The Effect of Electrical Stunning and Slaughter on the Electroencephalogram of Sheep and Calves

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SUMMARY

To study the application of electroencephalography (EEG) for the assessment of insensibility during stunning and slaughter, recordings were made on sheep that were slaughtered by throat cutting, electrically stunned head-only and allowed to recover, electrically stunned head-only followed by throat cutting or electrically stunned head-to-back. The same experiments were repeated on calves (1–6 weeks old) except some calves were stunned and allowed to recover before final stunning and throat cutting.

After the throat cut, sheep became insensible (i.e. EEG < 10 μV) at 8–22 s but the calf EEG did not fall below 10 μV until 79 s after the cut. With head-only stunning/recovery, high-amplitude EEG waves (electroplectic fit) continued for 47 s (sheep) and 33 s (calves) post stun. A quiescent period followed, which then developed into a period of moderate-amplitude EEG signals, so that, at 50 s post stun, the EEG usually exceeded 50 μV and often had bursts of 150 μV activity. After head-only stunning/throat cutting, the electroplectic fit was shortened (39 s in sheep, 23 s in calves) and the EEG took at least 50 s to fall below 10 μV. With sheep stunned head-to-back, which stops the heart, and thus should cause immediate, permanent insensibility, the EEG took longer to fall below 10 μV (52 s) than after throat cutting. These results suggest that electrical stunning of sheep and calves causes a prolonged increase in the post-stun EEG amplitude. Therefore, during slaughter, stunned animals have larger amplitude EEG signals than unstunned animals, and EEG criteria developed for judging the sensibility of unstunned animals cannot be used for those that are electrically stunned.
INTRODUCTION

From a humane aspect, animals must be insensible to noxious stimuli during slaughter. Identifying the onset of insensibility is difficult and is likely to be further complicated by the type of stun or slaughter treatment the animal receives. One set of criteria, proposed by Newhook and Blackmore (1982a), is that animals with electroencephalogram (EEG) amplitudes above 35 μV and below 10 μV can be presumed to be insensible. We explore the changes of the EEG produced by electrical stunning and discuss the validity of the criteria of sensibility based on EEGs.

Head-only electrical stunning has a short-term effect, producing insensibility for up to 42 s (Blackmore & Newhook, 1982). Newhook and Blackmore (1982a) showed that unstunned sheep lose sensibility (EEG < 10 μV) within 20 s of bilateral carotid severance. Thus, sheep would not recover from a head-only electrical stun before permanent insensibility supervened through exsanguination, provided it follows promptly. In the case of cattle, however, after bilateral carotid severance some blood continues to reach the brain via the vertebral arteries (Blackman et al., 1986). In one view this blood supply prolongs the period of sensibility of unstunned throat-cut calves, as shown by EEG studies (Newhook & Blackmore, 1982b); thus cattle electrically stunned head-only might recover from the stun before permanent loss of sensibility by exsanguination occurred (Blackmore & Newhook, 1982). If this is the case, slaughter of cattle by head-only stunning followed by throat severance may be inhumane. However, there are conflicting views based on EEG or electrocorticograms which suggest that insensibility occurs rapidly after exsanguination (Nangeroni & Kennett, 1963, Schulze et al., 1978; Gregory & Wotton, 1984). If this were true then problems of recovery from electrical stunning during slaughter of cattle would not arise.

An electrical stun considerably modifies the EEG and causes other physiological changes such as inhibition of breathing (Devine et al., 1986a,b). This raises some doubts about the use of EEG characteristics established for unstunned animals to interpret the state of sensibility of electrically stunned animals. For example, in EEG studies of unstunned sheep, bilateral severance of the carotid arteries results in insensibility in as little as 2–7 s and the trace becomes isoelectric in 10–43 s (Newhook & Blackmore, 1982a). In contrast, the EEG of sheep stunned head-to-back, which causes concurrent cardiac dysfunction and produces
immediate and permanent insensibility, does not become isoelectric until 40–51 s after throat cutting (Blackmore & Newhook, 1982). It is unlikely that head-to-back stunning would prolong sensibility over that of a throat cut animal; therefore, the unexpectedly prolonged period of apparent sensibility (EEG > 10 μV) must be due to other factors. The EEG amplitude therefore may not be a useful baseline for sensibility in these circumstances.

