Module 6

Assessing Animal Welfare Physiological Measures

This lecture was first developed for **World Animal Protection** by Dr David Main (University of Bristol) in 2003. It was revised by **World Animal Protection** scientific advisors in 2012 using updates provided by Dr Caroline Hewson.

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This module will examine

The relationship between welfare and the physiological responses

Stress response

- Chronic stress and welfare
- How measures of stress response may be used in the assessment of welfare
- Limitations

Immune responses

Neurobiological responses

Metabolic responses

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Animals' experience

Environment/Resources – Sensory input

↓

Brain evaluates – Emotions/sentience

↓

Body responds – Behaviour, physiology

↓

Animal adapts and survives – Homeostasis and allostasis

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The adaptive process

Animal adapts and survives

- homeostasis, eg maintain blood pH at 7.35–7.45
- allostasis, eg changes in physiology and behaviour in pregnancy and lactation

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Review: physiological responses

Stress response

Immune responses (eg white blood cell count)

Neurobiological responses (eg oxytocin)

Metabolic responses

Other, eg reproductive hormones

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Welfare and the stress response

What is stress?

In physics – the amount of pressure applied until the breaking point is reached

In biology: "Stress is an environmental effect on an individual which over-taxes its control systems and results in adverse consequences, eventually reduced fitness." (Broom & Johnson, 2000)

- Threats = internal and external (stressors)
- Homeostasis, e.g. pH of blood

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The stress response
(Mormède et al., 2007; Blache et al., 2011)

Autonomic nervous system

- sympathetic (adrenaline, noradrenaline)
- parasympathetic (acetylcholine)

Hypothalamo-pituitary-adrenal (HPA) axis

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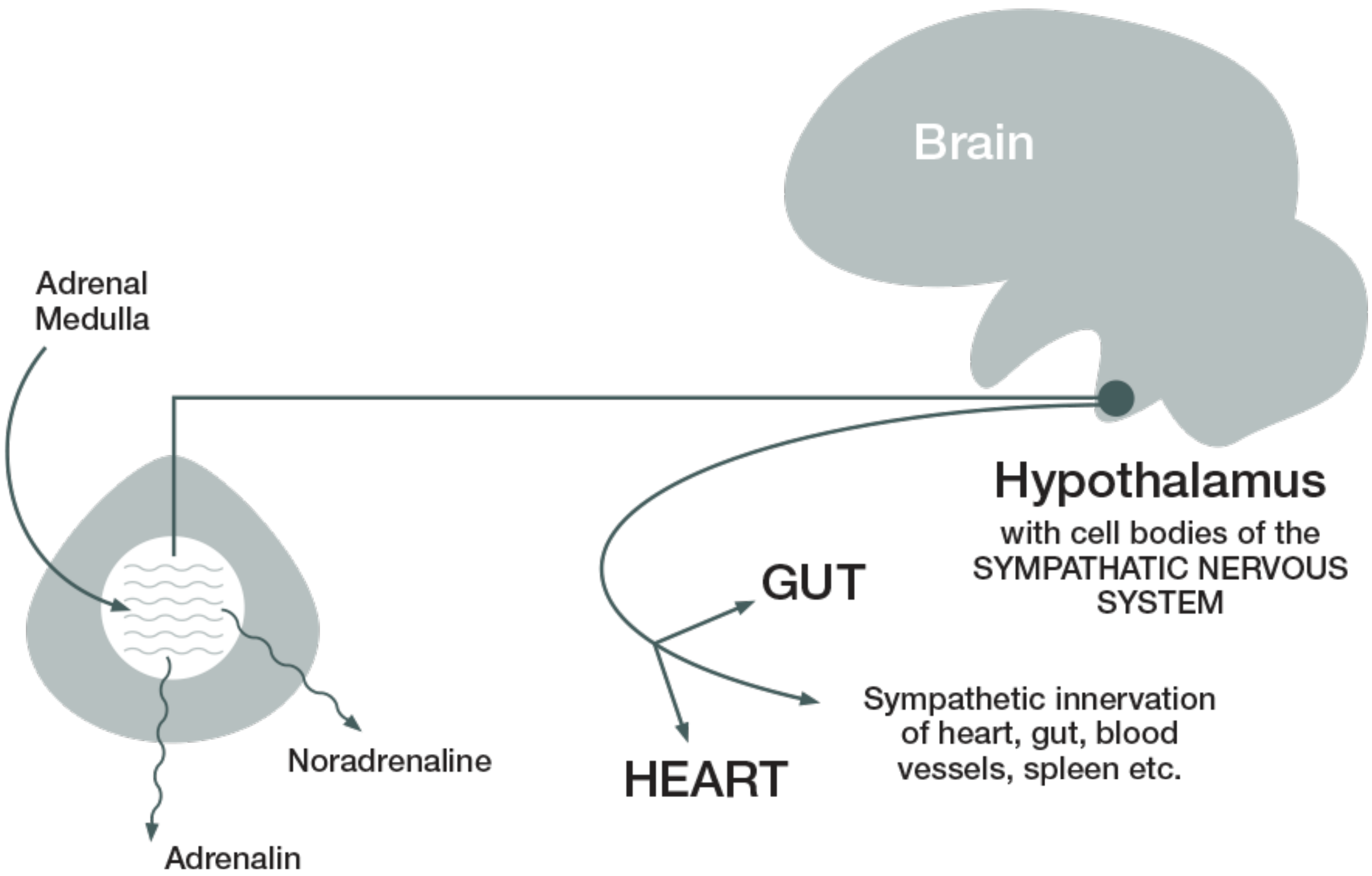
Sympathetic-adrenal-medullary (SAM) system



