1st WG1 meeting
“HPA axis, Cortisol and other Stress biomarkers”
14th-15th September 2015, Bern (CH)

Discussion of Session 3
Alternative Biomarkers for Stress
Chairman: Rob Smith
If a stimulus does not affect an important physiological process in it sufficiently stressful to consider?

Rob Smith
Stimulus

Activation
- General
- SNS
- HPA

Disruption
- Immunity
- Growth
- Reproduction
Hypothesis

• Reproduction affected if allostatic load too great

• If important function not maintained - welfare compromised
Transport reduces LH pulsatility

... changes pulse frequency - at hypothalamus
... effects most marked in first 2 hours

(OVX ewes mid-breeding season without prior steroid)
How is the hypoglycaemia-induced delay in LH surge mediated?

Expt design, relative to P removal and LH surge

- **CONTROL**
  - Insulin 38 + 40h Post Progesterone
  - Insulin 38 + 40h + Naloxone 37 to 49h Post Progesterone
  - Insulin 38 + 40h + RU486 37h Post Progesterone

- **INTACT**
  - Naloxone
  - RU486

Transmission

Activation/Transmission

Surge

Hours from oestradiol to surge peak
Discussion of Session 3
Alternative Biomarkers for Stress

Chairperson: Robert Smith, Liverpool, UK

3.1 Claudia Spadavecchia (Vetuisse Faculty, CH)
3.2 Bruno Stefanon (Dept. of Agricultural and Environmental Sciences, University of Udine, I)
3.3 Carlos Hernandez (Swedish University of Agricultural Sciences, S)
3.4 Geert Bruggeman (Nutrition Sciences N.V., B)
3.5 Ahmed Salama (Universitat Autónoma de Barcelona, Spain)

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C. Spadavecchia, Vetuisse Faculty, Bern

Outline

– Ongoing research

– General considerations from the literature
# Neuroendocrine Markers of Stress

## Kenneth M. Hargreaves, DDS, PhD

### Table 1. Peripheral Neuroendocrine Markers of Stress

<table>
<thead>
<tr>
<th>Endocrine</th>
<th>Increase Over Baseline Levels</th>
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<tbody>
<tr>
<td>1. Pituitary adrenal axis: (e.g., ACTH, B-END, B-LPH, cortisol)</td>
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<td>2. Prolactin</td>
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<td>3. Growth hormone</td>
<td>2–10</td>
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<td>4. Pituitary thyroid axis (e.g., TSH, T4, T3, rT3)</td>
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<td>5. Pituitary gonadal axis (e.g., LH, FSH, Testosterone, E2)</td>
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<td>6. Glucagon</td>
<td>0.5–1</td>
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<td>7. Insulin</td>
<td>0?</td>
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<td>8. Renin-angiotensin</td>
<td>2–3</td>
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<td>9. Aldosterone</td>
<td>2–3</td>
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<td>Neural</td>
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<td>(e.g., NE, NPY?)</td>
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<td>2. Adrenomedullary (e.g., EPI, DA, M-ENK)</td>
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<td>3. Vasopressin</td>
<td>2–15</td>
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<td>4. Primary afferents (e.g., SP, CGRP)</td>
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</table>

* Represents the stress-induced increase from baseline levels. See text for references.

### Table 2. Factors That Modify Physiologic Responses to Stress

**Extrinsic factors**

1. Type of stress:
   (e.g., psychologic vs physical)
2. Intensity
3. Time parameters
   - Duration
   - Frequency
   - Time of occurrence
4. Chronicity

**Intrinsic factors**

1. Age
2. Sex
3. Health status (e.g., concurrent disease)
4. Context (e.g., patient's control over stressor)
5. Concurrent drugs
Stress-induced analgesia

Ryan K. Butler, David P. Finn *

Department of Pharmacology and Therapeutics, NCBES Neuroscience Cluster and Centre for Pain Research, National University of Ireland, Galway, University Road, Galway, Ireland

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Fear
Rodent
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ABSTRACT

