Measuring consciousness in turbot (*Scophthalmus maximus*) and common sole (*Solea solea*) subjected to electrical stunning before slaughter

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Keywords: Electrical stunning; Fish; Slaughter; Animal welfare; EEG; Behaviour

In order to limit painful experiences all animals including fish should be rendered unconscious before slaughter. We subjected two species of flatfish, turbot (*Scophthalmus maximus*) and common sole (*Solea solea*) to dry (de-watered) electrical stunning. In one group the fish were exposed to a short stun (1 s) followed by a second, long stun (20 s) after 1 min of recovery. In the second group the fish were exposed to a long stun (20 s). The electrical potential difference for the short stun was set to approximately 110-120 V_{rms} for turbot and 150 V_{rms} for sole. For the long stun the potensial difference was set to 50 V_{rms}. This resulted in a short-stun current of 2.3 ± 1.0 A_{rms} in turbot and 1.2 ± 0.7 A_{rms} in sole, while the long-stun currents were 4.0 ± 1.3 A_{rms} for 1 s + 1.0 ± 1.0 A_{rms} for 19 s in turbot in group 1, and 1.2 ± 0.6 A_{rms} for 1 s + 0.4 ± 0.2 A_{rms} for 19 s for sole in group 2. The objective was to examine whether the fish lost consciousness immediately (within 1 s) and did not recover when submersed in ice water immediately after the long stun.

In order to assess consciousness we recorded electrophysiological (EEG and ECG) and behavioural responses (pain sensation, breathing and response to vibration). EEG and ECG recordings were measured using a DI-720 data recording module with a WinDaq Waveform browser (Dataq Instruments, Akron, Ohio, USA; 250 Hz sample frequency). The EEG electrodes (20 mm long and 1.5 mm diameter; 55% silver, 21% copper and 24% zinc) were placed percutaneously. The ECG electrodes (the same composition) were placed subcutaneously, ventrally and dorsally of the upper pectoral fin. The earth electrode for both the EEG and ECG was placed subcutaneously near the tail. Pain sensation was recorded as behavioural and EEG responsiveness to the application of three scratches with a needle to the dorsolateral skin. For breathing gill movements were scored subjectively on a scale from 0 (no breathing) to 5 (large gill movements). Vibration was applied by three soft to moderate taps on the wall of the polystyrene box which contained the fish in the ice water. Pain sensation, breathing and vibration were scored at 12 time points between 0.5 and 75 min after the long stun. To our knowledge this was the first study in which responsiveness to vibration was compared to the behavioural and electrophysiological responsiveness to pain in electrically stunned fish.

Based on the EEG recordings all except one turbot (n=26) and one sole (n=10) lost consciousness after the short stun, and all but one turbot (n=22+13) and all but perhaps one sole (n=9+22) remained unconscious after the long stun. During recovery after the short stun behavioural responsiveness of the turbot followed EEG-based responsiveness. All sole, however, remained behaviourally quiescent after the short stun, while the EEG recordings indicated recovery in more than 60% of the fish. For at least 15 min after the long stun breathing (gill movements) was observed especially in turbot, while responses to vibration were especially seen in sole. Heart rates did not decline following stunning. Notably, after the long stun the fish tended to be more responsive to vibration as compared to the nociceptive stimulation.

These findings indicate that behavioural measures are not sufficiently reliable to determine unconsciousness during electrical stunning. Furthermore, our findings suggest that flatfish may remain responsive to vibration for longer as compared to nociception when submersed in ice water after electrical stunning, Finally, we propose that tail-first electrical dry stunning may be developed into a humane killing method for slaughter of turbot and sole.