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HSA workshop report
Novel or improved methods of detecting un/consciousness in animals after stunning

Workshop held on Wednesday 18th June 2014

10:00am – 4:00pm

at:

British Veterinary Association, 7 Mansfield Street, London, W1G 9NQ, UK
The First Floor Boardroom

At the Humane Slaughter Association (HSA) workshop, it was evident that further scientific research and technological development must be carried out: to provide a clearer understanding of states of consciousness in animals during slaughter procedures; to enable the development of objective methods, and to determine more reliable behavioural indicators, for assessing animal consciousness in the 'field'. Specific points of interest identified at the workshop are described below:

What further questions does scientific research need to answer, regarding states of consciousness in animals after stunning? (This question applies to all animal groups including, for example, mammals, birds and fish.)

1. The bulk of research and technological development in assessing states of consciousness is concerned with human medicine. Human-based findings may not necessarily be relevant for other species and therefore may be less useful to animal welfare scientists; for example, typically, humans are not exposed to very deep levels of unconsciousness whereas animals must be for slaughter purposes.
2. Further research is needed to define the electroencephalographic (EEG) activity observed in different types of animals (e.g. species, ages, sex, anatomical composition/arrangement, condition – e.g. health), in various scenarios:
 - a. Further define a 'normal' conscious EEG and what parameters are associated with consciousness.
 - b. Further define animal sleep.
 - c. Following a range of different stunning scenarios (e.g. methods of stunning, modes of application), further characterise the brain conditions produced (e.g. frequency bands of activity, epilepsy, spike activity and 'flat' or isoelectric EEGs), the order and latency in which these conditions arise and the duration for which they persist.
 - d. Which parts of the brain survive longest and/or contribute the most to measured brain activity during consciousness, after stunning or bleeding?
 - e. Attempt to use multi-channel recordings to enable measurements of activity from more specific areas of the brain. (The possible number of electrodes will of course depend on the size of the animal and the recording equipment available.)

- f. Does stimulation of different brain structures, and with different stunning methods, cause different responses in brain activity?
 - g. Investigate the possibility of uncovering 'hidden' brain activity within 'isoelectric' EEGs and what this means for assessing animals' brain states. Equipment capable of sampling apparently 'isoelectric' EEG traces at 40 – 100 kHz is already in use in human medicine to identify 'hidden' activity during epileptic seizures.
 - h. Determine under what conditions brain activity may vary (e.g. immediate onset of isoelectricity in EEGs, instead of initial epilepsy, after application of the same type of stunning method). For example, gas stunning can induce a range of brain activity between different gas mixtures and with the same mixtures but between different animal types (EFSA, 2013a).
 - i. Animals can show behavioural signs of consciousness when the total EEG power content is as low as 26 – 33% of the pre-stun basal level (hens: Raj and O'Callaghan, 2004). Which parts of the brain are functional at this time and enable consciousness and sensibility? Might research be able to identify means of targeting specific brain structures during stunning, to increase the chance of effective stunning?
3. It was suggested that the effects of needle dimensions on the quality of the EEG signal should be investigated.
4. It was questioned whether some measures of un/consciousness are too conservative. For example, recording abolition of visual evoked potentials/responses (VEP/Rs) may be a more reliable means of measuring unconsciousness if EEG activity is ambiguous (EFSA, 2013a). But is it possible to increase the accuracy of such measures and determine if consciousness is lost before, for example, the VEPs are abolished? Can VEPs be examined further, to see if it is possible to distinguish between a VEP elicited during consciousness and a VEP elicited during unconsciousness?
5. EFSA (2013b,c,d,e) stated there is a lack of scientific publications involving simultaneous assessment of EEG indicators and welfare indicators, such as physical reactions and reflexes. Sometimes, scientific research has not found strong correlations between an animal's brain activity and the animal's behaviour (e.g. Llonch et al, 2011, 2013). In some cases, animal behaviour (e.g. corneal reflex) indicates a positive response to a test for consciousness whilst the animal's brain activity suggests it is not conscious; similarly, sometimes animal behaviour (e.g. response to a visual threat or to a noxious stimulus) indicates a negative response to a test for consciousness but the animal's brain activity suggests it is conscious. Examining animals for absence of certain behaviours is relatively reliable for determining unconsciousness. However, examining animals for loss of consciousness is not so reliable with the current behaviours used. Future scientific research needs to see if stronger correlations exist between brain activity and various animal behaviours (e.g. reflexes, muscle tension) and needs to reduce the risk of false positive and false negative test situations so that, both in the laboratory and in the 'field', observers can be sure they are looking at a truly unconscious animal or a truly conscious animal.
- a. Research needs to further examine the physiology and origins of certain behavioural reflexes.
 - b. How do different behaviours occur in different species and how might this affect the use of that behaviour in assessing brain states? (For example, breathing - avian air sacs versus mammalian lungs versus partial gaseous exchange through the skin in fish.)
 - c. Research needs to clarify what animal behaviour is typically observed in different species during consciousness and how those behaviours alter after a given procedure, e.g. what is 'normal', conscious breathing and what is 'rhythmic' breathing and does an intervention (e.g. stunning or slaughter without prior stunning) affect whether and how an animal breathes and can this be used to inform assessment of loss of consciousness?
 - d. Do different stunning methods affect behaviour differently? (It may be necessary to assess any influence of different restraint methods on the detection and/or reliability of behavioural parameters.)