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Effects of SAM activation

Sympathetic stimulation of sinoatrial node

increased heart rate and increased strength of cardiac muscle contraction lead to increased cardiac output

Increased blood flow to key organs

peripheral vasoconstriction and contraction of the spleen

Increased air intake

increased respiratory rate and relaxation of bronchioles in the lungs

Non-essential bodily activities are inhibited

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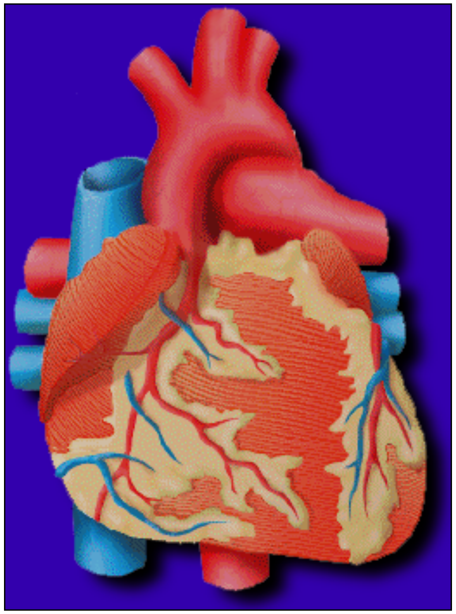
Parasympathetic nervous system

Regulates SAM system

Reduces cardiac output

- bradycardia via effect on sinoatrial node



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The stress response

Autonomic nervous system

- sympathetic (adrenaline, noradrenaline)
- parasympathetic (acetylcholine)

