Validation of indicators used in sheep to assess unconsciousness at slaughter
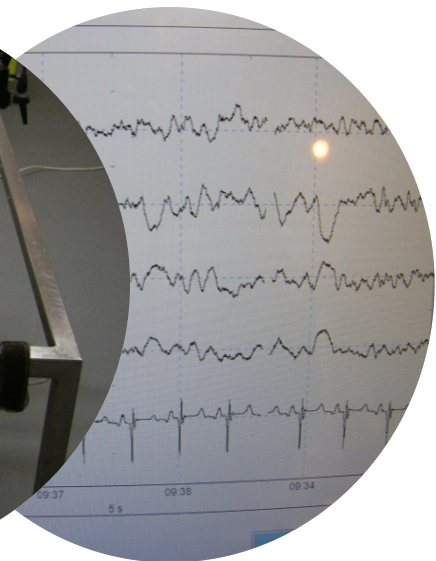
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Assessing unconsciousness at slaughter

"*A state of unawareness (loss of consciousness) in which there is temporary or permanent disruption to brain function. As a consequence of the disruption the animal is unaware of and unable to respond to normal stimuli, including pain*" [1]

Assessment important after stunning and slaughter [2,3,4]

No agreement on what is the best way to assess unconsciousness [5,6]

[1] EFSA, 2006; [2] GWvD, 1992; [3] EU Council Directive 93/119, 1993; [4] EU Council Regulation, 2009; [5] Gerritzen et al., 2009, [6] EFSA, 2013

Main objective of this study

Study the presence/absence of:

1. Eyelid reflex;

2. Pain withdrawal reflex;

3. Threat reflex;

4. Rhythmic breathing.

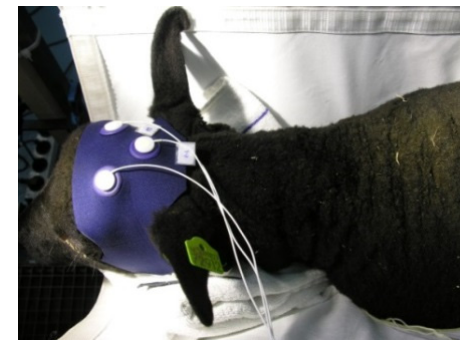**Figure 1**. Example of the threat reflex

Figure 2. Placement of the EEG electrodes

Use of electroencephalogram (EEG) as 'golden standard'

Example of the EEG

- Consciousness: high frequency, low amplitude waves[1]
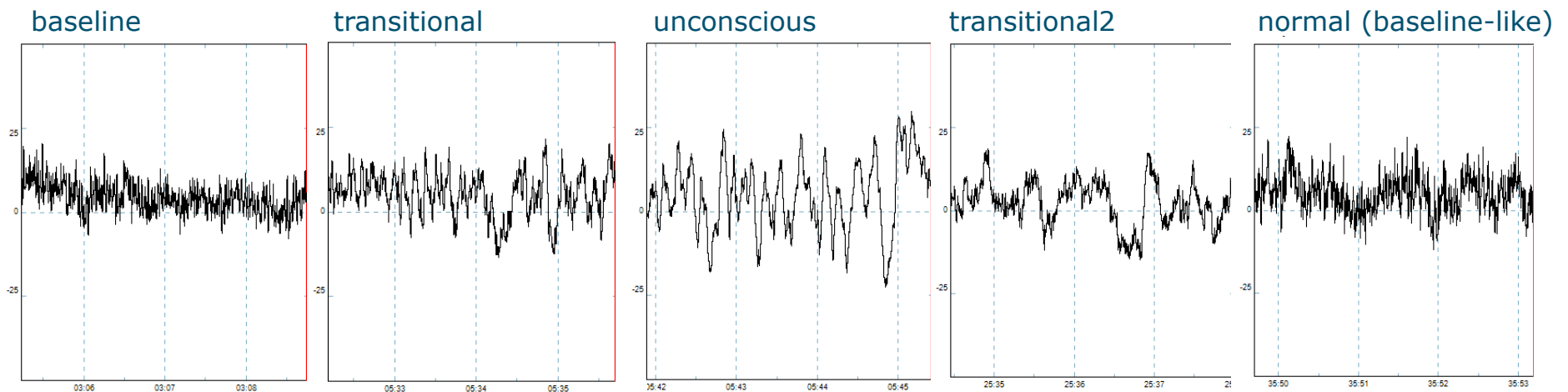
- Unconsciousness: low frequency, high amplitude waves[2]