- e. Identify the time it takes for different types of animals to physically recover from different stunning methods and stunning parameters. The sequence of behaviours, including provoked responses, observed during recovery should be determined to provide a timeline of when an animal is likely to reach consciousness, enabling personnel to plan appropriately for animal welfare and human health and safety. It is possible that sequences may vary between animal types; this will need to be investigated.
 - f. It will be important to establish whether a behavioural measure is an indicator of consciousness, unconsciousness and/or death.
6. It was suggested that an animal model might be a possible way forward for fundamental research to explore the EEG and the associated animal behaviour in further detail (including the points listed above), in a controlled environment and using different scenarios. A mammalian model species might be a rodent or lagomorph. Since adaptations (e.g. of anatomy, physiology) to different habitats/lifestyles can affect the behaviour of animals, including between species within the same class, it might also be necessary to work with a fish model and an avian model. For example, it was pointed out that the organisation of the brain may vary depending on animal type (e.g. birds and mammals differ in their neuronal structures) and it was suggested that literature sufficiently describing epileptiform activity in fish, including the specific frequencies observed, was needed. A model species might also be helpful in advancing the understanding of potential future methods of stunning animals, e.g. it was suggested that transcranial magnetic stimulation requires further data on interpretation of the outcomes of stuns.

What limitations are there to progress in determining states of consciousness in animals after stunning?

7. Stunning methods that involve a non-immediate induction of unconsciousness, e.g. gas stunning or low atmospheric pressure stunning, allow scientists to measure animal behaviour during the loss of consciousness. However, it was pointed out that the apparent immediacy of onset of unconsciousness caused by some stunning methods (e.g. captive-bolt and electrical stunning), prevents the identification of correlations between brain activity and animal behaviour *during the loss* of consciousness. Instead, the focus has been on correlations between EEG activity and animal behaviour during the stages of *recovery* of consciousness. However, it was pointed out that the correlations are of low strength. The number of animals potentially required to achieve strong correlations may be large and EEG research typically involves relatively small sample sizes, particularly if recording equipment is invasive, because of the ethics, cost and time in instrumenting large numbers of animals, because equipment often becomes detached from some animals during the experimental process or because 'noise' renders the signals unusable.
8. Indices of consciousness offer a single-value output for assessing a human's state of consciousness. The algorithms of existing equipment are based on humans, often in a relatively mild state of anaesthesia, and may not necessarily be appropriate for different types of animals, which may be in a state of deep insensibility. Although such indices are not solely relied upon in human medicine, methods like this are likely to also be useful in animal welfare science and so algorithms and equipment suitable for animals, should be developed.
9. Movement artefact within EEG traces remains a challenge even in human medicine and is significantly more problematic when recording free-moving animals which cannot be instructed to remain still, or which convulse involuntarily during the slaughter process. Some indexing equipment takes a dynamic mean of brain activity over a defined time period (e.g. 7 seconds) – any artefact within that time period may render that value unusable. Artefacts may be of the same frequency as delta wave brain activity and therefore ultimately require a scientist to make a less-than-ideal choice whether to risk including 'noise' within the EEG trace or to filter it and potentially also remove useful brain activity from the trace. However, the EEG and behaviour of free-moving birds (including whilst flying at approximately

50 km/h) and wild mammals has been studied using miniaturised neurologgers (e.g. homing pigeons: Vyssotski et al, 2006, 2009; barn owls: Scriba et al, 2012; sloths: Rattenborg et al, 2008); information exchange and collaboration with other areas of research may prove useful. Technology that can assist with characterising animal activities within the traces may enable the 'cleaning up' of signals.