Hypothalamo-pituitary-adrenal (HPA) axis

Chronic exposure to stressors

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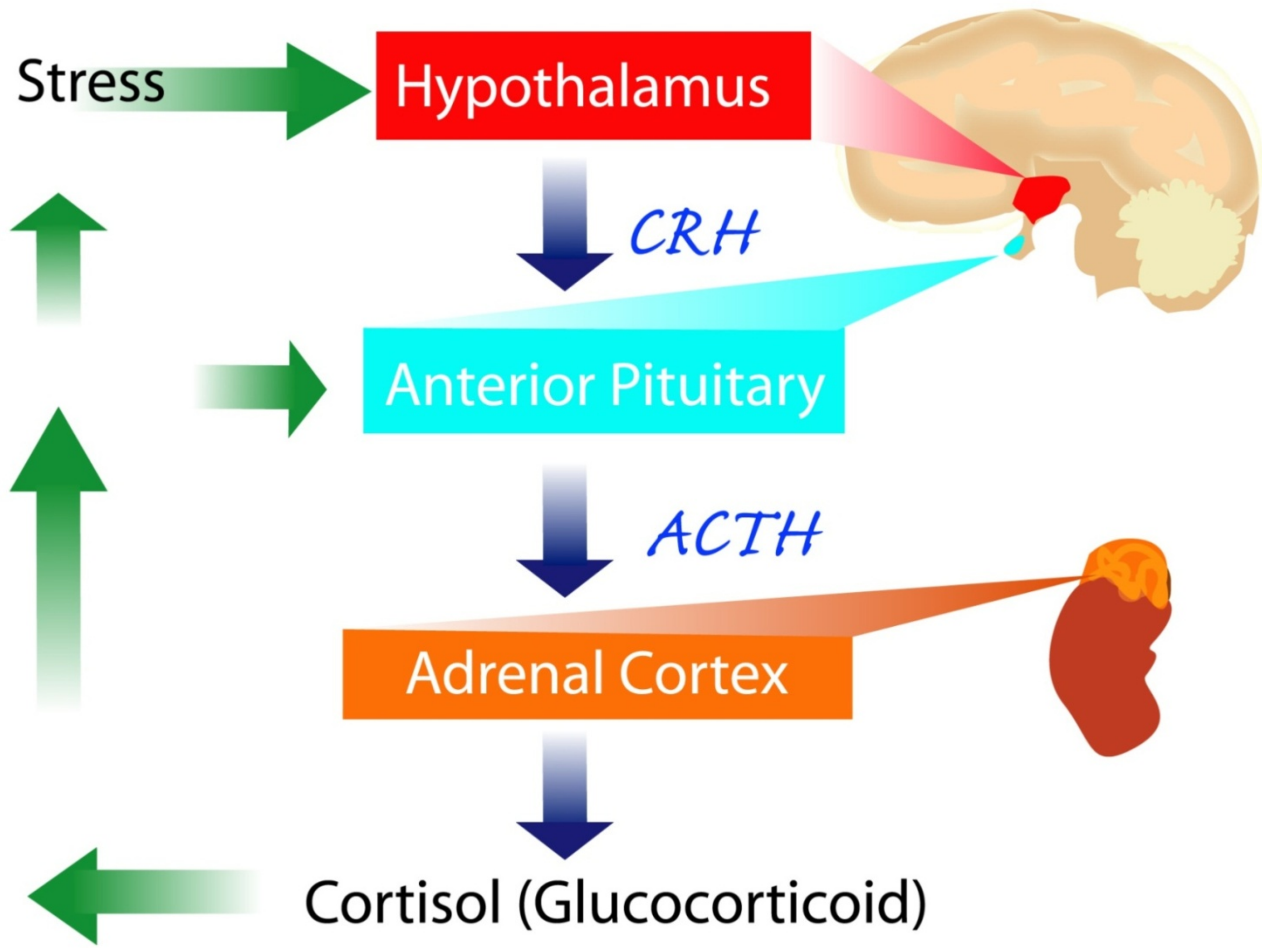
HPA Axis - Cortisol



CRH: corticotrophin releasing hormone   ACTH: adenocorticotrophin

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Cortisol

Mobilises energy stores in the short term

Stimulates glycogenolysis in the liver and suppresses insulin secretion

- Increases glucose levels in the blood

Self-regulating system

- Gives negative feedback to the pituitary gland and the hypothalamus, thereby indirectly to the adrenal glands. This modulates the stress response as the animal's situation improves

