Stress Physiology in Animals

Aims

- ***Stress measurement***
- ***Assessing different types of emotion***
- ***Brain regions and pathways***
- ***Research on Pain***
- **Problem solving**

When choosing stress indicators
be selective

Select the indicators that are most appropriate for the question or problem being examined

If a researcher is interested in assessing the frequency or severity of thirst in a group of animals, what would he/she measure ?

Behavioural measures

- no urination
- time to drinking
- amount consumed

Postmortem measures

- no urine in bladder
- sticky muscle
- difficult to remove skin

Clinical & physiological measures

- urine colour
- skin pliability
- dry mouth
- ↑ plasma protein
- ↑ plasma osmolality
- ↑ haematocrit
- [↑ serum sodium]

Emotions in people

Aggressive	Fascinated	Love
Amused	Flattered	Mistrust
Angry	Frightened	Nauseous
Annoyed	Frustrated	Passionate
Anxious	Glad	Pity
Bitter	Guilt	Pride
Bored	Happy	Relief
Brave	Hatred	Sad
Confused	Horror	Satisfied
Defiant	Humble	Scornful
Depressed	Hungry	Sexy
Despair	Hurt	Shy
Disappointed	Irritated	Sorry
Disgusted	Jealous	Thirsty
Elated	Lonely	Vexed
Excited	Longing	Weary

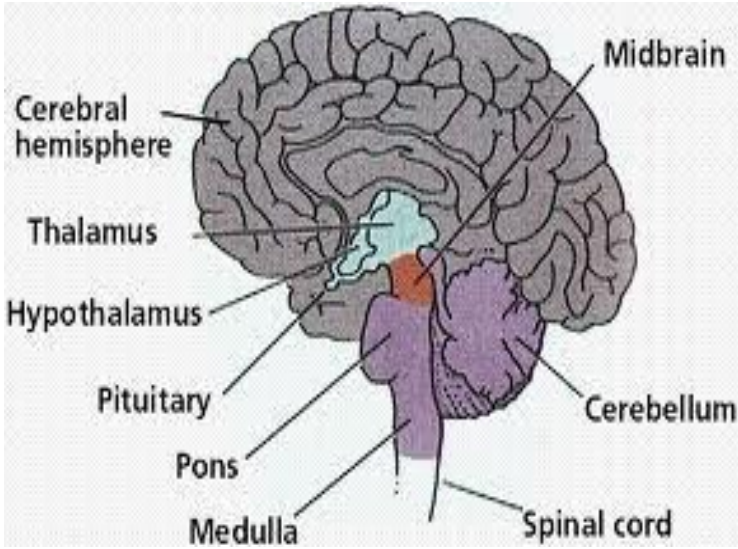
How can we measure these emotions ?

What are the mechanisms involved in emotions and their associated responses ?

To help answer this let us start with some simple brain anatomy

Anatomical subdivisions of the brain

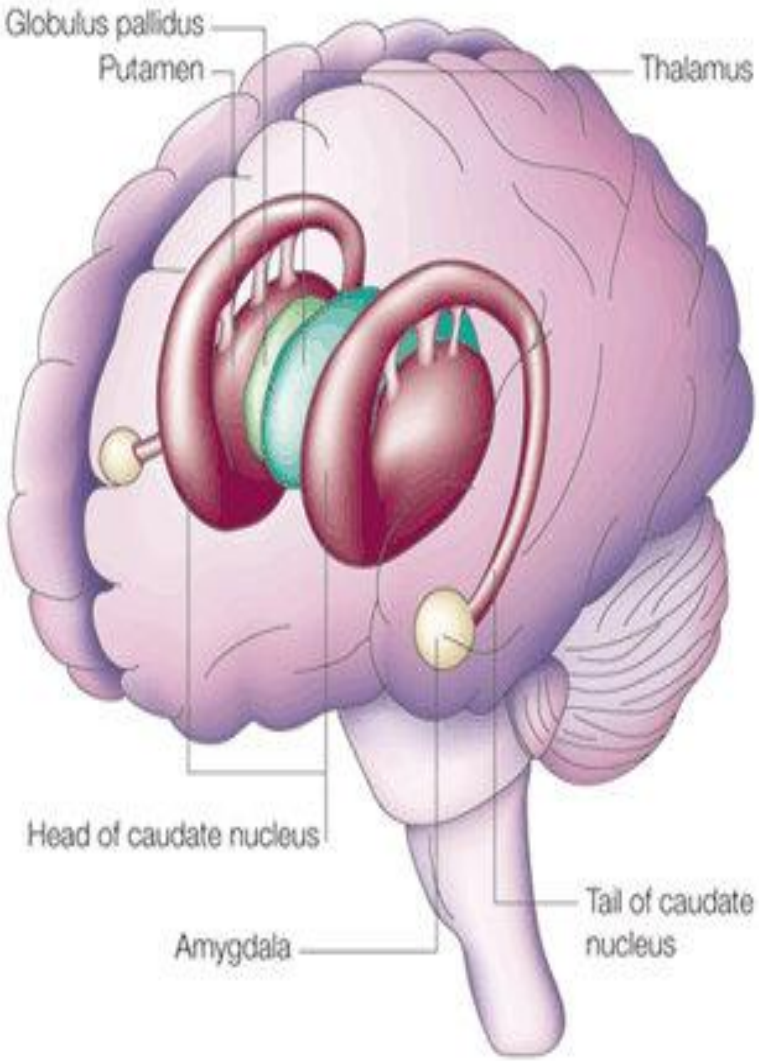
Major division	**Principal structures**
Forebrain (prosencéfalo)	Cerebral cortex, basal ganglia, limbic system (amygdala), Thalamus, hypothalamus.
Midbrain (mesencéfalo)	Tectum, tegmentum.
Hindbrain (rombencéfalo)	Cerebellum, pons. Medulla oblongata.