To provide a more realistic basis for deducing sensibility from EEG traces, we conducted experiments in which calves were head-only electrically stunned and allowed to recover. The calves were stunned and recovered a second time, enabling us to ensure that EEG results are repeatable for individual animals. After a delay of several days, the same calves were stunned and slaughtered. In this way, the EEG of an animal recovering from a stun was compared with the EEG from the same animal during stunning and slaughter. These results enabled us to re-examine the ‘window of sensibility’ (10–35 μV) proposed by Newhook and Blackmore (1982a). Similar experiments were conducted with sheep except that different experimental groups were used for each treatment. As we believe that adequate experimentation has been done on insensibility after throat severance of unstunned calves and of head-to-back stunned calves, we did not study these treatments in detail.

METHODS

EEG recording

Year-old sheep of mixed breed (approximately 30 kg liveweight) were purchased locally and held until required. Calves were obtained at 2–4 days old (approximately 30 kg liveweight) and were maintained for 1 to 6 weeks (approximately 35–55 kg) before experimentation.

For both species, EEG recordings were obtained by means of three stainless steel 18 gauge syringe needles. These needles, directed anteriorly, were inserted subcutaneously with the point of insertion made along a line parallel with, and 2–3 cm posterior to, a line joining the posterior edge of the orbit. Because care was taken to avoid piercing muscle, the actual placement varied somewhat, depending on breed and age. One needle was placed on the midline and the other two were placed approximately 2 cm either side of the midline; thus, transhemispheric EEG recordings were made. Electromyographic spikes from muscle
were rare but were identified by the more rapid rise times than that which occurred for EEG waveforms.

The needle electrodes were secured in place by suturing and collodion glue and the electrode cable attached to the skin or fleece to reduce the strain on the sutured electrodes. During electrode placement, lignocaine hydrochloride (Astra Pharmaceuticals Pty Ltd, North Ryde, Australia) was used as a local anaesthetic. The needles were connected via 3 m of screened cable to a Tektronix 5A 22N differential amplifier and a 5223 storage oscilloscope. The output of the oscilloscope was connected via a 50 Hz notch filter and pen drive circuit to an ultraviolet recorder (SE 3006, SE Laboratories Engineering Ltd, Great Britain). The upper and lower -3 dB points were 100 Hz and 10 Hz, respectively, the latter corresponding to a time constant of 0.016 s. The system gain was calibrated with a precision voltage source.

The method of securing the electrodes reduced the effects of animal and lead movement artefacts produced by vigorous head and body shaking to acceptable levels. As successful recordings were obtained it was not considered desirable to use implanted cortical electrodes over the 2 weeks’ duration involved with some of the calves in the stun-recovery experiments. By keeping the experimental procedures simple and consistent, we were able to use results collected over several months.

As frequencies below 10 Hz were progressively attenuated, the EEG baseline was comparatively straight. The EEG amplitude values were determined from representative portions of artefact-free trace. For prestun values, the maximum amplitudes were measured over approximately 3 s. The amplitude of the electroplectic fit was so variable that representative values taken from 10–15 s post stun were used. The end of the electroplectic fit was determined from the abrupt change that occurred in the peak to peak amplitude which was at least twice the prestun EEG and did not include the quiescent phase (Gregory & Wotton, 1984).

Video recordings were made of all calves and some sheep to enable an accurate correlation of animal movement and EEG recordings. An elapsed time indicator was activated at the moment of stun.

