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BRAIN MEASURES WHICH TELL US ABOUT ANIMAL WELFARE

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Abstract

Studies of the brain inform us about the cognitive ability of animals and hence affect the extent to which animals of that species are respected. However, they can also tell us how an individual is likely to be perceiving, attending to, evaluating, coping with, enjoying, or disturbed by its environment so can give direct information about welfare. In studies of welfare, we are especially interested in how an individual feels. As this depends upon high-level brain processing, we have to investigate brain function. Brain correlates of preferred social, sexual and parental situations include elevated oxytocin in the para-ventricular nucleus of the hypothalamus. Abnormal behaviour may have brain correlates, for example high frequencies of stereotypy with down-regulated mu and kappa receptors and dopamine depletion in the frontal cortex. Such results help in evaluating effects of treatment on welfare. Some brain changes, such as increased glucocorticoid receptors in the frontal lobes or increased activity in the amygdala, may be a sensitive indicator of perceived emergency. Some cytokine production in the brain depends on immune system function, mediated via vagal nerves, whilst some leads to sickness effects. Some aspects

of brain function can be temporarily suppressed, e.g. by opioids when there is severe pain, or permanently impaired, e.g. in severely impoverished environments or during depression. Coping attempts or environmental impact can lead to injury to the brain, damage to hippocampal neurons, remodelling of dendrites in the hippocampus or other brain disorganisation. The brain measures can explain the nature and magnitude of effects on welfare.

1. Introduction

When assessing the welfare of animals it is necessary to evaluate: the extent of any adverse impacts of the environment on the individual, the magnitude of difficulties in coping with such impacts and the degree of positive aspects of welfare (Broom 1988). Welfare encompasses the health of the individual and a wide range of feelings (Dawkins 1993, Fraser et al 1997), the feelings being a part of the various systems for coping with the environment (Broom 1998, 2001, Rolls 1999). Almost all of the coping systems are regulated by the brain and many adverse effects of the environment involve the brain so it is important to try to measure changes in the brain when assessing animal welfare. However, for many people involved in animal welfare research there is a moral problem concerning some brain monitoring techniques. We want to understand coping systems but there are limits as to what techniques we wish to use to discover how the brain is involved. There is a range of severity of effects associated with brain research. Some techniques involve no adverse effects on individuals, some involve nothing which would not occur in the absence of research, for example studies of farm animal brains after slaughter. Other studies are of animals which would not otherwise be kept in captivity, or of animals whose welfare is slightly affected, poor, or very poor as a result of the investigation of brain function. Individual researchers decide what they wish to do, or are restricted in what they do by national legislation or by the ethics committees of journals or Institutions. If results are obtained and published, they should be used, if necessary

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with a comment on moral aspects of the procedures used and on the desirability of further studies of this kind, otherwise the death or poor welfare of animals has been in vain. In this paper we exemplify and review some of the studies of brain function which help us to understand coping systems or to evaluate animal welfare. The involvement of the brain in coping systems is so widespread that only a sample of relevant work can be included but links to other welfare assessment methods are emphasised.

2. Cognitive ability in animals

Some brain studies, often in combination with experimental studies on learning, reveal substantial cognitive ability in animals. One example of such work is that of Kendrick and colleagues at the Babraham Institute in Cambridge (Kendrick and Baldwin 1987, Kendrick et al 1995).

When sheep were shown a variety of pictures of sheep and other animals, they were able to discriminate them and carry out an operant response. The sight of the stimuli was accompanied by firing in cells of the medial temporal and prefrontal lobes of the cerebral cortex. Some cells fired whenever the face of a sheep with horns was seen but there was little or no firing to a sheep with no horns and no firing to a picture of a pig, dog or human. Other cells fired most when a particular familiar individual sheep was seen.

In recent work (Kendrick et al 2001) sheep were trained to discriminate between 25 pairs of photographs and were sometimes heard to vocalise when they made the discrimination. The discrimination, with accompanying firing in cortical cells, could still be made by most sheep when they were tested 600 days later. Some decline in discrimination ability and concomitant firing in cortical cells occurred between 600 and 800 days after initial training. Many of the discriminations were for photographs of particular sheep faces but the sheep could also discriminate human faces and remember

them for the same amount of time. Cattle have also been shown to be able to discriminate among individuals of their own species (Hagen and Broom 2003).

3. Links between brain function and welfare

In order to use brain measures in the evaluation of animal welfare it is necessary to understand some of the ways in which the brain controls coping mechanisms or is affected by adversity. What is happening in the brain during feelings and other coping mechanisms? Where the relevant brain changes are detectable, it may be possible to find out the extent to which efforts are being made to try to cope or the magnitude of harm which is being done to the individual. Some of the key questions concerning these issues are listed in Table 1.