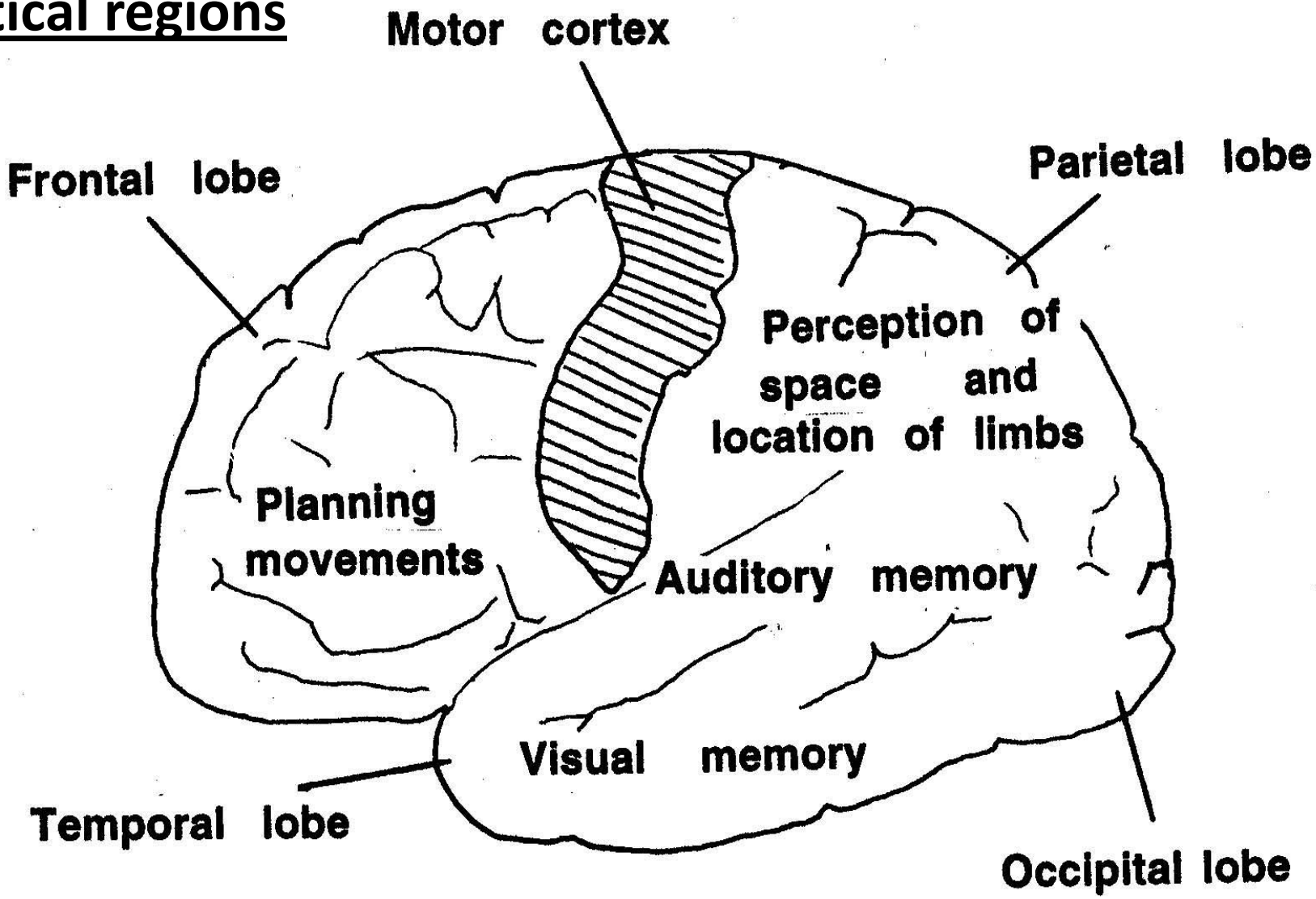
Differences in activity of the amygdala is probably the reason for differences in Temperament Score between Cattle

The AMYGDALA controls the strength of emotional responses; especially for fear and anger

It is part of the limbic system and is situated at the end of the hippocampus



Cortical regions



There are two parts to the cortex which are important in consciousness and emotions

the primary cortex
the association cortex

The primary cortex transmits signals to the association cortex where they are interpreted as sensations and emotions.

If the primary cortex is not working, the subject will be unconscious.

Typical effects of damage to the Association Cortex

Association cortex	**Typical effect of damage**
Visual	Unable to recognise objects by sight, but can recognise them by touch
Somatosensory	Difficulty in perceiving the shape of an object by touch alone
Auditory	Difficulty in perceiving or producing meaningful speech
Junction of visual, somatosensory + auditory association cortex	Difficulty in reading or writing

