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Corticosterone responses to captivity and sampling stress in the mallard (Anas platyrhynchos) and grey duck (Anas superciliosa)

A thesis presented in partial fulfilment of the requirements for the degree of

Masters of Science
in Physiology
at Massey University

Mark Forman
1994

Abstract

1. The aim of this study was to investigate the influence of capture and captivity stress on plasma corticosterone levels and breeding success in mallard and grey ducks. Measurements of plasma corticosterone levels were used to identify factors that cause stress and to identify individual birds with low stress responses and a greater likelihood of a successful breeding in captivity than their peers. The effect on corticosterone levels of the stress associated with the collection of blood samples was investigated and different sampling regimes for measuring corticosterone responses to stress were examined. The use of exogenous glucose administration and the placing of ducks into darkened boxes to lower corticosterone levels was also studied.

2. Corticosterone levels in wild mallards after capture were higher than levels in ducks held captive for 3 or 5 months. Corticosterone levels decreased in captive ducks in relation to the time spent in captivity and amount of contact spent with people.

3. Corticosterone levels repeatedly measured over 8 months varied between individual captive grey duck. The only 2 female ducks to rear ducklings, and their mates, all had lower corticosterone levels before the breeding season than the remaining 4 female and 3 male ducks.

4. The variation in corticosterone levels and responses between individual grey duck and the negative relationship between corticosterone levels and body weight may have been due, in part, to the existence of a dominance hierarchy amongst the grey ducks.

5. Corticosterone levels in winter may indicate potential breeding success and, can be used to identify stressful factors in the captive environment.

6. The observation of an increase in corticosterone levels with the sampling and handling of ducks depends on the magnitude of levels in the first sample obtained and on the frequency with which samples are obtained thereafter.

7. High corticosterone levels will decrease if a duck is placed in a darkened individual box or given an oral dose of 10 ml of 0.84M or 1.38M

glucose.

8. It is concluded that the measurement of corticosterone levels can be used to indicate factors that may affect the breeding performance of birds. Methods for minimising the stress associated with the capture and captivity of wild birds can also be identified from the measurement of corticosterone levels.

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Contents

Chapter 1:

General introduction

1. General introduction

Captive breeding of some native New Zealand birds occurs for conservation purposes. The removal of birds from predation should help to increase breeding success while guarantee a readily obtainable food supply. However despite the placement of birds in a captive environment which, as based on habitat studies, mimic natural habitats the birds may not breed at all or even die. In such situations the birds are unable to adapt to captivity and are said to be under stress.

In this study the stress associated with capture and captivity was measured in wild mallard ducks, with plasma levels of the adrenal gland hormone corticosterone used as a measure of stress. Ways to reduce capture and captivity stress were examined. The variation between individual mallards in their corticosterone responses to capture and captivity was noted and further investigated amongst a group of native New Zealand Grey duck over 8 months and discussed in conjunction with their breeding performance during this time.

Grey ducks were used in this study as they provided a species of waterfowl known to be reluctant breeders in captivity (Marchant and Higgins, 1990) and hence more likely to be influenced by any stress associated with captivity than their Mallard counterparts. Mallards were used for studies of, and ways to relieve, stress associated with capture and captivity as they provided a species of waterfowl abundant around the area of study and hence readily obtainable by trapping.

1.1 Stimuli, stressors, stress and distress

Before discussing stress associated with the capture and captivity of wild birds it is important to define the context in which the terms stress, distress and stimuli that are stressful (stressors) are used. Firstly, stimuli that elicit physiological responses that may include a rise in corticosterone levels are discussed. Next the variation in the range of physiological responses that various authors use to indicate stress, and hence stressors and distress, is

discussed. Finally the context in which stress is used in this study is explained.

Numerous stimuli, both artificial and natural, such as temperature extremes, starvation, disease organisms, electric shock, handling, repeated sampling, transportation, chasing and loud noises have been found to increase corticosterone levels in birds (reviewed by Mench, in press). Such stimuli have also been found to induce increases in the catecholamines, adrenaline and noradrenaline, released from the adrenal gland (reviewed by Harvey *et. al.*, 1984). Other responses exhibited by birds towards the above stimuli may be stimulus specific such as neurally mediated skin vasodilation in hot temperatures and withdrawal behaviour from the source of an electric shock (Siegel, 1971). Corticosterone, catecholamine and stimulus-specific responses are said to occur when the bird has interpreted the stimuli as threatening, or perceiving to threaten, its homeostasis or well-being.