For over 30 years, scientists have been investigating the phenomenon of pain suppression upon exposure to unconditioned or conditioned stressful stimuli, commonly known as stress-induced analgesia. These studies have revealed that individual sensitivity to stress-induced analgesia can vary greatly and that this sensitivity is coupled to many different phenotypes including the degree of opioid sensitivity and startle response. Furthermore, stress-induced analgesia is influenced by age, gender, and prior experience to stressful, painful, or other environmental stimuli. Stress-induced analgesia is mediated by activation of the descending inhibitory pain pathway. Pharmacological and neurochemical studies have demonstrated involvement of a large number of neurotransmitters and neuropeptides. In particular, there are key roles for the endogenous opioid, monoamine, cannabinoid, γ-aminobutyric acid and glutamate systems. The study of stress-induced analgesia has enhanced our understanding of the fundamental physiology of pain and stress and can be a useful approach for uncovering new therapeutic targets for the treatment of pain and stress-related disorders.

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HPA axis mediates SIA
Brain regions (amygdala, PAG, RVM)

Spinal cord and periphery
Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration

Johann F. Coetzee, BVSc, PhD; Brian V. Lubbers, DVM; Scott E. Toerber, BS; Ronette Gehring, BVSc, MMedVet; Daniel U. Thomson, DVM, PhD; Bradley J. White, DVM, MS; Michael D. Apley, DVM, PhD

Substance P showed a better association with nociception than cortisol
Stress-induced hyperalgesia

Elaine M. Jennings\textsuperscript{a,b}, Bright N. Okine\textsuperscript{a,b}, Michelle Roche\textsuperscript{b,c}, David P. Finn\textsuperscript{*,a,b}

\textsuperscript{a} Pharmacology and Therapeutics, School of Medicine, National University of Ireland, Galway, Ireland
\textsuperscript{b} NCBES Centre for Pain Research and Galway Neuroscience Centre, National University of Ireland, Galway, Ireland
\textsuperscript{c} Physiology, School of Medicine, National University of Ireland, Galway, Ireland

\textbf{A R T I C L E  I N F O}

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Human
Rodent
Anxiety
Brain

\textbf{A B S T R A C T}

The importance of the modulation of pain by emotion is now widely recognised. In particular, stress and anxiety, depending on their nature, duration and intensity, can exert potent, but complex, modulatory influences typified by either a reduction or exacerbation of the pain state. Exposure to either acute or chronic stress can increase pain responding under experimental conditions and exacerbate clinical pain disorders. There is evidence that exposure to chronic or repeated stress can produce maladaptive neurobiological changes in pathways associated with pain processing, resulting in stress-induced hyperalgesia (SIH). Preclinical studies of SIH are essential for our understanding of the mechanisms underpinning stress-related pain syndromes and for the identification of neural pathways and substrates, and the development of novel therapeutic agents for their clinical management. In this review, we describe clinical and pre-clinical models used to study SIH and discuss the neural substrates, neurotransmitters and neuromodulatory systems involved in this phenomenon.

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Chronic Stress →

ACC
- Increased activation

Insular cortex
- Adaptive changes remain unclear

Amygdala
- Altered opioid receptor binding
- Possible CRF-R1 involvement

PAG
- GFAP expression ↓
- EAAT2 expression ↓
- Possible CCK system involvement

RVM
- Tryptophan hydroxylase ↑
- μ opioid receptors activation
- Endocannabinoid system involvement
- Possible CCK system involvement
- Decrease in GFAP

Spinal Cord
- GABA signalling ↓
- Glutamate signalling ↑
- Transient increase in pro-inflammatory mediators

DRG
- CB1 receptor ↓
- TRPV1 receptor ↑

Enhanced pain signalling ←
The effects of acute psychological stress on circulating inflammatory factors in humans: A review and meta-analysis

Andrew Steptoe *, Mark Hamer, Yoichi Chida

IL6 and IL1β: robust effects
CRP: marginal effect
Elevated Inflammatory Markers in Response to Prolonged Sleep Restriction Are Associated With Increased Pain Experience in Healthy Volunteers