Emotions in animals

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Annoyed	Frustrated	Passionate
Anxious	Glad	Pity
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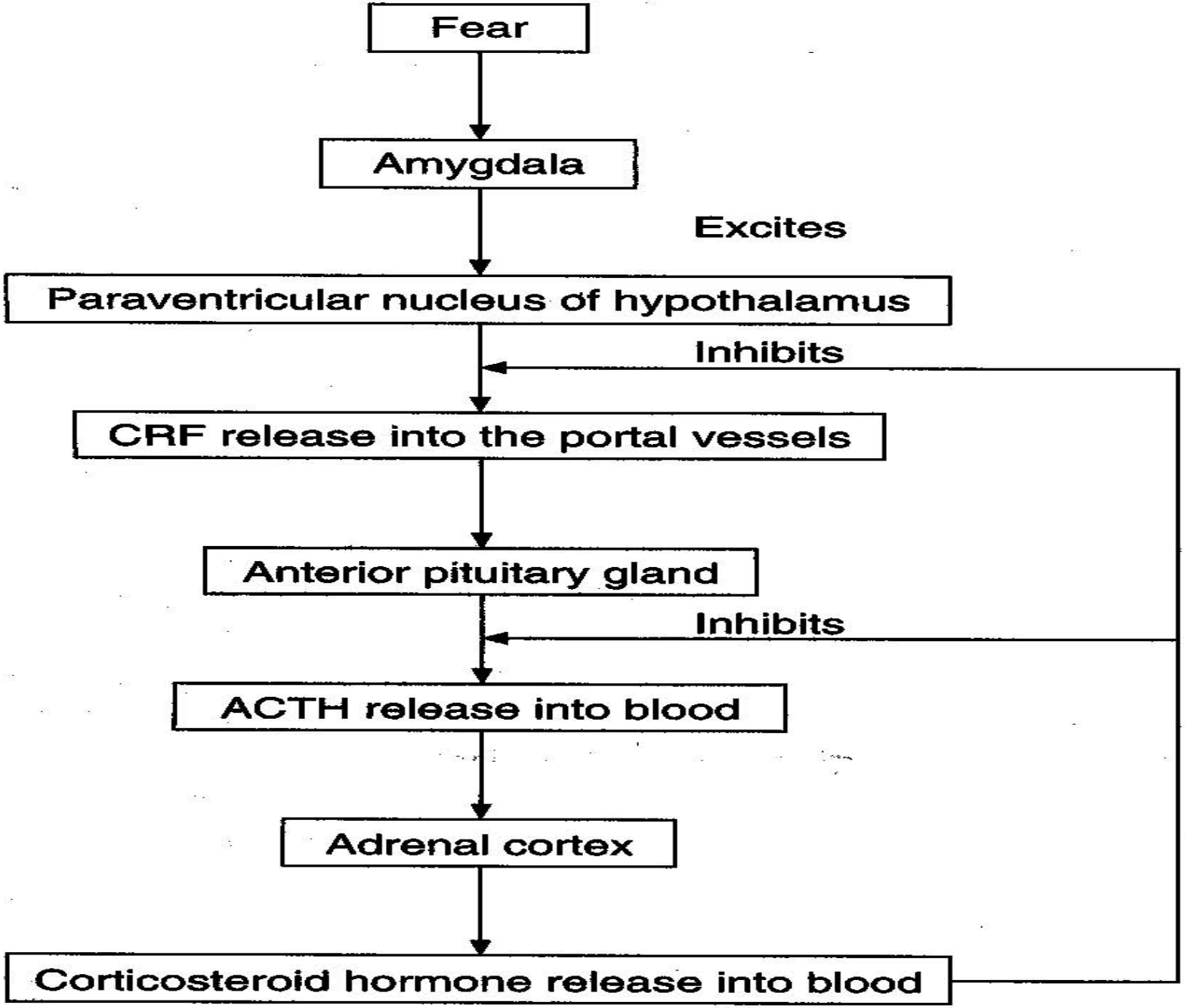
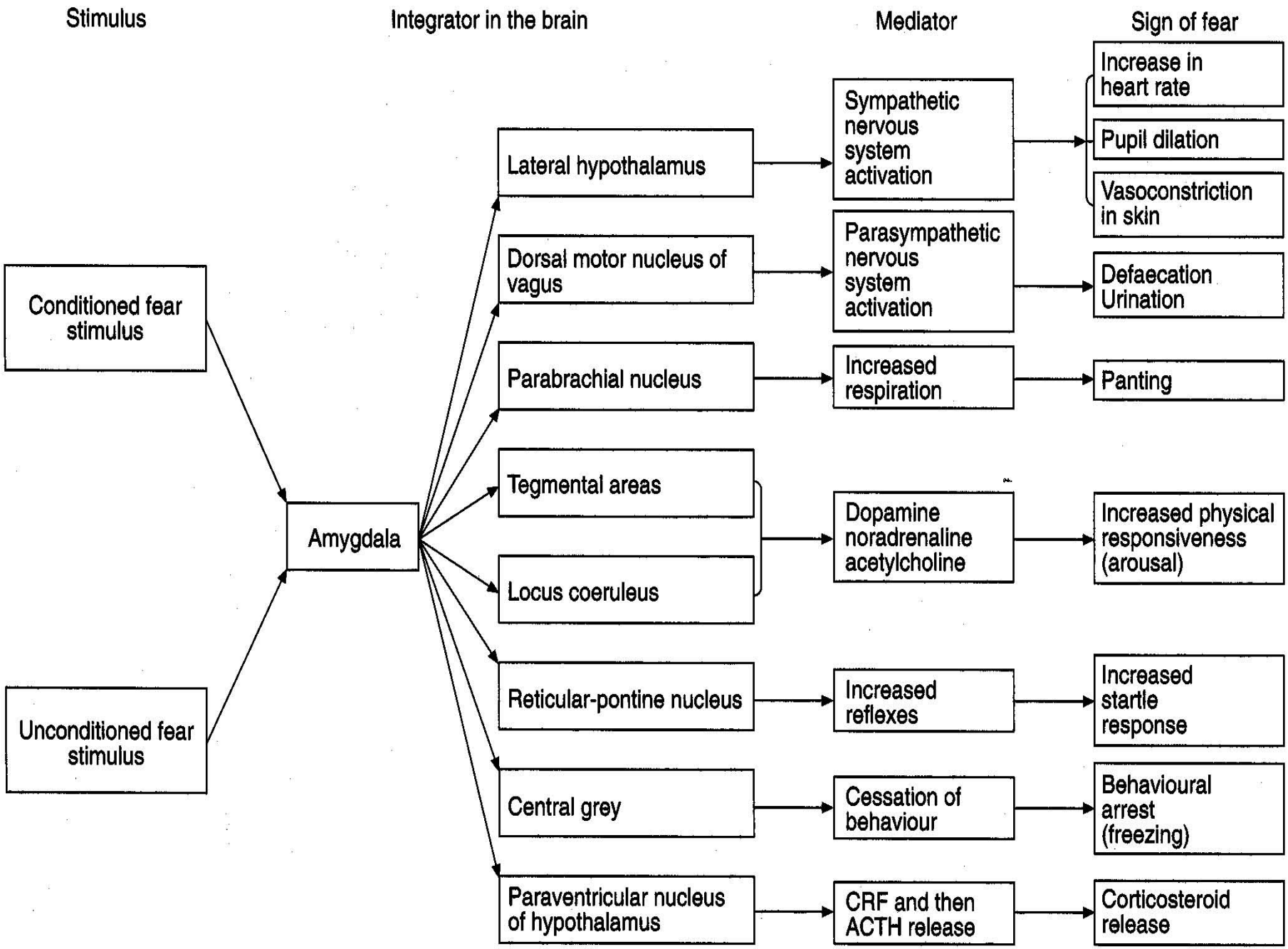
How can we measure these emotions ?

