Approche histologique et neurophysiologie de la douleur liée à la coupe de queue chez les porcelets

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Presentation overview

Approaches for assessing nociception and pain in pigs – tail docking/biting (FareWellDock Project)

- Tail histopathology (characterization, traumatic neuromas)
- Neurophysiology (peripheral nerve, dorsal root ganglia, spinal cord)
  - gene expression (pain neuropeptides)
- Behaviour (mechanical threshold testing)

Pig research at SRUC

- Heath & Welfare
  - Sow (dry, farrowing), piglets
  - Grower & finisher
FareWellDock project

- 3 year EC funded research programme (ANIWHA Era-Net initiative)
- 10 project collaborators from 8 different EU countries (plus 1 from USA)
- Address major welfare issues of tail docking and tail biting in commercial pig production in EU.

Aims: supply necessary information for quantitative risk assessment of tail docking and biting and develop towards a non-docking policy in the EU.
Tail biting is a major welfare and health issue
- up to 30% losses (NADIS, 2013)
- complicated multifactorial background

Tail docking is carried out as a measure against tail biting, but is a welfare issue in itself.
Objectives

Adverse effects: Hazard characterisation

• Assess the **short** (acute inflammation), **medium** (post-trauma repair) and **long term** (traumatic neuroma formation) pain associated with tail docking piglets

• Characterize the time course of **traumatic neuroma** development caused by tail docking in piglets

• To assess the effects of **tail resection** in older pigs on neuroma formation and stump pain sensitivity. **Provides a basis for assessing the pain associated with being tail bitten**
8th caudal vertebra – (3 day-old pig)
Pig tail - caudal nerves

Nerve cross section (Ca. 8th/9th caudal vertebrae)

*N. ventralis caudalis VIII*
Traumatic neuroma

Tail docking leads to neuroma formation in peripheral nerves

Can be associated with persistent pain or increased sensitivity to mechanical stimulation in tail stump
Sensory neurons - nociceptors

Physiological pain is the immediate response to noxious stimuli mediated through high-threshold receptors located on nociceptive sensory neurons.

**Fast pain** – small diameter, **myelinated** A-δ fibres

**Slow pain** – small diameter, **unmyelinated** C-fibres
Sensory receptors

Transduce painful stimuli into action potentials which is transmitted along primary sensory nerves to dorsal horn of spinal cord

Nociceptive nerve ending
Pain pathway

4 stages:

1. Transduction
2. Transmission
3. Modulation
4. Perception

Nociception

Pain
Tail histopathology

Characterize tail injury and peripheral nerve repair (pre, peri and post-neuroma development)

- Commercial docked pigs (3d)
- Assessment 1, 4, 8 and 16 weeks after tail docking (n=16)
- 200 sections (every 10, 20, 30 ……max 150)
- H&E and S100 immuno-localization of peripheral nerves
Tail histopathology

1 week after tail docking

A B

C D
Tail histopathology

4 weeks after tail docking
Tail histopathology

8 weeks after tail docking

A B

C D
Tail histopathology

16 weeks after tail docking

A B C D
Tail histopathology

- Tail docking causes significant tail injury
- Histopathological lesions that occur shortly after docking (one week & beyond) are not likely to induce or maintain pain
- Caudal nerve traumatic neuroma development is a consistent feature of this type of injury
- Neuromata axonal proliferation and dispersion is still ongoing 4 months after tail docking
- Not possible to confirm based only on histopathological assessment if this affects tail sensitivity
Gene expression studies – qRT PCR

Caudal DRG and spinal cord
• Collected 1, 4, 8 and 16 weeks after tail/sham docking (n=32)

Activating transcription factor 3 (ATF-3)
• a mediator of peripheral nerve axonal regeneration following injury (DRG)

Calcitonin gene related peptide (CGRP)
• a peptidergic sensory fibre marker specific to dorsal horn neurons involved in inflammatory pain processing

NMDA glutamate receptor 2B (GRIN2B)
• participates in the maintenance of chronic pain in the spinal dorsal horn
Gene expression studies