Figure 3. Typical example of the different EEG stages found in a sheep anaesthetised with propofol

[1] Seth et al., 2005; [2] Baars et al., 2003
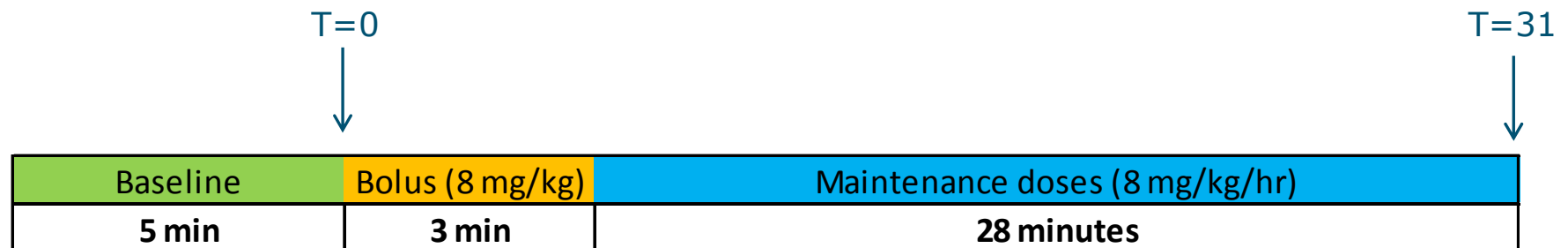
Part A: How to study the objective?

Propofol model

Day 1. N=10
Day 2. N=10

Figure 4a-4b. Placement of the sheep in the hammock

T=0   T=31

Baseline	Bolus (8 mg/kg)	Maintenance doses (8 mg/kg/hr)
5 min	**3 min**	**28 minutes**

Continuously: EEG

Reflex tested 2 minutes: Eyelid
                                       Pain withdrawal
                                       Threat

Part A: EEG and Indicators results (1)

Table 1. Onset (mean +/- SEM) of the different stages identified with visual assessment of the EEG and loss and regain of the different indicators during propofol anaesthesia in sheep.

	N	Onset (min)	
Onset of stages in the EEG[1]			
Transitional	20	00:33 ± 0:05	
Unconscious	20	00:43 ± 0:06	
Transitional2	20	23:54 ± 05:10	
Normal	20	28:27 ± 06:20	
Indicators		**Loss (min)**	**Regained (min)**
Threat reflex	20	01:57 ± 00:31	28:51 ± 06:14
Pain withdrawal reflex	20	02:48 ± 01:14	13:36 ± 05:02
Eyelid reflex	12[2]	04:40 ± 02:11	15:45 ± 05:31

[1] Propofol was administered from T=00:00 until T=31:00 min.

[2] During 8 out of 20 observations, the eyelid reflex was not lost.

Part A: EEG and Indicators results (2)

Table 2. Loss and regain of reflexes in relation to different EEG stages in sheep (N=20).

	Observed in # of animals	After unconscious EEG	Before normal EEG	After normal EEG
Loss of threat reflex	N=20	N=20		
Regain of threat reflex	N=20		N=6	N=14

	Observed in # of animals	After unconscious EEG	Before transitional2 EEG
Loss of pain withdrawal reflex	N=20	N=20	
Regain of pain withdrawal reflex	N=0		N=20
Loss of eyelid reflex	N=7	N=12	
Regain of eyelid reflex	N=21		N=12