- a. Can convulsive movements be strategically characterised (e.g. using multi-axial accelerometers), for different movement types and species and, on that basis, be used to filter the EEG signals to leave the remaining brain activity?
 - i. Investigate new techniques for removing artefacts from recordings or developing new filtering methods. An animal model may assist with this.
- b. Equipment is already being used to display 'live' EEG raw signal traces alongside electrocardiogram (ECG) traces and measures of breathing in animals. Ideally though, such equipment will automatically filter artefacts (e.g. mains 50 Hz frequency interference and artefacts caused by convulsions) and will automatically and immediately transform the signals to enable real-time observation and assessment of brain activity in the 'field'.

10. Conditions within slaughterhouses may obstruct accurate monitoring of livestock:

- a. The restraint equipment in slaughterhouses may sometimes interfere with the EEG electrode placement and sometimes the data produced.
- b. The speed of a slaughterline may limit the ability to obtain high-quality EEG signals because, after instrumentation and manipulation, there is insufficient time for the animal to stop moving *and* to record clear EEGs, before further processing.

11. Although some technology has been developed to measure EEGs less invasively and more rapidly (e.g. chicken EEG clamp: Coenen et al, 2007; IoC-view®: Llonch et al, 2013; Mobito: M. Verhoeven personal communication), further development of equipment is necessary, particularly to further hasten the speed of instrumentation and to, thereafter, reduce the time taken to obtain a useful signal, both in the laboratory and in the field.

12. The development of easy-to-use, quick-to-set-up/record, portable telemetric devices for objectively measuring brain activity in real-time could enable the recording of brain activity from large datasets of animals from a population that was already intended for slaughter. Minimally invasive technology, e.g. neuroimaging techniques, may offer potential solutions. Once sufficiently developed, telemetric devices may have the potential to be used by slaughterhouse personnel in routine checks for effective stunning.

Might there be ways of refining existing methods (e.g. electroencephalography or brain impedance recordings), or of exploring alternative or novel methods (perhaps from human medicine), to identify states of consciousness in animals?

13. It was considered that, currently, the EEG is still the most reliable method of assessing consciousness in animals. It was also said that abolishing VEPs was a guarantor of unconsciousness.

14. It was suggested that refinements in existing methodology might be achieved by producing guidance for scientists who investigate assessment of consciousness and the effectiveness of stunning animals. Such guidance can provide recommendations using a 3Rs approach to the recording, analysis and interpretation of data, as well as to the use of animals. (Much information is already provided in EFSA (2013a), but a guidance document specifically advising researchers on neurological (e.g. EEG) methodology was thought to be a further useful reference for those using these techniques to assess stunning.) Experts in EEG methods from outside the field of animal welfare science might be willing to peer-review the guidance and offer recommendations. It was made clear that whilst guidance should encourage performance of studies, and reporting of study parameters, in a manner that allows more useful scientific comparisons to be made between studies, such a guidance document should not

become a strict standard operating procedure or manual of requirements since this may stifle progress and innovation. Those who are interested in contributing to the production of such guidance should email Huw at info@hsa.org.uk Whilst avoiding duplication of EFSA (2013a) recommendations, the guidance might include the following:

- a. If the terms 'consciousness', 'unconsciousness' and 'death' are used, the way they are measured, as objectively as possible, should be clearly defined.
- b. Describe what the study aims to measure: 'consciousness', 'unconsciousness' or the 'loss of consciousness'?
- c. Encourage the detailed reporting of procedures and, where appropriate, encourage harmonisation of methods used, to improve data quality. For example:
 - i. anaesthesia protocols
 - ii. method of cleaning the recording electrodes
 - iii. the electrical properties of recording equipment. For example:
 1. electrode impedance (to assist with identification of the likely signal quality and calibration parameters)
 2. the type (shape, size) and number of electrode(s), which may vary depending on a species' skin characteristics, the conditions within the recording environment and the size of the animal/brain to be instrumented. (For example, some electrodes no longer require shaving of hair from the skin before attachment. For sheep, which have oily skin, wet sponge electrodes may be attached faster, may be more successful at remaining attached and are less-invasive than needle electrodes)
 - iv. invasive methods (e.g. attaching electrodes to the dura) typically provide better/clearer data than less-invasive surface electrodes, but some invasive methods (e.g. electrocorticogram) may disrupt recordings (e.g. by affecting electrical current pathways through the brain) and may require more time to implant and [after the animal recovers] begin recording
 - v. specify a minimum acceptable signal:noise ratio that should be met in order to obtain clearer signals with fewer artefacts, for all relevant methods (e.g. EEGs, ECGs, EMGs).
 - vi. input ranges for recording signals (for optimising the quality of digitisation)
 - vii. amplification coefficients
 - viii. method and location of placement of electrodes should include a description of which structure(s) in the brain the scientist implants the electrodes over and which structures s/he intends to record brain activity from. (Once research makes such information available, guidance on which parts of the brain to target for EEG recordings in specific types of animal (e.g. mammals, birds and fish) will be useful; different forms of brain may require different electrode placement.)
 - ix. describe the specific part of the brain that the stun is intended to target (and which part it actually targets on application)
 - x. orientation of the brain/animal to the applied stun (e.g. current/electric field)
 - xi. confirm that the voltage/current amplitude displayed on the stunning equipment is the same amplitude passing through the animal. Quality Assurance of this process will produce more reliable results on the effectiveness of stunning parameters
 - xii. when investigating electrical stunning, for all modes of application, a variable voltage, constant current electrical stunner is likely to offer more control and to standardise applied parameters because it removes much of the variation associated with a constant voltage, variable current stunner. It should also maximise the number of animals within the sample, which receive the intended current amplitude
 - xiii. specification of minimum requirements for accurate recordings may be necessary (e.g. for EEGs, at least 2000 Hz sampling rate was suggested; higher rates may reveal 'hidden' activity in an 'isoelectric' EEG)
 - xiv. the manifestations of brain activity observed including the frequencies of activity

- xv. there is no 'gold standard' for EEG interpretation. Visual assessment of EEG traces for high amplitude, low frequency activity indicating unconsciousness is subjective; training is required and traces do not always follow the expected pattern. It may be more objective to use software to analyse the raw brain activity (e.g. fast Fourier transformation of the EEG signal using spectral and power analysis). Ideally, before transforming the signals, equipment should be able to filter any artefacts (e.g. mains 50 Hz frequency interference as well as the lower-frequency 'noise' that may affect the outcome of power analysis)
 - xvi. scientists should be encouraged to publish traces of brain activity of individual animals, particularly if they do not match the observations of other individuals in the same scenario and outcome, to enable readers to consider the variation
 - xvii. where equipment produces a discrete, calculated value as an index of consciousness, scientists should also display a representative trace of a raw EEG signal and any associated noise.
 - 1. Artefacts can greatly affect index values so a range of parameters for assessing consciousness should be used to ensure accuracy.
 - xviii. visual EPs may be more reliable than somatosensory and auditory EPs
 - xix. when assessing recovery of consciousness describe, as objectively-as-possible, how any provoking stimuli were applied (e.g. if applying a nose- or footpad-prick to an animal to test it for sensibility, the stimulus should be applied in a controlled manner – e.g. report the pressure of the stimulus and the dimensions/properties of the implement used)
 - xx. provide data to describe which blood vessels were severed in the animals, to enable any associations with brain activity to be reported. (Reports of the type of cut performed (e.g. 'lateral cut') or reports of the vessels 'expected' to be cut, based on the type of cut performed, do not confirm the actual blood vessels that *are* cut and are therefore of a limited value.)
15. It was considered whether technology other than current use of EEGs might allow for progress to be made in different ways/areas. For example, refined and/or new methods of assessing unconsciousness/pain may be needed, particularly in attempts to correlate animal behaviour with brain activity. Brain impedance recordings were used to determine the extracellular volume (ECV) within the broiler chicken brain by Savenije et al (2002, 2000). The method gave promising results and was reported to be potentially useful in identifying ischaemia-induced brain damage in poultry, but "it remained unclear... which criteria trigger an immediate increase in brain impedance or a delay or what determines the rate of increase in brain impedance in individual chickens. In cases in which an immediate and progressive decrease in ECV was not found, brain impedance recordings were not conclusive about the state of consciousness of the animal." (Savenije et al, 2002). It was thought that brain impedance recordings might be used to further evaluate the effects of electrical stunning and that they might be performed on unanaesthetised animals. (However, the necessary stereotaxic equipment required insertion of a metal plate into the animal's mouth and metal probes into the ears to ensure an exact measuring point.) Another complicating factor is that the anatomy (e.g. as influenced by age and breed) of an animal might affect how the stereotaxic equipment is positioned, relative to the brain. Further studies may need to improve the ease and accuracy of positioning as well as determining the biochemical states of the brain, including during stunning and recovery.
16. Functional near-infrared spectroscopy (fNIRS) can detect haemodynamic and metabolic changes in specific locations of brain tissue. FNIRS is used clinically to assess the functional severity of brain lesions and the brain activity in non-verbal human patients, at their bedside, using wireless devices. FNIRS is a non-invasive method that can measure changes in oxygenation through an intact skull (where it is thin enough) and has been used to assess emotional reactions in non-sedated, free-moving sheep (Reefman et al, 2010). However, at present, fNIRS is capable only of measuring oxygenation of the cerebral cortex; deeper measurements would require a more intense light source which could

