Minimally Anaesthetised Model (MAM) & Electroencephalography (EEG) for pain studies in animals

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Overview

Excerpted from (Carbone, 2011; doi: 10.1371/journal.pone.0021578)

Researcher G wants to model myocardial infarction (MI) in mice to explore whether a proposed treatment with muscle growth factors has promise for humans suffering an MI, or “heart attack.”

An MI is surgically induced in mice by opening the chest and tying off a coronary artery so that a section of heart muscle loses its blood supply in a manner roughly comparable to the way that clots choke the blood supply to human heart muscle during a spontaneous MI.

Will pain alter the outcome of the experiment?

How much of which painkillers should these mice receive?
Overview

Excerpted from (Sabow et al., 2018; doi:10.1071/AN17486)

Researcher S wants to investigate whether electrical stunning would cause additional pain during slaughter.

The work is an extension of prior collaboration between Australian, Malaysian & New Zealander authorities, and industrial players to ensure animal welfare during slaughter, and in compliance to religious requirements during slaughter of animals.

Will the animal experience more pain when it is CONSCIOUS at the point of neck cut?

Can we separate sensory (nociception) from emotional pain?

Pain

“an unpleasant sensory and emotional experience associated with actual or potential tissue damage.”

First published by IASP in 1979, based on earlier work by Harold Merskey in 1964.

A complex emotional and sensory experience with many SUBJECTIVE components. Pain that has emotional, cognitive and social components, and yet required the understanding of biological principles to describe nociception (Williams and Craig, 2016; doi:10.1097/j.pain.0000000000000613)

The same article can also be found in AAALAC repository at https://www.aaalac.org/BOD/AdhocNewsletter/Updating_the_definition_of_pain_Pain2016.pdf
Pain

- is an aversive sensory experience – for protection
- caused by actual or potential injury that elicits progressive motor and vegetative reactions
- results in learned avoidance behaviour, and may modify species specific behaviour, including social behaviour

- An animal may even associate pain with a specific event through learning and memory!
- Central nervous system may even learn to exacerbate pain sensation leading to pain sensitization → failure of drugs….
- Can be described according to Duration, Intensity, Threshold and Area (DITA).
- The gate control theory of pain remained the most robust theory so far in our understanding of pain…..

Pathway in the production of painful sensations

From (Woodward, 2008; doi: 10.1053/j.tcam.2008.02.007)
Pain vs Nociception

- **Gate control theory** stated that non-pain sensation (which is conveyed via larger neural channels), can override pain sensation (conveyed by small neural channels) in the spinal cord → simple explanation as to why rubbing (or acupuncture) could relieve pain.
Pain vs Nociception

Before we go further …..

- **Noxious →** mechanical, thermal & chemical stimulus that damages or potentially damages tissue
- **Nociceptor →** primary AFFERENT neuron that senses noxious stimuli – typically the smaller-sized $A\delta$ and C fibres (many subtypes). The larger $A\beta$ fibres have lower threshold and respond to mechanical stimuli.

Nociception is a neural process that allows the detection and transmission of nociceptive information to the brain WITHOUT emotional or any other types of responses, a.k.a. “physiological pain”

...easier to “measure”, and thus very important to isolate it from other subjective measure of pain……
Pain vs Nociception

From (Woodward, 2008; doi: 10.1053/j.tcam.2008.02.007)

Pathway in the production of painful sensations

Why MAM?

Minimally anaesthetised model (MAM)

- The minimal anaesthesia model developed by Johnson and co-workers in the late 1990’s (Johnson et al. 2012; doi: 10.7120/096272812X13353700593888)
- Focus on the study of nociceptive response measured using electroencephalography (EEG) techniques.
- **Halothane is used** as it is not considered to have antinociceptive activity, and causes less depression on the cortex compared to isoflurane, sevoflurane etc….
- **End tidal Halothane tension 0.85-0.95 %**
- MAM state require constant monitoring and adjustments vis-s-vis baseline EEG response.
Why MAM?

Minimally anaesthetised model (MAM)

- Typical set up for cats, dogs and small ruminants at UPM

**Key features and benefits of MAM**

- Animals are *unconscious under light anaesthetic plane* but still able to demonstrate EEG responses to noxious stimulation.
- Animal need not to be subjected to pain, thus minimal issues of animal welfare.
- Possible to perform experiment with a *group of animals with no analgesia without ethical concerns*, as animals will be administered with analgesia before they recover from anaesthesia.
- Use of anaesthesia reduces interference from other stimuli and confounders such as movements, emotional component of pain etc → less animals required.
- **Validated** (Johnson et al. 2012; doi: 10.7120/096272812X13353700593888)
Why MAM?