Types of Fear in Cattle

- ***Novel fear (shadow)***
- ***Innate fear (isolation)***
- ***Learned fear (electric fence)***
- ***Signs of fear in others (stampede)***


Fear
Amygdala
Excites
Paraventricular nucleus of hypothalamus
Inhibits
CRF release into the portal vessels
Anterior pituitary gland
Inhibits
ACTH release into blood
Adrenal cortex
Corticosteroid hormone release into blood


Stimulus
Integrator in the brain
Mediator
Sign of fear
Conditioned fear stimulus
Unconditioned fear stimulus
Amygdala
Lateral hypothalamus
Dorsal motor nucleus of vagus
Parabrachial nucleus
Tegmental areas
Locus coeruleus
Reticular-pontine nucleus
Central grey
Paraventricular nucleus of hypothalamus
Sympathetic nervous system activation
Parasympathetic nervous system activation
Increased respiration
Dopamine noradrenaline acetylcholine
Increased reflexes
Cessation of behaviour
CRF and then ACTH release
Increase in heart rate
Pupil dilation
Vasoconstriction in skin
Defaecation Urination
Panting
Increased physical responsiveness (arousal)
Increased startle response
Behavioural arrest (freezing)
Corticosteroid release

What is the main <u>startle response</u> in each of these species :-

Rat
Cattle
Sheep
Horse
Pig

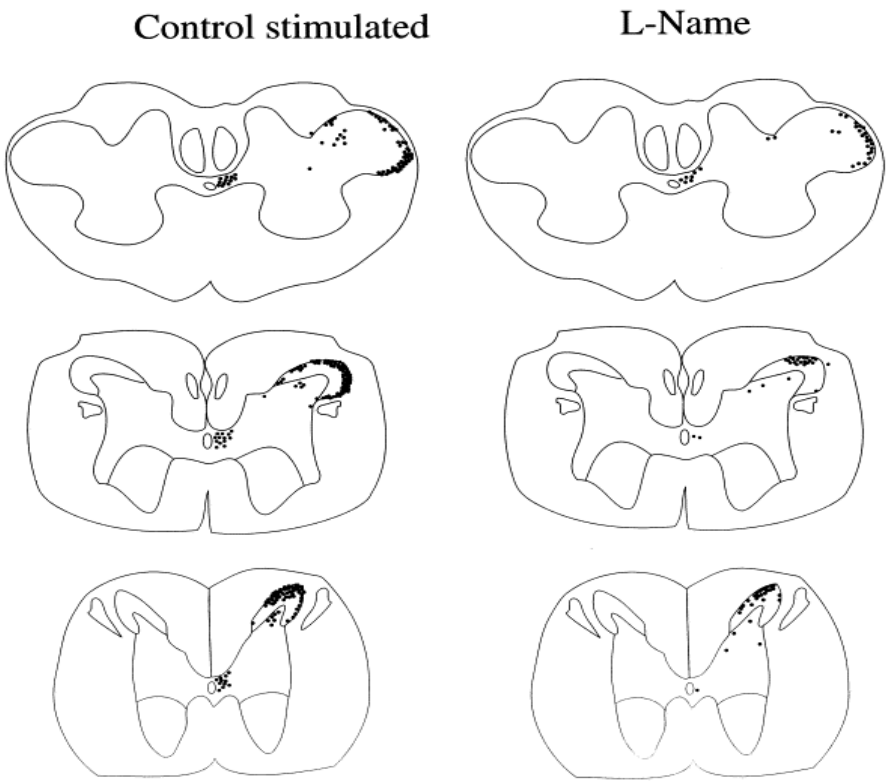
Can anything be measured in the brain when trying to assess stress ?

- ***c-fos protein expression***
- ***2-deoxy glucose uptake***
- ***functional MRI***
- ***brain pathology***

1. *C-fos protein expression in the brain*

C-fos is a protein that is rapidly induced (within 15 min of stimulation) and used as an indirect indicator of neuronal activity.

e.g. it can be used to confirm the activation of pathways involved in headache. Brain slices encompassing the trigemino-cervical nuclei are stained for c-fos protein.



5 examples of the effect of stress on c-fos protein expression

Stressor	**Brain region where c-fos is expressed**
Headache	Trigemino-cervical nuclei
Fear – isolation	Amygdala + nucleus accumbens
Fear – predator odour	Nucleus of stria terminalis
Physical restraint	Amygdala + dorsal hippocampus
Nausea	Nucleus of the solitary tract + area postrema

5 examples of pleasure associated with c-fos protein expression

Type of pleasure	Brain region where c-fos is expressed
Environmental enrichment	Hippocampal dentate gyrus
Social play	Medial prefrontal cortex to d. striatum
Learning birdsong	v. tegmental area + Substantia nigrosa
Suckling (infant)	Caudal region of nucleus of solitary tract
Sexual	Mesolimbic system (incl. ant. cingulate)

C-fos expression is a useful way of confirming the presence of a particular stress or pleasure, especially in situations where the cognitive effect is difficult to assess from behaviour alone.

C-fos expression has been used to quantify emotions in the brain.

Disadvantage:- The animal has to be killed to remove the brain, but in many studies this can be done on a small sub-sample.

Assessing c-fos along a pathway is more reliable than assessing it in one brain region alone.

