Module 6

Assessing Animal Welfare – Physiological Measures

Lecture Notes

Slide 1:

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.

Slide 2:

This module will cover the relationship between welfare and the physiological responses, namely: the stress response; immune responses; neurobiological responses; and metabolic responses.

Slide 3:

In the absence of a direct measure of an animal's experience, we can use physiological measures of welfare because they are an indirect measure of an animal's experience. In other modules grossly identifiable welfare outcome measures will also be discussed.

This slide shows the sequence that makes up an animal's overall experience:

1. An animal gets sensory input from his/her environment – e.g. he or she sees, hears, smells and feels events around him/her.
2. His/her brain evaluates this sensory input in accordance with the his/her experience, genetics, etc.
3. The brain's evaluation may, then, create an emotion that is negative (e.g. anxiety on seeing the vet or seeing a new animal), or positive (e.g. pleasure because of anticipated grooming or play). Or there may be no new emotion because the sensory input is neutral (e.g. the sound of the farm tractor).
4. The body responds to the emotion with behavioural and physiological responses. These enable the animal to adapt to its environment and 'survive' in a very general sense.

The adaptive process enables 'homeostasis' and 'allostasis' (which will be defined in the next slide) to be maintained.

Slide 4:

The adaptive process enables the animal to do two things:

- The first is to maintain homeostasis, i.e. preserve the internal cellular environment which is essential for life. An example of this is the pH of the blood: in dogs and cats, this is between 7.35 and 7.45 – if the blood becomes more acid or more alkaline than this, it starts to affect organ function and may become life-threatening.
- The second result of the adaptive process is that the brain now has new information against which to interpret future sensory input, so that the animal can respond appropriately to events in future. Those events might be part of the life cycle, such as pregnancy and lactation, or they might be a completely novel challenge such as the first time a cow experiences milking by a machine. In all cases, the animal has an expanding repertoire of physiological and behavioural adaptations that enable him/her to keep functioning within his/her environment. The name for this broader adaptive process is allostasis.

Slide 5:

This slide lists the main categories of physiological response that may contribute to homeostasis and allostasis.

Note that the stress response is complex and it affects and interacts with all the other responses.

Slide 6:

The term ‘stress’ was originally developed in physics, to describe the amount of pressure that an object could withstand before breaking.

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). This is a relatively recent definition of stress, and it builds on much earlier scholarship from the 19th and 20th centuries. Briefly, those studies led to the understanding that, at the cellular level, the body functions best around a set point. This is the concept of homeostasis. For example, the acidity, or pH, of the blood must be maintained within narrow limits because vital organs cannot function otherwise.

The threats that cause the stress response may be internal (e.g. when a ruminant eats too many grains or concentrates and develops acidosis) or external (e.g. when a vet approaches an animal to examine him/her). The general term for the threats is ‘stressor’.

The stress response is complex, and our understanding of stress physiology continues to evolve. Hence, while different measures of the stress response are helpful in the assessment of welfare, it is not generally possible to rely on physiological measures of stress alone when you are assessing welfare.

Slide 7:

As you see on this slide, the stress response has two main elements. One is the autonomic nervous system. The other is the hypothalamo-pituitary adrenal axis (HPA axis).

The autonomic nervous system provides the automatic control and regulation of tissues and key organs including the heart, blood vessels, bowel, kidneys and bladder. Those are life-sustaining structures, and the autonomic nervous system helps them to function optimally. For this reason, it responds very quickly to a stressful situation and we will look at it first.

Slide 8:

When the brain evaluates an event, and gives rise to emotions, the animal responds by engaging with the situation (e.g. fighting, approaching), freezing, or running away. These immediate responses are governed by the part of the autonomic nervous system called the sympathetic adrenal medullary system which is illustrated here.

The sympathetic adrenal medullary system comprises the sympathetic nervous system – which is neural – and the adrenal medullary system – which is hormonal.

As you see in the diagram, the system originates in the brain (in the hypothalamus) and innervates different structures in the body including:

- the adrenal medulla
- smooth muscle e.g. in blood vessels; the bowel; the sphincter of the bladder
- cardiac muscle

Note that because these sympathetic neurons innervate smooth muscle within tissues, the effect of the neuronal discharge there is relatively targeted. This contrasts with the adrenal medulla: when the neurons stimulate that, it releases adrenaline and noradrenaline into the bloodstream which are circulated everywhere, so their effect is more generalised.

Slide 9:

SAM activation prepares the body for immediate action. For example:

- there is increased cardiac output, because the sympathetic arm of the SAM system stimulates the sinoatrial node of the heart. This increases heart rate and the strength of cardiac muscle contraction
- there is increased blood flow to the muscles, because of peripheral vasoconstriction and contraction of the spleen
- there is increased air intake, because of increased respiratory rate and relaxation of bronchioles in the lungs.

These responses prepare the animal to run away, if necessary. In addition, non-essential bodily activities are inhibited, e.g. smooth muscle contraction in the small bowel is inhibited, peristalsis is reduced or stops, as does the secretion of digestive juices. Salivation may also be reduced or stop.

- You will encounter many of these signs as a vet in practice. For example, when you hospitalise dogs and cats, some may not urinate or defecate for 24 hours or longer even though they have no physical problems in that area. Instead, it is because of the SAM stress response. Similarly, when you are doing a physical examination, you may check an animal's mouth for dryness to help you assess if he or she is dehydrated. An animal's mouth may feel dry simply because his/her brain has evaluated you as a threat, and the SAM system has been activated and is inhibiting salivation.

The same may be true for increased heart rates and respiratory rates.

Slide 10:

The SAM stress response is designed to enable an animal to escape an undesirable situation or to make the most of a favourable one (both being considered acute stressors). Normally, when that is complete, the stress response is brought to an end by the parasympathetic nervous system.