**Sheep experiments**

Sheep were restrained in a V-shaped box insulated from the floor to reduce 50 Hz interference picked up via blood and water. The following
experiments were conducted:

(a) Throat severed swiftly with minimal animal agitation and movement (no pre-slaughter stun).
(b) Head-only electrical stunning (50 Hz, 400 V open circuit, current limited to 1.0 A, 4 s duration) and the animal allowed to recover.
(c) Head-only electrical stunning, and the carotid arteries, jugular veins, trachea and oesophagus severed (throat cut) at 10–14 s post stun.
(d) Head-to-back electrical stunning from the same current limited power supply as above (which causes cardiac arrest). The throat was not cut until approximately 5 min later.

Calf experiments

Initially, calves were restrained in the same V-shaped box as for lambs, but an improved restraint, in the form of a polypropylene net isolated from the floor, was later devised. This restraint allowed clear viewing of leg and body movement and minimized 50 Hz interference.

The same four experiments were conducted as for sheep, except that some calves were stunned and allowed to recover twice (the second time a week after the first); the throat was cut exactly 10 s after the head-only stun. The head-to-back electrical stunning was performed on two animals which had been stunned and allowed to recover only 5 min previously. This period was not long enough for recovery to be complete and the results can therefore only be used as a guide.

RESULTS

Sheep experiments

(a) After the throat was cut, all sheep became insensible (EEG < 10 μV) in less than 22 s (e.g. Fig. 1) and some became insensible as early as 8 s after the cut (Table 1). Immediately after the cut there were large amplitude EEG signals similar to those reported by Newhook and Blackmore (1982a).
(b) Sheep stunned head-only and allowed to recover had a large-amplitude (up to 500 μV) EEG trace, typical of an electropleptic EEG fit, averaging 47 s in duration (Table 1). The EEG then declined
to a low level, after which it rose again in amplitude so that, at 50 s, the EEG signal was 120 ± 97.5 μV.

During the stun the legs curled underneath the animal. After the stun the forelegs usually remained curled. Walking and paddling movements developed. Initially, these movements were slow, but they became extremely rapid, similar to movements described for stunned sheep by Croft & Hulme (1956). The walking and paddling movements were sometimes preceded by foreleg extension. The precise moment when sensibility was regained was impossible to determine visually: at about 60 s post stun lambs displayed behaviour consistent with sensibility, yet

**TABLE 1**

EEG Characteristics of Sheep after a Throat Cut, a Head-Only Stun Followed by Recovery, a Head-Only Stun Followed by a Throat Cut and a Head-to-Back Stun. Data are Given as a Mean ± SD for Representative Pre- and Post-Stun Amplitudes. The Time for the EEG to Fall Below 10 μV is Based on Maximum Amplitude Waves. The Time when the EEG Falls Below 10 μV is Measured from the Commencement of the Stun (a) or from the Time the Throat was Cut (b).

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>EEG amplitude (μV)</th>
<th>Duration of fit (s)</th>
<th>Time when EEG &lt; 10 μV (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-stun</td>
<td>Post-stun</td>
<td></td>
</tr>
<tr>
<td>Throat cut</td>
<td>10</td>
<td>33.5 ± 5.3</td>
<td>—</td>
<td>13.6 ± 7.4^b</td>
</tr>
<tr>
<td>Head-only stun,</td>
<td>6</td>
<td>21.0 ± 6.5</td>
<td>390 ± 167</td>
<td>46.8 ± 13.4</td>
</tr>
<tr>
<td>recovery</td>
<td></td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Head-only stun,</td>
<td>8</td>
<td>37.4 ± 10.0</td>
<td>316 ± 28</td>
<td>39 ± 14</td>
</tr>
<tr>
<td>throat cut</td>
<td></td>
<td></td>
<td></td>
<td>53.2 ± 8.1^a</td>
</tr>
<tr>
<td>Head-to-back stun</td>
<td>11</td>
<td>37.0 ± 7.9</td>
<td>266 ± 26</td>
<td>22.1 ± 3.8</td>
</tr>
</tbody>
</table>

...
Electrical stunning and EEG of sheep and calves

SHEEP

POST THROAT CUT

CALF

POST THROAT CUT

Fig. 1—contd.

the EEG signals were still of large amplitude (> 50μV). With long bursts of high amplitude EEG traces, muscle spasms occurred, but the EEG recordings did not have the spikes characteristic of electromyographic potentials. Whether or not these bursts of high amplitude EEG coincided with periods of insensibility could not be determined.