2. Magnetic Resonance Imaging MRI

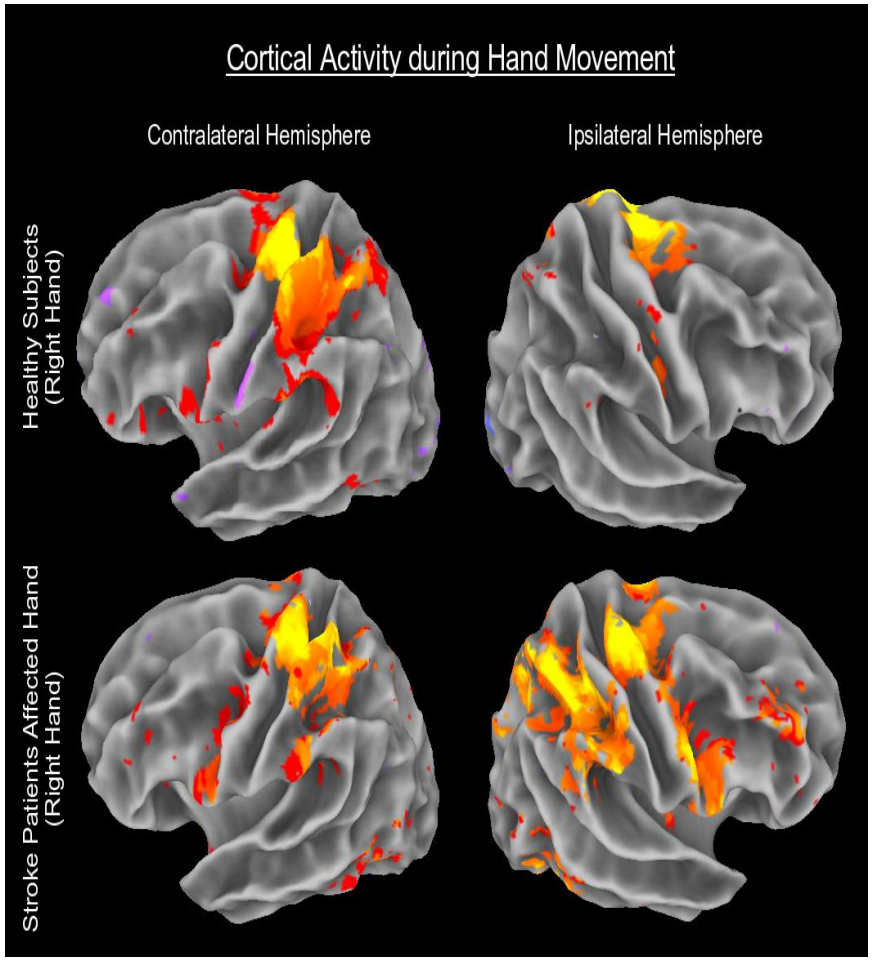
There are two types of MRI

MRI for pathology

Functional MRI (fMRI)

We will see some examples of MRI for pathology in the presentations on Stunning

Functional MRI



Measures brain activity from brain blood flow

5 examples of magnetic resonance images during pleasure

Type of pleasure	Brain region which is activated
Laughter from tickling	lat. hypothalamic-periaqueductal gray pathway
Massage	pregenual ant. cingulate cortex
Athletic success	ventral pallidum
Chocolate	dorsolateral prefrontal cortex
Music	left medial prefrontal cortex

Functional MRI has limited use in Animal Research because the animal has to keep still during the scanning.

However, based on studies in humans it has helped us understand which regions of the brain are involved in pleasant and unpleasant experiences. In the future it could form another basis for studying emotions in animals.

3. Brain pathology

Brain pathology can be useful in specific research topics.

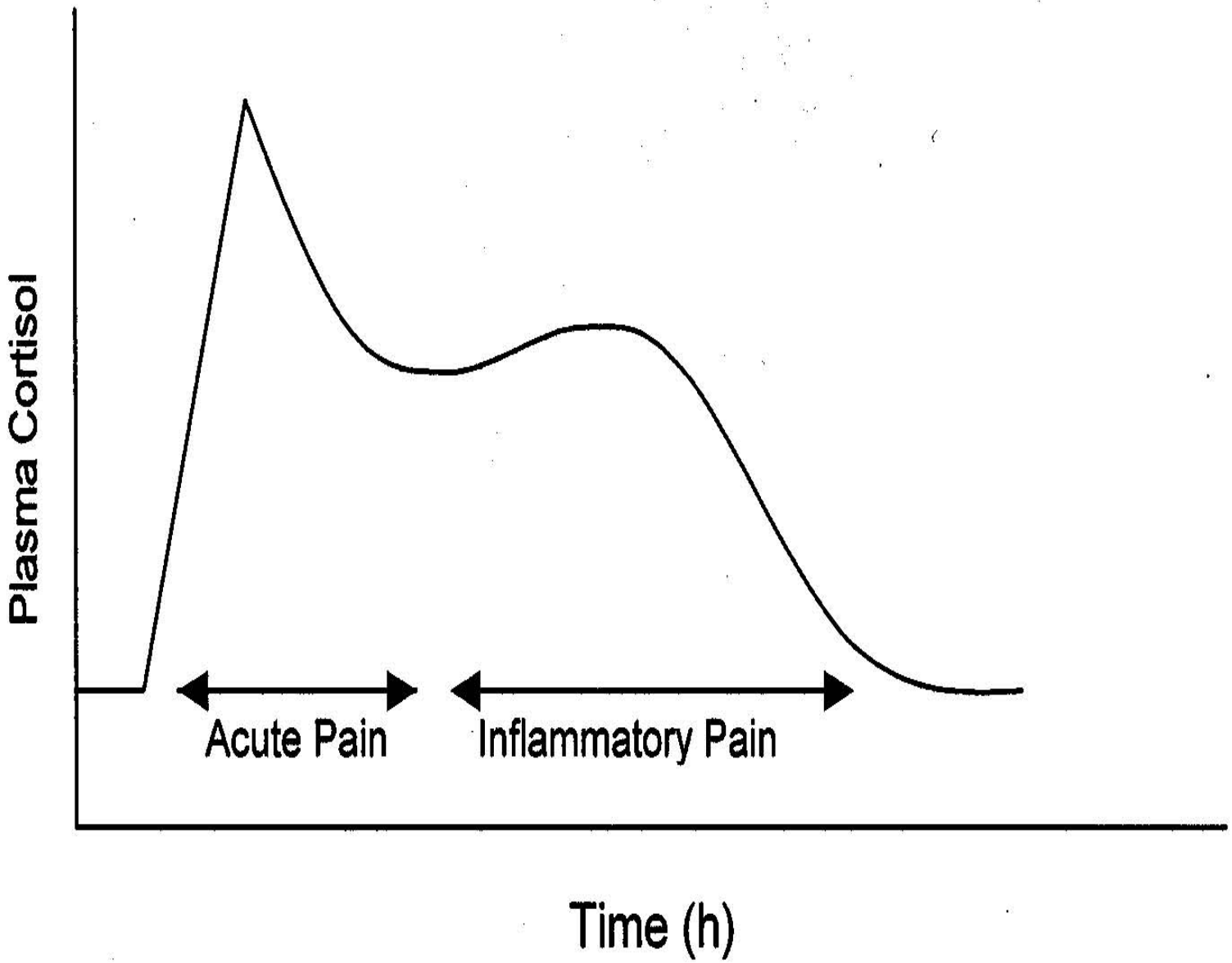
e.g. In Post Traumatic Stress Disorder (PTSD), stress cannot be assessed from adrenocortical hormones because the H-P-A axis is downregulated. Plasma cortisol levels are lower than normal. However, there is neurodegeneration and reduced size of the hippocampus, which can be assessed with brain imaging or postmortem pathology.

