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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 abilities 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, and so can give direct information about welfare. In studies of welfare, we are especially interested in how an individual feels. Since 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 are associated with down-regulated μ and κ receptors and dopamine depletion in the frontal cortex. Such results help in evaluating the 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. Active immunological defences lead to cytokine production in the brain, vagal nerve activity and sickness effects. Some aspects of brain function can be temporarily suppressed, for example, by opioids when there is severe pain, or permanently impaired, for example, 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 to other brain disorganisation. Brain measures can explain the nature and magnitude of many effects on welfare.

Keywords: *abnormal behaviour, adrenal, animal welfare, brain measures, coping, opioids*

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 the use of 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 in the severity of effects associated with methods used in brain research. Some techniques involve no adverse effects on individuals; some involve nothing that would not occur in the absence of research, for example, studies of farm animal brains after slaughter. Other studies are of animals that 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 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 the animals involved has been in vain. In this paper we exemplify and review some of the studies of brain function that 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.

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 & Baldwin 1987; Kendrick *et al* 1995).

When sheep were shown a variety of pictures of sheep and of other animals, their operant responses indicated that they were able to discriminate amongst these. The sight of the stimuli was accompanied by firing in cells of the medial

Table 1 Key questions concerning the possible links between brain function and welfare.

1. Are there links between having difficulties in coping and brain function?
2. Are there links between pleasure and brain function?
3. Do difficulties in coping 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?

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 when shown a picture of sheep with no horns, and no firing when shown 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 600–800 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 & Broom 2003).

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 mechanisms involved in coping? 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 that is being done to the individual. Some of the key questions concerning these issues are listed in Table 1.

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 in this paper. Section 1, 'Brain changes and good feelings', helps to answer questions 2 and 3. Section 2, 'Glucocorticoid binding and action in the brain', is relevant to question 1 and to questions 3–7, as, to a lesser extent, are Section 3, 'Opioid function in relation to abnormal behaviour' and Section 4, 'Immune system activity, depression and brain concomitants'. The recent use of 'Brain imaging in relation to happiness or sadness', the subject of Section 5, provides one level of answer to question 3.

1. Brain changes and good feelings

Early efforts to discover which parts of the brain were 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, which triggered administration of a small electric current to a region of their brain (Olds & Milner 1954). However, there was no simple pleasure centre because self-stimulation would be initiated when electrodes were placed 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 (Carter 2001). Oxytocin binds to receptors that regulate hypothalamo-pituitary-adrenal (HPA) axis activity, and increases in plasma oxytocin are associated with decreases in glucocorticoids and adrenocorticotrophic hormone (ACTH), proliferation of lymphocytes, increased γ-amino-butyric acid (GABA) and increased vagal tone (Carter & Altemus 1997; Altemus *et al* 2001; Redwine *et al* 2001). In rats, 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 (GP Moberg unpublished data). 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.

2. Glucocorticoid binding and action in the brain

Glucocorticoid production following activation of the HPA axis is part of an important emergency response. However, it is now clear that the glucocorticoids 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 (GR) (de Kloet 1991). In particular, many MR and GR have been found in the frontal lobes of the cerebral cortex, in the amygdala and in the hippocampus (Reul & 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 the cortex, hippocampus and amygdala activates the stria terminalis and thence the PVN

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(Nelson 2001). Indicators of activity in the PVN could be associated with fear and with other difficulties.

Mammals show rhythms of cortisol production with a peak in 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 (C Holzman *et al* unpublished data) 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* (unpublished data) have shown that an array of steroids and metabolites is present in the 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 & Mendl 2000). The cortisol is removed from the medium around the hippocampal cells only when the hippocampal cells themselves are present. In further experiments with early weaned and control piglets, which either were or were not subjected to 15 min of social isolation, whereas hippocampal glucocorticoids increased three-fold after the isolation of controls, they did not increase after the isolation of early weaned piglets (AJ Zanella *et al*, unpublished data). 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, their 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, piglets that were early weaned, but not isolated prior to the test, rapidly decreased their latencies to find the submerged platform after three or four trials out of a total of seven, with 10 min intervals between trials (K Laughlin *et al* unpublished data). However, early weaned piglets that were isolated for 15 min prior to the spatial task did not improve their performance over seven trials (Laughlin & Zanella in press). It would appear that the effects of the double problem (ie early weaning and social isolation stress) 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 of control animals, is currently being investigated using micro-arrays (Siegford *et al* 2003). 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, Siegford *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. Opioid function in relation to abnormal behaviour

Stereotypies are repeated sequences of behaviour with no apparent function (Broom 1983). Such behaviours are often associated with an inability to control interactions with the environment in a wide variety of animals, including humans (Broom & Johnson 1993). Some of the most dramatic stereotypies are shown by confined sows (Blackshaw & McVeigh 1984; Broom & Potter 1984; Cronin *et al* 1985). 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 (Broom *et al* 1995). When Zanella *et al* (1996) studied sows that displayed high levels of stereotyping, they were found, after slaughter, to have low μ and κ (opioid) receptor densities and low dopamine concentrations in the frontal cortex. Inactive, unresponsive sows, on the other hand, had more μ receptors in the frontal cortex. Stall-housed sows had higher dynorphin levels in the frontal cortex after slaughter than group-housed sows (Zanella *et al* 1998). Other brain studies of animals showing stereotypies include those of McBride and Hemmings (2001) who found that stabled horses that performed more stereotypies had more dopamine (D1) receptors in the nucleus accumbens than stabled horses that performed few stereotypies. It is possible that the reduction in frontal cortex cell membrane opioid receptors in pigs that 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.

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 include increased production of cytokines in the brain and related effects on brain and body that 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 has specific consequences for brain function. Sapolsky (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.

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 (PET) whilst the subject individuals have pleasant or unpleasant 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 the viewing of 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 welfare 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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