Eligibility Criteria for outcomes of stunning interventions

Mohan Raj

EFSA AHAW Panel Member

ONSET OF UNCONSCIOUSNESS

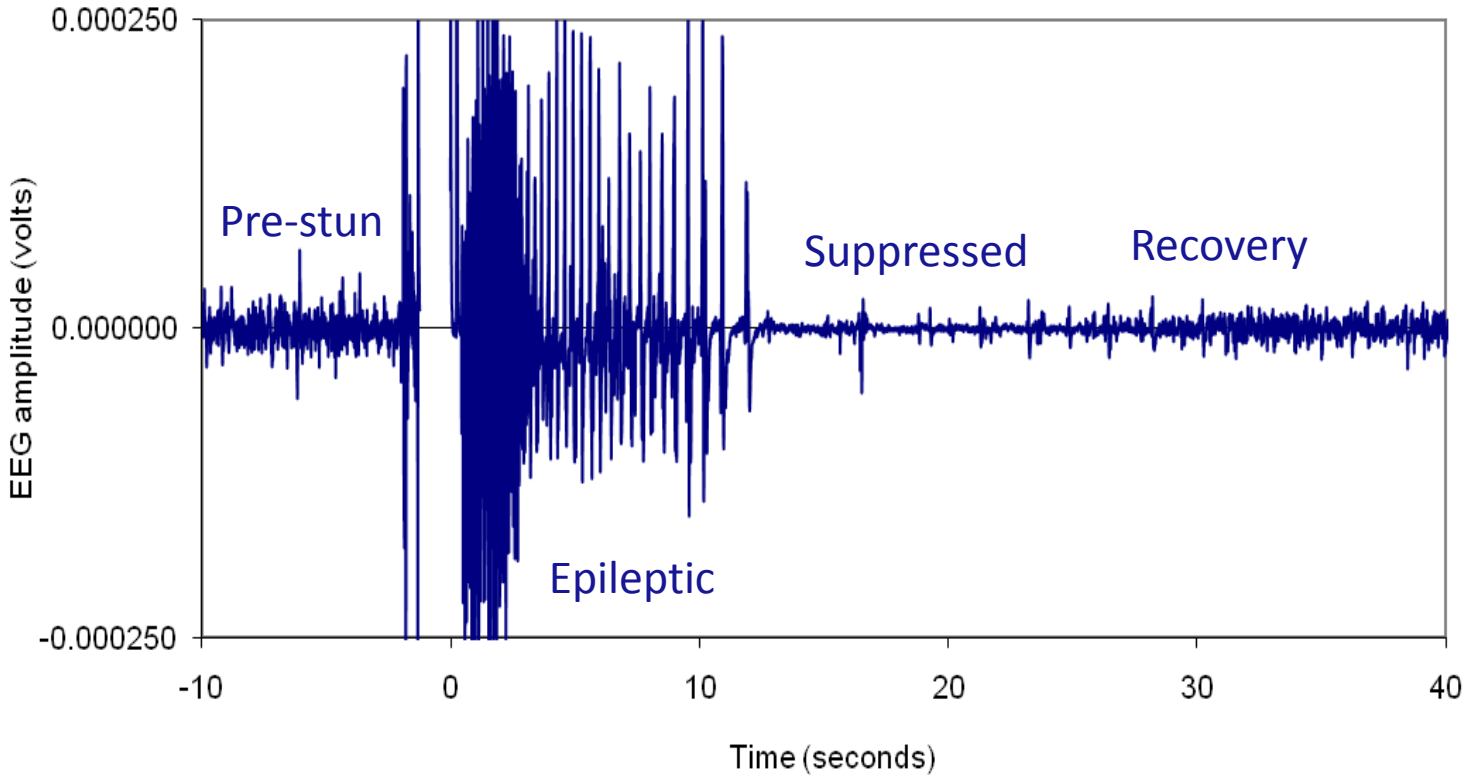
Studies should clearly provide:

- Description of brain mechanisms associated with induction of unconsciousness
- Demonstration of unconsciousness using EEG, ECoG and / or evoked potentials (SEPs, VEPs, AEPs)
  - Unique brain states that are incompatible with persistence of consciousness
- Assessment of correlation between EEG evidence and animal based indicators (as proxies) of the state of consciousness for monitoring in slaughterhouses