The parasympathetic nervous system balances the effects of the sympathetic adrenal medullary system. For example, when the stressful situation has passed, the parasympathetic nervous system:

- reduces cardiac output through direct innervation of the sino-atrial node by parasympathetic nerve fibres; this stimulates a decrease in heart rate (bradycardia)
- enhances gut motility and intestinal secretions so that digestive activity can resume.

Slide 11:

We have now seen the role of the SAM system in creating the stress response, and the role of the parasympathetic nervous system in ending the stress response.

You have also seen that the resulting signs of stress, like increased heart rate, can give you some insight into the animal's experience.

The other part of the stress response is the hypothalamo-pituitary-adrenal (HPA) axis. Next we will look at that, and then review what happens within both the HPA axis and SAM system if the animal is exposed to stressors chronically.

Slide 12:

The diagram here shows the chain of events in the HPA axis when the brain evaluates an event as threatening, attractive, etc.

- The resulting emotion causes the hypothalamus to release corticotropic releasing hormone (CRH).
- The CRH causes the pituitary gland to release adrenocorticotropic hormone (ACTH). You can see that the pituitary lies cranial to the hypothalamus.
- The ACTH enters the bloodstream and, on reaching the adrenal cortex, stimulates the release of glucocorticoids into the blood. In mammals and fish, the most common glucocorticoid is cortisol; in birds it is cortisone. In this lecture, we will refer to cortisol.

Note that cortisol exerts negative feedback on the release of CRH from the hypothalamus and ACTH from the anterior pituitary.

Slide 13:

Cortisol serves many functions in the body, helping it to cope with stressors. In the face of acute stress, the HPA axis is responsible for mobilising energy stores in the short term, so that the animal can run away from or fight off any threats, and therefore survive.

Glucocorticoids stimulate glycogenolysis in the liver and suppress insulin secretion. This increases the breakdown of glycogen to glucose and increases glucose levels in the blood. The raised glucose levels enable the animal to act rapidly to deal with the stressor e.g. by running away or fighting.

As we saw in the diagram, the HPA axis is normally a self-regulating system: the glucocorticoids provide negative feedback to the pituitary and the hypothalamus, thereby indirectly to the adrenal glands. This modulates the stress response as the animal's situation improves.

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The HPA axis does not respond as rapidly to changes in welfare as the sympatho-adrenal medullary system. For example, in most species plasma glucocorticoids become elevated within 2-10 minutes after exposure to an adverse event. In contrast, the sympathetic adrenal medullary response is almost instantaneous, e.g. increased heart rate.

Slide 15:

This slide summarises the stress response.

There is sensory input to the brain. The brain evaluates it and, if appropriate based on past experience, the stress response is activated. That is, there is communication with the hypothalamus, which:

- activates the SAM system, resulting in adrenaline and noradrenaline release from the adrenal medulla, and in stimulation or inhibition of smooth muscle in structures such as blood vessels and the gut and in stimulation of cardiac muscle; and
- releases CRH which stimulates the anterior pituitary to release ACTH, which causes the adrenal cortex to release cortisol.

Our job as vets is to help our clients care for animals in a way that minimises noxious sensory input, so that the stress response is not activated unduly.

Slide 16:

Note that many events in an animal's life can cause a stress response. The experience of stress is not automatically a welfare concern, because stress may also enable the animal to make the most of pleasant, positive experiences that enhance survival as well as avoid unpleasant negative experiences that may threaten survival.

Therefore, exposing animals to stressors only becomes a concern when the animal's body is stressed beyond the capacity of his/her emotional and physical systems to cope. This causes suffering, which may lead to adverse alterations in the body tissues which eventually becomes apparent as disease or in reduced production. In that case, the stress response is maladaptive. It has become maladaptive because animals are kept in conditions that cause ongoing stress and which they cannot avoid or adapt to. In that situation, the stressors become an ethical and a practical issue.

Slide 17:

The slide shows some examples of stressors and the feelings that result from the sensory input. The division into physical (environmental), physiological and mental stressors is loose, as many of the individual stressors overlap.

As vets, we are especially used to thinking about the first two groups, which concern clinical conditions and associated clinical signs of pain, nausea, weakness, etc. However, other important stressors are mental. Looking at the left-hand column, under physical stressors, confinement is an inherent physical characteristic of many husbandry systems. The advantage of animals being confined to us is that it enables us to monitor their welfare closely. However, confinement can be a significant cause of ongoing stress. For example, it may involve inflexible social groups (e.g. growing pigs of similar age and size) where it is difficult for a clear social order to be established. Another stressful aspect of confinement may be the housing itself, e.g. the flooring or the temperature. We will look more closely at this in other lectures about livestock.

Over-exertion is another stressor – where animals are pushed to produce beyond their metabolic or physical limits. This is seen in overloaded working animals or over-producing dairy cows.

Moving down to mental stressors, behavioural deprivation is another chronic stressor. This is related to confinement, such as when laboratory animals or pet birds are kept in small, barren cages without appropriate stimulation such as the presence of conspecifics. Another example of behavioural deprivation is found in sows and in the hens who produce broilers: because of the need for cheap human food, pigs and broiler birds have been bred to grow quickly and therefore to have large appetites. However, the breeding stock cannot be allowed to fulfil their appetite because it would be bad for their health and expensive. Consequently, they are relatively underfed. If their housing does not allow them to express food-seeking behaviour in response to feelings of hunger (e.g. if sows have no material to root in), the unrelieved hunger can be a chronic stressor.

Social stress is another chronic stressor, e.g. with overcrowding in pens, or mixing of unfamiliar animals, e.g. at markets.

Slide 18:

When an animal experiences negative stressors for a prolonged period, this can create pre-pathological states which can eventually become overt, i.e. manifest themselves in disease. This slide shows some examples of this.