Table 1. Questions about possible links between brain function and welfare

1. Are there links between having difficulties and brain function?
2. Are there links between pleasure and brain function?
3. Do difficulties or pleasure lead to detectable brain changes?
4. Are **there** effects, such as body system damage, mediated via the brain?
5. Are coping methods, and hence welfare indication, linked to brain function?
6. If any of these links do exist, which systems and which parts of the brain are involved?
7. Are factors during early development capable of organising or disorganising brain systems, thus altering coping responses?

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There is a large amount of evidence relevant to these questions. However, a relatively small proportion of this has been found to be of practical use. Some examples of such evidence will be presented. Section 3.1 on 'brain changes and good feelings' helps to

answer questions 2 and 3. Section 3.2 on 'glucocorticoid binding and action in the brain' is relevant to questions 1 and 3-7, as, to a lesser extent, are Section 3.3 on 'opioid function in relation to abnormal behaviour' and Section 3.4 on 'immune system activity, depression and brain concomitants'. The recent use of 'brain imaging in relation to happiness or sadness', the subject of Section 3.5, provides one level of answer to question 3.

3.1 Brain changes and good feelings.

Early efforts to discover which parts of the brain are associated with good feelings were not very successful. It was discovered that animals could be trained to carry out an operant response, such as pressing a lever, in order that a small electric current would be administered to a region of their brain. (Olds and Milner 1954). However, there was no simple pleasure centre because self-stimulation would be initiated when there were electrodes in very many different parts of the brain. More recent studies have shown how behaviour is related to brain reward systems (Spruijt et al 2001), that oxytocin is related to pleasurable stimulation and that happy situations elicit activity in specific brain regions.

Oxytocin concentrations in blood are elevated during several pleasant experiences, such as during: milk ejection and suckling in mammalian mothers, other maternal care, and social bonding. Oxytocin binds to receptors that regulate HPA axis activity and increases in plasma oxytocin are associated with: decreases in glucocorticoids and adrenocorticotrophic hormone(ACTH), proliferation of lymphocytes, increased gamma-

amino-butyric acid (GABA) and increased vagal tone (Carter and Altemus 1997, Redwine et al 2001, Altemus et al 2001). Systemic oxytocin treatment induces a decrease in the mRNA expression for glucocorticoid receptors in the hippocampus and an increase in the mRNA for mineralocorticoid expression in the same brain region in rats (Petersson and Uvnäs-Moberg, in press). Mineralocorticoid receptors, which have great affinity for glucocorticoid hormones, are important in the "buffering" of the stress responses.

Some brain effects can impair pleasure. If dopamine is lacking in the anterior cingulate gyrus it becomes more difficult to enjoy pleasurable stimuli.

3.2 Glucocorticoid binding and action in the brain.

Glucocorticoid production following activation of the hypothalamic-pituitary-adrenal cortex (HPA) axis is part of an important emergency response. However, it is now clear that cortisol and corticosterone have a much wider range of functions than was once thought. Glucocorticoid receptors have been found throughout much of the mammalian brain. At basal levels cortisol binds primarily to mineralocorticoid receptors (MR) but under stressful circumstances any excess of cortisol binds to glucocorticoid receptors (de Kloet, 1991). In particular, any MR and GR have been found in the frontal lobes of the cerebral cortex, in the amygdala and in the hippocampus (Reul and de Kloet, 1985). The roles of these brain regions in cognitive processing, memory and emotional response suggest that cortisol could be involved in these processes. The amygdala is known from a range of studies to be associated with fear responses (Panksepp 1998). It projects to the paraventricular nucleus (PVN) of the hypothalamus where corticotrophin releasing hormone (CRH) regulates ACTH. Information from cortex, hippocampus and amygdala activates the stria terminalis and thence the PVN (Nelson 2001). Indicators of activity in the PVN could be associated with fear and other difficulties.

Mammals show rhythms of cortisol production with a peak activity in the morning (Mendl et al 1992). The function of this diurnal rhythm, if there is a function, has been the subject of considerable debate. Recent studies (Holzman et al pers. comm.) show that pregnant women who are stressed in various ways show much less clear diurnal rhythms of plasma cortisol with no morning peak. Given the role of glucocorticoids in various brain mechanisms, this effect may link with important brain functions. It is surmised that animals whose environment has such an adverse impact upon them that they are unable to use cortisol in an adaptive way are subjected to chronic emotional overload.

When piglets are weaned from their mothers at an early age, for example at two weeks, they show many disturbances of behaviour and physiology (Worobec et al 1999). Zanella et al (in prep.) have shown that an array of steroids and metabolites are present in hippocampal tissue of early-weaned piglets. Hippocampal cells are capable of metabolising cortisol *in vitro*, as shown by incubating the cells with low or high concentrations of cortisol (Zanella and Mendl, 2000). The cortisol is removed from the medium around the hippocampal cells but remains there in controls without such cells. In further experiments with piglets, which were controls or subjected to 15 minutes of social isolation, whereas hippocampal glucocorticoids increased three-fold after isolation of controls they did not increase after isolation of early-weaned piglets (Zanella et al in prep.). It is suggested that the adaptive role of cortisol could not occur in the early-weaned animals. If animals, including humans, are unable to use this adaptive mechanism, welfare may be poor. Laughlin and Zanella (in press) found that these same early-weaned piglets had impaired spatial memory when subjected to social isolation stress prior to testing. In a test of learning to reach a submerged platform, the Morris-Water Maze test, (Laughlin et al in prep.) piglets which were early weaned, but not isolated prior to the test, rapidly decrease the latency to find the submerged platform by three or four trials out of seven exposures, with ten minutes of interval between

exposures. However, animals weaned early and isolated for 15 minutes prior to the spatial task did not improve performance over seven trials (Laughlin and Zanella in press). It would appear that the effects of the double problem interfered with the ability of the piglets to learn, possibly because of hippocampal malfunction.