- Validated & used for the following species (Johnson et al. 2012; doi: 10.7120/096272812X13353700593888)

<table>
<thead>
<tr>
<th>Species</th>
<th>Aspect investigated</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>Horse</td>
<td>Castration</td>
<td>Murrell et al. (2003)</td>
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<td></td>
<td>Effect of anesthetics</td>
<td>Murrell et al. (2005)</td>
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<tr>
<td>Sheep</td>
<td>Castration (ontological changes)</td>
<td>Johnson et al. (2005b)</td>
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<td>Johnson et al. (2009)</td>
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<td>Red deer</td>
<td>Velvet azier removal</td>
<td>Johnson et al. (2005a)</td>
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<tr>
<td>Dog</td>
<td>Effect of novel anesthetics</td>
<td>Kongara et al. (2010)</td>
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<tr>
<td>Rat</td>
<td>Ontological changes</td>
<td>Diedrich et al. (2009)</td>
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<td></td>
<td>Effect of different stimuli</td>
<td>Murrell et al. (2007)</td>
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<tr>
<td>Walabu</td>
<td>Ontological changes</td>
<td>Diedrich et al. (2010)</td>
</tr>
<tr>
<td>Pig</td>
<td>Castration</td>
<td>Hags &amp; Rasmuin (2005)</td>
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<tr>
<td></td>
<td>Tail docking</td>
<td>Kalls et al., in prep</td>
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<tr>
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<td>Ontological changes</td>
<td>Kalls et al., in prep</td>
</tr>
<tr>
<td>Cattle</td>
<td>Deharinging</td>
<td>Gibson et al. (2007)</td>
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<td>Slaughter</td>
<td>Gibson et al. (2009a)</td>
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<td>Gibson et al. (2009b)</td>
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<td>Gibson et al. (2009c)</td>
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<td>Gibson et al. (2009d)</td>
</tr>
</tbody>
</table>

Why MAM?

- Used at UPM in dogs, cattle, and goats since 2009 (only publications from the last 5 years shown). Cats, rodents and rabbits are ongoing:

  - DOI: 10.1016/j.meatsci.2015.02.004
Electroencephalography (EEG)

- EEG is an invaluable tool for nociception (& pain) research, and often used alongside animals that had been subjected to minimal anaesthesia.

- Briefly,
  - Electroencephalograph – machine
  - Electroencephalogram – recordings first recording made in 1924
  - Electroencephalography – the science/art of using electroencephalograph to obtain, analyze and interpret electrical recordings of brain’s neuronal activity (electroencephalogram).

Electroencephalography (EEG)

- Each neuron would generate electrical currents at a certain voltage when they are active or activated.
- Electroencephalograph record these SUMMATED neuronal signals through the scalp → cortical brain activity
- Signals can be picked up from electrodes placed strategically on the head.
- Different region of the scalp will have different wave intensity and wave components…see figure →

Kujala et al., (2013); doi : 10.1371/journal.pone.0061818
Electroencephalography (EEG)

- Electrode can be invasive (low impedance silver / alloy pin) or non-invasive (AgCl patch)

Conscious Goat & Cattle at UPM
Zygomatic process of the Frontal Bone (+ve)
Mastoid process (-ve)

Minimally anaesthetised DOG at UPM
Surface vs epicranial electrodes

Benefits of EEG

- EEG is an invaluable tool for nociception (& pain) research. It allows for:
  - detection of potential nociceptive response even during anaesthesia or at the point of slaughter, when it impossible to monitor pain related reflexes.
  - EEG monitors nociceptive response without the need for motor response.
  - EEG is silent and non-invasive – a passive recording method, can even record responses when subject did not pay attention to stimulus.
  - EEG registers instantaneous response as action potential takes around 0.5 to 150 ms to propagate through neurons – better than adrenaline, cortisol etc at least 3-12 sec.
  - Better tolerance to movement artefact as these can be excluded in frequency analysis.
Electroencephalography (EEG)

- EEG provides real-time cortical brain function at the point of evaluation.

- EEG is typically used to monitor/diagnose brain conditions and activity such as:
  - cognition, epilepsy, depth of anaesthesia, coma or brain death, and other encephalopathies (such as brain tumours) etc.

- EEG waves can be judged QUALITATIVELY based on wave morphology *(Qualitative EEG)*.
- Signals can also be processed and interpreted as Quantitative EEG (qEEG) where statistical and mathematical analysis are applied → useful in nociception research…..