- The first example is reduced immunity. Chronic exposure to glucocorticoids reduces the production of macrophages and monocytes and reduces circulating numbers of lymphocytes. This can make animals more susceptible to infection. An example of this is in young calves that are shipped to market, mixed with other calves during or after the sale, and then shipped on to their new farm. The continuous exposure to hours of stressors (transport, noise, mixing, novelty) makes them more likely to develop respiratory and enteric infections.
- Another pathology that can arise from chronic stress is hypertension (raised blood pressure). Signs of this may not be clinically obvious in the live animal, but can be detected post-mortem e.g. in the kidneys. Note that hypertension is well known as a sign of stress in humans.
- Another post-mortem finding in animals that are under ongoing stress may be an enlarged adrenal gland.
- Other signs of chronic stress that you can detect in the live animal—and that the owner may want you to investigate as a vet—are lack of growth and weight loss. Whilst these can also be caused by malnutrition and parasitism as a stress response they may be caused by the catabolic effect of cortisol and adrenaline / noradrenaline in response to other stressors such as bullying by other animals.
- Reduced fertility can also be a sign of stress.

- Chronic exposure to cortisol can result in ulcers in the stomach and small intestine because the cortisol causes cell death with thinning of the mucosal lining.

These examples illustrate how the stress response may contribute to reduced welfare in terms of physical functioning.

Slide 19:

We will now look more closely at how you might measure the signs of the stress response.

This slide gives examples of measures of the **acute** stress response. They are some of the directly detectable results of the SAM and HPA systems on the body, as well as the hormones concerned.

Note that these are only a few examples of measures of the stress response. There are at least 40 others, which reflects that the stress response is mediated through the circulation (via cortisol, adrenaline and noradrenaline) and through the autonomic nervous system which is very wide-ranging. Therefore the stress response produces measurable effects in many body systems and functions. However, today, we are confining ourselves to examples that are relevant to us as a vet doing clinical work in the field.

The column on the left gives the indices of the stress response.

The middle column indicates which part of the stress response system is involved—SAM for sympathetic adrenal medullary system, and HPA for hypothalamic pituitary adrenal axis.

The column on the right indicates some of the methods you could use to measure that index of the stress response in the field. You can see that you could measure many of them directly, e.g. you measure heart rate using a stethoscope, or by palpating the thorax. You can measure respiratory rate from a distance. Measuring blood pressure requires restraint and can then be done using a cuff on the limb, similar to human medicine.

Other parameters like glucocorticoids require laboratory measurement using samples of body fluids such as blood. These are more invasive measures and the laboratory analysis necessitates a delay before you can see the data: therefore you cannot use those measures to obtain an immediate (patient side) assessment of an animal's welfare.

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When we consider physiological measures of chronic stress in animals under our care, the HPA axis generally provides more useful measures of chronic stress than the SAM system. This slide gives examples of measures you might use.

- Glucocorticoids are eliminated in the urine where they are not subject to acute fluctuations. Accordingly, urinary cortisol can provide an index of stress levels over recent hours in many species.
- Cortisol is also found in saliva and milk and, again, the levels here are not affected by acute fluctuations in the blood and so can reflect recent stress. Samples of urine, milk and saliva are relatively easy to obtain, the sampling is generally non-invasive which may confer strong practical and welfare linked advantages.

The second way in which the effect of chronic stressors may be assessed is via ACTH:

- Ongoing stress can increase the responsiveness of the adrenal cortex to ACTH: therefore, after injecting ACTH, higher than normal levels of plasma cortisol may be recorded.

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We will now look at some examples of how researchers have looked at acute and chronic stress in relation to welfare.

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The first example concerns wild vicuñas in Argentina. The vicuña is a member of the llama family. They are ruminants; their hair is very soft, and it is highly desired for making clothes. Vicuñas may be farmed but many live wild in the Andes and are captured for shearing.

The study followed three methods of vicuña capture during ~2 years, involving almost 500 animals. Some animals were rounded up in the traditional way, by people on foot; others were rounded up using trucks and bikes; while others were rounded up by people on foot and vehicles together.

The authors compared the effect of these three different methods by using several measures of the acute stress response:

- Direct observations: heart rate, rectal temperature and respiratory rate. These all reflect activation of the SAM system.
- Blood samples: measures recorded included those which reflect the HPA axis and SAM system. The measures were: glucose, cortisol and two muscle enzymes. Muscle enzymes are released when an animal exerts his/her muscles considerably by fighting or running fast or for long distances, which happens in association with the activation of the SAM system.

Note that the authors did not measure adrenaline and noradrenaline. These markers have a very short half-life, meaning that their levels in a blood sample will decrease very quickly, often before you have a chance to measure them. The authors also measured behavioural responses such as fighting and vocalising.

Slide 23:

It was a complex study and we will look at just some of the results in brief.

There were sex differences in cortisol related to the capture method:

- Females who were captured by people on foot showed higher levels of cortisol compared with females who were captured by people and vehicles.
- By contrast, males captured by people on foot had lower levels of cortisol compared with males who were captured by people and vehicles together.

Cortisol levels also showed an effect of captivity over time:

- Animals were held in corrals before and after shearing, so that they could all be released safely together. Cortisol levels increased during the first 2 hours of captivity and remained high in both sexes over the next 24 hours of captivity. The animals' vigilance and alertness also remained high. All of these responses reflect activation of the HPA and SAM systems.

A third finding was that the longer that animals spent in captivity after shearing, the higher the muscle enzyme creatine kinase tended to be:

- In females, this was a statistically significant relationship. That may suggest a possible pre-pathological state, whereby the females could be at risk of developing stress-related inflammation of the muscles (myopathy) if they were held captive for a long time.

Based on these results, and on others not shown on the slide, the authors concluded that capturing vicuňas on foot was the best approach, so long as the animals were not kept long in captivity after that.

Slide 24:

Our next example concerns dogs who had been put into a re-homing shelter in the UK. It investigated whether kennelling was a chronic stressor.

Briefly, the researchers measured the urinary cortisol levels in 26 dogs who had entered the kennel either because their owners had given them up, because they were strays, or because they had been adopted from the kennel and the adoption had not worked out. The researchers also looked at behavioural measures of stress in the dogs.