In order to encompass the corticosterone, catecholamine and stimulus-specific responses of an animal exposed to stimuli affecting (or perceived to affect) its homoeostasis Selye (1936) used the term General Adaptation Syndrome (G.A.S.). The G.A.S. consists of three distinct sequential stages or reactions; the alarm reaction, the stage of resistance and finally the stage of exhaustion. Siegel (1980), cited Selye (1963) in further stating that the stimuli inducing such responses as outlined in the G.A.S. are stressors and stress is the general term used to describe the animal's defensive response to the stressor.

The alarm reaction involves responses such as catecholamine mediated vasodilation and stimulus specific behaviour such as ruffling of feathers to increase cooling in response to hot temperatures. The stage of resistance involves the release of corticosteroids while the exhaustion stage occurs when stimuli such as disease organisms cannot be either avoided or adapted to by corticosterone, catecholamine or stimulus-specific responses.

The problem with defining stress in terms of the G.A.S. is the presence of situations where only the alarm reaction occurs (Freeman, 1985). For example, the ruffling of feathers to increase cooling during hot temperatures

could be thought of as an alarm reaction and hence interpreted as indicative of stress. As stress is generally perceived as having a negative influence upon an animal's well-being the use of stress in describing an animal's defensive response to such environmental stimuli is confusing (Harvey *et. al.*, 1984; Freeman, 1985). However, Williams (1984) stated that the use of the term stress is generally positive and adaptive in that it describes reactions combating or accommodating stimuli.

Although still describing alarm reactions as indicative of stress, Henry (1993) cited Seyle (1974), in using the term "distress" to describe a response to a stressor that involves corticosterone release while stress without distress occurs in predominantly alarm reactions to stressors. Other authors further defined distress in describing corticosteroid responses to stressors only where the magnitude and duration of the response is above that exhibited by the animal in response to innocuous stimuli such a midday rise in ambient temperature (Ewbank, 1973; Harvey *et. al.*, 1984; Freeman, 1985). Hence distress is used to describe situations which are unpleasant, incur some cost or damage to the animal and possibly causes suffering (Ewbank, 1992).

In this study, the use of the word stress is limited to describing the presence of stimuli believed to have induced a rise in corticosterone levels in the birds studied. For example, "capture stress" is used to describe the stimuli associated with the capture of birds which elevated their corticosterone levels. The individual stimuli which may have contributed to the overall capture stress, such as the presence of humans or being contained within a trap, are labelled stressors. The use of the term distress is avoided in this study as it is not known whether any of the observed corticosterone responses of the mallards or grey ducks are any different from responses that could result from innocuous stimuli.

1.2 Physiological measurements of stress

The use of corticosterone levels as one physiological parameter in describing whether certain stimuli are stressful is based upon experiments

demonstrating elevation of corticosteroid levels in animals when they are exposed to stimuli labelled stressful e.g. restraint (Harvey *et. al.*, 1984). Corticosterone levels can be used to measure and compare the severity of stress in birds (Brown, 1961) as the magnitude and duration of any corticosterone response depends on the stressor applied (Harvey *et. al.*, 1984).

However, the use of parameters such as plasma corticosterone levels in quantifying the disturbance caused by certain stimuli means all stimuli which increase corticosterone levels, including egg laying in chickens, are termed stressors (Freeman, 1985). Ewbank (1992) avoids describing a relatively innocuous increase in corticosterone levels as being indicative of stress by comparing any increase in levels with previous corticosterone responses induced by stimuli that caused some damage or disadvantage to the animal, such as debeaking of chickens, that is presumably noxious.

Duncan (1981) suggests using more than one physiological parameter when deciding whether a stimulus is stressful to a bird. Situations could exist where the absence of a corticosterone response could be interpreted to indicate the absence of any disturbance created by certain stimuli, but the catecholamine response could be as marked as responses to other stimuli said to be stressful.

Despite the limitations of using only plasma corticosterone levels to detect stress, measurements of corticosterone levels could help to detect stimuli in the environment of birds which are stressful and that could be removed to improve the bird's welfare. For example, comparisons of the corticosterone responses of birds placed in solitary as opposed to communal confinement for transportation may reveal which type of confinement is the least stressful (Freeman, 1971). The maintenance of low levels of environmental stress is especially important in captive breeding programmes where greatest success will be achieved from captive environments in which the birds are exposed to as few stressors as possible.

1.3 Plasma corticosterone levels and the hypothalamo-pituitary-adrenal axis

In understanding why stressful stimuli inducing an increase in plasma corticosterone levels should be avoided among captive birds if possible, an appreciation of how corticosterone acts to resist or adjust to such stimuli within the bird is useful. Increased circulating levels of corticosterone can induce the mobilization of energy stores (gluconeogenesis), suppression of reproduction, immunosuppression and suppress inflammation (Siegel 1971, 1980; Harvey *et. al.*, 1984; Axelrod and Reisine 1984). The suppression of reproduction is of greatest concern in captive breeding programmes of wild birds.