Electroencephalography (EEG)

- Sample of EEG (dog)
Electroencephalography (EEG)

- **qEEG in conscious animal**
  - Measures PAIN

- **qEEG in anesthetised animals (MAM)**
  - Measures NOCICEPTION

- Based on Event Related Potentials (ERP’s) that are time-locked to a specific stimulus, i.e. record EEG at the point when animal is subjected to a needle prick…
- Requires extensive (off-line) computer processing power to determine values such as Median frequency (F50), Spectral Frequency 95% (F95), RMS voltages etc…

Electroencephalography (EEG)

- EEG wave are the result of millions of neurons synchronising their firing orientation IN A NETWORK OF NEURONS → summated oscillation (or wave) that can be recorded.
- EEG is attributed mostly to pyramidal neurons

(Lorentz et al., 2010; Handbook of Veterinary Neurology)

Fig. 1. Six layers of cerebral cortex are seen with three stains used to show different histologic features (stains, cell bodies, & white neurons). The six layers are numbered at the left and named at the right. P = pyramidal cell; S = stellate (molecular) cell.
Electroencephalography (EEG)

- Pyramidal neurons are also found in deeper centres of the brain such as hippocampus, thalamus etc......
- Different region of the brain = different signal intensity and frequency → the need standardised electrode placement.
- Imperative to find a spot on the scalp where signals are always available...

(EEG record predominantly cortical neuron activity as electrical signals from deeper part of the brain are lost rapidly when they arrive at the scalp)

(Bear et al., 2015; Neuroscience: Exploring the brain)

Electroencephalography (EEG)

- Sample of EEG (dog)

- Raw EEG signal being re-sampled at 1 kHz or 2 kHz to eliminate muscle tremor, ECG and other artefacts (that occurred at 25-35 Hz)

- Total Power (Ptot)
- Root Mean Square (RMS)
- Median frequency (F50)
- Spectral Edge Frequency (SEF) etc...
**Electroencephalography (EEG)**

**Wave classifications:**

<table>
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<tr>
<th>Wave Type</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td><strong>Gamma wave</strong></td>
<td>(&gt;30 Hz). High levels associated with stress and anxiety. Indicator of learning &amp; memory.</td>
</tr>
<tr>
<td><strong>Alpha wave</strong></td>
<td>(8 -12 Hz) : Prevalent when eyes are closed. Interpreted together with beta and delta waves as part of ratio. Associated with “calmness”. Suppressed during stress.</td>
</tr>
<tr>
<td><strong>Beta wave</strong></td>
<td>(12 – 30 Hz) : High levels associated with stress and pain. Prevalent in awake animals, associated with cognitive tasks.</td>
</tr>
<tr>
<td><strong>Delta wave</strong></td>
<td>(&lt;4 Hz) : Too high indicate dysfunctions. Optimal range reflect relaxation. Related to sleep and anaesthesia.</td>
</tr>
<tr>
<td><strong>Theta wave</strong></td>
<td>(4 - 8 Hz) : Too high indicate suppressed brain functions &amp; even depression. Related to relaxation, sleep &amp; anesthesia.</td>
</tr>
</tbody>
</table>

*The DOG subject is under anaesthesia!*

**Electroencephalography (EEG)**

- Interpretation of the wave frequency is meaningless without understanding their power and intensity.
- Important to know which wave component is contributing significantly to the overall EEG.

*...an OVERSIMPLIFICATION would be........*

- EEG waves with high total power **IN A CONSCIOUS ANIMAL**, and dominated by **beta waves** most probably indicate stress (and pain if there’s a pain stimulus)
- EEG waves with high total power **IN AN ANESTHETISED ANIMAL**, dominated by **delta waves** may indicate deep anaesthesia, insensible or unconscious state.
- EEG waves with higher than usual total power **IN AN ANESTHETISED ANIMAL**, with significant beta and gamma waves, could be due to noxious stimuli.
Electroencephalography (EEG)

• Apart from total power, two other parameters that are critical to the understanding of nociception (or pain) in anaesthetised animals.
• Median Frequency (MF or F50) – the frequency under which 50% of the total EEG power is found.
• 95% Spectral Edge Frequency (SEF95 or F95) – the frequency under which 95% of the total EEG power is found.

MF = 89 Hz
SEF95 = 170 Hz

Electroencephalography (EEG)

• Dog EEG, data from Kaka et al.,(2016) DOI: 10.1515/pjvs-2016-0086

Note the delay in the increase of heart rate following pain stimulus. This indicates that pain response mediated by adrenaline is slower than EEG, ± 3 s. (EEG Signal is delayed by approx 1 s)
Electroencephalography (EEG)

- Dog EEG, data from Kaka et al., (2016) DOI: 10.1515/pjvs-2016-0086

MF & Spectral Edge Frequency 95 values increased due to nociception!