The results indicated that dogs who had had no prior experience of kennelling showed higher levels of urinary cortisol over time than the group with strays and dogs who had experienced the kennel before. This illustrates the point discussed earlier about how prior experience can affect the stress response.

Broadly, the results suggested that the pet dogs were stressed by being left at the shelter. The results also suggested that stray dogs and dogs who already know the facility may adapt more readily and experience less stress.

Slide 25:

Our next example of using physiological measures of the stress response to assess the welfare of animals in field conditions concerns the capture of wild sardines.

This Portuguese study examined plasma cortisol (and other measures) in sardines who were captured during commercial fishing in the Atlantic Ocean. The sardines were captured with a purse seine (that is, a large circular net used to create a 'wall' around a group of fish; once enough fish have been encircled, the 'wall' is pulled tight, like a purse, so that the fish cannot escape). Fish may be encircled by the net, under increasingly crowded conditions, for one to two hours, or perhaps even longer.

The authors sampled 174 fish, over 10 fishing trips, and assessed the effect of the time that the fish spent in the net on welfare using measures such as cortisol. There was a marked effect of time on cortisol levels: the mean level of cortisol after fish had been in the net for one hour was significantly elevated.

However, based on several other physiological measures, the authors concluded that the acute stress response to being netted for up to two hours was not altogether bad: it was adaptive in some ways, but maladaptive in others.

This result illustrates how important it is not to rely on cortisol or other measures of the stress response in isolation, when making an assessment of welfare.

Slide 26:

Our final examples of using the stress response as a measure of animal welfare concern the castration of domestic animals.

In many countries around the world, the castration of animals such as piglets, lambs, calves and horses has typically been conducted without analgesia or anaesthesia. Since the 1980s, however, a growing number of studies have been carried out on the welfare implications of castrating animals like this. The studies have used acute and chronic measures of stress, most notably cortisol, as well as behavioural measures of pain. Overall, the studies indicate that castrating farm animals without analgesia is painful, regardless of their age or the method used.

Before we look at some of those, let us apply the basic framework of the animal's experience to predict the expected stress response in the case of castration. Moving down the slide:

- we know that castration by any method causes tissue damage. This results in the release of inflammatory substances locally, which activates the pain pathway. Therefore, castration without analgesia must be evaluated by the brain as noxious sensory input, as you see in bold on the slide.
- we would predict this to create feelings and emotions such as pain and fear. These would in turn cause a range of bodily responses, including pain behaviours and the stress response.
- we would therefore expect measures such as cortisol to be greatly elevated in animals who are castrated without analgesia.

Slide 27:

Here are some examples.

The castration of piglets

- Piglets are normally castrated in the first days of life while still suckling, and without analgesia. A French study of a total of 84 piglets found significant differences in a range of behaviours during the first four days after castration (without analgesia), in comparison with piglets who had had a 'sham castration' (by restraining them and handling the scrotum and testes), and piglets who had not been disturbed at all.
- However, the authors did not find a significant difference in the stress response between the groups: urinary corticosteroids and urinary catecholamines were similar in all groups at the time of castration and during the following days. This contradicts what we predicted on the previous slide. Several other studies have all indicated specific behavioural signs of pain (e.g. vocalisation frequency and pattern) that are markedly different in piglets who are castrated without analgesia and those who are not castrated. Therefore there is no doubt that the castration of piglets without analgesia is painful, and reduces their welfare for some time, but if we only relied on levels of urinary cortisol to measure stress and pain, we might be misled.

Slide 28:

The castration of dairy calves

This study supports what we hypothesised about castration using our framework of the animals' experience: cortisol levels were increased by all methods of castration, and systemic pain relief prevented the cortisol increase. This suggests that castration without analgesia is stressful.

Dairy calves are typically castrated when ~ 6 days of age, or when they are older—up to ~ 6 months of age. Some methods are surgical, some are not (e.g. rubber ring, latex band). A study in New Zealand compared these methods in 190 calves. The authors used blood cortisol to assess differences between the methods when the calves had no analgesia, local anaesthesia, or local anaesthesia plus ketoprofen (a non-steroidal anti-inflammatory drug).

The authors controlled the study in several ways:

- by simulating castration in some calves, by restraining them and handling the scrotum and testes ('sham castration')
- by using ACTH stimulation in some of those control calves, to provide a maximum cortisol level against which to compare their other results

They also controlled for any stress that injecting local anaesthetic might cause on its own.

Like the vicuña study earlier, this was a complex piece of research. Briefly:

- Handling the calves' genitals and injecting lignocaine did not, of themselves, cause significant changes in cortisol.
- When analgesia was not used, all the castrated calves had a marked cortisol response compared to calves in the control groups. Depending on the method of castration, the mean ranged from 56 to 101 nmol /L; for comparison, the ACTH group had a mean level of 99 nmol /L.
- When analgesia was not used, clamp castration caused the smallest cortisol response. That was reduced or eliminated by local anaesthetic or local anaesthetic plus ketoprofen. However, this method of castration was not always successful.
- Local anaesthetic alone did not reduce the cortisol response to surgical castration. However, when ketoprofen was given as well, this eliminated the cortisol response.

This study supports what we hypothesised about castration using our framework of the animals' experience: cortisol levels were increased by all methods of castration, and systemic pain relief prevented the cortisol increase. This suggests that castration without analgesia is stressful.

Slide 29:

The castration of water buffalo

Similar results were found in a study from Brazil that compared the effect of surgical castration and clamp castration (Burdizzo method) on the welfare of water buffalo. A control group was restrained and handled as if for castration, but was not castrated.

The researchers used several physiological and behavioural measures of welfare, including plasma cortisol and rectal temperature. Surgical and clamp castration affected cortisol levels for six and nine hours, respectively, after the procedure. As with the New Zealand study, which was carried out on younger calves of a different species, the cortisol response to clamp castration was somewhat lower than with surgical castration.

