IMMUNOLOGICAL MARKERS OF STRESS AND WELL-BEING IN DOMESTIC ANIMALS.

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INTRODUCTION

Stress has been recognised for over a century as a trigger for altered physiology. It has not been possible until recently to monitor neuroendocrine functions sufficiently critically to provide links between CNS function and systemic physiology and more recently, immune function. Claude Bernard (1878) described the physiological response of animals to produce homeostasis in response to environmental stimuli characterised by their variability and the 'milieu interieur' characterised by its constancy. Fraser et al (1975) described stress as "an abnormal or extreme adjustment in the physiology of an animal to cope with adverse effects of its environment and management".

When the rate or level of change, within the environment, demands a significant physiological response by the host the stimulus may be defined as a stressor. Physical stressors include extremes of temperature, restraint, transport, surgery, exposure to novel sounds, sights, odours, tastes or noxious chemicals. Behavioural stressors include overcrowding, capture of wild animals, hierarchical challenge, exposure to unfamiliar surroundings, or sensory deprivation through isolation. The stressors above are perceived as such by the animal, through cognitive recognition of the alterations in their external environment. This triggers a neurophysiological response, that impacts upon many target organs within the body (Figure 1), causing alterations in physiology which impact, on the rates of blood flow, metabolism and sensitive reproductive or immunological tissues.

Neurophysiology of Stress

The limbic system of the CNS is involved in adaptation and with neuroendocrine and emotional responses to stressful signals. The limbic system evaluates the stressful signals in the context of past experiences and stimulates an appropriate response in the visceral brain. The hypothalamus serves as the efferent arm which stimulates sympathetic activity and endocrine secretions