Monika Haack, PhD; Elsa Sanchez, BA; Janet M. Mullington PhD

Sleep, 2007

Figure 2—Change of plasma IL-6, serum CRP, and plasma sTNF-R p55 levels from baseline to the 11th day of sleeping either 8 h/night (grey bar, N=8) or 4 h/night (hatched bar, N=10 for IL-6, N=9 for sTNF-R p55). IL-6, CRP, and sTNF-R p55 were measured every 4 h and averaged across a 24-h period. Original values are presented, and statistics were based on log-transformed values. Asterisk indicates significant difference between sleep conditions.
Together, these studies indicate that there is no fixed, invariant stress response as originally proposed in Selye's General Adaptation Syndrome. Rather, the CNS selectively activates various neuroendocrine responses to stress under dynamic, flexible conditions which can be modified by numerous extrinsic and intrinsic factors.
Expression of NGF, BDNF and their receptors in subcutaneous adipose tissue of lactating cows

Monica Colitti, Juan J Loor, Bruno Stefanon

Highlights:

• NGF and BDNF are involved in fat metabolism and in activation of the sympathetic response.
• Immunohistochemistry revealed the localization of NGF and BDNF in subcutaneous adipocyte of lactating cows.
• Gene expression of NGF, BDNF and TrkB confirms their local production.
Expression of mRNA in bovine subcutaneous adipose tissue

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- NGF 188 bp
- BDNF 241 bp
- TRKB 215 bp
- ACTB 194 bp
Expression of NGF, BDNF and their high-affinity receptors in ovine mammary glands during development and lactation

Monica Colitti¹
Prepubertal stage

20 days before lambing

60 days of lactation

NGF

BDNF

TrkA

TrkB

*
Human Milk:
342.3 ± 51.1 pg/ml (Dagat et al., 2013);
13,010 ± 5.88 pg/ml (Li et al., 2011)
Other biomarkers of stress?

Can we use telomere length & attrition rate as indicators of stress in dairy cattle?

“Wear & Tear”
Potential for KRL as biomarker for monitoring animal health and welfare in ruminants

Katrien De Smet, Jeroen Krijnen & Geert Bruggeman
Nutrition Sciences NV, Belgium
Background
- KRL bio–assay = ‘Kit Radicaux Libres’ measuring anti-oxidant capacity of the blood, which is considered a parameter for welfare and health.

- KRL has a proven efficacy for assessing anti-oxidant effect in:
  - *in vitro* studies: anti-oxidant activity of natural or synthetic anti-oxidants
  - *in vivo* studies: in human and monogastric studies, assessing the effect of natural and pharmaceutical treatments

Aim of the study
Test and rank different plant-based functional feed ingredients for their effect on blood anti-oxidant activity in calves.
**Trial design:**
32 calves in 4 pens; 2 control pens; 2 treatment pens
Trial period: 4 weeks
Blood collection: day 1 + day 30
Weight collection: day 1 + day 30

<table>
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<tr>
<th>Treatment 8 calves</th>
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**Results/findings:** research is still ongoing
H NMR based biomarkers for mammary inflammation under heat stress conditions

S. Love, A.A.K. Salama*, A. Contreras, G. Caja

G2R of Universitat Autònoma de Barcelona, Bellaterra, Spain

First WG1 Meeting, Bern, Switzerland

- **Two climatic treatments:**
  - Thermal-Neutral (TN; 15-20°C, THI = 65-68)
  - Heat stress (HS; 28-35°C, THI = 75-83)

- **LPS treatment:**
  - LPS half: 10 µg in 2 ml 0.09% sterile saline
  - CON half: 2 ml 0.09% saline

- **Sampling of milk:**
  - Composition; 0, 2, 4, 6, 8, 10, 12, 24, 36, 48, and 72 h
  - Metabolomics; 0, 4, 6, 12, and 24 h

- **$^1$H NMR Reads:**
  - Low molecular weight compounds (< 1 kDa)
  - Biomarkers for HS and inflammation
Milk Composition

**Protein, %**

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<th>TN-LPS</th>
<th>HS-CON</th>
<th>HS-LPS</th>
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**Lactose, %**

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**Difference (%)**

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<th>HS-CON</th>
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Milk Metabolomics

- **Heat stress markers:**
  - Increment in 3-methylhistidine, PUFA, and Citrate
  - Decrease in lactose, galactose, and N-acetylcarbohydrates

- **Inflammation markers in TN conditions:**
  - Betaine
  - Hippurate
  - Acetate

- **Inflammation markers in HS conditions:**
  - Trimethylamine-N-oxide
  - N-acetylcarbohydrates
  - 3-methylhistidine