After 48 hours, the surgical group of water buffalo castrates still showed marked pain on palpation, whereas the clamp group no longer showed detectable pain on palpation.

The authors concluded that the clamp method was preferable to the surgical method, under conditions where no analgesia is used.

Slide 30:

We have now seen several examples of how cortisol has been used to help assess the effect of routine procedures on animals' welfare.

You can see that, when used with other measures, cortisol can be informative. However, there are many limitations in using adrenal activity measures to evaluate an animal's experience.

- The first limitation is that the measures do not tell us if the animal's brain has evaluated his/her situation as being positive or negative. In the examples we have just looked at, the logical expectation was that the various situations might be negative experiences for the animals. It would seem unlikely that the situations were positive experiences. However, if you did want to evaluate a putative positive situation, increases in cortisol would not tell you if an animal was having the positive experience you hoped for.
- The second, related, limitation is that some events cause the animal to become more active without being stressed significantly, i.e. the events are neutral with respect to their feelings and their welfare. For example, your potentially positive situation might cause an animal to be more alert, which would affect heart rate, blood glucose and cortisol, for example, but not affect his/her feelings in a positive or negative way.
- The third limitation of many measures of the stress response is that the act of measuring can itself be stressful for the animal. For example, animals have to be restrained for blood sampling. The restraint itself can produce an acute stress response: accordingly, if blood glucose is elevated, you cannot tell if this was because of an underlying stressor, or because taking the blood was stressful. In the studies of calf castration and piglet castration, this was controlled for.
- Even the presence of a human being can be stressful and affect results. For example, respiratory rate can often be measured from a distance. However, your presence while you make the measurement may distress the animal and cause an increase in respiration for that reason alone.
- To eliminate these effects on your measures, you may be able to use implanted computerised devices to obtain your data. This removes the stress that 'hands on' measurement may cause. However, it still does not tell you if an increase in heart rate, for example, is simply the result of an increase in activity or whether it reflects a situation of decreased or increased welfare. Also, implanting devices is invasive, and the devices are expensive.

You can see from this that measuring the stress response cannot tell us everything that we want to know about an animal's experience. You generally also need behavioural measures and you need to know how well the physiological measures mirror the behavioural measures. For example, in the case of the water buffalo, cortisol levels 48 hours after castration were not dramatically raised, but animals who had been castrated surgically still showed marked signs of pain on palpation.

Slide 31:

Other limitations of using adrenal activity measures as an index of animal welfare are shown on this slide. They include:

- Individual differences – normal values can vary quite widely between individual animals within a breed or species, and it is important to have a well-established normal range.
- The animal's sex can also affect their normal measurements and their responses to stress. The vicuňa study illustrated this with males showing higher cortisol levels than females when captured with vehicles.
- Prior experience affects the stress response – the dog study illustrated how previous experience of kennelling was associated with lower urinary cortisol, compared to dogs that had never been kennelled before.
- Related to this, the type and degree of response can vary between individuals exposed to the same situation, which is perhaps an innate, genetic difference between individuals.
  - For example, in a study of stress in laboratory rats, catecholamine levels in rats were consistently high in some animals ('high responders') and low in others ('low responders') during immobilisation aged three months and 16 months.

A more recent study, of Zebu cows in Mexico, showed that cows with low social status in their herd showed significantly lower cortisol levels than more high-ranking animals when repeatedly exposed to the stress of going through a chute. It seemed from this that low-ranking cows had a passive strategy that allowed them to have better control over the stressful event. In contrast, the high-ranking cows responded with higher cortisol levels associated with an active strategy for controlling stress – in this case, going through the chute first.

Slide 32:

Another limitation of adrenal activity measures is that physiological responses to ongoing stress may not continue in a linear and proportional way.

For example, repeated exposure to the same stimulus can lead to a decrease in catecholamine production on subsequent exposure to the stimulus. This is known as 'habituation' of the adrenal response, and it has adaptive value. However, sometimes repeated exposure to the same stimulus may not result in habituation of the adrenal response, and the response may be more marked, especially if the animal is then exposed to a different stimulus. This is known as 'sensitisation' and it may also be adaptive. The stress response may depend on the exact nature of the stressor.

For example:

- when weaned pigs were exposed to noise stress every day, they showed strong responses to ACTH stimulation during the first week only, and their adrenal glands were increased in size. This suggests that their response to the stress habituated because of constant exposure

- in contrast, pigs who were only exposed to noise stress three times a week continued to show strong responses to ACTH; this suggest that they did not get used to the stress of the noise and may have become sensitised by it (Mormède et al., 2007).

Another factor is circadian rhythms: in some species, glucocorticoids are secreted periodically throughout the day. So, an apparently high level may simply reflect the time of day at which you measured it, not the animal's response to any stressor.

Slide 33:

The next limitation is that, given all these limitations of using adrenal activity measures as an indicator of animal welfare, it might seem better to use combinations of physiological measures. This can help, yet combined results can also be difficult to interpret because they may conflict.

For example:

- in the vicuña study, we saw that the muscle enzyme creatine kinase was elevated during captivity, especially in females. However, another muscle enzyme, aspartate transaminase (AST), was not significantly affected. This made it difficult to know if significant muscle damage had really occurred, i.e. perhaps there was no pre-pathological state or risk of myopathy. Another possibility may have been that the muscles were damaged, but the samples were taken before the release of AST occurred (as it is recognised that CK levels respond more immediately to muscle insult than do AST levels);

Sometimes, studies of the same questions that use the same physiological measures may give conflicting results. This happened in some of the studies we have looked at:

- in the vicuña study, the authors reported that other studies had found significant increases in aspartate transaminase when vicuñas were captured
- in the case of piglet castration, that procedure is known to activate the HPA axis. However, the study we have just looked at did not demonstrate a clear effect on urinary corticosteroids.

Slide 34:

The stress response is linked to and affects other physiological responses, in various ways. We can see, then, that those measures can also be used to assess the welfare of animals. We cannot look at all of them in any detail, but we will review some of them in today's lecture.