influence the physiology of the subject, e.g. through thermal effects on the brain. fNIRS has been used to detect epilepsy (changes in haemodynamics of the brain during and prior to seizures, which can be seen prior to changes in the EEG), including to pinpoint the location of the seizure, and to detect pain but experiments must be well-controlled to avoid misinterpretation of data, e.g. following a noxious stimulus, respiration rate may increase and this may influence brain oxygenation, as well as any effect of the perception of pain. Anaesthetics may also have varying effects on oxygenation of the brain. Loss and recovery of consciousness can be measured using fNIRS, e.g. the Perturbational Complexity Index is a joint measure of integration and differentiation of the information within an EEG signal and is obtained using transcranial magnetic stimulation of the brain and recording the electrical response via EEGs. It has reportedly been used to good effect in humans.

- a. Trialling fNIRS may:
 - i. allow determination of which structures in the brain produce specific activity observed in EEGs during noxious stimulation, stunning, bleeding and recovery;
 - ii. allow less-invasive measurement of VEPs;
 - iii. indicate how much blood has been lost at slaughter and therefore an idea of which, and how many, blood vessels might be severed, or if false carotid aneurysms have formed?
- b. fNIRS equipment has the advantage that it can be portable and can measure subjects 'in the field'; it is also apparently relatively inexpensive. However, like other methods, it is susceptible to movement artefacts (including from respiration) and may not be suited to measuring oxygenation of large-brained and/or thick-skulled species due to a limited penetration depth of the emitted light.
- c. Multimodal neuro-monitoring (e.g. using fNIRS and EEG) was suggested to be a good option for future work but integration of other techniques will further improve data (e.g. fNIRS, EEG, skin conductance, heart-rate variability, signal modelling and signal fusion).

17. Arterial partial pressure of blood oxygen or pulse oximetry have also been suggested as measures of hypoxia, in addition to EEG recordings (EFSA, 2013a).

18. Magnetoencephalography is another potential technique but may be difficult to measure.

What funding sources are available for further necessary research?

19. Joint international research projects and collaborations between scientific centres of expertise in multiple countries may improve resources and maximise potential outcomes. There is a need for a structured approach to securing funding between countries. COST Action projects might be suitable for co-ordinating the efforts of multiple research groups, although COST do not provide funding themselves. Avoiding the need for a Home Office licence may allow applications to a wider range of funding bodies, particularly charities.