EXAMPLE: ELECTRICAL STUNNING

- Induces immediate unconsciousness through epileptiform activity in the brain
- Epileptiform activity ascertained from the occurrence of low frequency / high amplitude activity in the EEG and abolition of evoked potentials
- Epileptic state leads to immediate collapse, apnoea, tonic – clonic seizures and absence of eye reflexes
- Ineffective stunning ascertained from failure to collapse, vocalization, presence of rhythmic breathing / eye reflexes and escape attempts
- Duration of unconsciousness determined from the time to return of spontaneous / evoked activity in the brain and animal based indicators of the state of consciousness
- Estimated maximum stun-to-stick interval in order to ensure death occurs in unconscious animals
  - Blood vessels supplying oxygenated blood to the brain to be cut during slaughter

Epileptiform activity in the EEG



Duration of unconsciousness = duration of epileptic + suppressed EEGs

EXAMPLE: HEAD-ONLY ELECTRICAL STUNNING

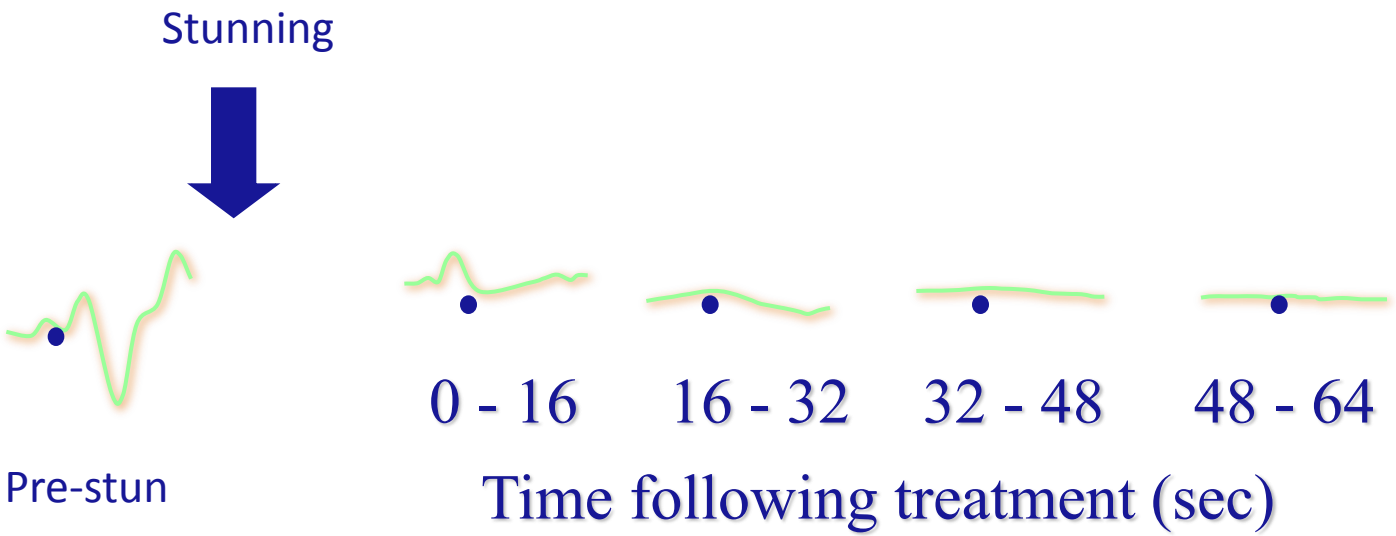
Animal based indicators:

Flexed hind legs
Extended fore legs
Fixed eye
Tonic-clonic seizures

EXAMPLE: MECHANICAL (CAPTIVE BOLT) STUNNING

- Induces immediate unconsciousness through brain concussion
- Brain concussion ascertained from the predominance of slow waves / low frequency activity in the EEG and abolition of evoked potentials
- Concussion leads to immediate collapse, apnoea, tonic seizures, fixed eyes and absence of eye reflexes
- Ineffective stunning ascertained from failure to collapse, presence of rhythmic breathing and eye reflexes, and head righting / body arching
- Duration of unconsciousness determined from the time to return of spontaneous / evoked activity in the brain and animal based indicators of the state of consciousness
- Estimated maximum stun-to-stick interval in order to ensure death occurs in unconscious animals
  - Blood vessels supplying oxygenated blood to the brain to be cut during slaughter

EXAMPLE: MECHANICAL (CAPTIVE BOLT) STUNNING



Captive bolt stunning leads to immediate abolition of visually evoked potentials (VEPs) in the brain

Daly et al. 1988

EXAMPLE: MECHANICAL (CAPTIVE BOLT) STUNNING

Immediate collapse

Flexed legs and muscle spasms

EXAMPLE (WBS): ASSESSMENT OF LAB STUDY

Table 2: Information provided in the laboratory study by the submitted study in relation to the onset of unconsciousness and insensibility (3.2.1 of EFSA guidance (EFSA AHAW Panel, 2013a))