Given the role of glucocorticoid hormones in altering the phenotype of cells, integrative measures of gene expression in animals subjected to different treatments may be very informative. The impact of early-weaning on the overall expression of genes in the hippocampus of pigs weaned at 12 days of age or control animals is currently being investigated (Poletto et al 2003) using micro-array. Micro-array experiments allow the detection of overall differences in gene expression. Using a collection of 880 genes sequenced from brain samples collected at slaughter from domestic pigs Poletto et al (2003) reported significant differences in the expression of genes as a result of weaning age and social isolation. Social isolation and early-weaning caused a decrease in the expression of genes associated with protein synthesis and an increase in the expression of genes associated with cell differentiation.

3.3 Opioid function in relation to abnormal behaviour.

Stereotypies are repeated sequences of behaviour with no apparent function (Broom 1993). Such behaviours are often associated with inability to control interactions with the environment in a wide variety of animals including humans. Some of the most dramatic stereotypies are shown in confined sows (Blackshaw and McVeigh 1984, Broom and Potter 1998, Cronin et al 1998). It seems that sows' needs are not met at all well in stalls and tethers so they show either substantial amounts of stereotypy or apathetic, unreactive behaviour. When Zanella et al (1996) studied sows which show high levels of stereotyping, they were found, after slaughter, to have low mu and kappa receptor densities and low dopamine concentrations in the frontal cortex. Inactive, unresponsive

sows, on the other hand, had more mu receptors in the frontal cortex. Other brain studies of animals showing stereotypies include those of McBride and Hemmings (2001) who found that housed horses which performed more stereotypies had more dopamine (DI) receptors in the nucleus accumbens than those housed horses which performed few stereotypies. It is possible that the reduction in frontal cortex cell membrane opioid receptors in pigs which show behavioural abnormalities could be a direct consequence of glucocorticoid receptor activation. The reason for the nucleus accumbens changes in the stereotyping horses is not clear but could be linked to the action itself rather than to any underlying poor welfare.

3.4 Immune system activity, depression and brain concomitants.

When immune system activity is high because the individual is encountering pathogen attack or tissue damage, there are various consequences (Dantzer 2001). One effect is on vagal nerve activity, presumably because of the various defensive responses associated with vagal activity (Porges 1998). Other changes following high levels of immune system activity are increased production of cytokines in the brain and related effects on brain and body which are associated with feelings of sickness and behaviours associated with sickness. The consequences of feelings of sickness are generally adaptive, even if they are unpleasant.

When people are depressed there are various negative effects on hippocampal and other brain function, as well as impairment in immune system function (Irwin 2001). Those who study animal welfare have much to learn from the literature on human depression and those who investigate, or try to treat, human depression have much to learn from work on the welfare of confined, defeated, or seriously frustrated pigs, cows, dogs, rats and hens.

A wide range of environmental impacts have specific consequences for brain function. Sapolsky et al (1992) and McEwen (2001) describe stressful events leading to impaired learning ability, impaired memory, damage to hippocampal neurons, remodelling of hippocampal dendrites, suppression of neurogenesis, changes in neurotransmitter distribution and disorganisation of brain function.

3.5 Brain imaging in relation to happiness or sadness. What is happening in the brain when individuals are happy or sad? It is now possible to monitor brain activity non-invasively using magnetic resonance imaging (MRI) or positron emission tomography whilst the subject individuals have more or less pleasant experiences. Sudheimer et al (2001) showed sad pictures to people whilst scanning their brains using MRI. A set of regions were found in which there was activity during the viewing of sad, but not during neutral or cheerful, situations. It is not surprising that animals' enormously important systems for coping with problems in life have specific brain system function as part of their mechanism. It is likely that the brain changes associated with happiness or sadness will be a major topic in medical and veterinary research in the immediate future.

Conclusions and animal welfare implications

The brain is involved in many different mechanisms for coping with adverse environmental impact. Hence there are links between measures of brain function and most other animal wefarte indicators. Investigation of brain function can help us to understand how animals cope, how much they are having to do in order to cope, and the extent to which damage is being done to the individual, Those conducting studies of animal welfare may gain valuable insights by the use of brain measures. However they should consider the adverse effects on welfare of some kinds of brain investigation when deciding to what extent they will use brain measures.

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