Today we will look at:

- Immune responses (e.g. white blood cell count)
- Neurobiological responses
- Metabolic responses

Slide 35:

The immune response enables animals to respond to foreign molecules and organisms. The organisms might be parasites, bacteria or viruses. The molecules might be released by such organisms, or come from other sources such as diet, or when the tissues within the body are damaged. The response of the immune system involves inflammation and other reactions.

Broadly, the immune response has two sections.

One is the 'innate' response, which serves to neutralise invading organisms (e.g. bacteria) or substances (e.g. suture material). The innate response is mediated by cells such as neutrophils and macrophages which engulf the foreign material. The response is also mediated by chemicals which, in the case of tissue damage, occur in a complex cascade including the complement system that creates inflammation. The innate response of the immune system is non-specific; it is not affected by vaccination.

The 'adaptive' immune response is mediated by lymphocytes. These are:

- T cells, which provide cell-mediated immunity. There are several different populations of T cells with differing functions such as removal of foreign proteins, and the priming and modulation of the rest of the immune system
- B cells, which produce antibodies in response to the proteins in foreign organisms or substances (e.g. viruses, pollens). This is known as humoral immunity.

In contrast to innate immunity, the adaptive immune response can be modified by exposure to the foreign organism or protein and it responds to vaccination.

The activation and operation of both parts of the immune response also involves numerous chemical messengers. One group is called cytokines. Two examples are Interleukin–2 and gamma-interferon (γ-interferon). Both these messengers are produced by the lymphocytes (T and B cells) and macrophages, and they help to induce certain populations of T cells and overall cellular immunity. However, there are many other chemical messengers besides these.

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The interaction between the stress response and the immune system is complex, involving changes in the immune cells' receptors and gene expression, and consequent activity.

The cells of the immune system develop in the immune tissues, i.e. bone marrow, thymus, spleen, lymph nodes and gut-associated lymphoid tissue. Many of the immune cells have catecholamine and glucocorticoid receptors on the cell walls. All of the immune tissues are also innervated by the sympathetic nervous system, and some immune cells produce adrenaline themselves.

When the stress response is activated, the immune tissues are exposed to noradrenaline from the sympathetic nerves. The tissues are also exposed to circulating adrenaline from the adrenal medulla and to cortisol from the adrenal cortex. The immune cells that are already in the blood stream are also exposed to adrenaline and cortisol in the blood.

There is ‘communication’ between the immune cells and cortisol and adrenaline. Research on this is ongoing.

Regarding the interaction with adrenaline, it seems that the SAM system can cause rapid and subtle alterations in the immune response: they can affect both the humoral responses, and the production of cytokines, and the action of some T cells. However, it is not yet clear when those effects are beneficial and when they are detrimental.

Much more is known about the interaction of cortisol with the immune system: broadly, cortisol is essential for a successful immune response to infection. However, if an animal has been under the influence of cortisol for some hours, days or longer, this may prevent him/her from mounting an adequate immune response.

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Cortisol can enhance or impede the immune response depending on the context.

When an animal is infected by a micro-organism, the initial local immune response causes release of several different chemicals into the bloodstream, e.g. leucotrienes.

These chemicals are detected by the brain, and this activates the stress response. Therefore, initially, infection is an acute stressor and this results in relatively moderate elevations of cortisol. This level of cortisol seems to help the initial immune response and thus enable the animal to recover from the infection.

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In contrast, very high levels of cortisol may have inhibitory effects on different parts of the immune response and therefore make animals vulnerable to infection.

Common features of animal husbandry can be stressors that give rise to very high or chronically elevated levels of cortisol. These stressors may be part of a natural process in the animal’s life cycle (e.g. parturition, lactation), or they may be modifiable (e.g. size of social group; time spent in transport without a break). However, whether they are natural or modifiable, such stressors create elevations in cortisol that can adversely affect the immune system.

Examples of the adverse effects of stress on the immune system include:

- an adverse imbalance in populations of T cells
- the inhibition of production of interleukin-2 and γ-interferon by lymphocytes and macrophages.

Such changes in the immune system in response to stressors tend to make animals more susceptible to disease. Note that the stressors do not cause the disease, but their effects on the immune system increase the animal’s risk of getting an infection. We will now look at some examples of this.

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The first example is mastitis in dairy cows. Mastitis is a very serious welfare problem for cows in countries where the demand for dairy products is high, and dairy farming is relatively intensive. As other countries also come to eat more dairy produce, by adopting more Western-style, processed foods, it is likely that mastitis will become a problem for cows there too.

Mastitis can occur at any time during lactation, and the levels of mastitis within a herd are affected by many factors. Among these factors is the physiology of parturition. Parturition is necessary for cows to lactate. High levels of cortisol are part of the endocrine mechanism that allows parturition to occur (as vets, we inject synthetic glucocorticoids to induce parturition). However, high levels of cortisol also induce an imbalance in populations of T cells and result in immune suppression which probably predisposes cows to mastitis. If husbandry is poor (e.g. poor nutrition, dirty bedding), and if the cows has other stressors such as pain due to lameness, they are at high risk of mastitis in the days just before and after calving.

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Another practical example of the interaction between stress and the immune response concerns how we handle young animals.

An Italian study looked at studied the effect of 5 minutes of gentle, daily human handling:

- in lambs who were left with their mothers
- in lambs who were taken from their mothers and reared artificially.

At three and 15 days of age, all the lambs were injected under the skin with a foreign antigen so that the researchers could monitor the antibody levels when the lambs were stressed subsequently (the antigen was hemocyanin from keyhole limpets).

The stressor was isolation: this is known to stress sheep because they are social. So, at 15 and 45 days of age, each lamb was put in a novel isolation pen for five minutes, then blood samples were taken.