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HPA characteristics

Measure of acute welfare changes

Response not as immediate as the SAM system

- eg plasma glucocorticoids elevated 2–10 minutes after stimulation

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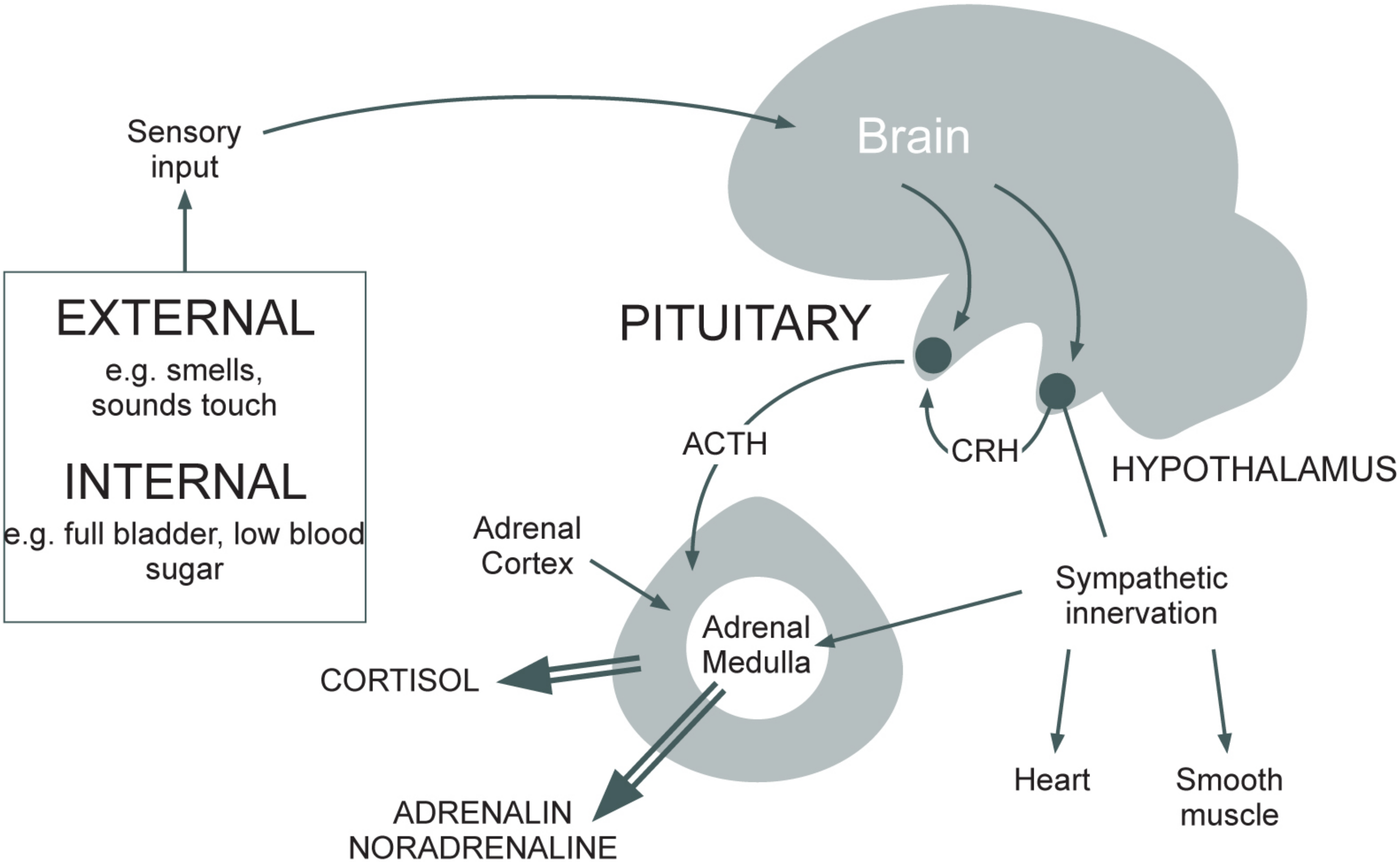
Summary of stress response



Welfare and the stress response

Any event in the animal's life can create a stress response

- Should be adaptive

Experiencing stress is a natural response

- Only a concern when animal is stressed beyond ability to cope ⇨ pathology
- Chronic, inescapable bad conditions ⇨ maladaptive

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Type	Stressor	Associated feelings
Physical (Environmental)	Heat/cold	Thermal discomfort
	Lack of food/water	Hunger/thirst
	Disease and injury	Pain, nausea, weakness
	Confinement	Frustration, boredom, pain
	Over-exertion	Fatigue, pain, anxiety
Physiological	Metabolic demands	Weakness
	Nutritional deficiencies	Weakness
Mental	Behavioural deprivation	Frustration, boredom
	Social stress	Anxiety, fear

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Welfare and chronic stress

Chronic stress can create pre-pathological states, eg

- Reduced immunity
- Hypertension
- Enlarged adrenal gland
- Lack of growth
- Weight loss
- Reduced fertility
- Gastrointestinal ulcers

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Examples of indices of acute stress response

Index	Stress system	Measure with
Heart rate	SAM	Stethoscope
Blood pressure	SAM	Cuff
Respiratory rate	SAM	Visual
Catecholamines	SAM	Blood sample
Glucocorticoids	HPA	
ACTH	HPA	

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Examples of measures of chronic stress response