(c) Stunned animals which had their throats severed had shorter electroplectic fits than animals allowed to recover. The average time for the EEG to reach 10μV was in excess of 50 s from the start of the stun (36-40 s from the time the throat was severed) (Table 1).

(d) With a head-to-back stun, the animals died through cardiac arrest. The electroplectic fit was very much shorter than that of animals stunned head-only and allowed to recover and much shorter than that of animals stunned head-only and slaughtered (Table 1). The time for the EEG to fall to less than 10μV was similar to that for animals stunned head-only, although the amplitude initially declined more rapidly than with animals stunned head-only.

Calf experiments

(a) In one calf, apparent insensibility (EEG < 10μV) did not occur until 79 s after the throat cut. This result is similar to that reported by Newhook & Blackmore (1982b) and for humane reasons further experiments were not conducted. At 45-48 s after the throat cut, the EEG indicated apparent sensibility, although it was different in its waveform from the EEG recorded before slaughter (Fig. 1).
TABLE 2

Typical Amplitudes of EEG Traces of Calves Pre- and Post-stun. Animals were Electrically Stunned Head-only and Allowed to Recover

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>EEG amplitude (µV)</th>
<th>Duration of electropleptic fit (s)</th>
<th>EEG at 50–52 s* (µV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-stun</td>
<td>Post-stun</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>35</td>
<td>600</td>
<td>31</td>
</tr>
<tr>
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<td>550</td>
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</tr>
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<td>15</td>
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<td>34</td>
<td>35</td>
<td>700</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>500</td>
<td>36</td>
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</tbody>
</table>

* Time from the commencement of the stun.

(b) For calves stunned and allowed to recover twice, the amplitudes of the pre-stun EEGs and EEGs during the electropleptic fit were not identical but the time course of the fit was similar (Table 2). In all animals but one, the electropleptic fit lasted from 24 to 39 s (mean, 33 s). The calf with the extended electropleptic fit in its first stun-recovery sequence had an extended period of continuous high amplitude bursts of EEG activity in the recovery phase. In subsequent stunning experiments, this calf was similar to the others. During and after the stun, the forelegs curled. After 8–10 s, the forelegs often extended as part of walking, paddling and running movements taking place while the electropleptic fit was still present. During the recovery phase the EEG amplitude declined to 10–20 µV and movement was reduced, after which there was a period of further leg movement and spasm associated with the bursts of high amplitude EEG activity.

The results suggest that the stun duration for each animal was reproducible and the EEG trace of a stunned animal would follow a similar course with each stun. At 50 s post-stun the EEG varied in amplitude from 30 to 350 µV (see Table 2; also Fig. 2) and consisted of periods of moderate amplitude followed by
Fig. 2. EEG traces of a calf (No. 29) showing a normal pre-stun trace, a stun-recover trace recorded 41–53 s after the stun and a stun-throat cut trace also recorded 41–53 s after the stun. In the stun-recover trace, the EEG amplitude is relatively constant compared with the stun-throat cut trace in which amplitude is declining. The time, shown in seconds, is from the commencement of the stun.
Fig. 3. EEG traces of a calf (No. 34) stunned head-only and allowed to recover and of the same calf stunned and throat cut. The time, shown in seconds, is from the commencement of the stun.
TABLE 3

