion_ahaw_02_ej45_stunning_report_v2_en1.pdf

Gregory NG 1998 Physiology of stress, distress, stunning and slaughter. In: Gregory NG (ed) *Animal Welfare and Meat Science* pp 64-92. CABI: Wallingford, Oxon, UK

Gregory NG 2008 Animal welfare at markets and during transport and slaughter. *Meat Science 80*: 2-11. http://dx.doi.org/10.1016/j.meatsci.2008.05.019

Gregory NG and Wotton SB 1984 Time to loss of brain responsiveness following exsanguination in calves. *Resource Veterinary Science 37*: 141-143

Guiterrez, G, Reines HD and Wulf-Gutierrez ME 2008 Clinical review: hemorrhagic shock. *Critical Care 8*: 373-381. http://dx.doi.org/10.1186/cc2851

Hemsworth PH, Fisher AD, Mellor DJ and Johnson CB 2009 *A scientific comment on the welfare of sheep slaughtered without stunning.* http://www.daff.gov.au/__data/assets/pdf_file/0018/1370331/welfare-sheep-slaughter.pdf

Kavaliers M 1989 Evolutionary aspects of the neuro-modulation of nociceptive behaviors. *American Zoology 29*: 1345-1353

Llonch P, Andaluz A, Rodríguez P, Dalmau A, Jensen EW, Manteca X and Velarde A 2011 Assessment of consciousness during Propofol anaesthesia in pigs. *Veterinary Record 169*: 496. http://dx.doi.org/10.1136/vr.d5643

Mellor DJ, Cook CJ and Stafford KJ 2000 Quantifying some responses to pain as a stressor. In: Moberg GP and Mench JA (eds) *The Biology of Animal Stress: Basic Principles and Implications for Welfare* pp 171-198. CABI: Wallingford, Oxon, UK

Mellor DJ, Gibson TJ and Johnson CB 2009 A re-evaluation of the need to stun calves prior to slaughter by ventral-neck incision: An introductory review. *New Zealand Veterinary Journal 57*: 74-76. http://dx.doi.org/10.1080/00480169.2009.36881

OIE 2009 *Slaughter of Animals, Terrestrial Animal Health Code, World Organization for Animal Health, 18th Edition.* OIE: Paris, France

Raj ABM, Johnson SP, Wotton SB and McKinstry JL 1997 Welfare implications of gas stunning of pigs 3. The time to loss of somatosensory evoked potentials and spontaneous electrocorticogram of pigs during exposure to gases. *The Veterinary Journal 153*: 329-340. http://dx.doi.org/10.1016/S1090-0233(97)80067-6

Revuelta M, Paniagua P, Campos JM, Fernández JA, Martínez A, Jospin M and Litvan H 2008 Validation of the index of consciousness during sevoflurane and remifentanil anaesthesia: a comparison with the bispectral index and the cerebral state index. *British Journal of Anaesthesia 101*: 653-658. http://dx.doi.org/10.1093/bja/aen245

Rodríguez P, Dalmau A, Ruiz-de-la-Torre JL, Manteca X, Jensen EW, Rodríguez B, Litvan H and Velarde A 2008 Assessment of unconsciousness during carbon dioxide stunning in pigs. *Animal Welfare 17*: 341-349

Silva A, Ferreira DA, Venâncio C, Souza AP and Antunes LM 2011 Performance of electroencephalogram-derived parameters in prediction of depth of anaesthesia in a rabbit model. *British Journal of Anaesthesia 106*: 540-547. http://dx.doi.org/10.1093/bja/aeq407

USDA 2010 *United States Department of Agriculture, Humane Methods of Slaughter Act, National Agriculture Library.* USDA: USA

Velarde A, Rodríguez P, Dalmau A, Fuentes C, Llonch P, von Holleben K, Anil H, Cenci Goga, Lambooij B, Pleiter H and Yesildere T 2010 *Religious slaughter: Evaluation of current practices.* Factsheet WP2.2 Religious slaughter: Evaluation of current practices. http://www.dialrel.eu/dialrel-results

Von Holleben K, von Wenzlawowicz M, Gregory N, Anil H, Velarde A, Rodríguez P, Cenci Goga B, Catanese B and Lambooij B 2010 *Report on good and adverse practices animal welfare concerns in relation to slaughter practices from the viewpoint of veterinary sciences.* WP1. Religion, Legislation and Animal Welfare: Conflicting Standards. http://www.dialrel.eu/dialrel-results

https://doi.org/10.7120/096272812X13353700593644 Published online by Cambridge University Press