A neurobiological basis for welfare assessment

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Introduction

Rapid urbanisation of society, and devout religious demands, have triggered a growing recognition of animal rights and welfare. Withi recognition has come a consumer, and moral movement, based drive to ensure the need for animals welfare is maximised. This nee encompassed such catch cries as 'quality of life', and the 'five freedoms', but unfortunately these provided little assessment ability (a thirsty an does not always drink when water is provided, and we should not be hasty in judging quality of life by our own confused standards).

Assessment cannot be taken lightly. Inadequate assessment, or lack of a suitable knowledge base to achieve it, can produce ignorance and wo a wanton disregard for the animals used in such assessment. Returning to the example of 'thirst' - a simple experimental design might examine whether cfos, an intermediate early gene product was expressed in osmoreceptive areas of the brain. This will tell us if those areas active in a period prior to examination. It of course has the beauty of being a non-invasive measure but requires sacrificing the animal alternative design would be to monitor the activity of this neural area (both electrically and neurohumorally) during different situations of 'pote thirst', and provision of water supply, correlate this with systemic measures such as circulating vasopressin, urine output and behaviour o reasonable period of time in a behaving, free field animal. At the completion of this the animal can be returned to a 'normal' life. The techno exists to do this type of experiment, albeit far more expensively than the first. The potential yield in terms of animal welfare data is howeve higher and morally more than justifies the additional cost. The first experimental design may lead to a guess, the second to a quantifiable prac The same number of animals would be involved, the welfare outcomes completely different. Robert Sapolsky, one of the world's lea endocrinologists, sums this approach up eloquently in his 1992 book (1) "Stress, the aging brain, and mechanisms of neuron death". I paraph this - "for the rats and the pain of these experiments may these pages (sic - results) justify the forced involvement of these animals". My own b is that as yet we do not have adequate tools, methodologies, or fundamental understanding needed to truly assess an animal's welfare wit rather circular qualifier being the prime need for a low enough methodological invasiveness that the method in itself does not compromis animal's welfare. Perhaps the real goal of the animal welfarist heading into the new millennium is not - "what are the welfare problems w our livestock systems? (which on the surface appear painfully obvious)" - but "how do we actually assess them?". As Clint Eastwood (2 succinctly put it - "a mans gotta know his limitations" - but perhaps be willing to exert considerable perspiration to extend them!

This assessment will obviously cover a broad number of sciences, many of which I am not qualified to comment on. As such within the con of this paper I attempt only to cover some of the new approaches towards assessment. Such a narrow approach is bound to reflect my pers biases and I apologise in advance to those in fields, of equal contribution to welfare, of which I do not know enough to give objective and bala representation.

Development of new Technologies

One of the key areas in approaching welfare is the development of the ability to assess physiological state in animals whilst they are freely (wi restraint) behaving and without the additional imposition of uncontrolled experiment(er) related stress (such as direct obtainment of b samples). The development of telemetric devices to log or transmit data concerning heart rate (and electrocardiographic cycle) and b temperature (3, 4) represents a major step in this direction. Longer term interpretation of this data, particularly the interdependence and integra of different body area temperatures and of cardiac events over time will require considerable attention.

The development of a remote sampling device to allow obtainment of blood samples from animals in the field represents a major advance This has led to some remarkably interesting and original observations in the deer model relating to chronic stress (6). Development of more n invasive measures of hormones , such as in faecal, urine or saliva samples (7, 8) also represent a major development in the area of stress stu These approaches have yielded fascinating insights into the importance of individual, species, hierarchy position per se and type of hierar resource availability, previous stress experience, and aggressive nature on determining stress responsiveness (8, 9, 10).

The advent of microdialysis development (11) produced new methodologies for the study of brain function in conscious animals. Early utilisa of these techniques enabled mechanistic approaches to quantifying welfare within preslaughter stun technologies (12, 13). Microdialysis pro have allowed approaches to the hypothalamo-pituitary axis and concurrent correlation between hormonal activity at this level and systemic (14). Further development should allow pituitary portal circulation monitoring without the extreme trauma of current cannulation methods (

Obtainment of data in real-time represents a considerable development as it allows both the measurement of factors that may degrade quickly samples (such as ACTH) and the experimental ability to manipulate at any point during a recorded physiological event. Real-time measure neurohumoral events have been achieved both within the brain and systemically, using implanted microprobes, during stress and are reveal of individual differences (15, 16, 17). The ability to be able to manipulate an event during its occurrence offers an approach to determining ca links. The low invasiveness and rapid microminiaturisation of these technologies offer advantages as does the removal of the need for ac withdrawal of the sample from the animal. Combining this technology with other methods of real-time measurement (15) may provide tools assessing how the central nervous system processes, perceives and responds to stress.