Other topics suggested for further investigation during the discussions:

20. Research into slaughter without stunning will likely be most feasible in a laboratory.
 - a. Further research is needed on behavioural indicators of pain. For example, explore whether an association exists between neck cutting and facial expression/grimaces in animals that are highly-restrained for slaughter without stunning. (It was acknowledged that facial expressions may be difficult to accurately and reliably investigate.)
 - b. Determination of the mechanism of formation of false carotid aneurysms, which might provide information as to why aneurysms are observed in cattle but not in sheep. Is the formation of an aneurysm influenced by the method and degree of restraint of the animal (e.g. the amount of tension in a restrained neck), the skill of the slaughter person, the animal's heart beat or

blood pressure, breed or age, the movement of the blood vessels once severed (e.g. if/how they retract within the neck)?

21. Stunning using:
 - a. very high amplitude currents at short pulse durations (e.g. mammals: Robins et al 2014; fish: Roth et al, 2003);
 - b. transcranial magnetic stimulation;
 - c. microwaves. The size of the generator/stun box is very large relative to the size of the animal to be stunned; technology will need to address this, or perhaps determine how to focally apply radiation so the microwaves target the brain specifically.
22. There is very limited published research on humane stunning of ducks and geese, particularly of the neurological effects of intended stunning methods and of the efficacy of electrical stunning.
23. Further research on humane stunning of water buffalo, including electrical stunning as a potential alternative to captive-bolt stunning which may be problematic.
24. Scientific information is lacking for the effective stunning of some types of animal, e.g. Vietnamese pot-bellied pigs.
25. Evaluation of slaughter persons' extent of knowledge of states of consciousness and the effectiveness of stunning, and the variation between staff at different slaughterhouses. How effectively do slaughter personnel apply that knowledge and to what degree is their level of knowledge associated with the level/quality of their training and the frequency of re-training? As well as their skills working with stunning equipment and animals, it will be useful to assess slaughter persons' understanding of recorded parameters (e.g. those required by European Regulation 1099/2009) and an individual's ability to review that information and decide whether action is necessary (e.g. to repair equipment).

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Report prepared by: Jade Spence 2 December 2014

Appendices

Agenda

09:45am: Registration. Refreshments available

10:15am: Welcome and introductions

10:20am: Presentations.

1. *Methods of assessing consciousness in different types of animals: advantages and limitations for determining the state of consciousness and for correlating with behavioural indicators of consciousness; what is needed to make further progress?* Dr Marien Gerritzen, Wageningen UR Livestock Research
2. *The extracellular volume in the broiler brain, as measured with brain impedance recordings: is there a need for further research?* Dr Bert Lambooi, Wageningen UR Livestock Research
3. *Near-infrared spectroscopy (NIRS): detection of pain, epilepsy, loss and recovery of consciousness; advantages and limitations for determining the state of consciousness, including compared to EEGs.*
Felix Scholkmann, University Hospital Zurich

Delegates (including speakers)***Humane Slaughter Association***

Dr Robert Hubrecht, Chief Executive & Scientific Director

Charlie Mason, Technical Director

Jade Spence, Technical Officer

Nathan Williams, Technical Officer

Dr Marien Gerritzen	Senior Scientist, Wageningen UR Livestock Research, Netherlands
Dr Troy Gibson	Lecturer in Animal Welfare Science, Royal Veterinary College, UK
Dr Bert Lambooj	Senior Researcher, Wageningen UR Livestock Research, Netherlands
Dr Joanna Murrell	Senior Lecturer in Veterinary Anaesthesia, University of Bristol, UK
Dr Simone Pauling	Coach Research, Marel Stork Poultry Processing, Netherlands
Dr Mohan Raj	Visiting Fellow, University of Bristol, UK
Dr Ana Roque	Researcher, Institute of Research and Technology for Food and Agriculture, Spain
Felix Scholkmann	Research Assistant, University Hospital Zurich, Switzerland
Dr Antonio Velarde	Director of Animal Welfare subprogram, Institute of Research and Technology for Food and Agriculture, Spain
Merel Verhoeven	PhD student, Wageningen UR Livestock Research, Netherlands
Steve Wotton	Senior Lecturer in Farm Animal Science, University of Bristol & Farm Animal Welfare Committee, UK