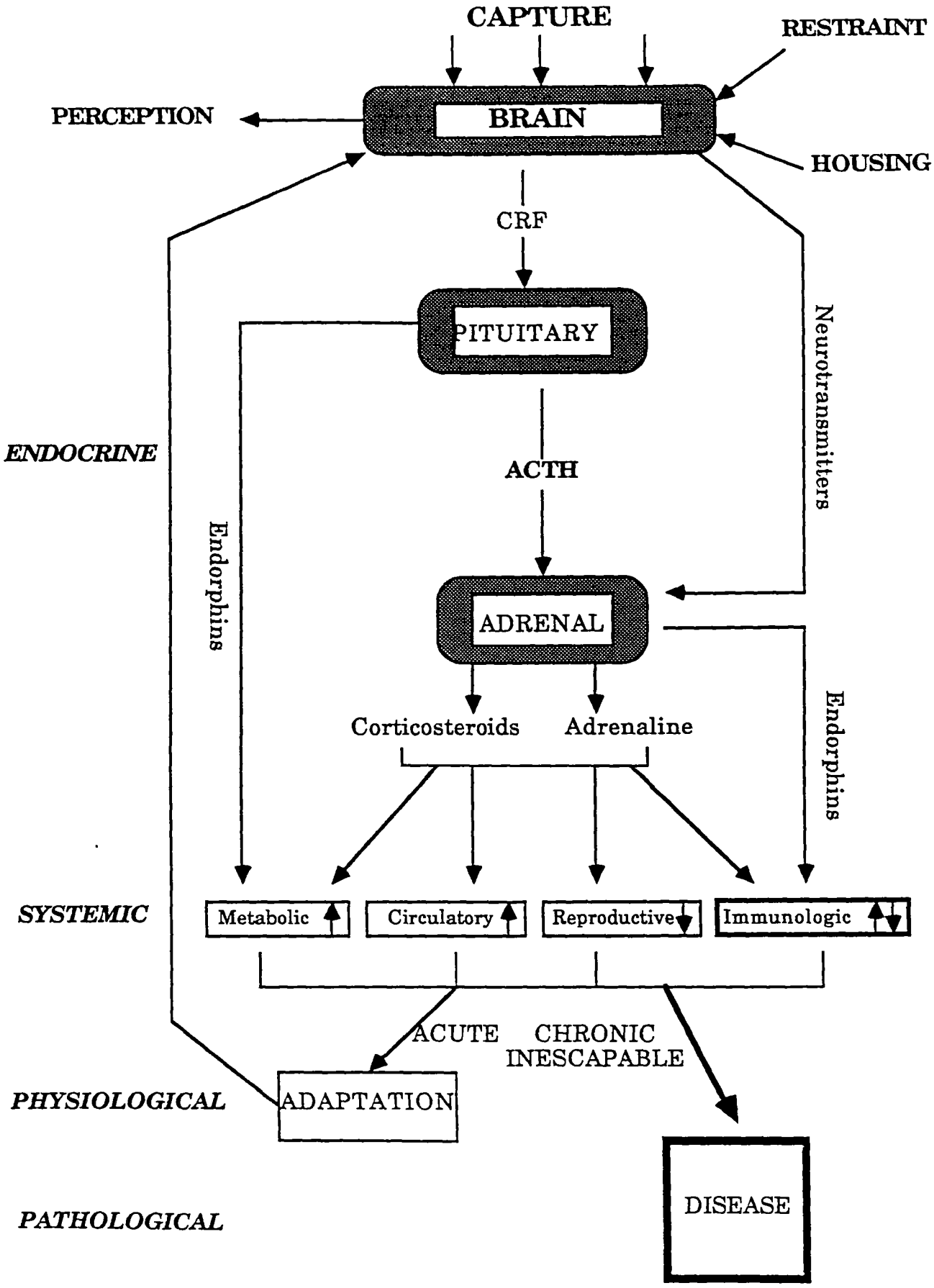

Figure 1

STRESS AND DOMESTICATION OF ANIMALS

including vasopressin, oxytocin and corticotropin-releasing factor (CRF). The production of CRF stimulates the pituitary to produce a cascade of hormones, central of which are adrenocorticotropins (ACTH). ACTH stimulates the adrenal cortex to produce corticosteroids and thus the cycle is completed. Simultaneously autonomic nervous stimulation activates production of catecholamines in the adrenal medulla. Apart from CNS production of CRF, certain primary lymphoid organs such as thymus, can produce CRF. Thymic hormones also increase production of ACTH. In addition to the hypothalamic-pituitary-corticoadrenal (HPA) and the sympathetic-adrenomedullary axis (SPA), a third axis involving neuropeptides (NP) such as opioids, enkephalins and endorphins, is activated in the stress response. In gross terms the three pathways of CNS activation, set up systemic responses which produce the general adaptation syndrome (GAS), first described by Selye (1946). The triology of responsiveness to stress, described by Selye, include the alarm reaction (Flight/Fight) (SPA), the resistance (Conservation) phase (HPA) and finally adaptation (NP) or exhaustion (SPA, HPA, & NP).

Neuroimmunological Links between CNS and LRS

In recent years a large body of data has established bidirectional links between the brain (CNS) and immune (LRS) system. Whereas the CNS responds to signals from the external environment, the LRS similarly responds to changes within the host, functioning as an involuntary sensory organ. Both CNS and LRS function as central organs which send signals to all tissues within the body, through an integrated communication system, involving endocrine factors and receptor recognition.

Lymphocytes produce ACTH, vasoactive intestinal peptide (VIP), TSH, Prolactin and endorphins and express receptors on their surface for ACTH, VIP, Substance-P, Prolactin, growth hormone, corticosteriods, catecholamines, acetylcholine, hormone releasing hormones and opioids (Griffin, 1989). Opioid and ACTH receptors have also been found on granulocytes, monocytes and platelets. All the neuroendocrine factors tested above have an impact *in vitro* on lymphoreticular cell function, which may result in immunosuppression or immunoenhancement, depending on the dosage involved (Spector, 1990).

Stress and Altered Disease Resistance

Pasteur's early studies (1878), which established the 'germ theory' of infection, used a hypothermia model to demonstrate increased susceptibility of fowl to anthrax (reviewed Griffin, 1989). Ishigami (1919) (Cited in Khansari et al, 1990) showed reduced phagocytic activity in tuberculous patients during episodes of emotional stress and he postulated that a stressful life led to immunodepression and increased susceptibility to tuberculosis. Perinatal stressors (Curtis, 1974) and hypothermia in piglets, (Blecha & Kelley, 1981) increase their susceptibility to infectious disease. Shipping fever associated with transport of cattle is a well documented infectious syndrome (Hoerling, 1980). Transport stressors caused increased susceptibility to disease in calves (Staples & Haugse, 1974) and weaner deer (Griffin et al, 1991). Stressors associated with capture, adaptation to farming, adverse climate and breeding have been associated with increased susceptibility of deer to acute bacterial (foot-abscess) and viral (MCF) disease and chronic infection (tuberculosis) (Griffin, 1989).