Glucocorticoids

- Urine, saliva, milk
- ACTH stimulation

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Stress in relation to welfare: practical examples

Capture of wild vicuñas for shearing

Effect of kennelling on dogs

Capture of wild sardines

Castration of farm animals

- Piglets
- Dairy calves
- Water buffalo

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Capture of wild vicuñas for shearing
(Arzamendia et al., 2010)

~500 wild vicuñas rounded up

Three methods used

- People on foot
- Vehicles only (trucks, bikes)
- Combination: vehicles + people on foot

Measured stress response

- Direct measures (SAM): heart rate, temperature, respiratory rate
- Blood samples (HPA and SAM): glucose, cortisol, muscle enzymes

Behaviours, eg vocalising, fighting

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Capture of wild vicuñas for shearing
(Arzamendia et al., 2010)

Cortisol: sex difference

- Females: captured by people on foot had higher levels of cortisol and more vocalising than females captured by vehicles and people
- Males: captured by people on foot had lower levels of cortisol than males captured by vehicles and people

Cortisol: effect of captivity

- Cortisol values remained high, as did vigilance and alertness

Creatine kinase increased with longer captivity, especially in females

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Effect of kennelling on dogs
(Hiby et al., 2006)

26 dogs entering shelter

Urinary cortisol measured on

- days 1, 2, 3, 5, 7 and 10 after admission

Half were from homes; half were either strays or returns to the shelter

Mean cortisol tended to decrease with time in the strays and returns, and to increase in dogs relinquished from homes

Capture of wild sardines
(Marcalo et al., 2006)

Commercial purse-seine fishing, Atlantic Ocean, Portugal

174 sardines

Effect of time spent in the net

- Marked elevation of cortisol after one hour

Credit: NOAA

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Castration of farm animals

Castration = tissue damage

Noxious sensory input

Brain evaluates input

Emotions and feelings, eg pain and fear/frustration as they try to escape and cannot

Body responds

Pain behaviour, stress response

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Example 1: castration of piglets

Piglets **(Hay et al., 2003)**

- 84 piglets; five days old
- No clear effect on urinary corticosteroids, catecholamines or growth. Clear effects on pain behaviours for up to four days afterwards

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Example 2: castration of dairy calves
(Stafford et al., 2002)

190 Friesian calves, up to six months old, four different castration techniques used

Simulated castration: no significant changes in cortisol caused by handling or lignocaine

When castration was carried out without analgesia

- All methods significantly increased cortisol to 56–101 nmol/l (comparison: ACTH response = 99 nmol/l)
- Clamp castration: smallest cortisol response.
  Analgesia eliminated it. However, castration not always successful

Surgical castration: local anaesthetic did not reduce cortisol response.

Surgical castration: local anaesthetic + ketoprofen eliminated cortisol response

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Example 3: castration of water buffalo
(Martins et al., 2011)

21 calves; 7–18 months old

Compared methods of castration

- Surgical vs. clamp vs. no castration
- No analgesia
- Measured cortisol

Results

- Surgical castration: cortisol elevated for ~6 hours; pain on palpation at 48 hours
- Clamp: cortisol elevated for ~9 hours; no pain on palpation at 48 hours

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Limitations of adrenal activity measures 1

Do not indicate if animal's experience is positive, negative or neutral

Increased activity from stress

Measurement itself may be stressful

Cost (eg implanted devices, some lab analyses)

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Limitations of adrenal activity measures 2

Individual differences eg

Species and breed (Mormède et al., 2007)

Sex (Arzamendia et al., 2010)

Experience (Hiby et al., 2006)

'High' vs. 'low' responders, e.g.

- Catecholamines in rats (Livezey et al., 1985)
- Cortisol in Zebu cows (Solano et al., 2004)

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Limitations of adrenal activity measures 3

Habituation or sensitisation

Depends on nature of the stressor

- Eg daily vs. occasional exposure

Circadian rhythms, eg glucocorticoids

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Limitations of adrenal activity measures 4

Interpreting combined measures?