- The results indicate the positive effect of the ewe on lambs' ability to generate an antibody response, and a similar positive effect of human handling on lambs who have been separated from the mother and reared artificially.
- The artificially reared lambs who had experienced daily gentle human handling and were then isolated showed much higher antibody levels than the lambs who had been artificially reared but did not experience any particular human handling. This suggested that positive handling of artificially reared lambs might help to ensure a strong immune response if the lambs were stressed by an infection. To verify that hypothesis, you would need to expose lambs to specific infections. However, the study illustrates that positive experience (a positive stressor) may tend to enhance the immune response.
- The naturally reared lambs also had high antibody levels, regardless of whether they were handled or not. The human handling did not seem to increase their antibody levels, probably because the ewe had such a significant role in the lambs' lives.

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The next examples show how a degree of stress may enhance the immune response, and how cortisol may be involved in this.

Briefly, it is well known that when young cattle are transported to market, sold, mixed with other calves, and then transported again to their new farm, they are at risk of developing respiratory disease. We also noted earlier that cortisol can enhance the immune response to infection. It is important to know how much transport animals can tolerate, before the stress becomes such that it predisposes them to disease.

One example studied gene expression in the neutrophils of ~eight-month-old beef bulls who had been transported by truck for nine hours. Neutrophils are an important part of the initial response against respiratory pathogens, and the researchers were investigating the relationship between the expression of relevant neutrophil genes with the plasma levels of cortisol and other adrenal steroids such as testosterone.

- They found that cortisol was significantly elevated during transport, and was associated with significantly raised levels of lymphocytes and neutrophils in the blood when compared to levels before transport.
- The transport was also associated with increased expression of certain neutrophil genes involved in immunity, when compared to their expression in the animals before transport. However, that gene expression was not clearly associated with the elevated cortisol levels alone. It seemed likely that it was the ratio of cortisol to some other adrenal steroids that was needed for optimal expression of the neutrophil genes and, therefore, a successful immune response.
- The study indicated that, for those bulls, nine hours of transport was stressful, but not necessarily so stressful that it reduced their immunity. On the contrary, the stress response seemed adaptive in terms of overall numbers of neutrophils and lymphocytes. However, the study did not detect a simple, linear relationship between the expression of neutrophil genes that might enhance immunity, and the levels of cortisol. (To understand this fully would probably require challenge studies, whereby animals are deliberately infected with respiratory pathogens and their clinical and cellular responses are assessed in relation to cortisol levels, transport duration and other factors.)

Our point today is not to draw definitive conclusions about the degree to which transport stress may predispose cattle to respiratory infection. It is simply to illustrate that stress –and cortisol in particular – is not necessarily bad for animal health, and to show that the interaction between cortisol and the immune system is complex.

To continue with our examples of this, we shall now look at a study of long-distance transport in cattle.

Slide 42:

There have been many studies carried out on the effect of long-distance transport on the welfare of farm animals in different countries.

One study measured gamma-interferon and many other immune and physiological measures in cattle who were being exported from Ireland to Italy. The animals were first transported by ship and truck for approximately 28 hours. They were then allowed to rest in lairage for 24 hours. They then travelled by truck for a further ~18 hours.

The study was complex but, briefly, immune and other measures indicated transient changes that were within the normal physiological range for the age and weight of animals involved. There was some immunosuppresssion in heifers compared to bulls, but none of the animals developed respiratory disease. The authors concluded that the 24-hour rest in the lairage, with hay and water freely available, allowed animals to recover substantially and adequately.

This suggests that, with appropriate management, journeys of up to ~28 hours may not adversely affect animal welfare. Modules 25 and 16 discuss practical aspects of the transport of livestock in more detail. We will see there that many factors can affect animals' welfare during transport and that, depending on those, a long time in lairage may not always enhance their welfare.

Slide 43:

We have now seen several examples of how positive and negative stressors can modulate the immune response of animals in the field. This is because of communication from the brain to the immune system via the SAM and HPA responses.

However, communication can go the other way, i.e. from the immune system to the brain. For example, there are glucocorticoid and cytokine receptors in the brain. This may be partly why infection – which is a stressor and generates both the stress response and an immune response – can cause a depressed mood.

So, not only can the stressor of infection or other illness activate the SAM and HPA systems and modulate the immune response, the resulting immune response can in turn affect the brain and emotions.

Moving away from the relation between the stress response, the immune responses and animal welfare, we shall now look at other brain-mediated responses.

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The brain is an extremely complex structure at the neurological and the neurochemical levels. Measurement is invasive, often requiring post-mortem tests. Therefore neurobiological measures are not a practical tool for welfare assessment in the field, only for research. However, we will still look at some of these measures briefly, because the animals you will see in practice will have this complex physiology occurring within their brains.

- Measurements can include brain activity, using electrophysiological measures or advanced imaging.
- The effect of potential stressors on learning and memory can also be assessed. Neurotransmitters and their receptors may also be measured.
- There are many neurotransmitters, each with a variety of receptors that may become more or less sensitive and more or less numerous, according to changes in the animal's feelings and situation. Today, we will briefly consider three examples of these many neurotransmitters. They are:
    - opioids
    - dopamine
    - prolactin.

Slide 45:

There are three types of endogenous opioids:

- **endorphins** – most commonly associated with welfare changes, and therefore used in welfare assessment
- **enkephalins** and **dynorphins** – not so clearly associated with welfare changes, and therefore less used in assessing welfare. However, both are involved in endogenous pain management, in the dorsal horn of the spinal cord and the brain.

Opioids seem to have three functions that are controlled by their release in the brain:

- stress-induced analgesia – opioids have a powerful analgesic effect. This analgesia may help animals to cope with some forms of adverse change in welfare, e.g. injury
- controlling the release of several hormones from the hypothalamus and pituitary glands which are involved in the stress response, including ACTH and prolactin
- the perception of pleasurable stimuli, such as the enjoyment of food.

There are different types of opioid receptor, and their relative concentration is affected by the presence of positive and negative stressors such as pain, confinement and pleasure.

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The examples on this slide indicate that the knowledge of opioids and their use in research are long-standing.