**ATF3 - caudal DRGs**

- Fold-change in expression relative to GAPDH
- Time after treatment (weeks)
- Sham docked
- Tail docked
- ***
- *

**CALCB - caudal spinal cord**

- Fold-change in expression relative to ACTB
- Time after treatment (weeks)
- Sham docked
- Tail docked
- ***

**GRIN2B - caudal spinal cord**

- Fold-change in expression relative to ACTB
- Time after treatment (weeks)
- Sham docked
- Tail docked
- ***
Gene expression studies

- Significant ATF3 upregulation in the caudal DRGs observed up to 8 weeks after tail injury, but not different from sham-docked 16 weeks after tail docking
- Spinal changes in CGRP expression mediating the induction and maintenance of inflammatory pain are relatively short lasting (TD+1 week), and are not different from sham-docked thereafter.
- Significant elevation spinal GRIN2B expression is only observed 1 week after tail docking

The effects of tail docking on injured peripheral nerve axonal activity are relatively short-lasting (consistent with histopathological data)

The possible painful consequences of tail docking linked to peripheral and spinal neuronal nociceptive processing appear to be resolved 4 weeks after tail injury
Behavioural Assessment

Approaches

• Spontaneous behaviours (pain-related, abnormal, desynchronized)
• Validate pain-related facial expressions in pigs (piglet grimace scale)
• Nociceptive mechanical threshold testing on tail (Pressure Application Measurement [PAM] device)
Nociceptive testing

Experimental set-up

- Adjustable test crates
- Habituation
  - Pairs of pigs – (1 session per day over 4 days, test day 5)
  - 5% sucrose solution
  - Testing bout duration (15 minutes)
Nociceptive testing

Pressure Application Measurement (PAM) device

- Force application and measurement system (Ugo Basile)
- Probe/force transducer linked to laptop
- Purpose built software
- Response measure – Tail flick
Nociceptive testing

Baseline thresholds in intact pigs (17 week-old)

- Three tail regions (dorsal tail), 3 stimuli applied (peak force at response - averaged)
- Significant difference ($P<0.01$) in thresholds of intact tail sensitivity across different regions.
- Region 1 - higher thresholds compared to regions 2 and 3
Tail resection

Simulation of effect of tail biting

- 3 treatment groups (16 gilts/group)
  - sham (intact)
  - 1/3rd tail removed
  - 2/3rd tail removed
- Surgical amputation at 16 weeks of age
- Assess short and long term consequences on tail sensitivity
Tail resection

Post operative mechanical thresholds

- Testing: pre operative (-1 day), post-operative (1 week)
- Significant decrease ($P<0.05$) in threshold sensitivities compared to pre-op in both $2/3^{rd}$ (short) and $1/3^{rd}$ (long) one week after tail resection

![Graph showing withdrawal threshold (gf) before and after surgery for short and long tail resections]
Summary (1)

• Tail docking piglets produces a significant tail lesion.

• Minimal evidence of histological features likely to induce or maintenance of pain (beyond 1 week after TD).

• Gene expression studies support histopathological observations:
  - peripheral nerve axonal activity, proliferation and peripheral and spinal nociceptive processing after tail docking are short lasting (resolved 1 month after tail injury).
Summary (2)

• Clear withdrawal responses to mechanical stimulation (PAM) were observed and allowed characterisation of thresholds of sensitivity in the pig tail before and after tail amputation.

• Tail resection (simulating tail biting in older pigs) induced increases in regional tail sensitivity to mechanical stimulation one-week post surgery, reflecting physiological events associated with the acute phase of injury.

• Further data on the thresholds of mechanical sensitivity several weeks post-surgery will provide information on the temporal nature of the change in mechanical sensitivity associated with tail amputation injury.