For stressful stimuli to induce an increase in plasma corticosterone levels, a series of hormonal events must occur within the bird before the presence of such stimuli in the birds environment leads to elevated corticosterone levels. The hypothalamo-pituitary-adrenal (HPA) axis modulates the corticosterone response to stress in birds. The release of corticotrophic releasing factor (CRF) and arginine vasotocin (AVT) from the hypothalamus triggers the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland which in turn releases corticosterone from the adrenal gland (Harvey and Hall, 1990).

CRF and AVT are released from paraventricular nuclei (PVN) neurons in the hypothalamus when they are stimulated by the catecholamine neurotransmitters adrenaline and noradrenaline and other ß-adrenergic agonists (Rees *et. al.*, 1985a). Serotonin and gamma-amino-butyric acid (GABA) are another two neurotransmitters that are released from neurons synapsing on PVN neurons and they may stimulate CRF and AVT release (Harvey and Hall, 1990).

Certain stimuli are implicated in increasing the turnover of the neurotransmitters that induce CRF and AVT release. For example, adrenaline and noradrenaline content and turnover in hypothalamic neurons is increased when birds are starved while serotonin turnover is increased during water deprivation but not starvation (Harvey and Hall,

1990). Hence it is the presence of stressful stimuli that triggers hypothalamic neural activity, HPA activation and finally corticosterone release.

CRF is secreted into the hypothalamic portal vasculature from CRF containing neurons while AVT containing neurons terminate on the posterior pituitary. CRF and AVT act synergistically to evoke the release of ACTH at the pituitary with CRF also inducing the transcription of pro-opiomelanocortin (POMC) mRNA, the precursor for ACTH synthesis (Harbuz and Lightman, 1992).

Although the release of ACTH from the pituitary is primarily controlled by levels of CRF and AVT, its release may be inhibited by hypothalamic somatostatin (SRIF) and by corticosterone negative feedback (Harvey and Hall, 1990). Corticosterone feedback regulation of ACTH is directed towards inhibiting a surge in ACTH release that occurs in response to stressors (Harbuz and Lightman, 1992). CRF levels are also controlled by a corticosterone feedback inhibition which regulates basal CRF levels rather than inhibiting any surge in CRF as seen in response to stressors (Harbuz and Lightman, 1992).

Corticosterone secretion is primarily stimulated by ACTH, though ACTH-like factors originating from the pineal or hypothalamic sites possibly also have a stimulatory effect (Holmes and Phillips, 1976; Harvey *et. al.*, 1984). ACTH causes the proliferation of adrenocortical tissue and induces concentration-dependent increases in the release and biosynthesis of corticosterone. Corticosterone release and biosynthesis may also occur autonomously from adrenal gland cells (Holmes and Phillips, 1976).

Other hormones that can stimulate corticosterone release include pituitary growth hormone (GH) and prolactin (PRL) from the pituitary gland (Carsia *et. al.*, 1984, 1985; Cheung *et. al.*, 1988), while parathyroid hormone (PTH) from the parathyroid gland can induce corticosterone biosynthesis in the same manner as ACTH (Rosenberg *et. al.*, 1988). Triiodothyronine (T_3) may reduce corticosterone release as hypothyroidism (low T_3) potentiates corticosterone synthesis (Carsia *et. al.*, 1985) while prolonging the half-life of corticosterone in plasma (Kovacs and Peczely, 1983). The catecholamines, adrenaline and noradrenaline, released from

adrenomedullary cells in the adrenal may potentiate ACTH induced corticosterone secretion (Rees *et. al.*, 1985).

The effect of gonadal steroids on plasma corticosterone levels is uncertain. Assenmacher *et. al.* (1975) state that testosterone increases plasma levels of both corticosterone and corticosterone binding proteins while Carsia *et. al.*, (1987) found that testosterone inhibits corticosterone biosynthesis. These variable effects of testosterone on corticosterone biosynthesis and release could be due to seasonal variation in the responsiveness of the adrenocortical tissue to gonadal steroids (Silverin, 1979).

Other factors that may cause increased plasma levels of corticosterone include alterations in adrenal blood flow (Harvey *et. al.*, 1984) and changes in the characteristics of corticosterone binding proteins (Gould and Siegel, 1978; Wingfield *et. al.*, 1984). Increased plasma levels of corticosterone decrease the sensitivity of adrenocortical cells to ACTH, demonstrating a negative feedback relationship of corticosterone on its own biosynthesis and release (Carsia *et. al.*, 1983).

In conclusion, while an increase in plasma corticosterone levels can be due to the presence of stress and subsequent activation of the HPA axis, other factors such as the plasma levels of gonadal or thyroid hormones may effect the magnitude and duration of any corticosterone response.