Behaviour - Neuroendocrinology - Immunology

To date a solid body of evidence suggests a bidirectional link between factors produced by the CNS and LRS. However, no functional links have been established which suggest how the systems interact *in vivo*. Although behaviour is used as a key indicator, of stress and well-being in animals, the links between neuroendocrinology, immunology and behaviour have yet to be established. Evidence from our laboratory suggests that behavioural responses may infer that an animal is extremely aversive to a given treatment. Restraint of 'needle shy' animals evoked an extremely aggressive behavioural response but still produced little physiological response and caused potentiation, rather than suppression, of immunocompetence (Griffin et al, 1988).

Adrenalectomised animals are shown to develop a significant level of immunosuppression unrelated to corticosteroids when subjected to severe acute stress (Keller et al, 1983). Other factors which may be of special relevance in altered immune function during stress are CRF and opioid peptides. CRF has been shown to be a potent immunosuppressive agent *in vitro* (Audhya et al, 1991). Shavit et al (1984) found that escapable foot-shock stress

mediated by non-opiate factors had no effect on immune function, whereas inescapable stress, which produced opiate mediated analgesic was consistently immunosuppressive. It has been shown subsequently that this pathway of suppression can be reversed by treatment with melatonin (Maestroni et al, 1988). Long term restraint (12hr) of mice has been shown to exert immunosuppressive effects on antibody production and CMI in mice (Okimura & Nigo, 1986). In contrast, limited restraint, which induced increased levels of corticosteroids and B-endorphin, did not have any effect on immunocompetence (Flores et al, 1990).

Early endocrine studies concentrated heavily on the role of corticosteroids as the key factors, of functional relevance during stress (Selye, 1973). The general inference was that stress induced steroid production, which in turn exerted an anti-inflammatory and immunosuppressive effect. Recent studies (Griffin et al, 1988) however, suggest that moderate levels of steroid production may produce immune enhancement rather than suppression. A recent model (Mason, 1991) advances the concept that steroids, produced as the end point of the stress response, are regulated by genetic factors within a species, which impact selectively on T-cell subpopulations. Steroid levels which suppress cell-mediated immunity (CMI) and inflammation have no influence on antibody production. Animals with a low stress status, produce low concentrations of steroids and have a vigorous CMI and high levels of resistance to intracellular organisms (parasites, viruses and Tb). In contrast, high stress status animals, have increased susceptibility to intracellular parasites and low CMI. This model suggests that the stress response may be a relevent experimental probe for disease resistance to certain infections. In a recent study in our laboratory, a transport and relocation paradigm, using red deer, compared lymphocyte proliferation, antibody production and corticosteroid levels, in transported and non-transported animals. Lymphoproliferation (expressed as radioactive counts per minute-CPM) to the T-cell mitogen, ConA, was significantly reduced up to 21 days post-transport (Figure 2A). The level at 7 days after transport, being reduced to 55% of the pre-transport level. Animals immunised with a novel antigen (keyhole limpet haemocynanin) pre-transport had significantly attenuated antibody responses, as measured by ELISA, when compared with animals immunised pre-transport (Figure 2B). Plasma cortisol concentrations fluctuated pre- and post-transport, with elevated levels at 1 and 7 days post-transport, that were not significant (Figure 2C).