• Gene expression analysis is currently being undertaken in tail resected pigs
SRUC Pig Facilities

- Two farms (Easter Howgate, Oatridge)
- Located South and West of Edinburgh City Centre

**Oatridge:**
100 sow farrow-to-finish
Conventional indoor unit
Focus on **education**

**Easter Howgate:**
100 sow farrow-to-finish
High welfare unit, conventional and research buildings
Focus on **research**
SRUC Pig health & welfare research

• **Dry sow**
  – Hunger in sows

• **Farrowing sow**
  – Social aggression and prenatal stress
  – Analgesia to reduce pain at farrowing
  – Developing free farrowing environments

• **Piglet**
  – Tail docking
  – Play

• **Grower & Finisher**
  – Social mixing aggression
  – Tail biting
  – Gastric ulcers
Hunger in sows

Rick D’Eath

- Ration feeding of dry sows resulting in hunger
- EU requirement to provide fibre
  - What do farmers provide?
  - Do sows eat straw bedding?
  - Which fibre types really help satiety?
Prenatal stress

Kenny Rutherford

• Stress during pregnancy affects progeny ability to cope with postnatal challenges

• Social stress caused by mixing gilts and sows

• Piglets from mixed sows have:
  – greater pain and stress responses
  – altered growth after weaning

• Mixed gilts have:
  – impaired social and maternal behaviour
  – altered immune and reproductive function
Pain at farrowing

Sarah Ison, Kenny Rutherford

- Farrowing may be painful for some sows
- PhD project:
  - survey of UK vets and farmers to understand attitudes and practices in relation to pain relief for sows
  - identify possible behavioural indicators of pain during and after farrowing
  - investigated use of analgesia (Ketoprofen) after farrowing
PigSAFE

Emma Baxter

- Free farrowing system
- Promoting better maternal behaviour
- Improved piglet survivability and productivity
Neurobiology of tail docking and biting

Dale Sandercock

- “FareWellDock” ANIWHA Era-Net Project
- Addresses major welfare issues of tail docking and tail biting in commercial pig production in EU
  - Does docking cause long term pain in the tail
  - Does chronic sensitivity and/or pain result from traumatic neuromas?
  - How does tail injury later in life (tail biting) affect sensitivity/pain?

Source: PROVIEH
Piglet play

Alistair Lawrence. Sarah Brown

- Growing emphasis on ‘positive’ welfare states
- Play is widely thought to be a positive welfare indicator
- Situations and environments which promote play in livestock are poorly understood
  - Litters differ in the amount of play
  - Fast growing litters play more

Breeding to reduce pig aggression

• Routine mixing results in social aggression:
  - injury, disease, activity (= poorer FCE), pre-natal stress
  - welfare, food intake, growth rate, reproductive success

• Mixing aggression is heritable ($h^2=0.3 – 0.4$)
  - breed against it using skin lesion scores
  - molecular markers might be even easier?

• Aggression in stable groups?
  - How does it relate to mixing aggression?
  - How can we breed to reduce mixing and stable group aggression?
Assessment strategies during aggression

Irene Camerlink

• Gaining a better understanding of why pigs fight (or not), who they fight and why they give up (or not)
• Game theory models studied through dyadic contests between pigs matched or unmatched for:
  – Body weight
  – Aggressiveness as personality trait
• Effect of experience/socialization on aggression
• Stakeholders’ perception of pig aggression
• Review of causes of tail damage
• Economic assessment of systems
• Can behaviour be used for early detection of tail biting?
  – Tail posture, activity, tail investigation
  – How can we use this to stop an outbreak?
  – PhD Helle Lahrmann (University of Copenhagen, Denmark)

• Developing practical enrichment methods to occupy pigs in fully slatted systems
  – Materials, presentation
  – PhD Jen-Yun Chou (Teagasc, Ireland)
Gastric ulcers

• Highly prevalent in many countries
• Main risk factor is feed structure and content
• Welfare relevance of different lesion severities is uncertain

• Current work is investigating whether ulcers are painful for finisher pigs
  – behavioural analysis
  – response to analgesia
The “FareWellDockers”

Newcastle University – Sandra Edwards, Matt Leach, Pierpaolo Di Giminiani, Mark Brett, Emma Malcolm
RDSVS Vet Pathology Unit – Neil McIntyre, Sionagh Smith, Dawn Drummond
Edinburgh Genomics/Roslin – Richard Talbot, Alison Downing, Stephen Meek
SRUC AVS – Jenny Coe, Sarah Hall

FareWellDock website: http://farewelldock.eu/