Some Stress Indicators in Livestock Welfare Research

Plasma cortisol has been one of the most valuable measures of stress in Livestock Welfare Research

Peak plasma cortisol responses in sheep µg / L

Shearing	**131**
Transport	**80**
Part shearing	**79**
Castration	**62**
Tail docking	**49**
Mustering	**48**
Restraint	**34**
Laparoscopy	**22**


Plasma Cortisol
Acute Pain
Inflammatory Pain
Time (h)

Measuring stress responses from activation of the Sympathetic Nervous System has also been valuable

The sympathetic nervous system can be divided into two branches, based on their pharmacological differences ……

Adrenergic effects (α and β)

α-Adrenergic effects	***β-Adrenergic effects***
Pupil dilatation	**Increase in heart rate**
Arteriole constriction	**Ateriole dilatation**
Contraction of gut sphincters	**Bronchodilation**
Reduced intestinal motility	**Reduced intestinal motility**
Contraction of bladder sphincter	**Trembling**
Piloerection and feather erection	**Muscle glycogenolysis**
Splenic contraction	**Lipolysis**
Liver glycogenolysis	
Salivary and lacrimal gland secretion	

More examples …..

- Haemorrhage Noradrenaline release
- Hypoglycaemia Adrenaline release
- Hypothermia Increase in noradrenaline release – decrease in adrenaline

HEAT	EXERCISE	EMOTION
p. Adrenaline ↗	p. Adrenaline ↗	p. Adrenaline ↗
p. Noradrenaline →	p. Noradrenaline ↗	p. Noradrenaline ↗
p. FFA →	p. FFA →	p. FFA ↗

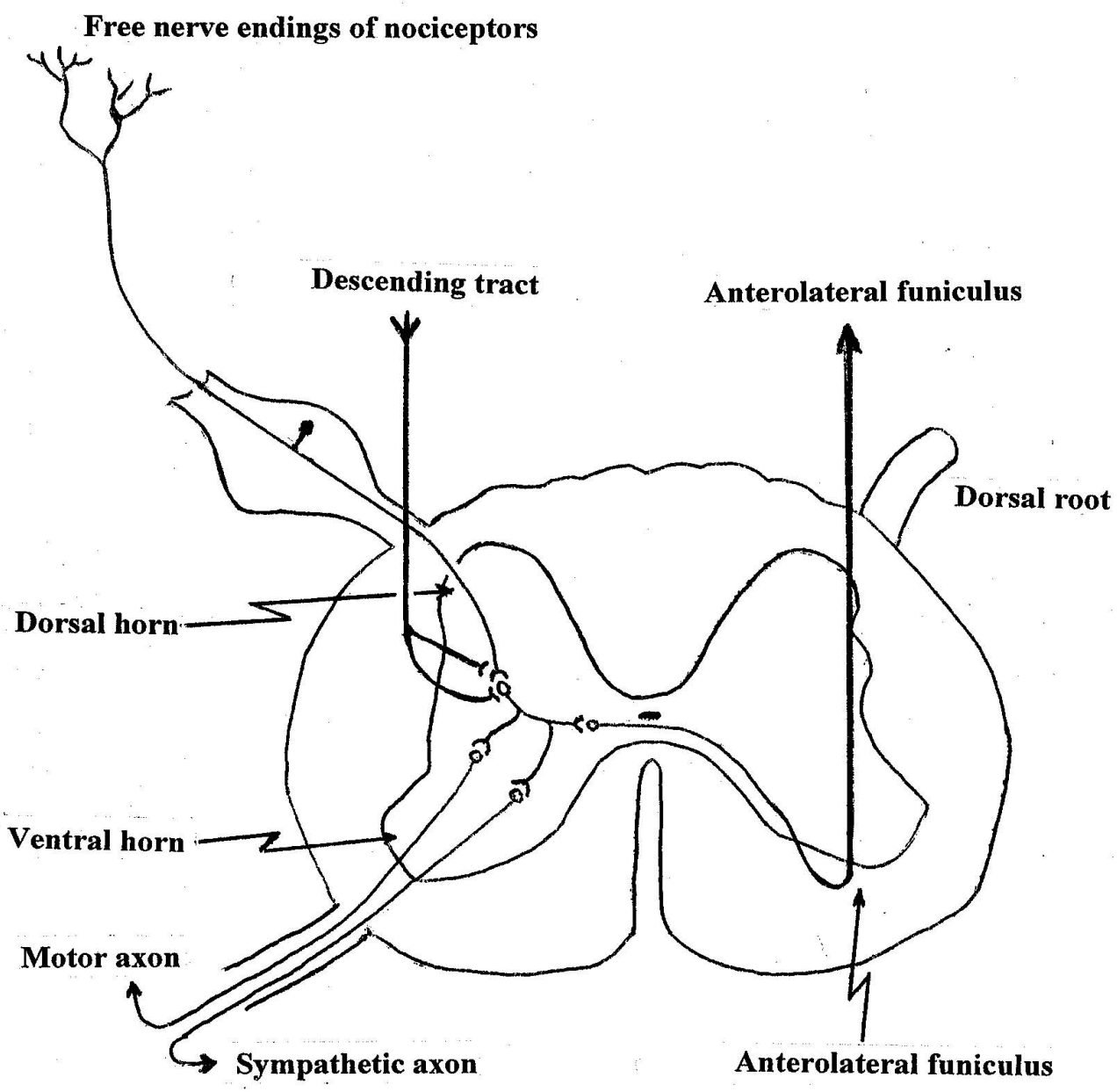
Potentially useful stress indicators

Stressor	*Indicator*
Fasting	**↓p. glucose, acetate (ruminants), liver glycogen**
	↑p. FFA, glycerol, urea, GLDH
Dehydration	
no feed	**↑p. protein**
with feed	**↑p. protein, osmolality, PCV**
Exercise	**↑p. PCV, adrenaline, noradrenaline, K^+, β endorphin, lactate (if anaerobic), CPK, meat pH_{ult} (chronic)**
Motion sickness	**↑p. cortisol, VIP**
Fear/alarm	**↑p. adrenaline, noradrenaline, ACTH, cortisol, glucagon, prolactin, β endorphin**
Heat	**↑p. ACTH, cortisol, adrenaline, β endorphin**
Cold	**↑p. noradrenaline, cortisol, PCV**

Is heart rate useful as a stress indicator ?