Part A: Conclusions

- Pain withdrawal reflex absent, animal is unconscious;
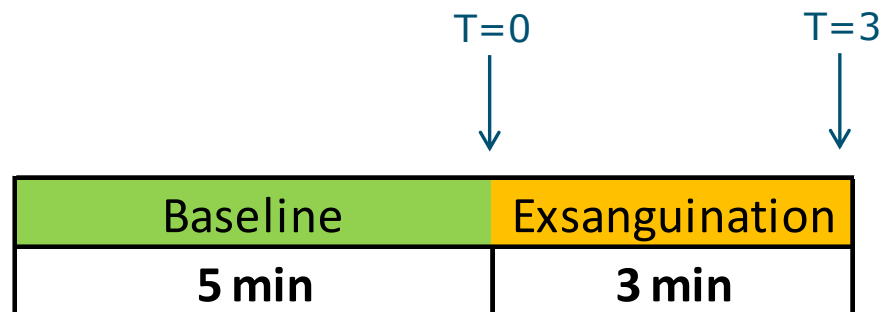- Pain withdrawal reflex present, not certain;

- Eyelid reflex absent, animal is unconscious;
- Eyelid reflex present, not certain;

- Threat reflex absent, animal is unconscious;
- Threat reflex present, not certain.

Part B: How to study the objectives?

Exsanguination

Day 1. N=21

T=0   T=3

Baseline	Exsanguination
5 min	3 min

Continuously: EEG, rhythmic breathing

Reflexes every 2 seconds: Eyelid,

                                 Pain withdrawal

                                 Threat

Part B: EEG and Indicators results (1)

Table 3. Onset (mean +/- SEM) of the different stages identified with visual assessment of the EEG and loss of indicators after applying a neck cut in 21 sheep.

	N	Onset (min)
Onset of stages in the EEG[1]		
Unconscious EEG	21	00:15 ± 0:04
Iso-electric EEG	21	00:27 ± 0:08
Indicators		*Loss (min)*
Threat reflex	7[1]	00:07 ± 0:01
Eyelid reflex	21	01:14 ± 0:17
Pain withdrawal reflex	0[2]	-
Rhythmic breathing	21	00:43 ± 0:12

Figure 5. Example of an iso-electric (flat) EEG

[1] In 14 out of 21 sheep, the ocular reflex to threat was not observed

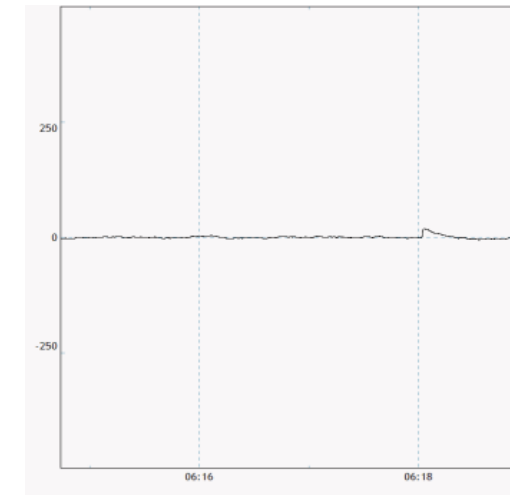[2] In 21 out of 21 sheep, the pain withdrawal reflex was not observed

Part B: EEG and Indicators results (2)

Table 4. Loss of indicators in relation to different EEG stages in sheep (N=21).

	Observed in # of animals	Before unconscious EEG	Before iso-electric EEG	After iso-electric EEG
Loss of rhythmic breathing	N=20		N=2	N=18
Loss of pain withdrawal reflex	N=0			
Loss of threat reflex	N=7	N=7		
Loss of eyelid reflex	N=21		N=1	N=20

Part B: Conclusions

- Pain withdrawal reflex absent, not certain;
- Pain withdrawal reflex present, not observed;

- Eyelid reflex absent, animal is unconscious;
- Eyelid reflex present, not certain;

- Rhythmic breathing absent, animal is unconscious;
- Rhythmic breathing present, not certain.

- Threat reflex absent, not certain;
- Threat reflex present, animal is conscious;

Overall conclusions

- No eyelid reflex and rhythmic breathing → unconscious;

- No pain withdrawal reflex and threat reflex → not necessarily unconscious;

- Eyelid, threat, pain withdrawal reflex and rhythmic breathing → not necessarily conscious;

- Different slaughter practices → different indicators

Thank you for your attention!

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