- In one study, when lambs underwent castration, tail-docking and mulesing procedures, without anaesthesia, the plasma ß-endorphin level was elevated between 3–10-fold. Mulesing is a controversial surgical procedure involving the removal of wool-bearing skin around the hindquarters to reduce the risk of fly-strike.
- In another study, sows who were tethered in single stalls for many months showed higher μ-receptor density in the frontal cortex than animals who had been group-housed for the same length of time. The μ-receptor density was positively correlated with the amount of time that the sows were inactive. In contrast, sows who actively responded to tethering – by developing repetitive (stereotypic) behaviours such as chewing the bars of their stall – had lower concentrations of μ-receptors and κ-receptors. This difference in receptor number and type might be mediated by glucocorticoids.

Slide 47:

The next neurotransmitter we will look at is dopamine, which is in the catecholamine group of neurotransmitters. There are a number of different dopamine receptors, and dopamine is involved in several pathways in the brain, including those that affect mood and voluntary movement. For the latter reason, dopamine has been studied in research on stereotypic behaviour (stereotypies).

Stereotypies are repetitive behaviours that are constant in form and serve no obvious purpose in the context in which they are performed. They are not typically seen in animals under natural conditions. However, they are well described in confined animals. For example, in some husbandry systems, sows are kept side by side in individual stalls. Many sows develop the stereotypy of biting the bar of their stall, and the earlier study of sows showed that those sows had relatively low number of D1 dopamine receptors in the frontal cortex.

The same has been found in stabled horses who performed a lot of stereotypies (Broom & Zanella, 2004).

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Prolactin is produced by the pituitary gland and is involved in milk production. However, it is also involved in modulating emotions and the stress response. For example, dogs with generalised anxiety are reported to have much higher prolactin levels than dogs who are less anxious, or normal.

Prolactin release is suppressed by several factors including dopamine from the hypothalamus.

In a clinical trial, researchers exposed hospitalised dogs to a synthetic, calming pheromone in their cages, while the dogs were in the clinic for routine surgery. The researchers measured several indices of stress in the blood including prolactin, immune measures and cortisol. They also measured the dogs’ behaviour.

The dogs exposed to the pheromone showed lower serum prolactin levels following routine surgery than dogs who had not been exposed to the pheromone. That result suggested that the pheromone preparation may have helped to reduce the dogs' anxiety. However, there were no clear effects of the pheromone on the dogs' behaviours or welfare overall, and it is not yet possible to say that pheromone preparations are valuable in helping to calm hospitalised animals.

Slide 49:

Next, we shall look briefly at metabolic responses to stressors.

Metabolic responses to stressors are the result of the activation of the SAM system and HPA axis, i.e. the stress response. The picture on the slide summarises this.

To review: the animal has internal and/or external sensory input to the brain. The brain evaluates the input and, if appropriate based on past experience, activates the stress response. That is, there is communication with the hypothalamus which:

- activates the SAM system, resulting in adrenaline and noradrenaline release from the adrenal medulla and in stimulation or inhibition of smooth muscle in organs such as blood vessels and the gut as well as in stimulation of cardiac muscle
- releases CRH which stimulates the anterior pituitary to release ACTH, which causes the adrenal cortex to release cortisol.

The combined results of this are increased metabolism and we can measure this.

Slide 50:

This slide gives some examples of metabolic responses to stress.

- Cortisol causes an increase in blood glucose through the breakdown of glycogen from the liver. Several of the studies we have mentioned included glucose in their measurements. Like cortisol, glucose is affected by the stress involved in restraint and venipuncture.
- Lactic acid results from the anaerobic metabolism of glucose, e.g. when an animal is running very fast to escape a threat, as part of the SAM response.
- Beta-hydroxy-butyrate is a ketone that is produced from the metabolism of fat when there is not enough glucose available, as can happen if animals are subjected to ongoing acute stress, e.g. in transport. This was one of the measures taken in the study of transport of cattle by ship which we looked at earlier.
- Haematocrit is the total red blood cell count. It is increased in preparation for running away, helping to ensure good oxygen delivery to the muscles, and is a result of activation of the SAM system and splenic contraction.
- Muscle enzymes are released into the bloodstream if animals are under extended physical stress. Earlier in this module we looked at a study of wild vicuñas, in which creatine kinase was elevated in the females when they were held captive for 24 hours following shearing.

- Metabolic hormones may also be used to assess an animal's metabolic response to a particular set of conditions. Examples are insulin and thyroid hormones. Insulin release is suppressed by cortisol following acute activation of the HPA axis.

Slide 51:

To sum up this lecture: you now know that when an animal's brain evaluates an event in his/her environment as being very different from past experience these events are called stressors and they can disrupt homeostasis. The animal responds with a number of physiological responses.

The stress response restores homeostasis which ultimately enables animals to survive. The stress response involves activation of the SAM system and HPA axis. They cause the adrenal gland to secrete catecholamines and glucocorticoids into the blood. The stress response also involves responses in other tissues that are innervated by the sympathetic and parasympathetic nervous systems. So we can see that the stress response has far-reaching effects throughout the body, and can influence various physiological systems. Chronic exposure to stressors can affect the expression of the stress response and its interactions with these other bodily systems.

You can measure some of the elements of the acute and chronic stress response quite easily, either by direct observation or using laboratory tests for plasma cortisol etc. However, you need to use measures of the stress response with caution because they have many limitations. Moreover, they cannot usually tell you if an animal's welfare is good or bad. Therefore, you need to use them in combination with other measures. The physiological measures are largely inter-related, and many of them directly reflect the stress response. They result from how the animal's brain has evaluated the sensory input that the animal is getting from its environment.

The various physiological responses to stress can strongly affected production traits (e.g. the stress of transport and handling at an abattoir can result in so much lactic acid production that the meat is affected.) We will consider the relationship between welfare and production in other lectures.

Other measures are needed to assess welfare and this will be covered in other lectures.