Conflicting measures within a study,
eg aspartate transaminase vs. creatine kinase
in vicuña study (Arzamendia et al., 2010)

Conflicting results between studies

Eg urinary corticosteroids in piglets
(Hay et al., 2003)

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Review: physiological responses

Stress response

- Autonomic nervous system
- HPA axis

Immune responses

Neurobiological responses

Metabolic responses

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Overview of the immune response

Innate response

- Cells, e.g. phagocytic cells such as neutrophils, macrophages
- Chemicals, e.g. the complement system

Adaptive response

- T cells – cell-mediated immunity
- B cells – humoral immunity (antibodies)

Role of chemical messengers, e.g. cytokines:

- Interleukin-2 and gamma-interferon (γ-interferon)

Interaction with stress reponse

Immune tissues all innervated by sympathetic nervous system (Bellinger et al., 2008)

- Bone marrow, thymus, spleen, lymph nodes, gut-associated lymphoid tissue

- Research is ongoing regarding the effect of noradrenaline, adrenaline and cortisol on the immune tissues and cells

- The role of cortisol

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Cortisol and infection

Initial immune response → chemicals released into the blood, e.g. leucotrienes

Detected by brain → acute stress response

Moderate elevations of cortisol can help with immune response

Infection is overcome

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Stress and the immune response

For example, parturition, social instability, long journeys

- **Cortisol already elevated → reduced immune capacity (e.g. decreased circulating lymphocytes, decreased phagocytosis, reduced production of cytokines)**

- **Infection not readily overcome**

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Parturition and mastitis in dairy cows

Parturition: high levels of cortisol

- Expulsion of foetus
- Milk letdown
- Also, adverse imbalances in T cells at risk of mastitis, especially if environment dirty, poorly fed, in pain

Credit: J. Sutcliffe / flickr.com

Handling and immunity in lambs (Caroprese et al., 2006)

64 Comisana lambs

Compared daily human handling and artificial rearing vs. daily human handling and natural rearing

- Lambs injected with mollusc antigen at 3 days and 15 days
- Lambs were put in a novel isolation pen at 15 days and 45 days
- Daily human handling had positive effect on antibody levels in artificially reared lambs but not in ewe-reared lambs
- Ewe had positive effect on lambs' immunity

Credit: J. Hynds / flickr.com

Transport and respiratory disease in cattle 1

Some transport may enhance the immune response (Buckham Sporer et al., 2008)

- Belgian Blue Cross bulls reared indoors
- Nine hours transportation by truck with rest stop after 4.5 hours
- Blood taken at intervals before, during and up to 48 hours after transportation
- During transport, cortisol was significantly elevated, as were lymphocytes and neutrophils in the blood
- No obvious adverse effect on neutrophil gene expression

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Transport and respiratory disease in cattle 2

How much transport? (Earley et al., 2012)

- Measured gamma-interferon, etc.
- Journey by sea and truck for ~28 hours;
  24 hours rest; then another 18 hours journey
- The rest ensured adequate recovery

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Neurobiological measures
(Ziemssen & Kern, 2007)

The brain can modify immune response via HPA and autonomic nervous system

Immune response can also affect the brain

- Cytokine receptors in brain
- Glucocorticoid receptors in brain

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Neurobiological measures
(Broom & Zanella, 2004)

Brain activity (MRI)

Learning and memory

Neurotransmitters, eg

- Opioids
- Dopamine
- Prolactin

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Opioids

Three types

- Endorphins
- Enkephalins
- Dynorphins

Functions

- Stress-induced analgesia
- Control hormone release
- Perception of pleasurable stimuli

Different receptors, eg

- Kappa (κ), mu (μ)

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Opioid examples

Lambs

- Increase in plasma ß-endorphin during castration, tail-docking and mulesing (Shutt et al., 1987)

Tethered sows (Zanella et al., 1996)

- Passive sows had higher μ-receptor density than group-housed sows
- Stereotyping sows had low κ- and μ-receptors
- Role of glucocorticoids?

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Dopamine

Catecholamine

Various receptors (D1, D2, etc.)