- **It is used clinically for assessing pain in cattle**
- **The rate of decline in heart rate after exercise is used for assessing physical fitness in horses and ponies**
- **Acute increases in heart rate indicate activation of the Sympathetic Nervous System (Stress), but if blood pressure also rises, heart rate quickly falls through reflex activation of the parasympathetic nervous system. If this does not happen, the animal can develop a crisis.**

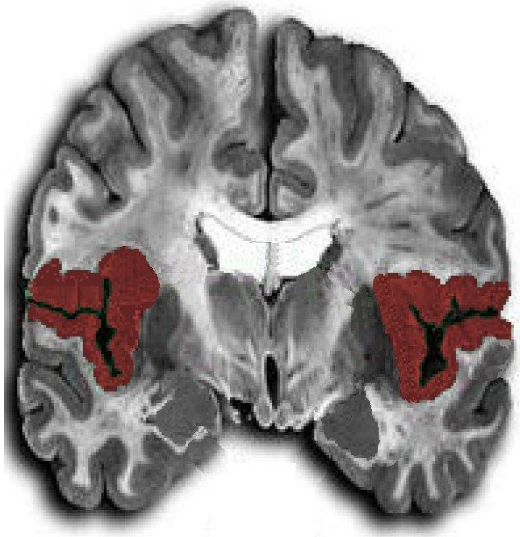
Painful stimulus



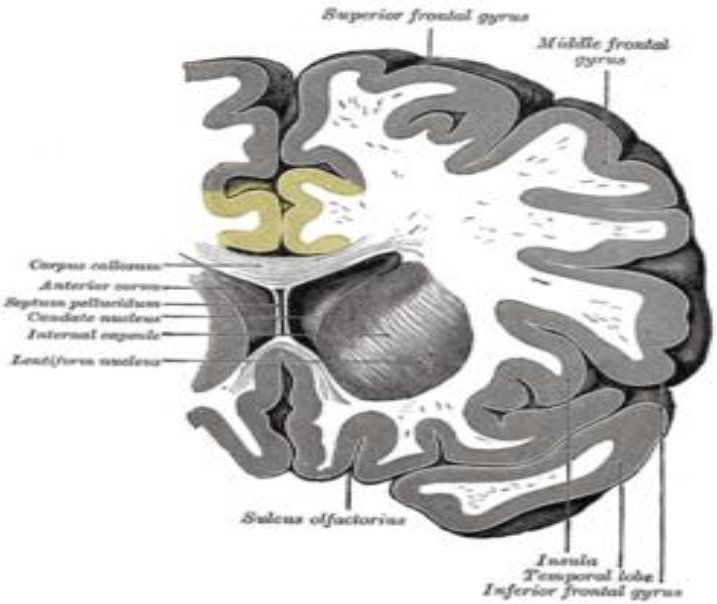
Pain

Signals which pass up the spinal cord enter the thalamus and branch to the cortex. The cortical regions mainly involved in pain are the

insular cortex



cingulate cortex

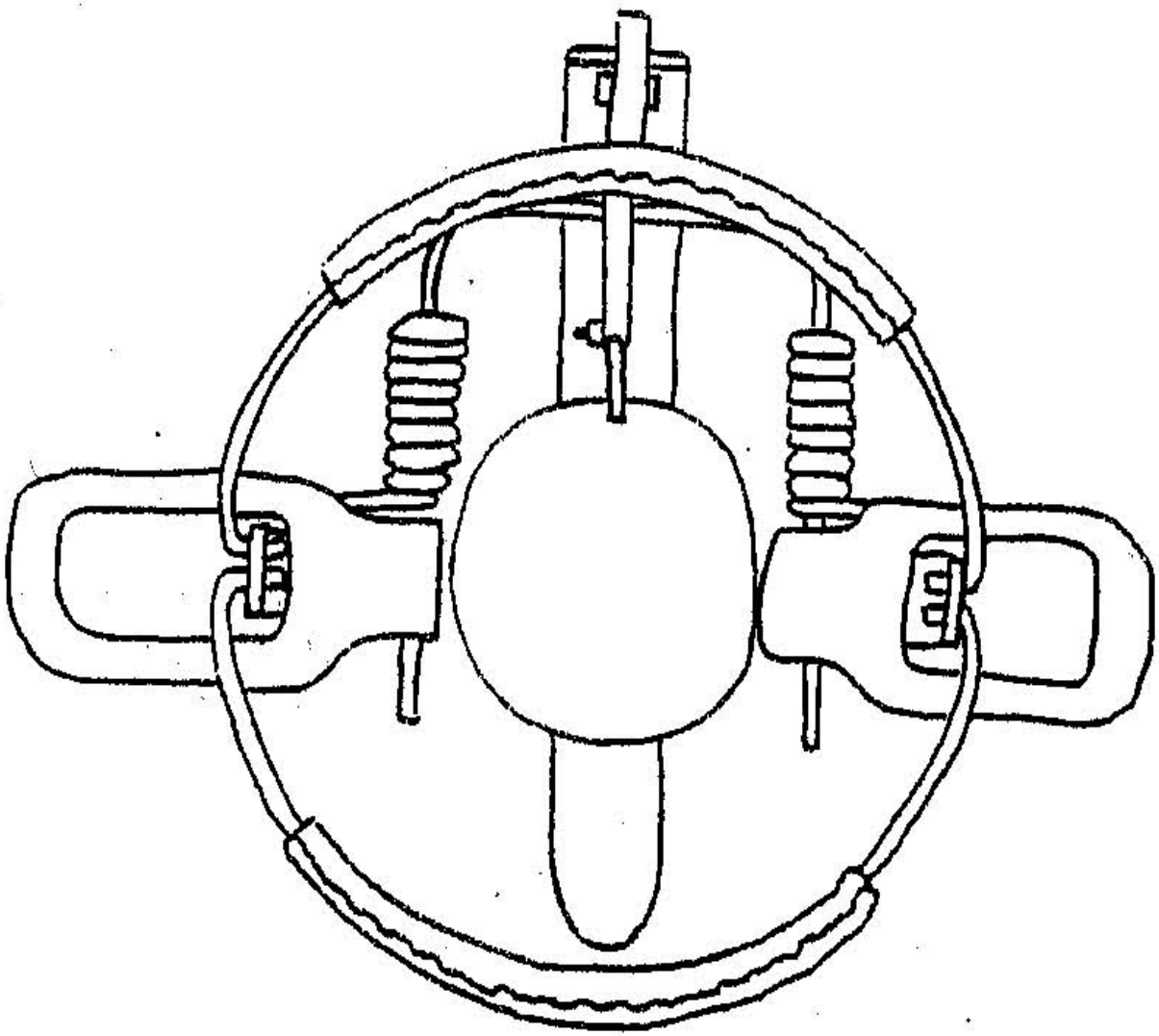


It is difficult to distinguish pain from other forms of distress when using physiological indicators alone.