The updating of classical evoked potential methodologies through the use of sophisticated technology has also opened opportunities to as cognitive processing prioritising during stress.

Presented in the following sections are a number of examples of the use of these types of technologies and their development in approach questions of welfare.

Preslaughter stunning, microdialysis and the evoked potential

At the turn of this century growing concerns about the ways in which animals were killed at abattoirs emerged. Stunning an animal prior to slaughtering if not only offered some increase in welfare of the animal but also of the human worker in terms of increased movement safety (18). With the advent of stunning came the need to ensure that the animal was unconscious during the subsequent slaughter procedure. Use of electroencephalograms (a measure of overall electrical activity in the brain) suggested that with electrical stunning (the most common employed method) a state similar to grand mal epilepsy in humans occurred immediately upon stun application (19). In humans there are no accounts of consciousness during this state suggesting strongly that consciousness is lost in animals following electrical stunning. A useful research tool to further support this was the use of evoked potential techniques. Evoked potentials consists of the electrical activity associated with the brain's perception and response to an external stimulus (20). These potentials are signal averaged out from the brain's background electrical noise by time locking to repeated stimulus delivery. Using this technology does not determine consciousness directly per se but can determine unequivocally insensibility to a particular stimulus (if the evoked responses are not present the stimulus is not being received). Using this procedure it appears that following electrical stunning there is a relatively long period of insensibility to pain (21). In the case of young calves individual animals can display a prolonged period from throatcut to loss of consciousness. Potentially this could result in an animal recovering from the preslaughter stun prior to throatcut induced anoxic loss of consciousness (22). To deduce this required an approach to measuring neurochemical response, and effect, following stunning and throatcut. This led to methodologies utilising microdialysis techniques. These suggested that the neurotransmitter glutamate was responsible for the epileptic type seizure and ultimately brain death (13). Stunning and throatcut had a synergistic action on glutamate causing much higher levels than either alone. Mimicing or antagonising these levels allowed a manipulation of state of consciousness suggesting causal effect as did the mirroring of the neurotransmitter profile to observed behavioural effects. A second neurotransmitter gamma-amino-butyric acid activated by the stun resulted in an analgesic state. Again these was a synergy between electrical stunning and throatcut. This synergy results in a longer period of unconsciousness, a greater analgesia and subsequently a better welfare related practice. This is a good example of where a welfare need led to a fundamental understanding which in turn provided tools to better assess a welfare need.

Evoked potentials have reemerged as of interest to welfare assessment because of developments that allow their obtainment in the field from unrestrained animals. An interesting hypothesis under test is that the processing of an innocuous stimulus may be re-prioritised in the face of competing stress related stimuli, and that such prioritisation could be reflected in the evoked potential. Prioritising different stimuli in terms of potential stressfulness would be a useful welfare assessment.

Assessment of pain

Pain, and its control, represents one of the most contentious welfare issues. As is has been reviewed extensively (23, 24, 25) I will only briefly dwell on it. In livestock studies cortisol has often been used as an assessor of pain response (26, 27) and while it does offer a useful approach to realistic treatment it also has its limitations. Lactating animals have a remarkably 'flat' cortisol response to stressors possibly attributable to suckling and milk letdown related pulsatility in oxytocin and prolactin (28). Other measures of stress, or pain, in these animals are however present including sympathetic changes (28). New vistas have now been made into both sex and individual differences in pain perception and the outcomes of this for effective therapeutic treatment (29). Understanding the mechanisms that control different opioid receptor expression and different pathways of analgesia in animals (30) also offers therapeutic and practical options including sensitive assessments. Interestingly in measuring analgesia in sheep, to a thermal stimuli, both cortisol and heart rate gave adequate measures of analgesia per se but behavioural measures gave a more sensitive measure of actual analgesic titre (29). In standard pharmacological assessment of analgesics in rats tail flick response offers a more sensitive assay of analgesia than systemic physiological measurements. This is a clear example of the need for a holistic science approach to welfare issues.