Mood, locomotion, voluntary movement

Stereotypies, eg

- Sows in stalls, bar-biting: low dopamine

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Prolactin

Pituitary gland

Modulates emotions and acute stress response

Hospitalised dogs (Siracusa et al., 2010)

- Appeasing pheromone associated with lower plasma prolactin
- Appeasing pheromone not associated with dogs' behaviour or welfare

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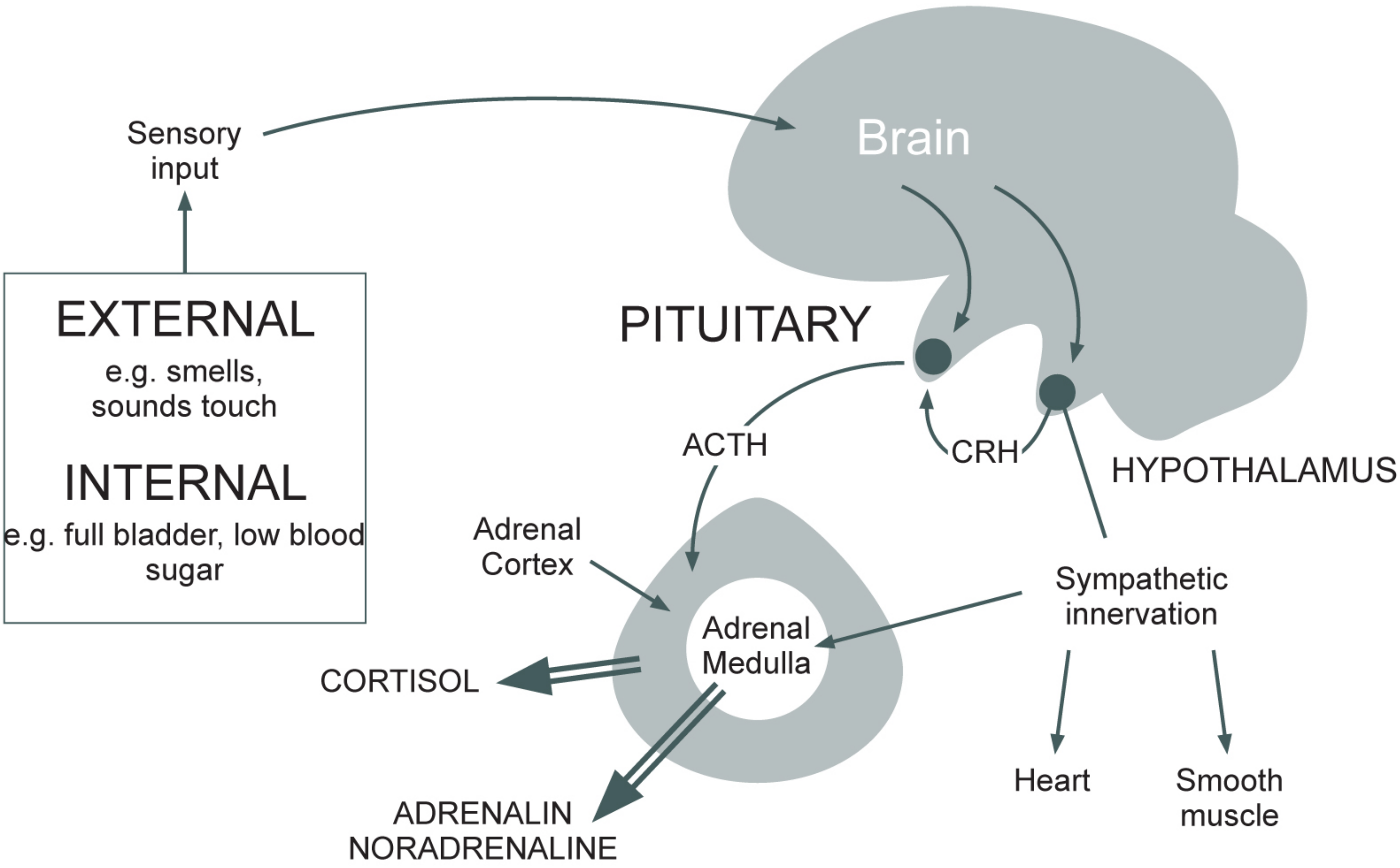
Metabolic responses



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Metabolic responses to stress

Glucose

Lactic acid

Beta-hydroxy-butyrate

Haematocrit

Muscle enzymes, eg creatine kinase

Hormones, eg insulin, thyroid hormones

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Summary

There are many physiological measures of welfare, and many reflect the stress response

Stress response – **SAM and HPA**

- Acute response to maintain homeostasis
- Chronic response may be maladaptive

All responses can affect production

Need for other measures as well

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- **What did you not like?**
- **Do you have any tips to share?**

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Your feedback will help other teachers like you

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