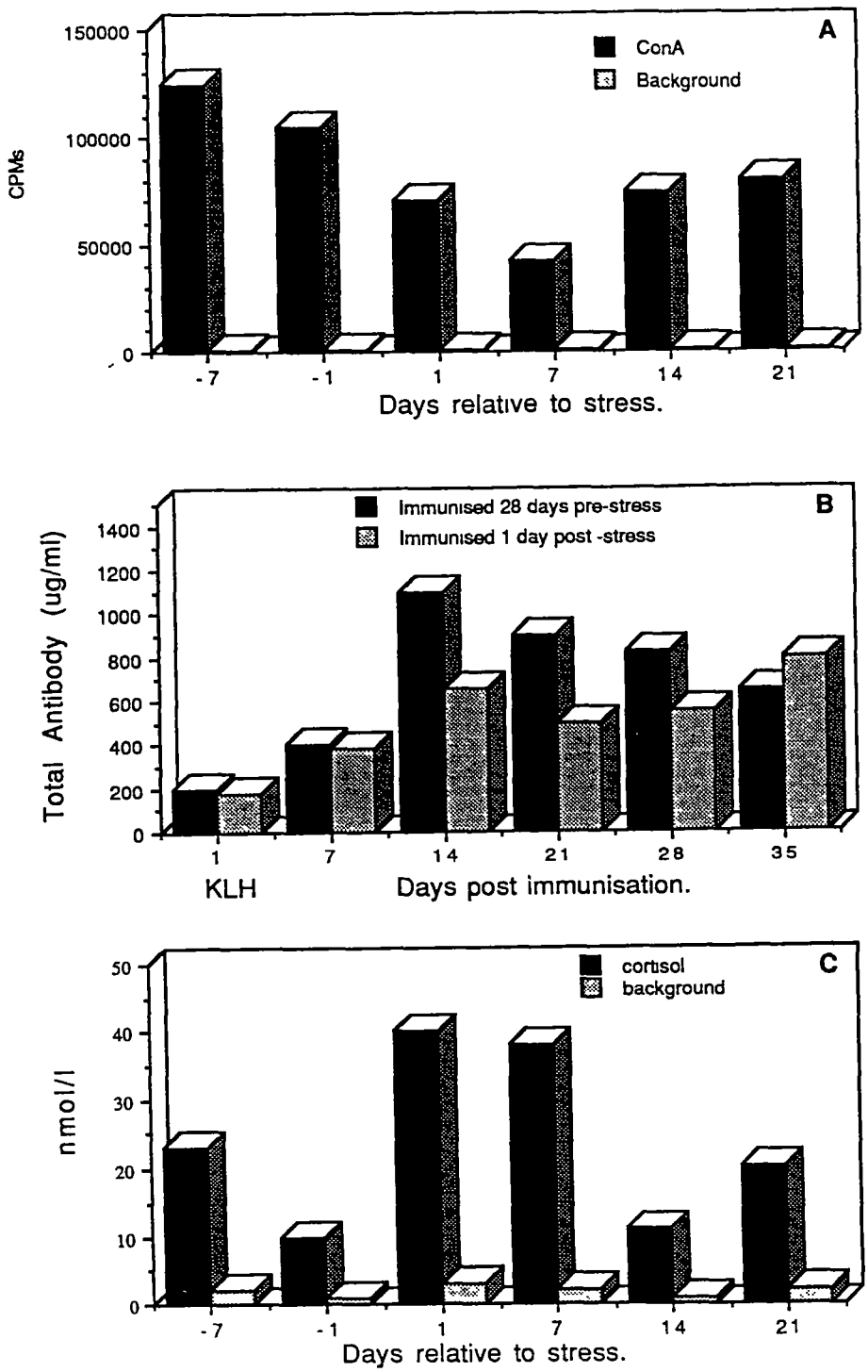

Figure 2

IMMUNOLOGICAL and NEUROENDOCRINE MARKERS OF STRESS: A-Mitogenic response to ConA in animals exposed to stress (Day-0); B-Levels of total immunoglobulin specific for KLH in animals exposed to stress; C-Levels of cortisol in animals exposed to stress (Day-0).

Stress and Well-being

Social expectations in civilised society demand that the animals used for food production or as companions, should be allowed to live their lives free from abuse. Not only does this serve to affirm our sacred right to assert dominion over animals, but pragmatism should demand that unless the conditions under which our animals live, are adequate, then the animals cannot express their full genotypic and/or phenotypic potential. An environment that is socially enriching and challenging and which allows for appropriate hierarchical interactions is necessary to sustain good levels of health in all animals. Well-being is compromised by inescapable or chronic stressors, whereas acute reversible stressors may be fortifying. Classic stressors such as transport, may be completely neutralised by sensitive human attention to animal well-being. We have transported 50 hinds, all in the last weeks of their pregnancy 700km to relocate them because of management constraints associated with an outbreak of Tuberculosis. One healthy fawn was born in transit and no adverse effect was seen in the remaining hinds, 47 of which produced healthy fawns within four weeks of relocation. This affirms that transport *per se* need not produce stress or evoke untoward physiological changes within a host, if the humans involved pay proper attention to animal well-being.

Most studies on stress in animals have used extreme stress models so there is little information currently available on neuroendocrine markers which typify low grade stressors. Using the array of sensitive receptors on LRS cell it may be possible in the near future to produce new immunoassays using lymphocyte receptor expression or activation to define the stress response and allow us to implement interventions which remove adverse stressors and return animals to a plane of well-being. Detailed longitudinal studies will be necessary to chart responses unique to individual species. The scope of such studies must be extended beyond corticosteroids to focus on hypothalamic, pituitary and opiatergic factors. Critical control of model systems, is necessary, to establish representative responses, which can accomodate an individual animal's perception of their environment, rather than those which fortify our prejudice, as to what humans perceive to be stressful, for animals.

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