Learning to adapt and the adaptative nature of social status stress

An animal's ability to learn avoidance or adaptation to a stress and ability to take that learning into other situations of stress is an extremely important determinant of its 'welfare state'. This ability can differ considerably with social status and change thereof. These differences are often expressed in basal and stress induced cortisol levels and in other changes within the hypothalamo-pituitary-adrenal axis (31). In primates dominant animals seem to fare much better than subordinates whereas in other species the reverse occurs (8, 9, 31). Dominants appear to fare less well in highly aggressive, resource scarce, social environments where the cost of maintaining dominance is likely to be high. In sheep (32), within stable flocks, animals in the mid order tend to display signs of stress more easily than dominant or subordinate counterparts. These same animals appear more active in aggressive interactions (both losing and winning!). If animals are subjected to an experimental stressor in which avoidance is possible through associative type learning animals tend to fall into a bimodal population grouping - the larger group being those that learn to successfully avoid, the other those that don't (10). Long term basal and stress related cortisol levels became different in these groups following these experiments (10). Neurochemical profiles in the brain also differed during the learning situation. In poor "learners" cortisol levels were much higher than successful "learners" and this influenced glutamate release during the learning situation and subsequent memory consolidation (33). Pharmacological manipulation suggests that there is an inverted U dose relationship between glutamate and cortisol in the hippocampus (an area of the brain) and successful "learning". Pharmacological manipulation during the learning situation can change previous patterns of success or failure even in animals that appear to be "longterm affected". Another area in the brain, the nucleus accumbens, also appears to show profile differences.

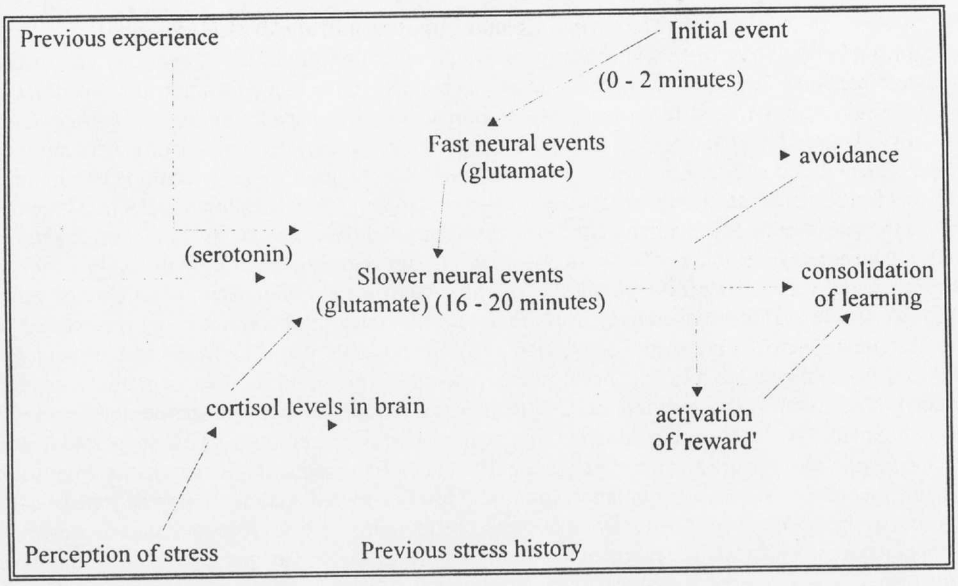

Figure 1.Schematic overview of stress related learning. Glutamate and serotonin are neurotransmitters in the brain. The events represnted in this figure appear to take place in the hippocampus and the nucleus accumbens, areas of the b

This area has been linked to addiction and reward behaviours and has been speculated as involved in "feelings" of pleasure following succes Animals that show successful learning exhibit high activity in this region 20-30 minutes after avoidance, poor animals do not (35). Ag pharmacological manipulation, within the appropriate window of time, the activity can be suppressed or enhanced and this has significant upon subsequent success and response to stress (39). In summary there are two major areas of contribution one integrating inherent memory (through the neurotransmitter glutamate) and perception of the stress situation (through their level of cortisol) into learning consolidation, th signalling the reward value to the animal possibly reinforcing the adaptive behaviour. I have not gone into detail to suggest a pleth pharmaceutical possibilities that arise from this work but rather to suggest the detail needed to begin to understand how to assess welfa use of a single animal in an experiment justifies "heroic" attempts to achieve that needed detail or we cannot with honesty express a reg animal welfare.

Summary

One of the key issues in animal welfare is the provision of adequate assessment. This requires a background of fundamental excelle experimental science combined with a genuine desire to achieve an ethical balance in animal use. Methodological advances mean that we ca 'eavesdrop' on the function of the brain in behaving animals with the necessity of only a low level of invasiveness. Such an approach may n help us in addressing Chalmer's (36) central philosophical problems of what is conscious mind - and what species does it exist in but also p a detailed and realistic means of approaching as assessment capability for animal welfare.

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