Typical Amplitudes of EEG Traces of Calves Pre- and Post-stun. Animals were Stunned Head-only and Their Throats were Cut 10 s Later

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>EEG amplitude (µV) Pre-stun</th>
<th>EEG amplitude (µV) Post-stun</th>
<th>Duration of electroplectic fit (s)</th>
<th>EEG at 50 s* (µV)</th>
<th>Time to 10 µV (s)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>30</td>
<td>500</td>
<td>26</td>
<td>50–60</td>
<td>160</td>
</tr>
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<td>31</td>
<td>15</td>
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<td>35</td>
<td>550</td>
<td>26</td>
<td>10–15</td>
<td>57</td>
</tr>
<tr>
<td>33</td>
<td>30</td>
<td>400</td>
<td>18</td>
<td>100–120</td>
<td>100</td>
</tr>
<tr>
<td>34</td>
<td>20</td>
<td>500</td>
<td>26</td>
<td>30–60</td>
<td>86</td>
</tr>
</tbody>
</table>

* Time from the commencement of the stun.

bursts of activity. These bursts of activity were more prevalent in calves Nos 31 and 32 than the others.

(c) When calves were stunned head-only and slaughtered, the amplitude of the electroplectic fit was similar to that of stunned animals that were allowed to recover, but fit duration (mean, 23 s) was shortened by almost 10 s (Fig. 3, Table 3). At 50 s the EEG amplitude was considerably reduced but was still above 10 µV (Fig. 2). The time to fall to 10 µV ranged from 57 to 160 s post-stun.

(d) Head-to-back stunning of two calves, which had been stunned head-only 5 min previously, produced a similar result to that of sheep, in that the electroplectic fit was shortened to 16 s and the time for the EEG to fall to < 10 µV was less than 20 s. The prior stun had an obvious effect on the electroplectic fit and fall to isoelectric, with the time to isoelectric being shorter than that reported by Blackmore & Newhook (1982). Therefore, these results are not useful in comparisons and reinforce the necessity of having adequate periods between successive stuns.

DISCUSSION

The immediate prestun EEG for sheep and calves was between 15 and 40 µV which is slightly higher than that reported by Newhook & Blackmore (1982a) but less than that recorded by Gregory & Wotton
(1984). As the experimental conditions are likely to be stressful for some animals, no importance was attached by us to the variation in upper limit of the prestun EEG. In fact, the prestun EEG values in the same calf can vary considerably over 2 weeks. With long periods of EEG recording the resting EEG amplitude fell to low levels.

It is unlikely that unstunned sheep and calves are sensible once the low voltage fast activity EEG waves fall below 10 μV (Blackmore & Newhook, 1982). In the present study, the EEG of unstunned sheep fell quickly after throat cutting, becoming less than 10 μV within 8–22 s of the throat cut. However, in the unstunned calf the EEG remained in the sensible range (> 10 μV) much longer after the cut, possibly due to some cerebral blood supply from the vertebral arteries. Both species had prolonged high amplitude EEG traces during recovery from a stun which were not dominated by electromyographic potentials. The EEG traces of sheep stunned head-to-back or stunned head-only and then slaughtered remained above 10 μV well beyond the time when the EEG of unstunned slaughtered sheep fell below 10 μV (present studies; Kuhne et al., 1979; Blackmore & Newhook, 1982; Newhook & Blackmore, 1982a). Thus, an EEG trace indicating apparent sensibility (amplitude > 10 μV) was present in stunned and throat-cut sheep at a time when similar unstunned, throat-cut sheep had traces clearly showing insensibility. A similar prolongation of elevated EEG amplitude would be expected in calves stunned head-only and then slaughtered by a throat cut. Therefore, basing the onset of insensibility on the time when the EEG amplitude falls below 10 μV is not valid for animals that have been electrically stunned.