Laboratory study	Information provided in the submitted manuscript	Is the criterion described/met adequately? (yes, no or not possible to assess)
Start and end of EEG measurement	In the submitted study the information is provided that the EEG was recorded 80 seconds before and 80 seconds after stunning before sticking (Section 2.1.1.1).	Yes
EEG measurement	For the EEG registration, the birds were individually implanted with 2 needle electrodes (55 % silver, 21 % copper, 24 % zinc of 10 mm x 1.5 mm diameter) positioned under the skullcap through the skin and skull onto the brain lobes (0.3 cm left and right of the sagittal suture and 0.5 mm toward an imaginary transverse line at the caudal margin of the eyes). The electrodes were sealed using adhesive tape.	Yes
EEG recording analysis	Information from last paragraph of Section 2.1.1.1: The EEG was collected at a sample frequency of 120 Hz and the digital signal obtained was processed using fast Fourrier transformation (FFT), implemented using the MATLAB tool to obtain the frequency spectrum of different stretches associated with different events on the EEG. Several successive artifact-free stretches were also analyzed and filtered into four frequency bands: delta (0.3-4 Hz), theta (4-8 Hz), alpha (8-12 Hz) and beta (12-30 Hz). Elliptical filters integrated onto a visual tool developed at the MATLAB® were used. In each of these frequency bands, the energy of the signal obtained was calculated using MATLAB® signal processing toolbox. Spike-and-slow-wave-complex (SASWC) (known as petite mal) and spike-or-sharp-wave (SOSW) (common to several different types of epilepsy) are referred in the results Section (3.1.1.1)	No
EEG results	In the submitted study one sample recording of epileptic EEG obtained after 15 seconds stunning is shown in Figure 5. The epileptic ictal stage has 12 seconds of duration. However the legend of the figure mentions 15 seconds of electrical exposure. Epileptic patterns in the EEG were detected by analyzing the spectral power density. The results show that the electrical parameters tested will induce epilepsy in all birds after 15 seconds of exposure to the electrical current and that the energy of the epilepsy generated depends on the time of exposure to the electric shock. In fact, the tested parameters did not cause epilepsy within 1 second in 100 % of the chickens. In fact an application of the chosen current parameters for up to 12 seconds failed to induce epileptiform EEG in all the birds. A minimum exposure time of 15 seconds is needed to result in unconsciousness – there is no evidence that the animals are not subjected to avoidable pain during the stun.	No

EXAMPLE (WBS) : ASSESSMENT OF SLAUGHTERHOUSE STUDY

Table 3: Information provided in the study under slaughterhouse conditions by the submitted study in relation to the onset of unconsciousness and insensibility (3.2.1 of EFSA guidance (EFSA AHAW Panel, 2013a))

Study under slaughterhouse conditions	Information provided	Is the criterion described/met adequately? (yes, no or not possible to assess)
Start and end of EEG measurement	In the submitted study it is stated that the same procedure as in the laboratory was followed.	No
EEG measurement	In the submitted study it is stated that the same procedure as in the laboratory was followed. A separate Section of the submitted study describes the continued evaluation of the EEG of chickens during bleeding.	No
EEG recording analysis	In the submitted study it is outlined that the EEG analysis considered the drop in power of the 2-30 Hz band in 1 second epochs and the drop in the ratio of the power in the Delta band compared to the other bands before and after stunning.	Not possible to assess
EEG results	In the submitted study data showed an average time of unconsciousness of 32 seconds with a standard deviation of 14 seconds. Such variation in is expected due to individual impedance variations which influence the current that the bird receives while immersed in the waterbath. The distribution of the time of chicken unconsciousness following stunning is shown in Figure 7. Results of continued evaluation of the EEG of chickens during bleeding show that the EEG of birds bled by a deep ventral cut (2T) after stunning in Figure 11. The EEG at the moment of the cut is also marked by epileptic forms. Following cutting, the amplitude of the EEG drops considerably. After 35 seconds, the bird's EEG reaches a very low amplitude value, providing evidence of death. In the submitted study, Figure 12 reports the distribution of time between stunning and death.	No
ABM to detect onset of unconsciousness	In the submitted study resumption of rhythmic breathing as a sign of return of consciousness. A paragraph about physical signs of unconsciousness after stunning is reported in Figure 9. Evidence of death through observation of dilated pupils, absence of breathing and relaxation of the neck and wings during bleeding was performed.	No

THANK YOU

Any questions?

efsa
European Food Safety Authority

www.efsa.europa.eu