AFTER STIMULUS

BEFORE STIMULUS

Electroencephalography (EEG)

- Dog EEG, data from Kaka et al., (2016) DOI: 10.1515/pjvs-2016-0086

We can even see the effects of local anaesthetics when it is working (50 mcg lidocaine)!

BEFORE STIMULUS  AFTER STIMULUS
Electroencephalography (EEG)

• Dog EEG, data from Kaka et al., (2016) DOI: 10.1515/pjvs-2016-0086
And show that ketamine at 50 mcg has poor analgesic properties.....

The heart rate no longer respond vigorously as the response has been attenuated, but EEG still show nociceptive response

Electroencephalography (EEG)

• Composited stunning & slaughter data from cattle (CSIRO-UPM Collaboration) (Zulkifli et al., 2014; doi: 10.1071/AN12128)

Penetrative stun & slaughter with EEG data showing different bands of activity (δ, θ, α, β, γ) and the transition to brain death.
Electroencephalography (EEG)

• Pain and responses from cattle (As reported by Johnson et al. 2012; doi: 10.7120/096272812X13353700593888)
• The same authors reported that if animals were subjected to slaughter without stunning, there will be nociceptive response (MAM model):

Median Frequency or F50 increased following neck cut indicating nociception

Electroencephalography (EEG)

• Pain and responses from cattle (As reported by Johnson et al. 2012; doi: 10.7120/096272812X13353700593888)
• The same authors reported that if animals were subjected to slaughter without stunning, there will be nociceptive response (MAM model):

SEF95 increased following neck cut indicating nociception
Electroencephalography (EEG)

• Pain and responses from cattle (As reported by Johnson et al. 2012; doi: 10.7120/096272812X13353700593888)
• The same authors reported that if animals were subjected to slaughter without stunning, there will be nociceptive response (MAM model):

Total power doesn’t change much even though it differed between left & right cerebral hemispheres, indicating the brain is still functioning.

Electroencephalography (EEG)

• Pain and responses from cattle (As reported by Johnson et al. 2012; doi: 10.7120/096272812X13353700593888)
• BUT if PENETRATIVE mechanical stunning is used, a successful stun will suppress EEG activity instantaneously → extensive brain damage, and may be making the animal brain dead & unable to sense pain….

Where debate on whether stunning should be allowed in Jewish & Islamic slaughter rages on….
MAM & EEG

WHY DID WE DO ALL THESE AND WHAT ARE WE HOPEING TO ACHIEVE?

- The need for extended post surgical pain relieve & adequacy of pain relieve during procedures
- Understanding pain and stress during slaughter, and necessity of stunning – improving welfare & meat quality
- Basis for animal welfare guidelines in research, patient treatment, general handling of animals and even litigation

Future directions

• Ultimate aim of ACCURATELY measuring pain to improve Animal Welfare
• MAM & EEG as a tool to determine analgesic requirements and analgesia in animal experiments
• Minimise the necessity of certain procedures, and MINIMISE the need to inflict pain on conscious animals
• Efficacies screening for pharmacological agents in mitigating animal pain
• Potential usage of EEG and related technology to mitigate or prevent pain…
• Development of standards / code of practice to ensure animal welfare during slaughter & other procedures
Myth or Reality

Long distance (>5000 km) BRAIN TO BRAIN COMMUNICATION:
Grau et al., (2014); PLoSOne doi:10.1371/journal.pone.0105225

Basis for a revolutionary tool to answer the old question
"Can animal feel pain...?"
Pain vs Nociception

Nociception is a neural process that allows the detection and transmission of nociceptive information to the brain WITHOUT emotional or any other types of responses, a.k.a. "physiological pain" …easier to "measure", and thus very important to isolate it from other subjective measures of pain.

http://vanat.cvm.umn.edu/NeuroLectPDFs/Pain_vs_Nociception.pdf

VR1=Vanilloid (heat) receptor (now called TRPV1)
P2X3 = Purine (chemical) receptor, sensitive to ATP
mDEG/BaNaC=Degenerin/epithelial (mechanical) sodium channel

Pain vs Nociception

Inhibit Perception
• Anesthetics
  • Opioids
    • µ-opioid agonists
    • Benzodiazepines
    • Phenothiazines

Modulation of Spinal Pathway
• Local anesthetics
• µ-opioid agonists
• Tricyclic antidepressants
• Cholinesterase inhibitors
• NMDA antagonists
• NSAIDs
• Anticonvulsants

Inhibit Transmission
• Local anesthetics
• µ-opioid agonists

Transduction
• µ-opioid agonists
• Corticosteroids

Moderate Pain

http://vanat.cvm.umn.edu