The EEG traces in Fig. 2 clearly illustrate these points. The post-stun, post-throat-cut trace might be considered to indicate sensibility (amplitude >10 μV). However, this elevated amplitude is clearly the result of the stun. The fact that the EEG amplitude has fallen to just above 10 μV (i.e. borderline insensibility), even though stunning causes the amplitude to be increased, confirms that recovery of sensibility is unlikely. Note that electrical stunning changes the perspective from which sensibility, or the lack of it, is deduced. When an unstunned animal has its throat cut, the EEG trace indicates a shift from sensibility into insensibility; when an animal that has been electrically stunned has its throat cut, however, that animal is already insensible. Conclusions regarding its sensibility are impossible, if based on EEG amplitude criteria developed for unstunned animals.
The EEG still provides useful information for assessing insensibility, however. The greatest gains can be made in comparing the duration of the electroplectic fit of calves stunned head-only and allowed to recover with that of calves stunned head-only and then slaughtered. The electroplectic fit, possibly producing its anaesthetic effect through exhaustion of the brain (Gregory & Wotton, 1985), is dramatically shortened from approximately 33 s for calves allowed to recover to approximately 23 s for calves suffering blood loss from throat severance. This suggests that the intense electrochemical metabolism required to sustain the hypersynchronous electrical activity of the electroplectic fit is high, and after throat cutting it cannot be met, presumably due to cerebral anoxia from the greatly reduced blood supply to the brain. This suggests also that the vertebral artery is not sufficient on its own to allow recovery. Electrical stunning also inhibits breathing for at least 20 s, so during that time reoxygenation of the blood does not occur. Thus, the small blood supply to the brain via the vertebral artery in calves is of little or no advantage for some period after a stun.

After electrical stunning and slaughter, the electroplectic fit is only a partial indicator of insensibility. When the fit is shortened (this study, Kuhne et al., 1979), probably through cerebral anoxia after carotid severance, it is not reasonable to expect animal insensibility to be also shortened. Rather, insensibility would be prolonged, as cerebral oxygen levels would be below that necessary for recovery of brain function.

Animal variation is such that some animals can have very high amplitude EEG during recovery from a stun. Such animals may have higher than 'normal' EEG amplitudes after stunning and throat severance. These high amplitudes should not imply that sensibility is regained.

The important question is whether the inevitable decline in brain function that occurs after stunning and throat severance is enough to ensure continued insensibility. The contribution of blood by the vertebral artery in stunned and slaughtered calves may well be responsible for the prolongation of EEG activity beyond that of similarly treated sheep, but the continued presence of EEG signals above 10 μV is no indication of sensibility in these animals. Studies on rats, following electroconvulsive shock, have shown prolongation of high amplitude EEG waveforms; this was shown to be mimicked by intracerebroventricular injection of β-endorphin, thus suggesting that the release of such substances by the shock elevated the EEG (Holaday et al., 1981). The high amplitude
Coupled with adequate bleeding, the continued decline in EEG amplitude is consistent with continued insensibility.

Could sensibility be recognized if it did occur? We believe so. In one case, a stunned calf was bled poorly because inadvertently only a single carotid artery was severed. With this animal the EEG remained at a high level and fell only slowly (Devine et al., 1986a). This type of EEG response after a single carotid has been severed has also been demonstrated for unstunned sheep (Newhook & Blackmore, 1982a), where the vertebral artery plays no major rôle in the maintenance of sensibility. A rapid blood loss is therefore very important for a rapid decline in EEG amplitude and continued insensibility during head-only stunning and slaughter.

The conclusions regarding maintenance of insensibility in calves following electrical stunning and exsanguination will also apply to adult cattle where only limited studies have been made due to experimental difficulties (Devine et al., 1985, 1986b). Other consequences of the electrical stunning such as movements during the clonic phase of the stun, as well as reflex anoxic leg and body movements, can be abolished by low voltage electrical immobilization, thus allowing electrical stunning to be used routinely in the slaughter of adult cattle (Gilbert, 1984).

REFERENCES