However, superimposing an analgesia sub-treatment helps separate pain from the other forms of distress.

Using physiology to answer welfare questions …..

When leghold traps are used for foxes in Canada, should they be padded ?



Leg hold trap (padded)

Effects of leghold traps in red foxes

Plasma indicator	***Shot***	***Padded 2 h***	***Padded 8 h***	***Unpadded 8 h***
Log CPK IU/L	6.6[a]	6.9[a]	10.8[b]	10.1[b]
Phosphorus mg/dL	6.9[a]	7.4[a]	8.8[a]	11.3[b]
Bilirubin mg/dL	0.4[a]	0.4[a]	1.0[b]	1.0[b]
GGT IU/L	1.5[a]	1.0[a]	2.0[a]	23.4[b]
Protein g/dL	4.8[a]	5.3[a]	5.1[a]	5.3[a]

Choice of stress indicator depends on ...

- Type of stress
- Chronic or acute stress
- Diagnostic/relative
- Specific/general
- Half-life of the stress indicator
- Species

You are designing an experiment on long distance transport in cattle.

Which physiological indicators would you select ?

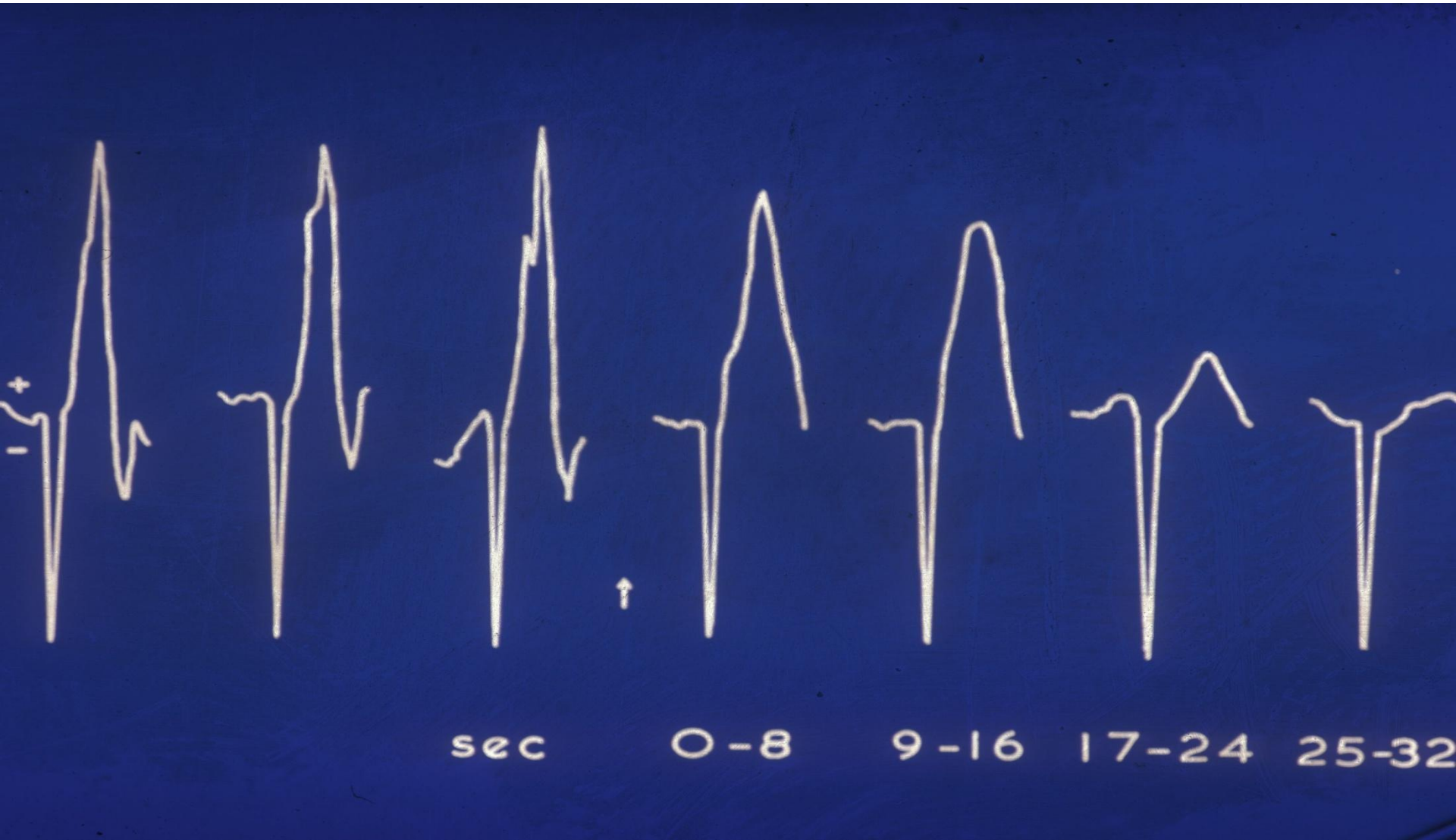
What would each parameter indicate ?

Comment on any limitations in each parameter.

Stress during Preslaughter Transport

- Water deprivation
- Feed deprivation
- Physical fatigue
- Sleep deprivation
- Social disruption
- Injuries
- Unpleasant motion
- Noise
- Heat stress
- Wind chill

Response tests



Select the indicator that is most appropriate for the question or problem being examined